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Identification and Controlling Verticillium Wilt Infecting *Parkia roxburghii* Seedlings in Manipur India

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ABSTRACT

A study was conducted to control *Verticillium* wilt disease of *Parkia roxburghii*. It was isolated from the infected roots of the seedlings collected from Forest Nursery of Chandel district, Manipur, India. Four fungicides viz. Bavistin 50% WP, Dithane M-45, Dithane Z-78 and Fytolan 50% WP were screened against the pathogen at different concentrations using poison food technique *in vitro* and then in field-conditions using soil drenching technique. The results revealed that Bavistin 50% WP was most effective at 75 ppm with 100% growth inhibition followed by Dithane M-45, Dithane Z-78 and minimum efficacy was observed by Fytolan 50% WP with 31.27% growth inhibition in laboratory conditions. All selected fungicides were evaluated against pathogen in field conditions at 0.1 and 0.2% concentrations. Bavistin 50% WP was reported to be the most effective at 0.2% concentration. Thus, the present study recommends soil drenching with Bavistin 50% WP at a minimal concentration of 0.2% for management of the disease.

Key words: Disease management, *Parkia roxburghii*, *Verticillium dahliae*, wilt

INTRODUCTION

Parkia roxburghii G. Don is a tall evergreen tree of family Fabaceae with straight tree trunk. The seed pod is a sough after vegetable in Manipur and Mizoram (India). Its wood is usually supplemented as fuel-wood. The increasing demands of seedling support its popularity as an important species of today's avenue plantation. Verticillium species have been reported to cause wilt disease in various trees species (Bilgrami *et al*., 1991; Ligoxigakis, 2000). It has also been reported to cause disease in fruit crops (Harris and Yang, 1996; Stapleton *et al*., 1993) forest trees and woody ornamental plants (Ligoxigakis, 2000; Pegg and Bradly, 2002; Levin *et al*., 2003), legumes (Krikun and Bernier, 1987), vegetables (Subbarao *et al*., 1995; Nagao *et al*., 1997). The disease is expressed as a complex function of the species and strain of the pathogen as well as degree of host resistance and environmental conditions (Sanei and Nassrollahnejad, 1995; Ligoxigakis and Vakalounakis, 1997; Sanei *et al*., 2008).

V. dahlia has been reported as a soil-borne pathogen (Pegg and Bradly, 2002) and many authors have listed the host plants (Ligoxigakis, 2000; Pegg and Bradly, 2002) and its prevalence and density (Dervis and Bicici, 2005). The methodology for estimation of inoculums density in soil has also been worked out (Lopez-Escudero and Blanco-Lopez, 2005).

Verticillium wilt causes decrease in fiber quality and yield in cotton (Erdogan *et al*., 2006) and various control measures have been worked out by several authors worldwide (Ozbay and Newman, 2004; Goicoechea, 2006; Shaigan *et al*., 2008; Ajibesin *et al*., 2008; Mutty and Hossenkhan, 2008; Ikeura *et al*., 2011).

During nursery disease survey, 80% mortality in *Parkia roxburghii* seedlings was noticed in Forest Nursery at Chandel, Manipur, India. Despite the economic and ecological importance of plant pathogenic fungi, much remains to be learned about their biology, particularly their host and geographic ranges. Even for common, easily found pathogens such as *Verticillium dahliae*, it appears that the pathogens are much more diverse and their host relationships are more complex than commonly assumed. A first report often provides important information for increasing our knowledge and understanding about these emerging plant pathogens. Hence, we felt it was imperative to study the incidence of *Verticillium* wilt on *P. roxburghii*.

MATERIALS AND METHODS

Diseased samples along with infested soil were collected from Forest Nursery at Chandel district, Manipur in 2008 and brought to Rain Forest Research Institute, Jorhat in sterile polyethylene bags for conducting detailed pathological studies.

Isolation of *V. dahliae* from affected seedlings: For isolation, the infected seedlings, root, stem and leaf tissues exhibiting vascular discoloration were rinsed thoroughly in tap water and air-dried for 5-10 min. Infected tissues were aseptically cut to dimensions of approximately 5-10 mm long and surface-disinfested in 0.525% NaOCL solution for 2 min, rinsed twice in sterile filter papers. Disinfested tissues were plated on potato dextrose agar (PDA, Difco) and ethanol agar (20 g of agar, 6 mL of 95% ethanol, 1 L of distilled water) amended with streptomycin sulphate at 100 μg mL^{-1} to inhibit bacterial growth. Petri plates were incubated at 23-24°C in the dark for 5-7 days. After incubation, hyphal plugs of each growing isolate colony were transferred to petri dishes (6 cm diameter) of PDA amended with the same concentrations of antibiotics and incubated in the dark at 23°C for 10 days. Colonies forming microscletoria were identified as *V. dahliae* according to the taxonomic features of the fungus via microscopic examination. Single-spore isolates of *V. dahliae* were obtained on water agar medium amended with 100 μL mL^{-1} streptomycin sulphate and maintained in vials containing PDA at 4°C.

Isolation of *V. dahliae* from infected soil: Infected soil samples were collected from Forest Nursery at Chandel, Manipur India and were brought to the laboratory using plastic bags. Samples were air-dried homogenized using a revolving jar mill and stored at 4°C. For isolation of *V. dahliae* from soil the wet sieving procedure (Huisman and Ashworth, 1974) was followed.

Pathogenecity test: Seeds of *P. roxburghii* were collected from the same district for conducting nursery experiments at RFRI, Jorhat. Two hundred and fifty seedlings of *P. roxburghii* were raised from seeds in germination trays using sterilized soil mixture (sand: soil: FYM = 1:1:1) and transferred to polyethylene bags containing 1 kg sterilized soil mixture. All polyethylene bags were arranged in 5 replicates of 50 seedlings each, out of which one replicate served as control. For pathogenecity test three fungal microsclerotia were randomly selected and cultured on C Zapeck solution agar medium. After 7-15 days, a fungal suspension with the concentration of 10^6 spores per mL was prepared for inoculation of *P. roxburghii* seedlings. The pathogenecity of the organism was confirmed by inoculating healthy seedlings with fungal spore suspension prepared from 15 days old culture and each replicate of 50 healthy seedlings of 6-8 cm height was drenched with 25 mL of fungal spore suspension. Control seedlings were treated only with distilled water. Diseases incidence was evaluated five to six weeks after inoculation according to the procedure described by Hamdollahzadeh (1993).

The efficacy of four fungicides viz. Bavistin 50% WP, Dithane M-25, Dithane Z-78 and Fytolan 50% WP were evaluated *in vitro* using poison food technique (Dhingra and Sinclair, 1985) against *Verticillium dahliae*. For this a stock solution of selected fungicides was prepared. The requisite amounts of the fungicides were mixed with PDA medium just before pouring to get desired concentrations of 25, 50, 75 and 100 ppm and gently shaken for thorough mixing. The PDA plates containing the fungicides were inoculated aseptically with pathogen by transferring five mm diameter agar disc from fresh cultures. Three replications were maintained for each treatment. PDA without any fungicide served as control. All the inoculated petri plates were incubated at 25±1°C. The radial growth of the test fungus in the treated plates was measured after five days and compared with control. The percent inhibition of fungal growth was estimated using the formula given by Vincent (1947):

$$I = \frac{C\text{-}T}{C} \times 100$$

Where:

I = Percent inhibition
C = Colony diameter in control
T = Colony diameter in treatment

To work out the control of the disease in field conditions the fungicides were tried at 0.1 and 0.2% concentrations for their comparative efficacy against the pathogen. Soil was drenched with 50 mL of 0.1 and 0.2% solutions of fungicides after 15 days of pathogen inoculation leaving the control. The plants were regularly observed and watered as and when required. The number of diseased/dead and healthy seedlings was noted after pathogen inoculation as well as fungicidal treatment. Final readings for diseased/dead plants were recorded by observing the symptoms, histopathology and isolation of pathogen and data was analyzed statistically.

RESULTS AND DISCUSSION

Almost 55% germination of seedling stock was observed on the germination trays. All the media plates inoculated with diseased root bits exhibited pure colonies of pathogen and confirmed the presence of organism in the seedlings of *P. roxburghii*.

The diagnostic characteristics of the pathogen are as follows:

Colony colour cottony white; conidiophores erect, hyaline, sparsely septate, 120-140 µm long and 2-3 µm wide, bearing 1-3 verticils of phiahides, phialides 3-5 in each verticals, acicular, 15-17.5 µm long and 1.5-2.5 µm wide; conidia acrogenous and borne at the tips of phialides, ellipsoidal-oblong, hyaline, 1-celled, 7.5-10.0×3.5-5.0 µm; sclerotia, intense brown in mass, 130-25.6 µm in diameter.

Identification key to the species:

- Conidia not brick red in coloured in mass
- Conidia ellipsoidal oblong or subcylindrical, 4-7×1.5-2 µ
- Black microsclerotia present. Perfect state not known

The pathogen was identified by microscopic analysis following standard procedures and taxonomic guides (Barnett and Hunter, 1972; Subramanian, 1971).

Verticillium wilt has been reported on nearly 300 cultivated plant species of widely diverse types, such as cereals, pulses, vegetables, fruits, ornamentals and shrubs (Ligoxigakis, 2000; Pegg and Bradly, 2002; Westcott, 1971). Browne (1968) and Tattar (1978) have also reported it to cause wilt disease in trees of various species. But so far Verticillium has not been reported to cause seedling wilt disease of forest tree species in nurseries.

The artificially inoculated healthy seedlings also exhibited wilt symptoms from 15 days of pathogen inoculation onwards and re-isolation of pathogen confirms Koch's postulates. In the control mortality due to wilt reached up to 80%.

In vitro screening of the fungicides using poison food technique revealed that all of the tested fungicides had inhibited the growth of pathogen and efficacy of inhibition increased with the concentration. Among the fungicides, Bavistin 50% WP was most effective showing complete inhibition of pathogen growth at the concentrations of 75 and 100 ppm followed by Dithane M-25, Dithane Z-78 and minimum inhibition was observed by Fytolan 50%WP (Table 1).

Working with olive trees, (Lopez-Escudero and Blanco-Lopez, 2000) found that the efficacy of soil solarization can be influenced by particular environmental conditions such as solarization together with soil fumigation may increase the rate of propagule death (Goldberg, 2003). The disease can be controlled by Bavistin 50% WP to a significant level. Jones (1987) also reported which support our finding that Verticillium wilt can be controlled by drenching the plant with benzimidazole fungicides. Both Bavistin and Topsin-M have a common fungitoxic principle, methyl benzimidazole-2-Yl-carbamate. It is evident from Table 2 that in field conditions, out of the two

Table 1: *In vitro* screening of fungicides on the growth of *Verticillium dahliae*

		Growth inhibition at different concentrations of fungicides (%)			
Fungicides	Control (growth in mm)	25 ppm	50 ppm	75 ppm	100 ppm
Bavistin 50% WP	40.3	31.27 (27.7)[a]	54.59 (18.3)[a]	100.00 (0.00)[a]	100.00 (0.00)[a]
Dithane M-25	41.0	20.24 (32.7)[b]	51.95 (19.7)[b]	72.44 (11.3)[b]	88.54 (4.7)[b]
Dithane Z-78	39.7	18.64 (32.3)[b]	24.43 (30.0)[c]	40.30 (23.7)[c]	48.87 (20.3)[c]
Fytolan 50% WP	40.3	8.93 (36.7)[c]	13.90 (34.7)[d]	31.27 (27.7)[d]	34.74 (26.3)[d]
SEM±0.34					
CD at 5% = 0.99					

Values in brackets indicates the growth of pathogen in mm. The columns sharing common letters are not statistically significantly different from each other at 5% significance level

Table 2: Efficacy of fungicides against *Verticillium* wilt in field conditions

	Seedling mortality at different concentrations (%)	
Fungicides	0.1	0.2
Bavistin 50% WP	20.38[a]	13.82[a]
Dithane M-25	38.25[b]	22.12[b]
Dithane Z-78	50.10[c]	31.33[c]
Fytolan 50% WP	76.13[d]	72.22[d]
control	78.00[d]	80.00[e]
SEM±2.256		
CD at 5% = 6.79		

The columns sharing common letters are not statistically significantly different from each other at 5% significance level

concentrations i.e., 0.1 and 0.2%, all fungicides were comparatively more effective at 0.2% concentration. Soil drenching with 0.2% Bavistin (13.82%), Dithane M-45 (22.12%) and Dithane Z-78 (31.33%) were found significant over the control (80.00%) and Bavistin 50% WP was found to be the most effective among them followed by Dithane M-45 and Dithane Z-78. At 0.2% concentration all the treatments were reported to be statistically significant ($p<0.05$), whereas, at 0.1% Fytolan-50WP was found to be non significant.

CONCLUSION

A wilt disease of *Parkia roxburghii* caused by *Verticillium dahliae* has been reported. It is a soil borne disease causing 80% mortality of seedlings. The disease can be managed by soil drenching with 0.2% Bavistin 50%.

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