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The first report of *Pestalotiopsis* sp. causing crown rot disease on strawberry (*Fragaria* X *ananassa* Duch.) in Bangladesh and evaluation of fungicide activity

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Abstract

This study was conducted to identify the crown rot disease in strawberry caused by an uncommon fungus. However the study also focused to evaluate efficacy of plant extracts and chemical fungicides against this fungus. Results from isolating 200 samples of infected plant parts collected from 15 main strawberry cultivating areas in Rajshahi, Bangladesh. Based on morphological and cultural characteristics the *Pestalotiopsis* sp. was found as fungal pathogen against strawberry and Koch's methodology confirmed it. Five different concentrations (50, 100, 250, 500 and 1000 ppm) of plant extracts (Neem, Tulsi, Bottlebrush, Arjun and *Aloe vera*) and commercial fungicides (Bavistin, Tilt, Hayconazole, Score, Rovral, Antracol, Dithane, Ridomil, Folicur, Cupravit, Secure, Thiovit and Sulcox) were used for efficacy study against *Pestalotiopsis* sp. in both *in vitro* and field condition. The percent inhibition of radial growth of the fungus was increased with the increasing concentrations of fungicide. Bavistin (1000 ppm) showed 100% inhibition of growth of this fungus in *in vitro* condition and in field condition. Bavistin treated plants showed only 21.5% disease incidence, where control was 85%. Number of fruits/plant was 10.88±0.23, fruit weight/plant was 148.56±0.81 (g) whereas in control these were 5.33±0.23 and 63.88±0.26 respectively. This is the first report on the association of *Pestalotiopsis* sp. causing crown rot disease of strawberries in Bangladesh.

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Introduction

Strawberry (*Fragaria x ananassa* Duch.) is a high value crop of economy and nutrition (Dung *et al,* 2016). The fruit of strawberry contains higher vitamin C concentration than that of orange or lemon (Ara *et al.,* 2013). It also contains significant levels of Ellagic acid, which is thought to be an anti-carcinogenic agent (ICAR news, 2005). Bangladesh being an underdeveloped country the 85% of children could not get proper nutrition due to poverty. Nonetheless, she has a great possibility of strawberry cultivation in winter season (November to March) (Karim *et al*., 2015). On the other hand, during hot summer and humid rainy season almost all of the plants are perished due to different diseases (Ara *et al*., 2013). A large number of strawberries have been destroyed every year due to strawberry pre and post-harvest diseases (Khatun, 2014).

Many pathogens of strawberries have been recorded over the world (Dung *et al,* 2016). They cause mainly crown, stem, root and fruit diseases (Dung *et al,* 2016). Particularly, crown rot disease caused by several types of pathogens, such as *Phytophthora cactorum*, *Colletotrichum acutatum*, *C. gloeosporioides* and *C. fragaria* (Dung *et al,* 2016). The first disease on strawberries was recorded in Florida in Brooks's report in 1931. In 1935 it was discovered that *C. fragaria* was responsible for wilting of strawberry (Dung *et al,* 2016).

Subsequently, in 1960, *C. gloeosporioides* had also been found to cause similar symptoms and eventually discovered *C. acutatum* (Delp and Milholland, 1981; Michael and Ellis, 2008; Bonde *et al.,* 2009; Smith and Black, 1990). Recent research also showed that *Verticillium* sp. is the causal agent of wilting symptom on strawberry foliar (Thomas *et al*., 2009). However, the effect of disease owing to the lack of effective control measures discouraged farmers to grow strawberry or turn to cultivation of other crops grudgingly.

Mostly the fungicides and different chemicals are applied to control of strawberry diseases (Khatun, 2014). Generally use of different chemicals is effective for pre harvest control of strawberry diseases (Khatun, 2014).

The coper fungicides alone or in combination with other fungicides are used worldwide for diseases control (Arauz, 2000). During winter season different strawberry diseases infections are controlled by using different chemical fungicides two or three times. These chemicals have adverse effects on human health and the environment also. This led to the intensive worldwide research efforts to develop alternative control strategies that are eco-friendly (Jabbar *et al.,* 2011). Recently scientists shifted their focus to eco-friendly methods (Faiz *et al*., 2016).

These include use of plant extract such as Arjun (*Terminalia arjuna*), Dhutura (*Datura metel*), Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*) against the mycelial growth of different diseases causal fungal pathogens (Khatun, 2014 and Jarin, 2016). In another study, Pereira *et al.* (2011) used *Melaleuca* sp. oil against the pathogen *Pestalotiopsis longisetula* both *in vitro* and field condition. The symptoms of disease observed on the field were similar to the studies reported in the world. The aim of this study is to identify the pathogen at least the taxonomic level genus and management of strawberry diseases by using different plant extracts and commercial fungicides under *in vitro* condition and field condition.

Materials and methods

The etiological study of crown rot disease on strawberries was conducted as the steps of the Koch's postulates (Koch, 1884).

Observation symptoms and sampling

The typical symptoms were recognized by field surveys and farmer interviews. Sampling was conducted of strawberry variety Festival at the age of 1-3 years at main strawberry growing regions of Rajshahi, Bangladesh in 2015-2016. At each site, 20 plants with typical symptoms of the disease as described by farmers were sampled. The samples were then packed into a sterile polythene bag already lined with soft paper and taken to the Plant Pathology and Microbiology, Department of Botany, University of Rajshahi, Bangladesh for isolation of causal agent of diseases and further studies.

Isolation of pathogens

The isolation of microorganisms from different parts of the infected plant, including leaves, flowers, stems, stalks and roots as described (Ocean, 1988). However, those were collected from all three different sampling areas. The fungus was isolated from infected strawberry leaves and petioles following the standard procedures of Agostini and Timmer (1992). Tissue was cut from the advancing margin of the lesion, surface sterilized with 0.1% sodium hypochlorite solution and washed in three times in sterile distilled water. The sterilized tissue was dried on sterile filter paper on a clean bench, plated on potato dextrose agar (PDA) and incubated at 28℃ for 10 days. The frequency of microorganisms was determined 5 days after incubation. The microbial isolates were identified through morphological structures observed under a microscope with a magnification of x10 and x40.

Pathogenesis test

Pestalotiopsis sp. observed mainly in all the isolated parts. The inoculation through spore suspension was prepared (15-day-old, on PDA) by following the method of Stall and Walte (1965). The free-disease strawberry variety "Festival" at 3 weeks old, planted in plastic pots (10 cm in diameter) with sterile soil inoculated by spraying spore suspension of *Pestalotiopsis* sp. (10^7spores/ml). At the same time, strawberries of control treatment were sprayed with tap water only. The treated plants were sprayed with tap water, monitoring and recording symptoms on the leaves were examined at 12, 24, 48, and 72 hours post inoculation. Disease symptoms were observed and evaluated 7-day post inoculation. The symptomatic strawberries were also re-isolated to fulfill the steps of etiology as Koch's postulates. The experiments were repeated three times independently.

Evaluation of botanical fungicides and chemical fungicides against Pestalotiopsis sp. under in vitro and field condition

The locally available medicinal plants (as botanical fungicides) were collected from Rajshahi city. Five concentrations (50, 100, 250, 500 and 1000 ppm) of plant extracts of *Eucalyptus obliqua* (Eucalyptus), *Azadirachta indica* (Neem), *Swietenia macrophylla* (Mehogoni), *Terminalia arjuna* (Arjun), *Callistemon linearis* (Bottlebrush) and Bavistin, Tilt, Hayconazole, Score, Rovral, Antracol, Dithane, Ridomil, Folicur, Cupravit, Secure, Thiovit and Sulcox were used for *in vitro* efficacy study against *Pestalotiopsis* sp. Plant extracts preparations were followed the method of Rathod *et al.* (2015) and commercial chemical fungicide solutions preparation were followed the method of Akhter *et al.* (2012).

Fungicide suspensions of 50 mg/L, 100 mg/L, 250 mg/L, 500 mg/L and 1000 mg/L were prepared by dissolving requisite quantities of each fungicide in autoclaved and cooled PDA just before pouring into Petridishes. Twenty ml of fungicide-amended media was poured into each 90mm sterilized Petri dish. Three replicates were performed for each concentration of each fungicide. Medium without fungicide served as a control. Mycelial discs (5 mm) were cut from 7-days-old cultures using a cork borer, placed in the center of each Petri dish after solidification of PDA and incubated at 28℃. Percent inhibition of growth of fungus was recorded using the following formula:

% inhibition= C-T/C X 100, Here, C = Average radial growth in control and T = Average radial growth in treatment.

Disease incidence, no. of fruits/ plant and fruit weight/ plant of treated plants were measured and % increased by using fungicides were also calculated.

Data analysis

All data collected from survey and laboratory studies were processed by Excel 2010 and SPSS software.

Results and discussions

Symptomatology

The typical symptoms observed from diseased strawberry were the drying begins from the edge of the leaves. Lesions appear first on the leaves and spreads down the crown. The severely infected plants had completely dried leaves and flowers, then turned dark in color, stems and roots are black (Fig. 1A-B). The disease also spread to all runners. Diseased plants may appear magenta stolon and leaves. The disease was relatively so common that incidence area has been increasing rapidly.

Identification and characterization of fungal pathogens of strawberry

The results of isolating from 200 samples of diseased parts with typical symptoms from the fifteen strawberry growing areas (Namobhadra, Shamshadipur, Horian, Parila, Rajshahi University Botanical garden, IBSC Research field, National strawberry field, Kashindaparila 1, Nowhata, Mohanpur, Kashindaparila 2, Akafuji field, Nowhatabajar field, Puthia field and Binodpur field) was made. Among them 57-77% plants were infected by *Pestalotiopsis* sp. and 22-42% infection caused by other pathogens (Table 1).

On PDA media, the colonies of *Pestalotiopsis* sp. were layered with concentric circles, slightly wavy edges and had a chrysanthemum-shaped. Colony was white to whitish from the edge to the center of colony and cottony, darker with age. Fungal spores formed and concentrated in the black water droplets on the surface colony after 12 day of inoculation on PDA media (Fig. 2C.).

The black water droplets with spores were bigger over time. Conidia formed earlier and more abundant in the region close to the center of the colony. The conidia were clavate-fusiform, straight or slightly curved, with 4 septa, three median cells were dark brown. Basal cells and apical hyaline cells were transparent, and apical cells with 2-5 tails (70% with 3 tails), basal cells with 1-2tails (2 tails less common) (Fig. 1, E).

After inoculation of healthy strawberry plants, the formation of white mycelia clumps was observed on the leaves and also black gelatinous mass. Similar symptoms were observed in strawberry in Minas Gerais State, Brazil (Teixeira *et al.,* 2015) and mangosteen attacked by *Pestalotiopsis cruenta* (Bastos *et al.,* 2011.) Morphological characterization tests showed that the pathogenic fungus belongs to the genus *Pestalotiopsis*. *Pestalotiopsis* genus is complex and its classification to a specific level is severely hampered by the enormous variation in morphology accepted for different species of the genus (Kruschewsky, 2010). Traditionally, the taxonomic characterization of this fungus has been based on conidial morphology (Jeewon *et al*., 2004). This study reports time that *Pestalotiopsis* sp. could attack strawberry crops in Bangladesh.

Not with standing this disease previously diagnosed in Brazil (Teixeira *et al.,* 2015), Egypt (Embaby, 2007), Hawaii (Keith *et al.,* 2006) and Morocco (Mouden *et al.,* 2014). *Pestalotia* (*Pestalotiopsis*) rot of strawberry fruits caused by *P. longisetula* Guba, was first ever recorded in Isreal (Howard, 1973), in USA Howard and Albregts (1973), in India (Shitole *et al*., 2000), in Vietnam (Guba, 1961) and NagRai (1993). Moreover, it caused rot disease on strawberry in Israel (Howard and Albregts, 1973). However, the effect of such causal agent on strawberry crowns had investigated extensively.

Pathogenesis test

After isolation and determination of the fungus frequency on strawberries with the typical symptoms, *Pestalotiops*is sp. was of the highest frequency in all three surveyed areas. The young leaves started to appear drying at the leaf edge 48 hours post inoculation of *Pestalotiopsis* sp. The lesions continued to grow rapidly over time. After 72 hours of inoculation, the lesion on the young leaves developed up to half the area of leaves, the symptoms were also observed on old leaves with green leaf edge.

Further, after 7 days of inoculation, the plants completely wilted, lesions began to turn dark-brown with symptoms similar to those on the survey fields. The controlled plants (sprayed with tap water only) meanwhile remained in normal development, and similar diseased symptoms were not observed.

To fulfill the test, the diseased leaves were incubated and the agent caused the symptoms were also re-isolated in PDA. Similar result was found as previously reported by Dung *et al.* (2016). These results revealed that *Pestalotiopsis* sp. was responsible for observed symptoms of crown rot in the strawberries in Rajshahi, Bangladesh.

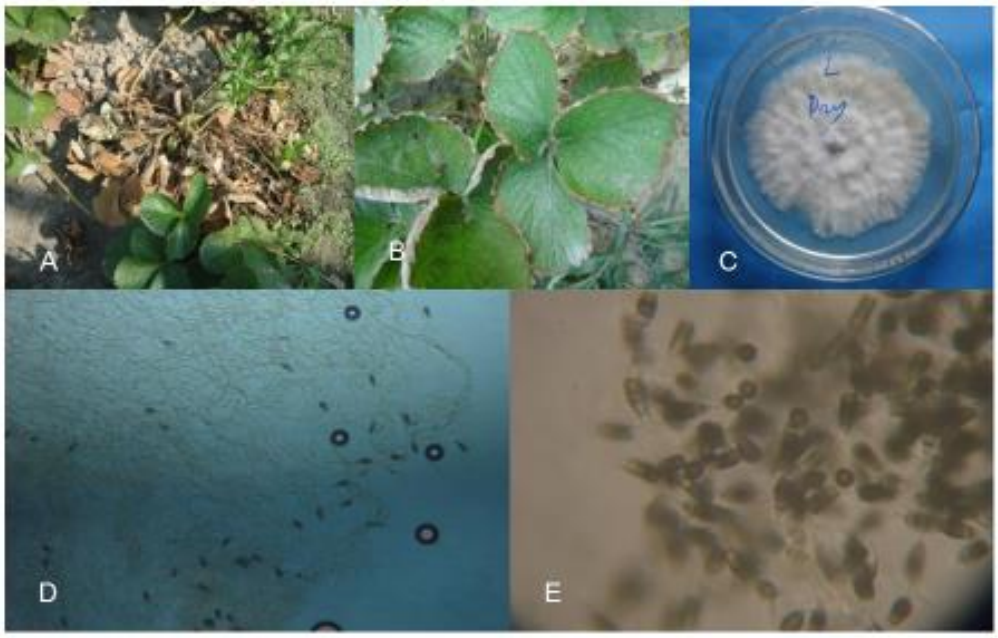

Fig. 1. Disease characteristics of *Pestalotiopsis* sp. in Strawberry.
Diseased symptoms in the field (A). Diseased on the plant leaf (B). Morphological features of *Pestalotiopsis* sp. post 7 days inoculation (C) on PDA media. Mycelia of *Pestalotiopsis* sp. with conidia under microscope (D) and enlarged view of fusiform conidia with tails at apical cells and basal cells (E).

Evaluation of botanical fungicides and chemical fungicides against this fungicide under in vitro and field condition

A progressive increase in percent inhibition of radial growth of the pathogen was observed with the increase in concentration of the plant extracts (Table 2) and commercial chemical fungicides (Table 3). In this experiment Neem extract (1000 ppm) was found more effective than other plant extract and inhibited 82.5% growth of *Pestalotiopsis* sp. Nonetheless, prior to this the neem leaf of (25%) extract showed 41.02 % inhibition of the fungus *Colletotrichum gloeosporioides* causal pathogen of crown rot in strawberry in Bangladesh (Khatun *et al.*, 2014).

One study also showed somewhat similar trend of antifungal activity of eucalyptus, neem and garlic (Sahi *et al.,* 2012). They proved that eucalyptus extract to be the best plant extract inhibiting the growth of fungus *Bitryodiplodia theobromae;* the causal agent of quick decline of mango Khatun *et al.* (2014). Faiz *et al.* (2016) also used eucalyptus, neem and garlic extratct for controlling *Colletotrichum gloeosporioides,* the causal pathogen of mango blossom blight and anthracnose.

Eucalyptus camaldulensis could be used as an alternative to chemical fungicides that exhibited highly prominent antifungal potential against fungal pathogens (Bajwa, 2005). In another study papaya, neem and garlic extract were found against the growth of *Aspergillus flavus,* a causal agent of fruit rot disease of tomato (Tijjani *et al.* 2014). Furthermore, Pereira *et al.* (2011) used *Melaleuca* sp. oil for the control of *Pestalotiopsis longisetula* in strawberry. Importantly, we used *Callistemon linearis* for control of crown rot disease of strawberry and it showed only 44.8% growth inhibition of this fungus. The same plant extract was found effective for *Aspergillus niger*, *Sacharomyces cerevacae, Candida albicans* (Haque *et al.,* 2013).

Among the chemical commercials the Bavistin DF was the most effective fungicide in this experiment for both in vitro and in field (Table 3 and 4). Growth of the fungus was totally checked using all five concentrations of Bavistin, Tilt, Hayconazole, Score, Rovral, Antracol, Dithane, Ridomil, Folicur, Cupravit, Secure, Thiovit and Sulcox. 1000 ppm Bavistin showed 100% control of this fungus in *invitro* condition.

Interestingly similar results were found against *Colletotrichum gloeosporioides* the causal pathogen of crown rot disease/anthracnose of strawberry in Bangladesh Khatun (2014) and Akhter *et al.* (2009). Chemicals like Bavistin DF, Dithane M-45 and Tilt 250 EC were found the most effective fungicides of *Bipolaris sorokiniana* at 500 µg/ml to 2,500 µg/ml and 1/10 to 1/1000 ml Alam *et al.* (2000). Ahmed *et al.* (1991) evaluated eight fungicides and found the Dithane M-45 manifested the best control of anthracnose (*Colletotrichum gloeosporioides*). Alam *et al.* (2000) reported that 100% germination inhibition occurred with the application of Bavistin and Thiovit after 15 minutes and 25 minutes of immersion in 0.25% Cupravit.

In field condition, Bavistin (1000 ppm) showed best result than other botanical fungicides and commercial chemical fungicides. In 1000 ppm Bavistin treated field number of fruits/plant was 10.88±0.23, fruit weight/plant was 148.56±0.81 (g) where in control these were 5.33±0.23 and 63.88±0.26 respectively (Table 4).

These results are similar with findings of Khatun (2014). As she also found best performance of Bavistin in field condition for the control of *C. gloeosporioides* the causal pathogen of crown rot of strawberry in Bangladesh.

Fig. 2. Inhibition of *Pestaloptipsis* sp. proliferation.
Inhibition of *Pestaloptipsis* sp. treated with different botanical fungicides and chemical fungicides (1000 ppm) after 12 days of culture (A-C). A= Bavistin, B= Neem and C = control.

Table 1. Incidence (%) of *Pestalotiopsis* sp. isolated from infected crown of strawberry plants of different places of Rajshahi, Bangladesh.

Name of places	Number of infected crowns	Incidence(%) of *Pestalotiopsis* sp. and other pathogens	
		Pestalotiopsis sp.	Other pathogens
Namobhadra, Rajshahi	25	76.00	24.00
Shamshadipur, Rajshahi	18	72.22	27.78
Horian, Rajshahi	20	58.80	41.20
Parila, Rajshahi	35	77.77	22.23
Rajshahi University Botanical garden	21	71.42	28.57
IBSC Research field	10	70.00	30.00
National strawberry field, Rajshahi	8	62.50	37.50
Kashindaparila 1, Rajshahi	9	66.66	33.34
Nowhata, Rajshahi	10	59.20	40.80
Mohanpur, Rajshahi	7	57.14	42.86
Kashindaparila 2, Rajshahi	8	75.00	25.00
Akafuji field, Rajshahi	6	66.66	33.34
Nowhatabajar field, Rajshahi	5	60.76	39.24
Puthia field, Rajshahi	9	77.14	22.46
Binodpur field, Rajshahi	5	60.00	40.00

Table 2. Effect of different leaf extracts on radial mycelial growth of *Pestalotiopsis* sp.

Name of the Plant	Concentrations (ppm)	Radial mycelium (mm) in different incubation period (days)				Inhibition of mycelial growth in different incubation period (days)			
		3	6	9	12	3	6	9	12
Azadirachta indica (Neem)	50	6.3	12.5	21.3	33.6	67.7	66.7	62.2	54.8
	100	3.3	10.3	21.0	23.3	83.1	72.5	62.7	68.6
	250	2.6	9.6	17.3	22.6	86.7	74.4	69.3	69.6
	500	2.3	9.3	12.3	17.3	88.2	75.2	78.2	76.7
	1000	2.0	8.3	11.0	13.0	89.7	77.9	80.9	82.5
	Control	19.5	37.5	56.3	74.3				
	F Value and LSD	16.83*, 11.65							
Ocimum sanctum (Tulsi)	50	19.5	28	44.5	53.3	36.4	34.1	26.7	31.8
	100	18.7	27.2	43.6	52.1	38.8	36	28.2	33.4
	250	18.1	26.7	43	51.8	40.9	37.2	29.2	33.8
	500	17.8	26	42.9	51.2	41.9	38.8	29.4	34.5
	1000	16.2	24.9	41.1	49.1	47.2	41.4	32.3	37.2
	Control	30.6	42.5	60.7	78.2				
	F Value and LSD	37.31*, 4.13							
Aloe indica (Alovera)	50	17.5	38.6	46.4	65.5	26.5	17	22.9	7.2
	100	16	36.7	44.5	59	32.8	21	26.1	16.4
	250	14.6	32	42	57.2	38.4	31.2	30.3	18.9
	500	13.1	31.5	39	55.9	44.9	32.3	35.2	19.4
	1000	12.5	29.1	37.5	55.3	47.5	37.4	37.7	21.7
	Control	23.8	46.5	60.2	70.6				
	F Value and LSD	38.49*, 3.36							
Callistemon linearis (Bottlebrush)	50	19.5	28.6	39.1	58.8	22.3	33.7	40.2	22.2
	100	17.6	25.8	37.1	55.5	29.9	40.1	43.3	26.6
	250	13	24.1	35.2	45.1	48.2	44.2	46.2	40.3
	500	11.5	22.2	32.1	44	54.2	48.6	50.9	41.8
	1000	9.1	20	28	41.7	63.7	53.7	57.2	44.8
	Control	25.1	43.2	65.4	75.6				
	F Value and LSD	23.57*, 6.99							
Terminalia arjuna (Arzun)	50	8.33	15.1	24.2	35.1	70.9	63.2	57.1	50.9
	100	7.20	14.2	22	33.6	74.9	65.4	61.1	52.9
	250	6.75	11.7	20.8	31.2	76.4	71.3	63.2	56.4
	500	3.9	9.33	13.4	16.8	86.4	77.2	76.3	76.5
	1000	3.3	8.7	12.5	15.1	88.5	78.8	77.9	78.9
	Control	28.6	41.0	56.5	71.5				
	F Value and LSD	17.6*, 10.9							

*= significant at 5% level

Table 3. *In vitro* evaluation of different fungicides against mycelial growth of *Pestalotiopsis* sp.

Fungicides	% inhibition of mycelial growth at concentrations (ppm) (Mean ±SE)						LSD at 5 %
	50	100	250	500	1000	($\overline{X}$ ±SE)	
Bavistin	90.8±0.26	100	100	100	100	98.17±0.05	0.675
Tilt	53.6±0.23	56.8±0.26	64.7±0.22	70.8±0.26	80.2±0.22	64.64±0.24	
Hayconazole	8.6±0.23	12.0±0.28	13.7±0.27	17.8±0.26	20.5±0.24	14.42±0.25	
Score	71.8±0.26	74.6±0.23	80.4±0.24	85.1±0.26	100	81.44±0.19	
Rovral	81.0±0.28	84.0±0.28	86.6±0.23	89.3±0.16	90.1±0.26	86.17±0.24	
Antracol	24.7±0.22	28.0±0.28	31.1±0.26	34.1±0.20	34.7±0.22	30.51±0.23	
Dithane	90.2±0.22	100	100	100	100	98.04±0.04	
Ridomil	42.1±0.26	46.6±0.33	50.2±0.22	55.3±0.16	63.2±0.22	50.97±0.23	
Folicur	17.7±0.27	20.2±0.22	26.1±0.20	30.4±0.17	33.1±0.20	25.33±0.21	
Cupravit	77.1±0.26	81.1±0.26	84.6±0.23	90.2±0.22	100	85.97±0.19	
Secure	26.0±0.23	30.4±0.17	35.2±0.22	41.2±0.22	45.7±0.22	35.44±0.21	
Thiovit	20.2±0.22	22.4±0.29	25.7±0.22	26.7±0.27	29.8±0.26	24.82±0.25	
Sulcox	25.3±0.16	34.5±0.24	41.2±0.22	44.2±0.22	47.3±0.33	38.35±0.23	

Table 4. Disease incidence and yield contributing characters of treated plants under field condition.

Treatments (1000 ppm)	Crown rot disease incidence (%)	% increase above that of the control	No. of fruits/plant	% increase above that of the control	Fruit weight/ plant (g)	% increase above that of the control
Neem	62	27.05	9.20±0.23	72.60	81.00±0.57	26.80
Arjun	81	4.70	9.22±0.23	72.40	105.96±0.61	65.87
Alovera	81	4.70	8.80±0.23	65.10	80.4±0.79	25.23
Bottlebrush	77	9.41	6.28±0.23	17.82	85.0±0.79	33.06
Tulsi	81	4.70	6.28±0.23	17.82	80.4±0.79	24.23
Bavistin	21.5	74.70	10.88±0.23	104.12	148.56±0.81	132.25

Treatments (1000 ppm)	Crown rot disease incidence (%)	% increase above that of the control	No. of fruits/plant	% increase above that of the control	Fruit weight/ plant (g)	% increase above that of the control
Rovral	32	62.35	9.33±0.23	75.04	134.86±0.88	111.11
Dithane	27.5	67.64	8.88±0.26	66.60	123.96±0.98	94.05
Cupravit	32	62.35	8.80±0.23	65.10	119.20±0.98	86.59
Control	85	--	5.33±0.23	--	63.88±0.26	--

Conclusion

Pestalotiopsis sp. were the fungi with the highest frequency on all parts of strawberry plants with symptoms of crown rot disease in the sampling area., This study revealed that *Pestalotiopsis* sp. were causal agent of crown rot disease on strawberries in Bangladesh and it could be controlled by using fungicides. This is the first report on *Pestalotiopsis* disease of strawberries in Bangladesh.

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References

Ahmed HU, Hossain MM, Alam SMK, Huq MJ, Andossain M. 1991. Efficacy of different fungicides in controlling athracnose and sooty mold of mango. Bangla. J. Bot **4,** 155-159.

Akhter MS, Alam S, Islam MS, Lee MW. 2009. Identification of the Fungal Pathogen that Causes Strawberry Anthracnose in Bangladesh and Evaluation of *In Vitro* Fungicide Activity. Mycobiology **37(2),** 77-81.

Alam S, Akhter N, Begum M, Alam MS. 2000. Effect of fungicides and plant extracts on the inhibition of *Bipolari sorokiniana* Sacc. Rajshahi Univ. Stud. Part-B, J. Sci. **28,** 15-23.

Ara T, Islam R, Hossain M. 2013. Varietal improvement in strawberry. LAP- LAMBART Academic Publishing Germany.

Arauz LF. 2000. Mango anthracnose: economic impact and current options for integrated management. Plant Diseases **84,** 600-611.

Bajwa R. 2005. Antifungal activity of allelopathic plant extracts VI: In Vitro control of fungal pathogens by aqueus leaf extracts of Eucalyptus. Mycopath (Pakistan).

Bonde MR, Peterson GC, Maas GL. 1991. Isozyme comparisons foridentification of *Colletotrichum* spp. pathogenic to strawberry. Phytopathology **81,** 23-1528.

Bostos CL, Bezerra JL, Santos AO. 2001. Ocorrencia de *Pastalotiopsis cruenta* em mangostao. Fitopathologia Brasileira **26(4),** 779.

Delp BR, Milholland RD. 1981. Susceptibility of strawberry cultivars andrelated species to *Colletotrichum fragariae*. Plant Dis **65,** 21-23.

Dunga L, Dienb NT, Daic PH, Tuana PN. 2016. The First Report of *Pestalotiopsis* sp. Causing Crown Rot Disease on Strawberry in Dalat. Dalat University Journal of Science **6(3),** 364-367.

Embaby EM. 2007. *Peatalotia* fruit rot on strawberry plants in Egypt. Egypt Journal Phytopathology **35,** 99-100.

Faiz H, Iram S, Rasool A. 2016. Management of mango diseases anthracnose and blossom blight by ecofriendly methods. Int. J. Agri. Agri. R **8(4),** 111-119.

Haque A, Siddiki MMA, Rahman AFMM, Chawdhury AMS, Hasan CM. 2013. Antimicrobial and cytotoxic activities of the crude extracts of *Callistemon linearis*. Int. J. Biosci **3(3),** 129-133.

Howard CM. 1973. Strawberry fruit rot caused by *Pestalotia longisetula*. Ibid **63,** 443.

Jabbar A, Malik AU, Islam-ud-Din, Anwar R, Ayub M, Rejwana IA, Amin M, Khan AS, Saeed M. 2011. Effect of combined application of fungicides and hot water quarantine treatment on postharvest diseases and quality of mango fruit. Pakistan Journal of Botany **43,** 65-73.

Keith LM, Velasquez ME, Zee FT. 2006. Identification and characterization of *Pestalotiopsis* spp. Causing scab disease of guava, *Pisidium guajava* in Hawaii. Plant Disease **90,** 16-23.

Khatun R. 2014. Fungal diseases of strawberry in Bangladesh with special references of crown rot disease. Ph. D Thesis, Institute of Biological Sciences, University of Rajshahi Bangladesh.

Kruschewasky MC. 2010. Taxonomia e ecologia do grnero *Pestalotiopsis* no Brasil, com enfasepara a Meta Atlantica do sul da Bahia. Dissertacao (Mestradoem Producao Vegetal). Univer-sidade Estadual de Santa Cruz, Iheus, Bahia.

Miah S. 2006. "Rajshahi Division". In Sirajul Islam. Banglapedia: National Encyclopedia of Bangladesh. Asiatic Society of Bangladesh.

Michael AE. 2008. Strawberry leaf disease: The Ohio State University.

Mouden N, Benkirane R, Ouazzani TA, Douira A. 2014. Pathogenic capacity of *Pestalotiopsis longisetula* Guba reported for the first time on strawberry (*Fragaria ananassa* Duch) in Morocco. Int. J. Pure App. Biosci **2(4),** 132-141.

Mukherjee A, Khandkar S, Islam MR and Shahid SB. 2011. Efficacy of some plant extracts on mycelia growth of *Colletotrichum gloesporioides.* Journal of Bangladesh Agricultural University **9,** 43-47.

Nagadesi PK, Arya A. 2016. Biocontrol of timber decaying fungi by botanical pesticides an ecofriendly technology. World Scientific News **44,** 206-223.

Pereira C, Teixeira MA, Vieira RF, Pereira GC, Faria JP. 2011. Evaluation of *Melaleuca* sp. oil in the control of *Pestalotiopsis longisetula*. A phytopathogenic fungus in strawberry cultivation in field condition. An Abstracts published in Resumos apresentados no V Simposio de Microbiologia Aplicada. Volume 11-Numero 1-Suplemento 1.

Rathod MC, Godhani VJ, Dhale DA. 2015. Antifungal Activity of Some Medicinal Plant Material Extract against Fungus *Aspergillus niger*. World Journal of Pharmacy and Pharmaceutical Sciences **4(10),** 1323-1332.

Shitole DM, Patil UR, Pawar NB. 2000. *In Vitro* evaluation of chemicals and antibiotics against important fruit rooting of strawberry. J. Maharasgtra Agric. Univ **25,** 179-181.

Teixeira MA, Martins RMS, Vieira RF, Vildoso CI, Adami MAV, Ferreira AC. 2015. *In vitro* Identification and control of *Pestalotiopsis longisetula* fungus, pathogens strawberry crop. Revista Agrogeoambienta, Pouso Alegre **7(3),** 59-65.

Tijjani A, Adebitan SA, Gurama AU, Haruna SG, Safiya T. 2014. Effect of some selected plant extracts on *Aspergillus flavus*, a causal agent of fruit rot disease of tomato (*Solanum lycopersicum*) in Bauchi State. Int. J. Biosci **4(12),** 244-252.

Vincent JM. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. Nature **159,** 850.