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Phytophthora parasitica causing *Catharanthus* blight and its biological control

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Abstract: The present investigation was undertaken to identify *Phytophthora parasitica* causing *Catharanthus* blight at Mymensingh ($24^0 75^/$ N latitude and $90^0 50^/$ E longitude). *P. parasitica* was consistently isolated from affected plants which was subsequently confirmed as the cause of the disease through pathogenicity test. *Pseudomonas* spp. contaminated the isolate *in vitro* that was confirmed by biochemical test and it inhibited the growth of *P. parasitica.* The radial mycelial growth of *P. parasitica* was naturally inhibited by *Pseudomonas* spp. The disease symptoms appeared in the leaves and flagging or wilting of the entire plants were identified as typical symptoms of *Catharanthus* blight. The effect of antagonist in controlling the disease was also studied. Antagonistic activity of *Pseudomonas* sp. was conducted on Carrot Agar (CA) medium against *P. parasitica*. The *Pseudomonas* isolate caused the growth inhibition on CA against *P. parasitica*.
Key words: *Phytophthora parasitica*, *Catharanthus* blight, Biological control

Introduction

Catharanthus roseus known as Madagascar Periwinkle is native to the island of Madagascar (Simpson *et. al.* 1986). It has been widely cultivated for hundreds of years as an ornamental and medicinal plant. It grows worldwide, especially in Asia, South Asia, Europe and North America. Though *Catharanthus* has few disease problems, a foliar blight caused by *Phytophthora parasitica* is very destructive for it (Daughtrey *et. al.* 1995). Considerable losses in the production of *Catharanthus* as transplants and potted plants have also been observed (McGovern, 2000). It has limited the use of this plant in many landscapes including Bangladesh. The pathogen causes stem rot and aerial blight, which under most conditions, results in death of the plant. Van Breda de Haan (1986) named the causal fungus *Phytophthora nicotianae* but he described a mixed culture and failed to give a Latin description for the organism (Shew *et. al.* 1991). Dastur (1916) described a similar organism pathogenic to castor bean and named the organism *Phytophthora parasitica* in India. Tucker renamed the fungus that attacks tobacco as *Phytophthora parasitica* Dastur *var. nicotianae* (Breda de haan) Tucker, and this is the name currently used by most Tobacco Pathologists (Shew *et. al.* 1991). Although information is available in abroad, there is no report on *Catharanthus* blight causing by *Phytophthora parasitica* in Bangladesh. So, it is our growing interest to study on *Catharanthus* blight causing by *Phytophthora parasitica*. The purpose of the study was to isolate the causal agent of *Catharanthus* blight and to identify the pathogen, determination of its host range or pathogenicity and its control by biological methods.

Materials and Methods

Detailed study was carried out in the Seed Pathology Centre, IPM laboratory and green house of Plant Pathology Department of Bangladesh Agricultural University (BAU), Mymensingh during January-November, 2006. The *Catharanthus* blight affected diseased samples were collected from Horticulture field of BAU. Samples were collected and then inoculation and isolation was done successively. Different types of symptoms were studied on the collected affected diseased portion. The specimen was dipped in sterilized water and examined daily for sporangial production.

Isolation, Purification and identification: Fresh culture plates of *Phytophthora* were prepared into Carrot Agar by transferring hyphal tip from RPARH medium. The fungi were identified by observing colony characters, linear growth, colour and sporulation.

Pathogenicity test: The pathogenicity of the isolated organism *Phytophthora parasitica* was tested on *Catharnathus* plant and placed in the net house in Plant Pathology Department in BAU, Mymensingh.

Biological control of *Phytophthora parasitica* by Antagonists: The study was conducted *in vitro* to find out the antagonistic effect of bacterial isolates against *Phytophthora parasitica.* With this view, they were cultured on King's medium as described by Robert (2006). Several biochemical tests were performed for confirmation of bacteria which were sugar fermentation test, Potassium hydroxide solubility test, Arginine dihydrolase test, fluorescence test, Temperature test etc.

Antagonistic effect of *Pseudomonas* spp *in vitro* against *Phytophthora parasitica*: 4mm diameter fungal block was cut from the edge of actively growing fungal colony of *Phytophthora parasitica* with a cork borer. A 4mm diameter *Phytophthora parasitica* culture was placed at the middle of the Petri plate containing Carrot Agar (CA) medium. Then a loop full of bacterial cultures was placed on both the side of the fungal block.

Results

Symptoms study: Sudden flagging of one shoot or wilting of the entire plant was identified as typical symptoms of *Catharanthus* blight (Plate 1). Water-soaked, gray-green lesions were seen at the base of the wilted shoots which was followed by sunken, reddish brown cankers that girdled the main stem or lateral shoots.

Isolation of *P. parasitica:* Isolation of *Phytophthora parasitica* was made from blighted *Cathranthus* plants. The fungus obtained from artificial inoculation subculture several times in RPAR medium and finally transferred to CA medium showed characteristics cottony type colony of *Phytophthora parasitica* isolates (Plate 2).

Pathogenicity: In the present study pathogenicity test of *Phytophthora parasitica* carried out on *Catharanthus*

seedling. All seedlings of *Catharanthus* inoculated with *Phytophthora parasitica* (Plate 3)

Biological control of *Phytophthora parasitica:* The characters of *Pseudomonas* spp isolated from contaminated *Phytophthora parasitica* culture plate were tested that inhibited the colony growth of *Phytophthora parasitica*. *Pseudomonas* spp are gram negative, rod shaped bacteria. They produced cream colour colonies on KB medium. Morphological characteristics (shape, size, surface texture, colour etc.) developed after 24 hours of inoculation. For identification, a series of biochemical tests especially selective for *Pseudomonas sp.* were performed.

Plate 1. Symptoms of blight infected *Catharanthus* plant caused by *Phytophthora parasitica.* a and b) Blight infected *Catharanthus* plant in the field; c and d) Sunken, reddish cankerous lesion developed on the stems and lateral shoots.

Plate 2a. Pure culture of *Phytophthora parasitica* on CA culture media.

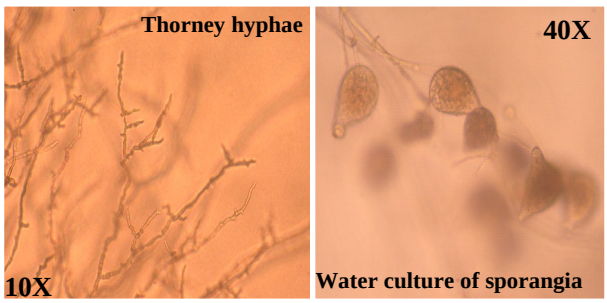

Plate 2b. Microscopic structure of *Phytophthora parasitica*.

Sugar fermentation: Results of sugar fermentation test showed that the antagonist 1 gave only positive reaction with Dextrose and negative reaction with other sugars (Table 1). Incase of antagonist 2, it showed negative reaction with all the sugars (Table 1).

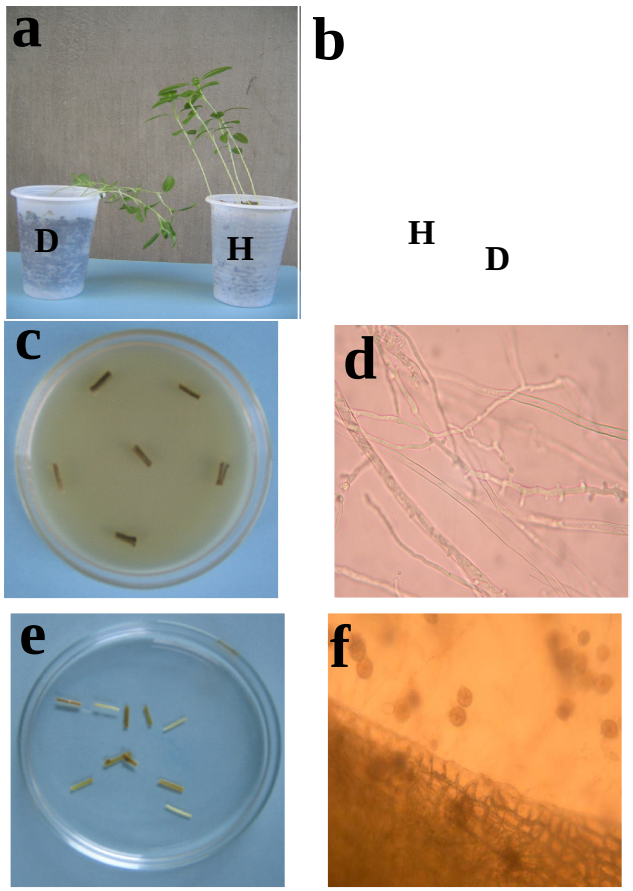

Plate 3. a and b Healthy (H) and inoculated seedling (D); c) Collection of artificially infected stem for isolation of the pathogen; d) Mycelium of *Phytophthora parasitica*

from infected stem; e) Incubation of causal organism and f) Sporangium of *Phytophthora parasitica*

Table 1. Biochemical tests for identifying antagonists

Antagonists Name	Sugar fermentation test's					KOH solubility test	Kovac's Oxidase test	Arginine dehydrolase test	Levan test	Fluorescence test	Temperature test			Inference
	Dextrose	Sucrose	Lactose	Maltose	Manitol						4^0C	30^0C	80^0 C	
N_1	+	-	-	-	-	+	+	±	±	+	-	+	-	*Pseudomonas sp.*
N_2	-	-	-	-	-	+	+	+	-	+	-	+	-	*Pseudomonas sp.*

Legend:
N_1 = Antagonist 1; N_2 = Antagonist 2; + = Positive reaction; - = Negative reaction; ± =Variable reaction

Effect of antagonist against *Phytophthora parasitica in vitro*: *Pseudomonas sp*. was effective for controlling *Phytophthora parasitica*. Both the antagonist inhibited the colony growth of *Phytophthora parasitica in vitro* (Plate 4).

Plate 4. a and b) Bacteria contaminated culture plate; c and d) N_1 and N_2 antagonists culture in KB medium; e) Antagonist activity of *Pseudomonas sp.* against *Phytophthora parasitica*, N_1= Antagonist 1; N_2= Antagonist 2

Discussion

Catharanthus roseus is very rewarding for the novice gardener because of its tolerance of extreme heat, humidity, drought, pests, urban environments and poor soil. Despite of its notorious durability, *Catharanthus* has occasionally been severely damaged by foliar and stem blight caused by *Phytophthora parasitica* (Schubert *et. al.* 1986). The disease had been reported previously from India (Dastur, 1916) and from California in naturalized plantings (Gill *et. al.* 1977), in nursery stock (Keim, 1977). In the present study, it was observed that *Phytophthora parasitica* was responsible for *Catharanthus* blight. The samples collected from infected *Catharanthus* plants showed foliar blight and wilting symptoms. The colony character observed in this investigation supported the result of Erwin and Robeiro (1996).

Identification of *Phytophthora parasitica* was also based on the morphology of chlamydospore. According to Mehrotra and Aggarwal (2001) terminal as well as intercalary chlamydospores 23.3×11.5-34.0 µm in diameter, which may be as large as 77 µm on pea seed agar medium was found. In the present study average size of chlamydospore range from 109.45-114.43 µm on carrot agar medium. The diameter of chlamydospores are greater than those of previous findings reported by Mehrotra and Aggarwal (2001).

The pathogenicity of *Phytophthora parasitica* was determined on seedlings of *Catharanthus* which were susceptible to *Phytophthora parasitica* infection and developed clear blighting and wilting symptoms on artificially inoculated seedlings. The infectivity of *Phytophthora parasitica* was inhibited by antagonist *Pseudomonas sp*. This result agrees with the findings of Sao Paulo (2006). Sao Paulo found that *Serratia marcescens* R_{35} isolated from citrus rhizosphere suppressed by *Phytophthora parasitica* more than 50 percent and promoted the plant growth.

This investigation on *Phytophthora parasitica* associated with *Catharanthus* blight is first of its kind in Bangladesh. So, further studies at molecular level of *Phytophthora parasitica* for determination of strains of field isolates are needed.

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