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Trichoderma asperellum suppresses viral diseases and promotes the growth and yield of country bean

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Viral diseases are the main adversaries of country bean (*Lablab purpureus* Lin.) production in Bangladesh. Potyviruses and cucumber mosaic virus (CMV) have been reported in country bean leaves that displayed virus-like symptoms. This study looked at the growth and yield of country bean plants that had been treated with *Trichoderma asperellum* to control country bean viruses. *T. asperellum*-treated plants exhibited decreased disease incidence up to 91% and a drop in the vector population up to 96%, when compared to control plants. Plant growth was enhanced in soil drenched with *T. asperellum* suspension, with an increase in the number of leaves per plant, pods per plant, root length, weight of dried pods/plant, and weight of dried seeds/plant. Finally, our findings suggest that *T. asperellum* could be an effective treatment for controlling viral diseases of the country bean in Bangladesh.

KEYWORDS

bio-control, diseases, bean, ecofriendly management, virus

Introduction

Country bean (*Lablab purpureus* Lin.), a member of the Leguminosae family and the Papilionaceae subfamily, is regarded as an economically significant pulse crop or vegetable in Bangladesh (Biddle and Cattlin, 2007; Jayasinghe et al., 2015). This is primarily grown in almost all districts of Bangladesh during the winter season (Chowdhury et al., 1989; Sibiko et al., 2013; Bangladesh Bureau of Statistics (BBS), 2020). The bean crop improves soil fertility by establishing a symbiotic relationship with rhizobium bacteria in order to fix atmospheric nitrogen (Karla, 2009). Country bean seeds and pods are edible and high in

protein, carbohydrates, essential nutrients, and vitamins, while the rest of the foliage is used as fodder. As a result, it has a significant nutritional impact on both rural and urban Bangladeshis (Rehana, 2006).

Viruses cause serious threats to bean crop production in Bangladesh (Akhter et al., 2019). These viruses can be transmitted through seeds, nematodes, and insects (Hema et al., 2014). Potyviruses such as bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCNMV) cause "common mosaic" symptoms in bean plants, which are also associated with symptoms such as green vein banding and leaf deformity, as well as systemic necrosis and plant mortality (Morales, 2003; Schwartz et al., 2005). Another potyvirus, Bean Yellow Mosaic Virus (BYMV), reduces photosynthesis by reducing pigment content in leaves, causing severe mosaic, mottling, and crinkling, as well as size reduction (Mojca et al., 2001; Miteva et al., 2005). CMV causes symptoms in bean plants that range from minor mosaic in the leaves to severe plant deformity, with 5% to 75% production losses (Morales, 2003; Schwartz et al., 2005). Various serological and molecular methods are used for detecting viruses in bean plants. For example, immunostrips are used in the field to detect potyviruses and CMV in a quick, easy, and practical manner (Chiemsombat et al., 2014).

In Bangladesh, field control of viral diseases in bean plants is difficult due to a lack of direct chemical therapies and effective resistance cultivars. Therefore, the integration of traditional control measures such as cultural management, vector control, and the use of virus-free planting material is encouraged to deal with viral diseases in country beans (Akhter et al., 2019). Biological control agents, such as *Trichoderma* spp., on the other hand, have been shown to have the potential to inhibit viral diseases by inducing plant defense responses such as systemic resistance against pathogenic viruses, as well as to improve plant growth and development (Harman, 2006; McLean et al., 2012; Vitti et al., 2015). Trichodermin, a trichoderma product, has antagonistic properties and promotes plant germination, seedling emergence, height, weight, and yield (Tverdyukov et al., 1994). Bhardwaj and Kumar (2017) found volatile secondary metabolites in *T. asperellum* culture filtrate with diverse actions ranging from anti-pathogenic to plant growth stimulation. Thus, the purpose of this research is to find out the effectiveness of the *T. asperellum* PMILN51 isolate in controlling viral diseases and promoting the growth and yield of country bean.

Materials and methods

The current study looked at the efficacy of a *T. asperellum* PMILN51 isolate in suppressing viral diseases of country beans and promoting growth and yield. The research was conducted between November 2019 and March 2020 at the Seed Pathology Centre (SPC) and Plant-Microbe Interaction Laboratory (PMIL), Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. Sheem Kartika, a high-yielding native variety of country bean in Bangladesh, was used in the pot experiments. The plastic pots in the experiments were filled with 5 kg of 4% formalin-treated soil mix. Twenty-four pots were prepared for eight treatments, including the control, and each treatment was replicated three times. On November 4, 2019, ten seeds were sown in each container. To maintain the optimal plant population (3 plants/pot), thinning was performed 21 days after sowing (DAS). Fertilizers were administered to each pot according to the Fertilizer Recommendation Guide's suggested dose (BARC (Bangladesh Agricultural Research Council), 2012). Weeding and irrigation were done on a regular basis in all of the pots as needed. Treatments listed in Table 1 were used to assess the impact of the *T. asperellum* PMILN51 isolate on the incidence and severity of country bean viral infections, as well as the growth and yield contributing features of country beans. Pure culture suspension and formulation of *T. asperellum* PMILN51 isolate,with strong antagonistic activity were prepared as reported previously (Islam, 2018) and used for biopriming, foliar spray, and soil soaking (Vitti et al., 2016).

The incidence of country bean viral diseases was determined in each pot (Degu et al., 2020) using following formula –

$$Disease\ incidence\ (\%) = \frac{\text{Number of infected leaves}}{\text{Total number of leaves}} x\ 100$$

In this study, potyvirus and cucumber mosaic virus (CMV) detecting immunostrips (Agdia, USA; www.agdia.com) were used to check the presence of potyvirus and CMV following manufacturer instructions. Insect population densities were visually counted from each pot (Alao et al., 2017). Plant height (cm), number of healthy leaves per plant, total number of pods per plant, total quantity of seeds per plant, root length (cm) after harvest, dry pod weight (g) per plant, and dry seed weight (g) per plant were all measured in both healthy and diseased plants. The experiment was carried out in a completely randomized design with three replications. The data on various parameters were statistically analyzed using the Analysis of Variance (ANOVA) technique to determine the degree of significance (Gomez and Gomez, 1984). Duncan Multiple Range Test (DMRT) was used to compare the treatment means at a 5% level of significance. SAS (University Edition version 3.71, basic edition) was used to analyze the obtained data.

Results and discussion

T. asperellum is an effective biocontrol agent that boosts plant defence mechanisms against viral infection (Elsharkawy et al., 2013). The current study found that spraying country bean plants with a foliar application of a pure culture suspension of *T. asperellum* PMILN51 isolate (T6) reduced incidence of viral diseases in country bean plants. In the control plants, symptoms of three different diseases: common mosaic, yellow mosaic, and leaf curl were detected (Table 1). On the infected country bean leaves, insect vectors such as aphids (*Aphis gossypii*) and whiteflies (*Bemisia tabaci*) were also observed (Table 2). The lowest incidences of common mosaic disease were found in T6 at 45 DAS (8.87%), 55 DAS (10.40%), and 65 DAS (13.57%), respectively (Table 1). Country bean leaf samples with typical mosaic symptoms

TABLE 1 Effect of different treatments on incidence of viral diseases of country bean.

Treatments	% Disease Incidence								
	Common Mosaic			Yellow Mosaic			Leaf Curl		
	45 DAS	55 DAS	65 DAS	45 DAS	55 DAS	65 DAS	45 DAS	55 DAS	65 DAS
T_0	35.78 a	41.35 a	50.54 a	25.20 a	27.98 a	31.23 a	20.88 a	24.63 a	30.24 a
T_1	17.50 b	18.23 b	20.45 b	9.66 c	13.22 c	14.34 c	3.94 d	5.81 d	9.30 c
T_2	12.20 cd	14.55 c	16.44 c	15.04 b	18.55 b	21.65 b	5.55 c	7.75 c	10.05 c
T_3	13.23 c	14.26 c	17.23 c	8.97 c	10.80 d	13.40 cd	3.02 de	5.02 d	6.75 d
T_4	15.63 b	18.77 b	20.34 b	7.00 d	9.79 de	12.56 d	12.61 b	13.49 b	17.65 b
T_5	12.14 cd	14.23 c	17.45 c	6.56 d	8.77 ef	10.73 e	2.18 ef	3.04 e	4.40 e
T_6	8.87 e	10.40 e	13.57 d	6.17 d	7.53 f	9.76 e	1.85 f	2.53 e	3.02 ef
T_7	10.65 de	12.37 d	13.61 d	4.38 e	5.23 g	5.56 f	0.00 g	1.00 f	1.00 f
LSD (0.05)	2.09	1.51	1.41	1.07	1.28	1.15	1.12	0.81	2.23

Here, T_0- Control (only water spray), T_1- Seed biopriming with *Trichoderma asperellum* PMILN51 isolate formulation, T_2- Seed biopriming with pure culture suspension of *T. asperellum* PMILN51 isolate, T_3- Soil drenching with *T. asperellum* PMILN51 isolate formulation, T_4- Soil drenching with pure culture suspension of T. asperellum PMILN51 isolate, T_5- Foliar application with *T. asperellum* PMILN51 isolate formulation, T_6- Foliar application with pure culture suspension of *T. asperellum* PMILN51 isolate, T_7- Foliar application with Shobicron 425EC (Profenofos+ Cypermethrin).
DAS, Days After Sowing; Data represents the means of three replications.
LSD (0.05): Least Significant Difference.
In a column, means followed by same letter(s) are statically similar at 5% level by DMRT.

tested positive for both potyvirus and CMV. According to Li et al. (2015), an increasing number of tricho-suspension sprays produce a higher concentration of trichodermin, which improves plant growth and the plant's immune defense mechanism against pathogens. We observed the occurrence of yellow mosaic disease in bean plants in this study, and interestingly, all treated plants with trichoderma or insecticides had a reduced incidence of yellow mosaic disease from 4.38% to 21.65% (Table 1). Plants treated with foliar application of pure culture suspension of *T. asperellum* (T6) had the lowest yellow mosaic disease incidence rates of 6.17 percent, 7.53 percent, and 9.76 percent, respectively, at 45 DAS, 55 DAS, and 65 DAS (Table 1), followed by T5 (foliar application with formulated *T. asperellum*). However, at 45 DAS, 55 DAS, and 65 DAS, control plants had the highest yellow mosaic incidence of 25.20 percent, 27.98 percent, and 31.23 percent, respectively (Table 1). Country bean plants with yellowing symptoms, on the other hand, exhibited a positive reaction solely to the CMV detecting immunostrip and a negative reaction to the potyvirus detecting immunostrip. Besides, common mosaic disease and bean yellow mosaic disease symptoms, bean plants in this study also

TABLE 2 Effect of different treatments on vector population of country bean.

Treatments	Number of Aphid (*Aphis gossypii*)/Plant			Number of white fly (*Bemisia tabaci*)/Plant		
	45 DAS	55 DAS	65 DAS	45 DAS	55 DAS	65 DAS
T_0	110.67 a	121.33 a	134.67 a	25.33 a	31.67 a	37.33 a
T_1	59.67 b	65.67 b	70.00 b	11.33 c	13.33 b	14.33 c
T_2	29.67 c	39.33 c	50.33 c	12.33 b	13.00 b	14.33 c
T_3	30.67 c	36.00 d	40.67 d	8.33 d	9.67 c	11.33 d
T_4	20.67 d	30.67 e	39.00 d	11.00 c	14.67 b	16.33 b
T_5	7.33 e	10.67 f	12.33 e	4.33 e	5.00 d	6.33 e
T_6	4.67 f	5.33 g	5.67 f	1.66 f	2.67 e	4.33 f
T_7	0.67 g	2.33 h	3.00 g	0.00 g	1.00 e	1.33 g
LSD (0.05)	1.64	1.87	1.71	0.86	1.69	1.69

Here, T_0- Control (only water spray), T_1- Seed biopriming with *Trichoderma asperellum* PMILN51 isolate formulation, T_2- Seed biopriming with pure culture suspension of *T. asperellum* PMILN51 isolate, T_3- Soil drenching with *T. asperellum* PMILN51 isolate formulation, T_4- Soil drenching with pure culture suspension of *T. asperellum* PMILN51 isolate, T_5- Foliar application with T. asperellum PMILN51 isolate formulation, T_6- Foliar application with pure culture suspension of T. asperellum PMILN51 isolate, T_7- Foliar application with Shobicron 425EC (Profenofos+ Cypermethrin).
DAS, Days After Sowing; Data represents the means of three replications.
LSD (0.05): Least Significant Difference.
In a column, means followed by same letter(s) are statically similar at 5% level by DMRT.

showed leaf curl disease symptom. In agreement with the reduction of common mosaic disease and bean yellow mosaic disease, treatment T6 also lowered the incidence of leaf curl disease at 45 DAS, 55 DAS, and 65 DAS, respectively (Table 1), followed by T5. Reduction of diseases by trichoderma-based treatment could be due to trichodermin-produced sesquiterpene antibiotics that bind to viral proteins and that inhibit protein synthesis of potyviruses, CMV, or other viruses. According to Elsharkawy et al. (2013), *T. asperellum* is an effective biocontrol agent that boosts plant defence mechanisms against viral infection. Harman et al. (2004) reported that several *Trichoderma* species added to the rhizosphere protect plants against a wide range of plant pathogens, including viral, bacterial, and fungal pathogens, indicating the induction of resistance mechanisms similar to the hypersensitive response (HR), systemic acquired resistance (SAR), and induced systemic resistance (ISR) in plants.

The vector population, such as the number of aphids and whiteflies, was reduced in *T. asperellum* PMILN51 isolate sprayed plants (T6 and T5) compared to other trichoderma-treated plants and non-treated controls in the current study (Table 2) . This is consistent with previous findings in which Trichoderma acts as an entomopathogen directly through parasitism and the production of insecticidal secondary metabolites, antifeedant compounds, and repellent metabolites (Poveda et al., 2020). Trichoderma can effectively parasitize insect bodies, using them as a source of nutrients for the formation of new conidia. Trichoderma produces many volatile compounds that can act as insect repellents, according to Siddique et al. (2011). Insecticides like Shobicron 425EC (Profenofos + Cypermethrin) are harmful to the environment. So, eco-friendly *T. asperellum* PMILN51 isolate could be a viable alternative to chemical treatment. However, further experiments need to be carried out in order to assess the field efficacy in controlling viral diseases of country bean plants with *T. asperellum* PMILN51 isolate and to conduct metabolic and gene expression studies in order to understand the resistance mechanism of *T. asperellum* PMIL-N51 isolate sprayed plants.

In this study, soil drenched with a pure culture suspension of *T. asperellum* PMILN51 isolate (T4) showed higher plant growth and increased numbers of leaves/plant, pods/plant, root length, weight of dry pods/plant and weight of dry seeds/plant compared to other treatments, including control plants (Tables 3, 4). *Trichoderma* spp., according to Pereira et al. (2014), can promote common bean plant growth by increasing root as well as foliar areas and size when compared to plants grown without it. Yedidia et al. (2001) reported a much stronger effect on cucumber plants treated with *Trichoderma spp*, which increased by 75% the length of the root, 95% aerial parts, 80% dry weight,and 80% the size of the blade relative to the untreated control. Abd-El-Khair et al. (2010) found that the average fresh weight of pods in beans was increased in the case of *Trichoderma* treatment, compared to the control plant. In this study, the yield of country beans was higher in *Trichoderma* treated plants than in non-treated plants. Hoyos-Carvajal et al. (2009) found that the ability of *Trichoderma* spp. to promote plant growth was initially attributed to its antagonistic activity against harmful microorganisms living in the rhizosphere or soil. Vitti et al. (2015) found that *Trichoderma* performs mycoparasitism, competition for nutrients with other pathogens, the release of extracellular hydrolytic enzymes, antagonism against plant pathogens, colonization of the rhizosphere and phyllosphere, production of secondary metabolites that are toxic to plant pathogens, promotion of plant growth and root development, and induction of systemic resistance against different pathogens.

All this information supports the findings of this study, which recorded lower disease incidence and greater growth and yield contributing parameters in *Trichoderma* treated plants. The use of *Trichoderma* spp. as bio-control agents induced the accumulation

TABLE 3 Effect of different treatments on plant height and no. of leaves/plant of country bean.

Treatments	Plant height (cm)			No. of leaves/plant		
	45 DAS	55 DAS	65 DAS	45 DAS	55 DAS	65 DAS
T_0	90.67 g	100.33 g	109.33 f	42.00 f	55. 33 f	76.00 f
T_1	112.67 d	121.00 cd	130.00 bc	75.33 e	94.00 e	117. 33 e
T_2	120.67 b	129.67 b	138.00 a	84.00 c	105. 67 c	122. 33 d
T_3	115.33 c	123.33 c	132.33 b	79.00 d	100. 33 d	121.00 d
T_4	123.17 a	132.67 a	139.83 a	89.33 a	111.00 a	134.33 a
T_5	105.67 f	112.67 f	121.00 e	83. 33 c	106. 67 bc	129.33 b
T_6	110.33 e	117.00 e	126.33 d	86.33 b	108. 67 b	129.00 b
T_7	113.33 d	120.33 d	128.00 cd	84.67 bc	106.00 c	126. 67 c
LSD (0.05)	1.54	2.44	3.11	1.77	2.13	

Here, T_o- Control (only water spray), T_1- Seed biopriming with *Trichoderma asperellum* PMILN51 isolate formulation, T_2- Seed biopriming with pure culture suspension of *T. asperellum* PMILN51 isolate, T_3- Soil drenching with T. asperellum PMILN51 isolate formulation, T_4- Soil drenching with pure culture suspension of *T. asperellum* PMILN51 isolate, T_5- Foliar application with T. asperellum PMILN51 isolate formulation, T_6- Foliar application with pure culture suspension of T. asperellum PMILN51 isolate, T_7- Foliar application with Shobicron 425EC (Profenofos+ Cypermethrin).

DAS, Days After Sowing; Data represents the means of three replications.

LSD (0.05): Least Significant Difference.

In a column, means followed by same letter(s) are statically similar at 5% level by DMRT.

TABLE 4 Effect of treatments on different agronomic characters of country bean.

Treatments	Root length (cm)	Number of pods/plant	Weight of dry pods(g)/plant	Weight of dry seeds(g)/plant
T_0	15.33 e	14. 67 e	12.00 d	9.17 d
T_1	21.17 d	20. 33 d	19.17 c	14.00 c
T_2	22.17 d	21. 33 d	19.00 c	14.50 c
T_3	26.00 c	20. 67 d	18.50 c	15.00 c
T_4	35.50 a	34. 67 a	29. 67 a	20.50 a
T_5	23. 33 d	29. 33 c	24.83 b	17.33 b
T_6	26.83 c	32. 33 b	27.50 a	19.00 ab
T_7	31.67 b	33.00 b	28.50 a	19.83 a
LSD (0.05)	2.22	1.33	2.29	2.11

Here, T_0- Control (only water spray), T_1- Seed biopriming with *Trichoderma asperellum* PMILN51 isolate formulation, T_2- Seed biopriming with pure culture suspension of *T. asperellum* PMILN51 isolate, T_3- Soil drenching with *T. asperellum* PMILN51 isolate formulation, T_4- Soil drenching with pure culture suspension of *T. asperellum* PMILN51 isolate, T_5- Foliar application with *T. asperellum* PMILN51 isolate formulation, T_6- Foliar application with pure culture suspension of *T. asperellum* PMILN51 isolate, T_7- Foliar application with Shobicron 425EC (Profenofos+ Cypermethrin).
DAS, Days After Sowing; Data represents the means of three replications.
LSD (0.05): Least Significant Difference.
In a column, means followed by same letter(s) are statically similar at 5% level by DMRT.

of some enzymes such as chitinase, peroxidase, and polyphenol oxidase, which play an important role in plant defence mechanisms against pathogen infection, and the enzymatic activity in treated bean plants increased more than in untreated ones. Peroxidase and resistance development in plants have positive relationships, and peroxidase has been shown in experiments to play a defensive role against invading pathogens (Caruso et al., 2001; Nawar and Kuti, 2003).

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

All authors contributed to the conception and design of the manuscript. The first draft of the manuscript was written by IH and all authors commented on submitted version of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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