CROWN ROT INCIDENCE OF STRAWBERRY IN BANGLADESH AND ITS BIOLOGICAL CONTROL

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ABSTRACT

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An investigation on crown rot of strawberry was performed in three districts viz. Rangpur-Sadar, Lalmonirhat and Mymensingh of Bangladesh during 2010-2011. Crown rot was found epidemic in the nurseries of northern districts of Bangladesh that cause severe wilting and sudden death of the strawberry seedlings. The highest crown rot incidence was recorded in Rangpur district. Crown rot pathogens *Phytophthora* sp. and *Rhizoctonia solani* were isolated and identified by microscopy and culturing on PARPand PDA media respectively. A his to pathological study revealed that crown rot pathogen spreads both in between and within the cells of cuticle, endodermis and cortex layer that resulted disintegration of cell wall, disorganisation of cortical cell layer and rottening of collar region of strawberry plant. The results of both pot and field experiments revealed that *Trichoderma harzianum* based bio-pesticide @ 64kg/ha effectively controlled crown rot pathogens as compared to non-treated treatments.

Key words: Crown rot, strawberry and biological control

INTRODUCTION

Strawberry (*Fragaria ananassa*) is known as the most delicious and refreshing fruit of the world to millions of people. The fresh ripe fruit is a rich source of vitamins and minerals with delicate flavor. It is an important high-value crop and its cultivation is increasing in Bangladesh. The amateur farmers in Bangladesh have been showing interest to cultivate strawberry due to its higher economic return. As strawberry culture is in expansion in these regions, so many problems are emerging out that affect its yield. Strawberry production is greatly influenced by agronomic practices, among which, planting date is the most important factor that greatly influences the growth and yield of strawberry (Laugale *et al*. 2004). However, very early planting may expose the plants to high temperature and late planting may expose the plants to high rainfall (Dennis and Hossein 2008).Moreover, disease constrain is one of the major problems**,** for its low yield production. Among the diseases, it has been reported that crown rot has occurred as an epidemic in many fields throughout the country including Mymensingh, Lalmonirhat and Rangpur districts (Hossain 2011). So far, no research work was done about the incidence, causal agent and severity of crown rot disease of newly adapted strawberry and its control in Bangladesh. On the other hand, biological control represents a natural and ecological approach to controlling diseases that reduces chemical inputs and their effects (Mukhopadhyay 1994). The *Trichoderma* based biopesticides have gained considerable recognition as biological agent.Several strains of *Trichoderma* have been found to be effective as bio-control agent of various soil borne plant pathogenic fungi such as *Fusarium, Sclerotium, Rhizoctonia*etc. (Chet and Inber 1994). *Trichoderma harzianum* isolate shows low ability in coiling round hyphae of *Sclerotiumrolfsii*, but it is very effective in penetrating or growing inside them. *T. harzianum* adversely affect them even without penetration (Ferrata and D'Ambra 1985). For biological management of plant diseases, *Trichoderma* based IPM Lab Biopesticide was formulated and marketed in small scale by IPM Lab, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. This IPM Lab biopesticide has been demonstrated to control a wide range of soil borne plant pathogens including the genus *Phytophthora*, *Rhizoctonia* and *Sclerotium.* Therefore, considering the above facts, the present research work was undertaken to isolate and identify the causal agent of crown rot of strawberry and its biological control through *Trichoderma* based biopesticide.

MATERIALS AND METHODS

The experiments were conducted in the plant disease clinic and net house of the Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh and farmers field during the period of January 2011 to April 2012.The incidence of crown or foot rot infected strawberry plants were recorded through visiting different nurseries of Mymensingh, Lalmonirhat and Rangpur districts of Bangladesh. Percent disease incidence (DI) was calculated by following the formula of Mansoor *et al.* (2007). Bio-pesticide formulated from *Trichoderma harzianum* was collected from IPM Laboratory, BAU, Mymensingh. *In-vitro* bioassay of *Trichoderma harzianum* against *Phytophthora* sp. and *Rhizoctonia solani* were done according to Hossain (2011). Crown rot pathogens were isolated by tissue planting methods. The collected samples were washed in tap water to make them free from soil and sand. Infected stem and roots were cut into small pieces from the junction of healthy and diseased tissues. These pieces were rinsed in sterile water and blotted to dry. Then the tissue segments were placed on corn meal agar medium amended with PARP (Pimarcin-0.4ml, Ampicillin Na-salt 250 mg, Rifampicin-1.0ml and PCNB-50 mg dissolving in 1 L) medium (Robert 2008). PARP medium was used for isolation of *Phytophthora*from infected crown rot tissues and allowed for incubation at 22ºC in the dark place. Then mycelial tips from the edge of the growing colony of *Phytophthora* was transferred on carrot agar medium. To get the sporangium, a mycelial block of *Phytophthora* was cultured into double distilled water for overnight at 20ºC with sufficient light. A commercial agar medium (PDA) was used to isolate *Rhizctonia* as described by Robert (2008). Microscopy was done following the Plant Disease Manual (Robert 2008) to identify the pathogen from carrot agar medium.

Histopathology of the diseased tissue was studied by sectioning (Hand Section) and microscopic examination was done following the methods of Johansen (1940). *Trichoderma harzianum* CP, a bio-agent, was tested for fungal antagonism by dual culture method on PDA medium (Rahman *et al.* 2009). The requisite amount of bio-pesticide substrates (lentil bran, peat soil) were thoroughly mixed in a 500 ml Erlenmeyer flask and autoclaved at l2lºC for 15 minutes for sterilization. The sterilized substrates were allowed to cool down and then inoculated with 5mm diamycelial disc of 7 days old *Trichoderma* culture. Ten discs were used in one flask for inoculation and the inoculated flasks were incubated at room temperature (25ºC+2). After incubation for 25 days, the contents were taken out from the flasks, air dried in laminar airflow cabinet and ground in a Moulinex blender. The ground materials were kept in polythene bag with proper labeling and treated as *Trichoderma* formulated biopesticide where its population (1.4x 10^6CFU / g) was measured by dilution plate technique (Nabi 2010). Pathogenicity tests were done in earthen pots drenched with *Trichoderma harzianum* based bio-pesticide. Twenty days aged strawberry seedlings were transplanted into pot soil amended with cow dung and fertilizer (BARC 2012). For pathogenecity test,30 days aged strawberry seedlings were inoculated with crown rot pathogens @ 100ml suspension of *Phytophthora* sp. (10^4 sporangia/ml/plant) and 100 ml suspension of *Rhizoctonia solani*/plant. *Rhizoctonia solani* suspension was prepared by using 12 days aged 2 culture plates dissolved in 100 ml water. Five treatments viz. T_1 = *Trichoderma harzianum* biopesticide (BP) + *Phytophthora* sp. +*Rhizoctonia solani*, T_2 = BP+ Cow dung+ *Phytophthora* sp. + *R. solani*, T_3= *Phytophthora* sp. + *R. solani*,T_4 = *Phytophthora*sp. and T_5 = *R. solani* were used in this experiment with three replication laid out in CRD.

For field experiment, the plots were prepared as described by Nabi (2010) and the land was drenched with bio-pesticide @ 64kg/ha. After 7 days of soil drenching strawberry seedlings were transplanted into the prepared land following the treatments T_1 = Control, T_2= Cowdung, T_3= Bio-pesticide and T_4=Bio-pesticide with Cowdung. Data were collected on healthy and crown rot infected plants, total fruit wt./plant and total yield/plot. The experiment was laid out in randomized complete block design. The size of the individual plot was $20m^2$ with 40 x 60 cm plant spacing. Data were tabulated and analyzed through a standard computer package of statistical procedure by WASP-2 (the first web based agricultural statistics software package).

RESULTS AND DISCUSSION

The youngest infected strawberry plant wilted suddenly and completely collapsed down within few days. Infected plants often broken down at the upper part of the crown when lifted from the soil. When cut lengthwise, the crown appeared brown or sometimes rose pink throughout the collar region (Plate 1A-1D). Significantly the highest crown rot incidence was recorded in Rangpur district while lower incidence was recorded at Kaliganj upazilla of Lalmonirhat district (Table 1).

Table 1. Incidence of crown rot of strawberry in different districts of Bangladesh

Thana (Districts)	(%) Disease Incidence
Rangpur	85 a
Rajshahi	66 ab
Mymensingh	50 bc
Lalmonirhat	40 c
LSD>0.05	22.8

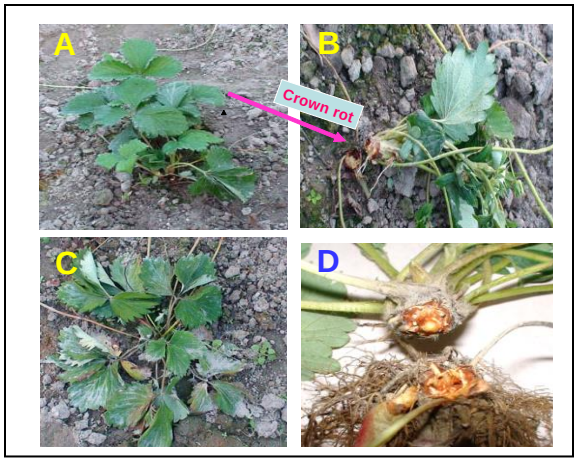

Plate 1. Symptoms of crown rot on strawberry plants. **A:** Diseased plant looks apparently healthy, **B:** Diseased plant after breakdown in collar region, **C:** Wilted plant, **D:** Brown necrotic tissue in collar region

The crown rot infected strawberry plants were observed under microscope by sectioning and two pathogenic structures were found on the infected tissues such as *Phytophthora* sp. and *Rhizoctonia solani* identified as manual of Robert (2008). In case of *Phytophthora,* hyphae were irregular, homothallic, hyaline and coenocyticbearing sporangium. Sporangia were microscopic sack-like structures that form and release zoospores. On the other hand, constriction and characteristics septation was found just before the origin of the lateral branches in case of *R. solani* (Plate 2).

Histopathological observation of affected collar zone of strawberry revealed that the mycelia of the pathogen on reaching the collar zone of the plants aggregated and settled on cuticular surface, adjacent to soil surface from where penetration of plant cuticles followed by infection occurred. Cuticles were discolored, and then rotting started with longitudinal progress along the collar region as well as circular progress around the base and thus covering entire cell mass of the cortex with an interwoven mycelial network. As a consequence of penetration, cell wall disintegrated, cortical cell layers disorganized and rotten (Plate 3). Rotting of cortical cell layers with large parenchymatous cell was rapid. In massive attack of collar zone (under microscope) showed that the cortex tissue had been sloughed from the vascular cylinder, fungi remain embedded in the sloughed cells (Plate 3). In another *in-vitro* experiment, it was observed that *Trichoderma harzianum* completely inhibited the growth of *Phytophthora* sp. and *R. solani*.

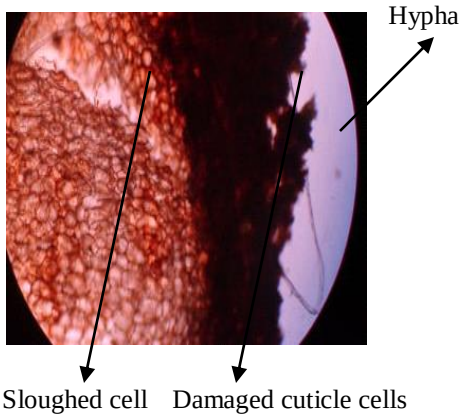

Plate 3. Histopathological study of crown rot pathogens. Hypha dissolving the endoderm cells. Cortex tissues has been sloughed from vascular cylinder, fungi remains embedded in the sloughed cells.

Pathogenecity test revealed that 11% strawberry seedlings were survived when *Trichoderma* (T_1) alone or *Trichoderma* and cowdung (T_2) was drenched with the soil (Table 2). But all the strawberry seedlings were wilted and died when these two isolated crown rot pathogens were sprayed.

Table 2: Effect of various treatments on strawberry seedlings (pot)

Treatments	No. of plants treated	No. of plants survived
T_1	10	10
T_2	10	10
T_3	10	0
T_4	10	0
T_5	10	0

T_1=*Trichoderma*+Cowdung+*Phytophthora*+*Rhizoctonia,*
T_2=*Trichoderma*+*Phytophthora*+*Rhizoctonia,*
T_3=*Phytophthora*+*Rhizoctonia,*
T_4=*Phytophthora*sp**.** and
T_5= *R. solani*

In the field experiment, *Trichoderma* based bio-pesticides howed significantly different effect on crown rot disease of strawberry. No plant was killed in treatment T_4 by crown rot pathogens where field soil was treated with *Trichoderma* and cowdung. The highest strawberry plant was killed (60%) by crown rot in case of Treatment $T_1$and it was followed by Treatment T_2 (20%) and there was no *Trichoderma* in both these treatment (Table 3). The minimum number (2%) of strawberry plant was killed in treatment T_3. Treatment T_4 yielded significantly the highest strawberry fruits (304.0 g/plant) followed by treatment T_3 (212.3 g/plant) and T_2 (180.0 g/plant). The lowest strawberry yield was recorded in treatment T_1(Table. 3). Significantly the highest strawberry yield (kg/ha) was observed in treatment T_4 (13333.3 kg/ha) followed by T_3 (9127.7 kg/ha) and T_2 (7826.0 kg/ha) respectively. The lowest strawberry yield was recorded in treatment T_1 (3662.7 kg/ha).

Table 3: Effect of *Trichoderma* based Bio-pesticide (BP)for the management of crown rot disease and yield ofstrawberry (Field experiment).

Treatments	% plant killed	Yield (g/plant)	Yield (kg/ha)
T_1	60	147.3 d	3662.7 d
T_2	20	180.0 c	7826.0 c
T_3	2	212.3 b	9127.7 b
T_4	0	304.0 a	13333.3 a
LSD>0.05	-	11.453	21.251

T_1= No Cowdung and *Trichoderma*
T_2= Cowdung without *Trichoderma* based bio-pesticide (BP)
T_3= BP without Cowdung and
T_4= BP with Cowdung

Strawberry plant infected by crown rot often wilted and breakdown in the collar region. In a massive attack by this disease, the collar region of the plant become necrotic and develops brown to rose pink color. Zveibil and Freeman (2005) found that a strawberry plant infected by crown rot develops necrotic and crown rot accompanied by plant wilting and leaf chlorosis. Latorre and Viertel (2004) also observed that crown rot in strawberry develop root and crown rots, particularly when plants are kept for a long period in cool conditions. Phillips and Golzar (2006) observed outstanding leaf symptoms of the *Rhizoctonia* disease of strawberry i.e. wilting under certain conditions and purpling of the veins. In this study incidence of crown rot of strawberry in the field varied from 40-85%. Aviles *et al.* (2008) and Santos *et al*. (2002) collected infected crown rot samples of strawberry plants and diagnosed in Plant Disease Clinic where, *Phytophthora* and *Rhizoctonia solani* were isolated from the infected strawberry plants and observed their hyphae under microscope and identified as well. This finding supports the research work done by Belisario *et al*. (2007), Rebollar *et al*. (2007) and Eikemo*et al.* (2004). Belisario*et al.* (2007) found seven species of *Phytophthora*, i.e. *P. cactorum, P. nicotianae, P. cinnamomi, P. hedraiandra, P. palmivora, P. cryptogea* and a *Phytophthora* sp. to be associated with decline and death of different species of plants grown in nurseries including strawberry. Phillipsand Golzar (2006) observed that Phytophthora crown and root rot caused by *Phytophthora cactorum* was a disease of long-standing importance in strawberry. From this study it was revealed that two pathogens such as *Phytophthora* and *Rhizoctonia solani w*ere involved in this devastating disease. This result was similar with the results obtained by Watehouse and Waterston (1964), Irzykowska *et al*. (2005), Latorre and Viertel (2004); Eikemo *et al* (2004), Parikka (2007) and Duncun (2002). They found that crown rot or leather rot of strawberry was caused by *Phytophthora nicotianae, Phytophthora cactorum* and *Phytophthora citricola.* Strawberry seedlings were found highly susceptible to these two pathogens. This finding was agreed with the results of many research workers such as Parikka (2003), Toyoda *et al*. (2001), Eikemo *et al.* (2004). Parikka was evaluated the susceptibility of 55 strawberry cultivars to crown rot, caused by *Phytophthora cactorum*, under greenhouse conditions. Toyoda *et al.* (2001) inoculated crown-rot pathogen by needle-prick inoculation method. In the field experiment, *Trichoderma* based biopesticide was used to control crown rot pathogens (*Phytophthora* sp. and *Rhizoctonia solani*). The highest strawberry fruit yield was obtained when the soil was pretreated with formulated *Trichoderma* (IPM Biopesticide) @ 64 kg/ha followed by cowdung as cultural control (Table 3). The lowest fruit yield was obtained when the soil was not drenched with both *Trichoderma* and cowdung (Table 3). This result indicated that soil drenched with *Trichoderma* and cowdung completely suppressed the crown rot pathogens especially *Phytophthora* sp. and *Rhizoctonia solani* and resulting higher strawberry yield. These findings were in agreement with many research workers as described by Anand and Zeller (2008), Anand and Singh (2004), Mukherjee *et al.* (1995), Mukhopadhyay (1995), Chet and Inbar (1994), Duncan, (2002), Ashokand Purthi (2007), Tanaka *et al.* (2002) and Jacobs and Kamon (1986). Chandrasekhar *et al*. (2005) found that *Trichoderma harzianum* in *in-vitro* condition completely suppressed the growth of collar rot causing pathogen. Anand and Singh (2004) observed that the

antagonistic activity of *Trichoderma harzianum* and *T. virens* against collar rot causing

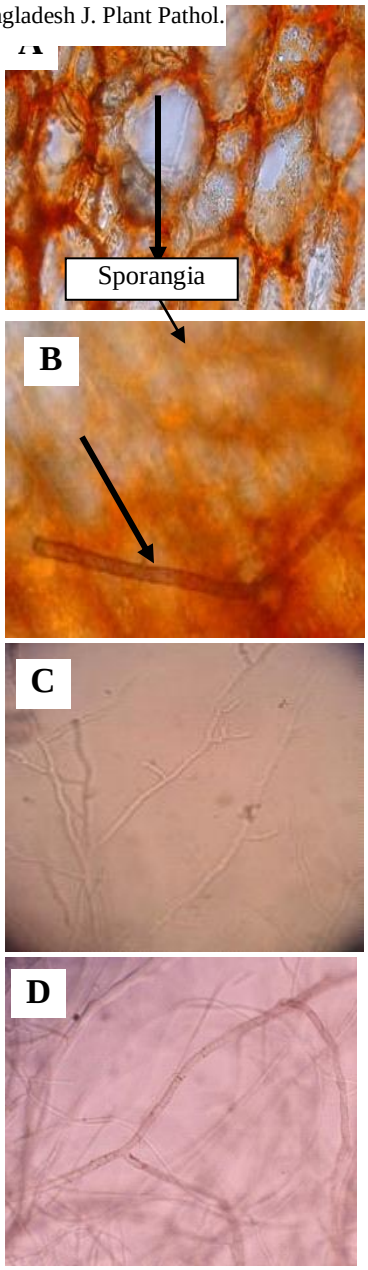

Plate 2a. Sectioning and microscopic observation of infected crown rot tissues of strawberry

A. Hypha of *Phytophthora* sp in infected tissue
B: Hypha of *Rhizoctonia solani* in infected tissue
C.Myceliun of *Phytophthora* sp. from culture medium
D.Myceliun of *Rhizoctonia solani* from culture medium

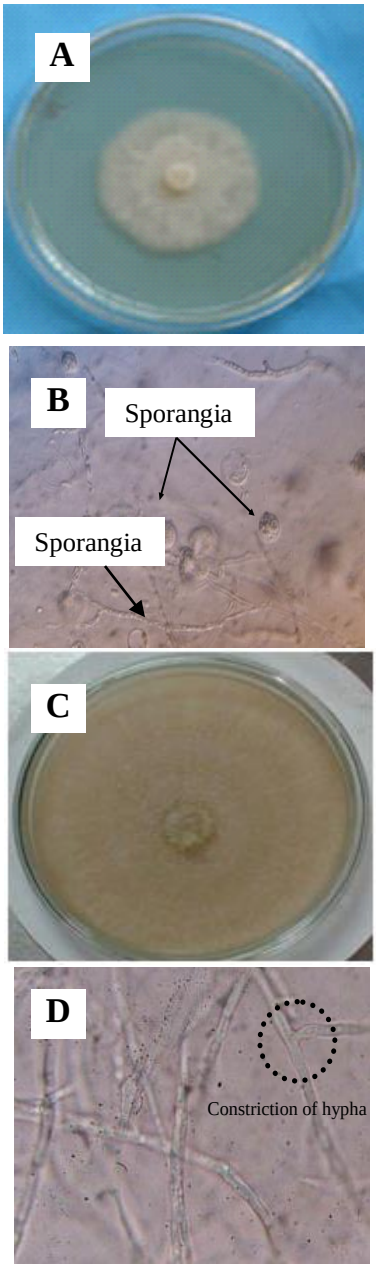

Plate 2b. Pure culture and different structure of *Phytophthora* sp. and *Rhizoctonia solani*.

A: Pure culture of *Phytophthora*sp. on PARP medium
B: Sporangia and zoospore of *Phytophthoa* sp.
C: Pure culture of *R. soalni* on PDA medium
D: Structures of *Rhizoctonia solani.*

pathogen *Mentha* spp. and also found that 66.67 to l00% reduction in disease was accompanied with significant increase in herb and oil yield. Prasad *et al.* (2003) observed that the efficacy of isolates of *Trichoderma* spp. in suppressing the growth of collar rot pathogen of cauliflower by dual culture method. Mukherjee *et al.* (1995) observed that *Trichoderma harzianum* was effective in suppressing *Sclerotiumrolfsii* and *Rhizoctonia solani*. *Trichoderma harzianum* was found to be effective in destroying the sclerotia of both fungi. Mukhopadhyay (1994) used *Trichoderma harzianum* for protection against a wide range of soil borne pathogens viz. *Sclerotium rolfsii, Fusarium oxysporum* and *Rhizoctonia solani.* Jacobs and Kamon (1986) found that *Trichoderma harzianum* produced cell wall lysing enzymes which antagonized against plant pathogens and improved biological control. Sharma *et al*.(2001) were found that the effect of farmyard manure compost (0, 124, 250, 275 and 500 g) on the yield of strawberry where compost at 500 g gave the highest number of flowers (5.33), number of fruits (5.00) and fruit weight (112.72 g). Tanaka *et al.* (2002) and Michel *et al*. (2006) found that the disease severity increased when the cow manure was not added.

Crown rot incidence was found more prominent in the strawberry nursery in the northern district of Bangladesh. This investigation revealed that IPM approach such as biological control measure using bio-control agents like *Trichoderma harzianum* along with cultural practice *i.e.* soil drench with cowdung significantly reduced the crown rot of strawberry in the field.

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