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ECO-FRIENDLY MANAGEMENT OF FRUIT ROT DISEASE CAUSED BY *PHOMOPSIS VEXANS* IN EGGPLANT FOR SEED PRODUCTION

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ABSTRACT

Phomopsis fruit rot is a major constraint to the quality seed production of eggplant in Bangladesh. A series of experiments were strategically designed to select the most effective treatments for eco-friendly management of *Phomopsis vexans* in eggplant seed production. The organism causing fruit rot in eggplant was identified as *Phomopsis vexans* based on its key characteristics (CMI Description No. 338). To manage the disease, a bioagent (*Trichoderma harzianum*), three fungicides—Companion, Autostin 50WDG, and Tilt 250EC—four plant extracts—Allamanda leaf extract, garlic clove extract, turmeric rhizome extract, and onion bulb extract—and Krishibid Organic Fertilizer were evaluated. Foliar spraying with *Trichoderma harzianum* ("Biotech Care Trichoderma Suspension") was found to be the most effective in controlling the incidence and severity of fruit rot caused by *Phomopsis vexans*, increasing fruit yield by 142.05% and seed yield by 146.84% compared to the control. Seed germination of harvested seeds increased by 88.41% over the control. Soil amendment with Krishibid Organic Fertilizer combined with Autostin 50WDG spraying was also effective, increasing fruit yield by 137.39%, seed yield by 142.54%, and seed germination by 93.56% compared to the control. Among the plant extracts, garlic bulb extract and turmeric rhizome extract were found to be the most effective, increasing fruit yield by 112.16% and 96.93%, and seed yield by 112.15% and 100.05%, respectively, over the control.

Keywords: *Phomopsis vexans*, eggplant, fruit rot, eco-friendly, *Trichoderma harzianum*

INTRODUCTION

Eggplant (*Solanum melongena* L.), also known as Brinjal or Aubergine, is an important solanaceous crop of tropics and sub-tropics in the world. The crop is ranked 4th as a vegetable in the world, and in Bangladesh, it is one of the most common, popular, and principal vegetable crops grown throughout the country. Its position in terms of production is first in winter vegetable crops, where the total cultivable area of eggplant is 78,458 acres with a total annual production of 3,40,150 metric tons (BBS 2017). It is described as the 'King of vegetables' due to its versatility as food (Choudhary and Gaur 2009). It is nutritious, being low in calories, fat, and sodium, and is a non-starchy fruit that is cooked as a vegetable. It is also known to have ayurvedic medicinal properties and is good for diabetic patients. The shape of fruit varies from ovoid, oblong, obovoid, or long cylindrical, and seeds are borne on the fleshy placenta, and the placentae with the seeds completely fill the locular cavity. It provides a continuous source of income, which brings solvency to the eggplant growers as well as contributing to our national economy.

The production and quality of eggplant are highly affected due to its susceptibility to a wide range of pests and pathogens, especially in our country, where eggplant is known to suffer from 12 diseases. Among them, fruit rot and leaf blight, caused by *Phomopsis vexans*, is a major disease reducing brinjal production in terms of fruit yield by affecting seed germination, seedling mortality, killing the plants, rotting of fruits, and spoiling the fruit quality (Beura 2008). The causal organism of the disease is both externally and internally seed-borne and remains viable for about 14 months in soil with plant debris and in the seed from infected fruits (Sekara *et al.* 2007). *Phomopsis vexans* is a pycnidial fungus with an apparent sexual form in the genus *Diaporthe*, particularly seedborne and producing large numbers of conidia. It causes disease in *Solanum melongena*, its only significant host, causing poor seed germination and damping off of seedlings, leaf and stem lesions, and fruit rot, both in the field and after harvest (Chalkley 2010). Seeds of vegetables are more vulnerable to attack by pathogens and quickly deteriorate during storage. Quality and healthy seed is crucial for sustainable crop production, and pathogen-free seeds are regarded as the vital factor for optimum plant population as well as a good harvest. Seed production of eggplant is difficult because the plants are kept in the field for a much longer period for proper maturity. During this extended period, the fruits become more susceptible to pathogenic attack, and thus seed production is severely hampered (Islam 2005).

To control this disease for commercial production, a number of synthetic pesticides are being used indiscriminately on a large scale. However, indiscriminate use of chemical pesticides in the long run leads to residual toxicity, induced resistance to pathogens, environmental pollution, and side effects on human and animal health (Avinash and Hosmani 2012). Improper use of pesticides has led to residue accumulation on eggplants (Chowdhury *et al.* 2013). Consequently, importing countries have imposed restrictions on vegetable exports, especially eggplants from Bangladesh (Rahman 2016). The negative impact of excessive and indiscriminate use of harmful pesticides has brought changes in attitude towards uses of pesticides in agriculture. Thus, biological control of pathogens is an environmentally sound method and avoids other threats posed by the use of chemicals, such as health hazards and the development of resistance in pathogens (Kumar *et al*. 2017).

In this context, the present investigation was undertaken to screen out and evaluate the potential management options that will be effective against *Phomopsis vexans* to develop compatible, effective, economic, and environment-friendly practices for the management of this disease for seed production.

MATERIALS AND METHODS

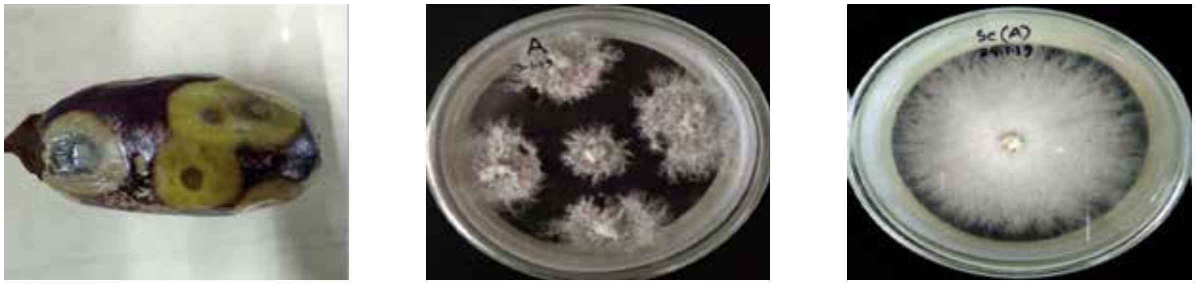
The experiment was conducted in the M. S. Laboratory, Department of Plant Pathology and in the Central Farm, Sher-e-Bangla Agricultural University, Dhaka-1207. The efficacy of four botanicals, viz., Allamanda (*Allamanda cathertica*) leaf extract (1:2 w/v), Garlic (*Allium sativum*) clove extract (1:2 w/v), Onion (*Allium cepa*) bulb extract (1:2 w/v), Turmeric (*Curcuma longa*) rhizome extract (5%); one biocide, *Trichoderma harzianum* ("Biotech Care Trichoderma suspension"); three synthetic pesticides, viz., Companion (0.2%), Autostin 50WDG (0.2%), Tilt 250EC (0.05%), and one Krishibid Organic Fertilizer (as soil amendment) combined with Autostin 50WDG spraying were tested, and an untreated control plot was maintained for comparisons. The experimental field was divided into three blocks, each representing a replication. The unit plot size was 4.5 sq. m. (4.5 m x 1 m). Plot to plot distance was maintained at 0.5 m, and block to block distance was 1 m. The *in vitro* experiments were conducted with three replications laid out in a Completely Randomized Design (CRD). The field experiment was also conducted with three replications and with Randomized Complete Block Design (RCBD)

Sample collection, isolation, and *in vitro* experiment of *Phomopsis vexans*

Eggplant (*Solanum melongena* L.) fruits showing initial stages of the symptoms were collected from Kawran Bazaar Vegetable Market, Dhaka. The infected tissue (fruit) was cut into small pieces (2–3 mm) with a margin of healthy tissue around it and placed in PDA media in sterilized petri dishes (Figure 1). The dishes were incubated at 25±2°C to allow the pathogen to grow, and when the growth of the fungus was visible, the mycelia of the organism was transferred into several fresh PDA plates by the hyphal tip culture method to prepare a pure culture of the pathogen and preserved at room temperature. For the laboratory experiment, three discs (5 mm) of the medium were scooped by the cup method from three places on a PDA plate, maintaining an equal distance from the center, by a sterilized disc cutter. One millimeter solution of fungicide, plant extract, or bioagent was put into each hole. In the case of control, only sterile water was poured into the cup. After 7 days, different redial mycelium growth was observed.

Inoculation, Application, and Seed Health Test

Spore suspension of *Phomopsis vexans* containing 5×10^6 spore/ml was sprayed 20 days after fruit formation. After inoculation, the plants were covered with polythene bags for 24 hours to ensure a favorable microclimate for the successful infection by the pathogen (Figure 2). Among the treatments, Krishibid Organic Fertilizer was mixed with soils of the root zone of the crop during transplanting. Spore suspensions of *Trichoderma harzianum*, plant extract, and fungicide solutions were applied at the fruiting stage of the crop. The pre-inoculation sprays were applied one day before inoculation, which was followed by two subsequent sprays with a 7-day interval at 50 ml/plant. The spraying was done by a hand sprayer to cover the whole surface of the fruit. Precautionary measures were taken to prevent drifting of spray materials to the neighboring plants using a polythene barrier (Figure 2).



Infected with Phomopsis rot | Tissue planting on PDA media | Pure culture of the pathogen

Figure 1: Isolation of the fungus by Tissue planting method

A. Seedling Stage | **B.** Transfer of mature plants | **C.** Covered with polythene bags for successful inoculation

D. Polythene barrier to prevent treatment spray drift | **E.** Fruiting stage | **F.** Harvesting Stage

Figure 2: Different stages of Eggplant in field experiment

Seeds arrangement | Incubation at room temperature | Germinated seeds

Figure 3: Seed health testing in blotter method

Data collection, analysis, and calculations

The brinjal variety 'BARI Bt Begun 2' was used for the experiment, and the seeds were collected from BADC (Bangladesh Agricultural Development Corporation), Dhaka. All collected data were tabulated and analyzed following standard procedure (Gomez 1984). Treatment means were compared by the LSD (Least Significant Difference) value. The Statistix 10 computer package was used for performing the statistical analysis. After inoculation and spraying of fungicides, plant extracts, and spore suspensions of bioagent, data were taken on the following parameters: % fruit infection, % FAD (fruit area diseased), fruit weight, and seed weight of individual plots. Inhibition of radial growth was calculated after 7 days of incubation based on colony diameter on the control plate using the following formula (Vincent 1947): $I = \frac{C-T}{C} \times 100$, where, I = percent inhibition of mycelial growth over control, C = radial growth of pathogen in control, and T = radial growth of pathogen in treatment applied. Disease severity, or Percent Disease Index (PDI), was calculated by using a 0–5 rating scale and the following formula developed by McKinney (1923): $PDI = \frac{\text{Sum of total disease rating} \times 100}{\text{Total no. of observations} \times \text{The highest grade in the scale}}$

RESULTS AND DISCUSSION

Isolation and identification of the pathogen

In the PDA media, the pathogen produced whitish mycelia, which later turned grayish white. In around 30 days, globose to irregular-shaped, black pycnidia appeared, having hyaline, elliptical, one-celled alpha conidia (Figure 4). The pathogen was compared with the literature described by Punithalingum and Holliday (1972), Islam (2005), Anwar *et al.* (2017), and CMI description No. 338.

In vitro evaluation of bioagent, fungicides, and plant extracts against radial mycelial growth of *Phomopsis vexans*

In the laboratory, redial mycelial growth of *Phomopsis vexans* varied significantly among the treatments. The lowest mycelial growth was recorded in cases of *Trichoderma harzianum* spore suspension (4.333 mm) and Autostin 50WDG (4.567 mm). Companion (7.167 mm) also inhibited the mycelial growth, and the highest mycelial growth was observed in the control (87.3 mm). Among the plant extracts, garlic clove extract was found most effective in reduction of mycelial growth, followed by allamanda leaf extract and turmeric rhizome extract. Both onion bulb extract and tilt 250 EC reduced the mycelial growth of the pathogen, which was comparatively lower than the other treatments but significantly higher compared to control. The inhibition percentages of mycelial growth of *Phomopsis vexans* are presented in Figure 5. The present result is nearly similar to the findings of other researchers. Jadeja (2003) reported that the antagonist *Trichoderma harzianum* had shown promising performance in inhibiting the growth of *Phomopsis vexans*. He also reported that garlic clove extract highly suppressed the mycelial growth of the pathogen.

Evaluation of bioagent, fungicides, and plant extracts on disease incidence and disease severity (PDI) of fruit rot of eggplant caused by *Phomopsis vexans*

The characteristic symptoms of pomopsis fruit rot appeared ten days after inoculation. On the infected fruit surface, the symptoms first appeared as pale, sunken, round to oval light brownish spots and then gradually increased in size. Later, a concentric ring with a brownish halo appeared, and finally, black, pimple-like pycnidia appeared on the fruit surface.

The treatments applied for the management of phomopsis fruit rot of eggplant in the field condition significantly reduced the disease incidence and disease severity (Table 1). Spraying of *Trichoderma harzianum* spore suspension was found most effective with the lowest disease incidence and severity, which was statistically similar to Autostin 50WDG. Krishibid Organic Fertilizer combined with Autostin 50WDG spraying also reduced the disease incidence and severity. Among the plant extracts, garlic clove extract was found highly effective, followed by turmeric rhizome extract. The highest disease incidence and severity were observed in the control plot. Ghosh (2017) applied *Trichoderma harzianum* for the management of Phomopsis fruit rot of eggplant. The result showed spraying of *Trichoderma harzianum* suspension at the interval of fifteen days after the initiation of fruits was highly effective.

Effect of different treatments on fruit yield (t ha^{-1}), seed yield (kg ha^{-1}), and seed health of eggplant

All the treatments significantly influenced the fruit yield, seed yield, and seed health as compared to control plots (Table 2). The plot treated with *Trichoderma harzianum* spore suspension resulted in the highest increase of fruit yield and seed yield, followed by soil amendment with Krishibid Organic Fertilizer combined with Autostin 50WDG spraying. However, the highest increase in seed germination of harvested seed was recorded in the case of soil amendment with Krishibid Organic Fertilizer combined with Autostin 50WDG spraying, followed by foliar spray of *Trichoderma harzianum.* Garlic clove extract (18.67 t ha^{-1}) was the most effective in increasing fruit yield. Islam (2005) stated that *Trichoderma harzianum*, Bavistin (50% carbendazim), and garlic bulb extract were found effective for the management of phomopsis fruit rot. Srinivas *et al.* (2005) reported that the formulation of *Trichoderma harzianum* was effective in reducing the *Phomopsis vexans* infection and increasing seed germination in comparison with control.

A. Pure culture of *Phomopsis vexans* with pycnidia

B. Alpha conidia of *Phomopsis vexans* under compound microscope (40×)

Figure 4. Pure culture and sporulation (alpha conidia) of *Phomopsis vexans*

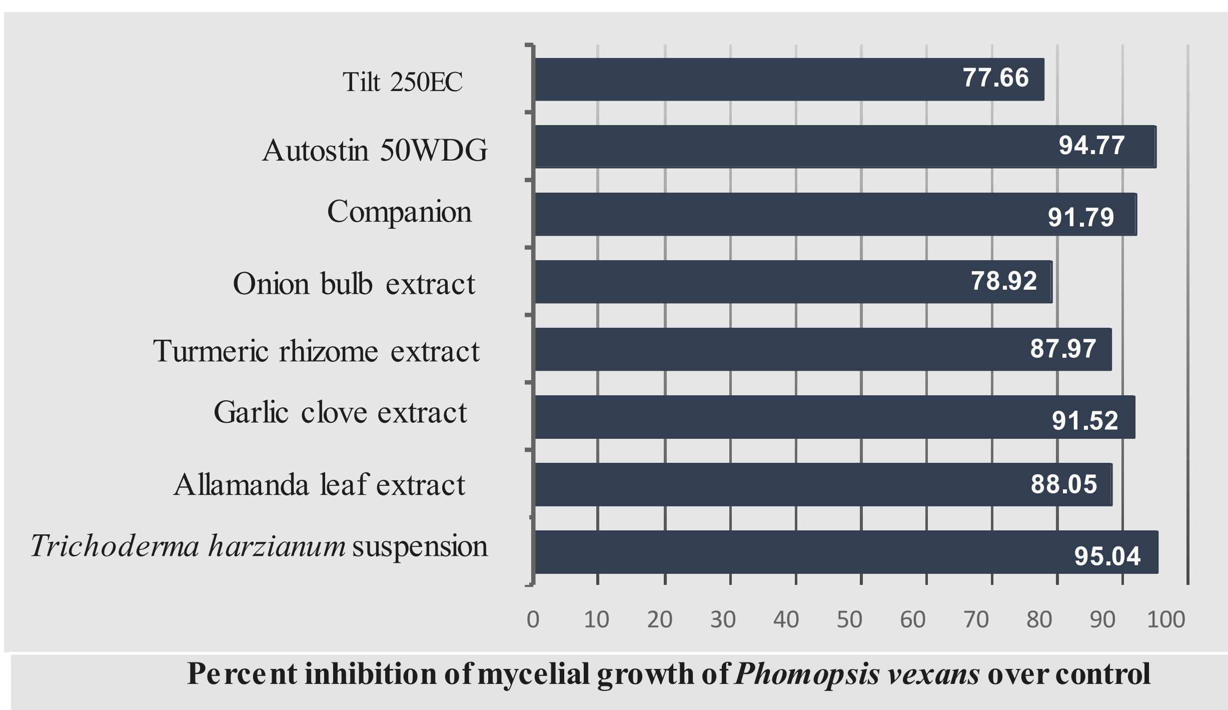


Figure 5. In vitro evaluation of bioagent, fungicides and plant extracts against mycelial growth of *Phomopsis vexans*

Table 1. Evaluation of different treatments on disease incidence and disease severity (PDI) of fruit rot of eggplant caused by *Phomopsis vexans*

Treatment	% Disease Incidence	% reduction of disease incidence over control	% Disease Index (PDI)	% reduction of PDI over control
T1 = *Trichoderma harzianum*	6.95 f	77.32	4.43 g	83.38
T2 = Allamanda leaf Extract	14.69 bc	52.07	11.97 c	55.10
T3 = Garlic clove Extract	11.32 c-e	63.06	9.31 de	65.08
T4 = Turmeric rhizome Extract	12.54 cd	59.09	10.64 cd	60.09
T5 = Onion bulb extract	17.38 b	43.30	15.46 b	42.01
T6 = Companion	13.37 cd	56.38	9.75 c-e	63.43
T7 = Autostin 50WDG	8.25 ef	73.08	6.62 fg	75.17
T8 = Tilt 250EC	18.15 b	40.78	16.67 b	37.47
T9 = Krishibid Organic Fertilizer with Autostin 50WDG	10.85 de	64.60	7.74 ef	70.97
T10 = Control	30.65 a	-	26.66 a	-
LSD (0.05)	3.6595		**2.2505**	
CV (%)	14.80		**11.00**	

Table 2. Effect of different treatments on fruit yield, seed yield, and seed health of eggplant

Treatment	Fruit Yield (t ha^{-1})	Seed Yield (kg ha^{-1})	Seed Yield increase over control (%)	Seed germination (%)	Incidence of *Phomopsis vexans* in seed (%)	Increase of seed germination over control (%)
T1 = *Trichoderma harzianum*	21.3 a	92.44 a	146.84	92.47 ab	2.67 g	88.41
T2= Allamanda leaf extract	16.04 b-d	70.48 c-e	88.20	84.87 d	11.00 cd	72.92
T3= Garlic clove Extract	18.67 a-c	79.45 a-c	112.15	88.67 c	6.33 f	80.67
T4= Turmeric rhizome extract	17.33 a-d	74.92 b-d	100.05	88.67 c	8.33 e	80.67
T5= Onion bulb Extract	15.11 cd	65.68 de	75.38	83.60 d	11.67 c	70.33
T6= Companion	17.78 a-d	75.65 b-d	102.00	88.67 c	9.33 de	80.67
T7= Autostin 50WDG	19.98 ab	84.42 ab	125.42	91.20 bc	2.67 g	85.82
T8= Tilt 250EC	13.30 d	57.21 e	52.76	78.53 e	19.00 b	60.00
T9= Krishibid Organic with Autostin 50WDG	20.89 a	90.83 a	142.54	95.00 a	1.33 g	93.56
T10= Control	8.8 e	37.45 f	-	49.08 f	38.33 a	-
LSD (0.05)	**4.4862**	**13.494**		**1.9169**	**2.9094**	
CV (%)	**15.46**	**10.80**		**10.17**	**2.03**	

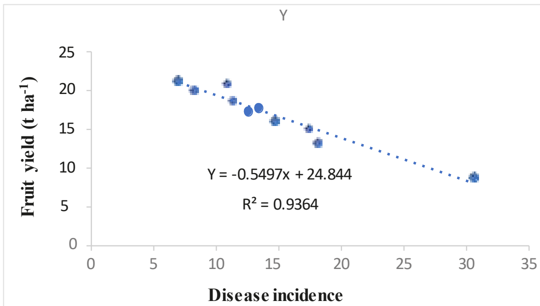


Figure 6. Relationship between disease incidence of Phomopsis fruit rot and fruit yield (t ha^{-1})

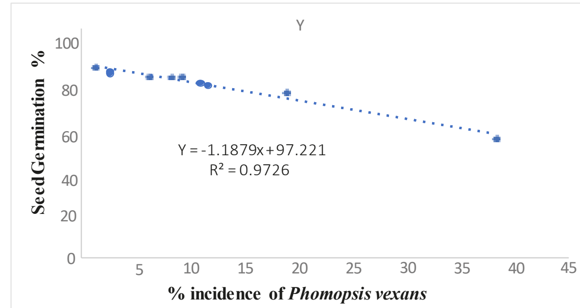


Figure 7. Relationship between % incidence of *Phomopsis vexans* in seed and % seed germination

Correlation and Regression

A correlation and regression study were done to determine the relationship between disease incidence and fruit yield (t ha^{-1}) and seed germination (%) of eggplant. Significant and negative correlations were observed for both cases.

CONCLUSION

Fruit rot of eggplant caused by *Phomopsis vexans* is one of the most destructive diseases of eggplant and is regarded as the main barrier to quality seed production in Bangladesh. From the present study, it may be suggested that foliar spray of *Trichoderma harzianum* ("Biotech Care Trichoderma Suspension"), soil amendment with Krishibid Organic Fertilizer combined with spraying of Autostin 50WDG, garlic clove extract, and turmeric rhizome extract could be effective as an eco-friendly approach for the management of fruit rot of eggplant for seed production. The results might be recommended at the field level to boost eggplant and its seed production by decreasing phomopsis rot incidence in Bangladesh.

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