ECO-FRIENDLY MANAGEMENT OF FOOT AND ROOT ROT DISEASE OF BETEL VINE CAUSED BY *SCLEROTIUM ROLFSII*

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ABSTRACT

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Efficacy of five plant extracts namely garlic (*Allium sativum*), onion (*Allium cepa*), ginger (*Zingiber officinale),* neem (*Azadirachta indica*) and allamanda (*Allamanda cathertica*) and two bio-agents *Trichoderma harzianum* and *Pseudomonas fluorescens* against the devastating foot and root rot disease (*Sclerotium rolfsii)* of betel leaf (*Piper betle* L.) was studied *in-vitro* in the Plant Pathology laboratory of Sher-e-Bangla Agricultural University and *in-vivo* in the betel vine orchard of Malonchi Upazila of Pabna district. A remarkable inhibition of mycelium growth and sclerotia formation of *Sclerotium rolfsii* was achieved by treatment with bio-agent *Trichoderma harzianum* (42.77%) and garlic clove extract (25.56%) on potato dextrose agar (PDA) medium. The eco-friendly approaches i.e. garlic clove extracts reduced both the incidence (30.44 - 40.21%) and severity (41.03 - 44.02%) of foot and root rot disease of betel vine in the betel vine orchard and increased betel leaf yield up to 30.15%.

Key words: Plant extracts, bio-agents, *Sclerotium rolfsii,* food poisoned technique, incidence, severity, Betel vine

INTRODUCTION

The betel leaf (*Piper betle* L.) belongs to the family Piperaceae cultivated widely for its leaves, which is an important cash crop of Bangladesh. Betel vine having the heart-shaped deep green leaves is an important horticultural crop of aesthetic and commercial values, cultivated under shady areas. Betel leaf contains vitamins, enzymes, thiamine, riboflavin, tannin, iodine, iron, calcium, minerals, protein and essential oil (Sharma *et al*. 1996).

The betel vine is highly susceptible to diseases, pests and natural calamities (Sayeduzzaman 1988). Humid and moist shaded conditions are favorable for betel vine growth, which also favor a variety of root and foliage disease development (Goswami *et al*. 2002). Thus the betel vine growers incurred huge loss due to different diseases of betel vine. The most important diseases of betel vine plants are foot and root rot disease, leaf spot disease, powdery mildew disease and leaf rot disease. Among the diseases foot and root rot caused by *Sclerotium rolfsii* is the most devastating disease, which decreases the production of betel leaf to a great extent. Farmers growing *Piper betle* in three upazilas of Rajshahi incured a huge loss as foot rot disease damaged about 60% of the cultivation in the year of 2004 (Islam 2005).

In the third world country like Bangladesh, farmers seldom follow the appropriate methods in handling chemicals, which created health hazards. The indiscriminate use of chemicals is not only hazardous to living being but also break the natural ecological balance by killing the beneficial and/or antagonistic microorganisms.

Biological control of soil borne pathogens offers environmentally safe, durable and cost effective alternative to chemicals (Papavizas and Lumsden 1980, Mukhopadhyay 1994). Many species of fungi and bacteria are reported to be effective bio-control agents against soil borne plant pathogens (Papavizas 1985, Mukhopadhyay 1994). *Trichoderma* spp. are known antagonists of plant pathogenic fungi and have been shown to be very potential bio-control agents of several soil borne plant pathogenic fungi under both greenhouse and field conditions. Especially, *Trichoderma* spp. was found to be effective against different sclerotia forming fungi including *Rhizoctonia solani* and *Sclerotium rolfsii* (Hadar *et al*. 1979).

Hence, efforts have to be made to retain pathogen activity below economic threshold level by choosing methods of biological control only. So, the present experiment was undertaken to study the effect of some bio-agents and botanical extracts on the

growth and sclerotia formation of *Sclerotium rolfsii* and also to control the foot and root rot disease of betel vine.

MATERIALS AND METHODS

The laboratory experiment was conducted at the Department of Plant Pathology of Sher-E-Bangla Agricultural University (SAU) during June 2012 to December 2012 while the field experiment was conducted in the betel vine orchard of Maloncchi Upazila of Pabna district during the period from January 2013 to July 2013 under natural condition.

Collection of diseased specimens

Diseased stem samples of betelvine (*Piper betle* L.) were collected from different "betel vine orchard" of Pabna district. The collected samples were put in polyethylene bags immediately after collection and were preserved at 4°C in refrigerator for further use.

Purification and preservation of the pathogen

Pure culture of the *Sclerotium rolfsii* isolates were prepared following hyphal tip methods (Tuite 1969, Mian 1995) and subsequently transferred to fresh potato dextrose agar (PDA) slants in test-tubes and petridishes. Petridishes and test-tube slants containing pure culture of *Sclerotium rolfsii* were stored at 4°C.

Preparation of plant extracts

The plant extracts were prepared by using the method of Ashrafuzzaman and Hossain (1992). For preparation of plant extracts, collected leaves were weighted in an electric balance and then washed in water. After washing the big leaves were cut into small pieces. The weighted plant parts were blended in an electric blender and then distilled water was added into the jug of the blender. The pulverized mass was squeezed through 3 folds of fine cotton cloth. For getting 1:2 (w/v) ratio extract, 200 ml of distilled water was added with 100g plant parts.

Bioassay of plant extracts using growth inhibition technique

Groove/Cup method: From a PDA plate 5 mm discs of the medium were scooped from three places maintaining an equal distance from the centre by a sterilized disc cutter. One milliliter of plant extract was put into each hole and the plates were stored overnight in refrigerator for diffusion of the input in the medium around the hole before resumption of fungal growth. Mycelial block (5-mm) of 7 days old culture of *S. rolfsii* was placed at the centre of each PDA plate. The linear growth (cm) of mycelium of *S. rolfsii* was recorded at 24 hr. interval until the control plates were filled in (Nene and Thapliyal 1979).

Plate 1. A) Foot rot disease sample of betel vine, B) Infected vine segment on moist blotter paper and C) Pure culture of *S. rolfsii* showing immature sclerotia.

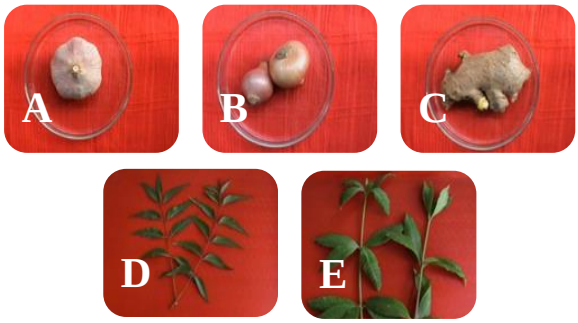

Plate 2. Plant parts used to test antifungal activity against *Sclerotium rolfsii* A) Garlic (*Allium sativum*), B) Onion (*Allium cepa*), C) Ginger (*Zingiber officinale)*, D) Neem (*Azadira chtaindica*) and E) Allamanda (*Allamanda cathertica*).

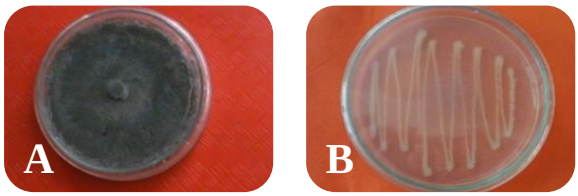

Plate 3. Bio-agents used to test antifungal activity against *Sclerotium rolfsii* A) Pure culture of *Trichoderma harzianum* and B) Pure culture of *Pseudomonas fluorescens*

Isolation/collection of biocontrol agents

Biocontrol agents *Trichoderma harzianum* were collected from Bangladesh Agricultural University and *Pseudomonas fluorescens* were collected from Laboratory of the department of Plant Pathology, Sher-e-Bangla Agricultural University.The fungal antagonists were cultured in Potato Dextrose Agar

(PDA) medium and the bacteria in Nutrient Agar (NA) medium.

Dual culture method for screening bio-agent against *Sclerotium rolfsii*

The culture discs (7 days old) of the bio-agents and pathogen were cut separately with the help of sterilized cork borers (5 mm). The culture discs of pathogen and bio-agent were aseptically transferred and placed them at the periphery of petriplate containing the medium at 2 to 3 cm apart in opposite direction. The culture disc of the pathogen alone in the petri plates containing PDA serves as control. The inoculated petri plates were transferred into the incubator and incubated at 25℃. The growth of the pathogen and antagonist in petri plates was observed periodically and measure the colony growth (diameter) in each petri plate.The percent inhibition of the pathogen was calculated by the bio-agent when the growth of the pathogen is full in the control plates.

Counting of sclerotia

After 30 days of culture, the sclerotia of each petridish were separated by using camel hair brush and number of sclerotia of each petridish was counted manually. The sclerotia of control plates were also counted and used to compare with those of treated plates.

Field experiment

The field experiment was conducted in the field of Malonchi Upazila of Pabna district during the period from January 2013 to July 2013 under natural condition.The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The field was divided into seven blocks with three unit plots in each. Each block contains one hill of betel vine and each hill contains three plants. The mycelial suspensions of *S. rolfsii* were mixed with soil and were incorporated at the base of each 21 hills @ 200 g soil/hill.

The plant extracts were sprayed to the betel vine plants at 7 days intervals up to 60 days after transplanting. Spraying of BAU-biofungicides i.e. bio-agent, a formulated product of *Trichoderma harzianum* developed by Prof. Dr. Iismail Hossain, Disease Resistance Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh was sprayed at 2% solution at 7 days interval for two times also done to the betel vine plants in the betel vine orchard.

The data were recorded on percent disease incidence, percent disease severity or percent foot area disease and yield (t/ha).

Plate 4. A) Plantation of betel vine B) Field view of experimental plot and C) Spraying of plant extract in the field of betelvine

The percent disease incidence and percent disease severity were calculated using the formula as: Percent disease incidence $=$

$$\frac{\text{Number of diseased plant}}{\text{Number of total plants observed}} X100$$

Percent disease severity $=$

$$\frac{\text{Area of stem tissue infected by disease}}{\text{Total stem area inspected}} X100$$

The percent values were finally transformed and analyzed statistically using a computer package program (MSTAT-C). The significant difference of the treatment means was compared by Duncans Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

In vitro* efficacy of plant extracts and bio-agents on sclerotia formation of *Sclerotium rolfsii

Effect of plant extracts and bio-agents in controlling foot and root rot disease of betelvine caused by *Sclorotium rolfsii* was assessed both *in vitro* and *in vivo* method. All the tested plant extracts and bio-agents showed strong lethal effect on the sclerotia production of *Sclerotium rolfsii* in culture media. The highest percent reduction of number of sclerotia was recorded in case of *Trichoderma harzianum* (74.99%) which was preceded by garlic extract (60.49%) at 4 days after inoculation (Table 1 and Plate 5). The findings of the present study were well supported by Lin and The (1990). They reported that isolates of *Trichoderma harzianum* inhibited the growth of *Sclerotium rolfsii* upto 67% in dual culture on malt agar and upto 100% using a cellophane overlay technique at 20± 1.5^{0}C.

Table1. ***I****n-vitro* efficacy of plant extracts and bio-agents on sclerotia formation of *Sclerotium rolfsii*

Treatments	Number of **s**clerotia	% Reduction of number of sclerotia over control
Garlic extract	209.0 j	60.49
Onion extract	349.7 c	33.89
Ginger extract	353.3 b	33.21
Neem extract	214.0 i	59.55
Allamonda extract	318.3 d	39.88
Trichoderma harzianum	132.3 k	74.99
Pseudomonas fluorescens	232.3 h	56.09
Control	529.0 a	0.00
LSD (0.01)	2.913	-

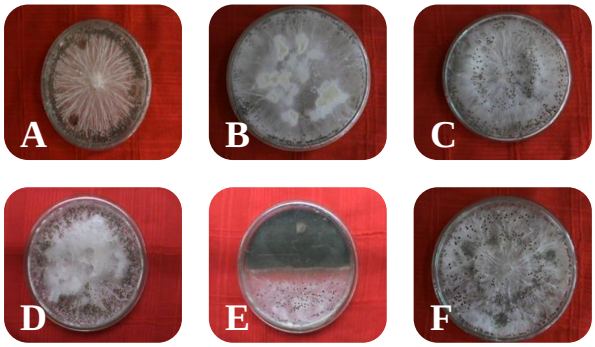

Plate 5. Sclerotia formation under different treatments A) Garlic extracts, B) Neem extracts, C) Allamonda extracts, D) Onion extracts, E) *Trichoderma harzianum* and F) Control

Field Experiment

Efficacy of plant extracts and bio-agents on the incidence of foot rot disease of betel vine in field condition

The effects of plant extracts and bio-agents recorded at different days after transplanting (DAT) differed significantly as compared to control (Table 2 and Plate 6). The result showed that the spraying of garlic clove extracts gave the lowest disease incidence that was (4.88%) at 120 DAT followed by *Trichoderma harzianum* (5.67%). The highest disease incidence was recorded in untreated control treatment which was (8.16%) at 120 DAT. Among all the treatments, garlic clove extracts was the best for reducing percent disease incidence (40.21%) of root rot of betel vine (Table 2).

Plate 6. Mycelium and sclerotia formation of *Sclerotium rolfsii* in the infected vine in control treatment

Efficacy of plant extracts and bio-agents on percent foot rot area diseased (%FAD) of betel vine in field condition

The lowest percent foot rot area diseased of betel vine was found with *Trichoderma harzianum* (1.60%) followed by garlic clove extract (1.69%) and the highest in the untreated control (2.287%) condition at 120 DAT (Table 3). Among all the treatments, *Trichoderma harzianum* was the best for reducing percent foot rot area diseased (44.02%) of betel vine. The present research result was supported by Mehrotra and Tiwari (1976) showed that dipping of cutting in a *Phytophthora parasitica* cell suspension effectively reduced the foot rot disease of betel vine. Ellil *et al.* (1998) stated that, *T. harzianum* reduced root rot infection 6.7-45.0% in bean. In other study Mauthamilan and Jeyarajan (1996) reported that *T. harzianum* reduced groundnut root rot caused by *Sclerotium rolfsii*.

Efficacy of plant extracts and bio-agents on yield of betel vine in field condition

The highest yield of betel leaf was recorded (2.26 t/ha) from the *T. harzianum* treated plot and the lowest yield (1.74 t/ha) was obtained from the untreated control plot (Table 4 and Plate 7). Maximum of 30.15% yield increase of betel leaf over control was produced by *T. harzianum* treated plot

Table 2. Efficacy of plant extracts and bio-agents on percent disease incidence of foot rot disease of betel vine in field condition

Treatments	% Disease Incidence			Reduction of disease incidence (%) over control
	60 DAT	90 DAT	120 DAT	
Garlicextract	11.11 (2.39)bc	33.33(4.88)abc	33.33(4.88)abc	40.21
Neem extract	11.11 (2.39)bc	33.33(4.88)abc	44.44(5.67)ab	30.44
Trichoderma harzianum	0 (0.71) c	22.22(4.08)abc	44.44(5.67)ab	30.44
Control	22.22(4.08)abc	55.55(7.36) a	66.66(8.16) a	0.00
LSD (0.05)	4.45	4.45	4.46	-

Data in parenthesis denotes the transformed values
In a column, DAT = Days After Transplanting

Table 3. Efficacy of plant extracts and bio-agents on percent foot area diseased (%FAD) of betel vine in field condition

Treatments	Percent foot area diseased (% FAD)			Reduction of foot area (%) over control
	60 DAT	90 DAT	120 DAT	
Garlic extract	1.04 (1.10)cd	1.87 (1.44)bcd	2.87 (1.69)bc	41.03
Neem extract	0.96(1.08)cd	1.9(1.47)bcd	3.12(1.75)bc	39.04
Trichoderma harzianum	0(0.71)d	1.0 (1.29) cd	2.49(1.60)bc	44.02
Control	2.08(1.50)bcd	4.6(2.26)ab	7.74 (2.87)a	0.00
LSD (0.05)	0.85	0.85	0.852	-

Data in parenthesis denotes the transformed values
In a column, DAT = Days After Transplanting

Table 4. Efficacy of plant extracts and bio-agents on yield (ton / ha) of betel vine in field condition

Treatments	Yield (ton/ha)	Yield increase over control (%)
Garlic extract	2.25 c	29.29
Neem extract	2.12 d	21.82
Trichoderma harzianum	2.26 c	30.15
Control	1.74 e	0.00
LSD(0.05)	0.07380	-

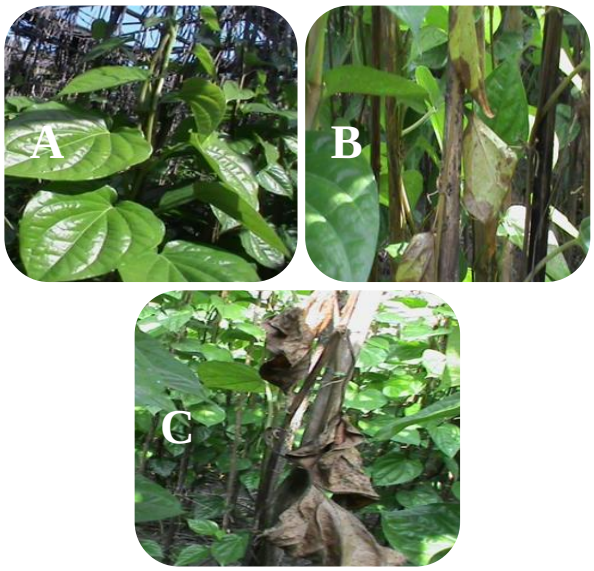

Plate 7. A) Healthy plant, B) Foot and root rot diseased wilted plant and C) Foot and root rot diseased with dead plant

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