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# Trichoderma and agrochemical induced defensive metabolites for suppressing rust disease of faba bean in Bangladesh

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**Research Article**

**Keywords:** Trichoderma, Defensive metabolites, Faba bean, Rust disease, Control, Yield

**Posted Date:** April 6th, 2026

**DOI:** https://doi.org/10.21203/rs.3.rs-9187735/v1

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**Additional Declarations:** No competing interests reported.

# *Trichoderma* and agrochemical induced defensive metabolites for suppressing rust disease of faba bean in Bangladesh

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**ABSTRACT**

Research investigations were conducted both in *in-vitro* and *in-vivo* with three potential strains of *Trichoderma* isolates (*Trichoderma harzianum* AB935952, *T. asperellum* AB935955, and *T. viride, Tv6*); three agrochemicals *viz.*, Bion, Chitosan and Amistar Top (as positive control) during two cropping season (2019-2021) to manage rust disease of faba bean in Mymensingh district of Bangladesh. *In vitro* assay clearly revealed that 60% culture filtrate of *T. viride;* bion and Amistar top @ 60 mM inhibited 100% inhibition of uredial germination of *Uromyces viciae fabae* followed by *T. harzianum and T. asperellum*. Chitosan showed significantly the lowest inhibition of urediospore germination. Spraying of each Tricho-suspension (@$10^8$cfu/ml) and agrochemicals on faba bean plants in the greenhouse significantly decreased rusty spot severity where, Amistar top, *T. viride* and Bion were found to be the most effective treatments followed by *T. harzianum* and *T. asperellum*. Five treatments were found significantly effective in reducing the rust disease severity of faba bean comparing with control treatment under field conditions. In both cropping seasons, field experiments revealed that spraying of *Trichoderma* spp., Bion and Amistar Top on faba bean plants significantly reduced the rust disease severity and increased the agronomic parameters *viz*., plant height (cm), no. of pods /plant, fresh wt (g), dry weight (g) and seed yield (kg/ha) compared with the control treatment. Among the three *Trichoderma* spp*., T. viride* showed the best performance in reducing rust disease severity (8.20% and 10.20%) with increasing faba bean yield (3240 and 3331 kg/ha) followed by Bion. Analysis of induced biochemicals, Bion resulted the highest activity of peroxidase (POX), polyphenoloxidase (PPO), and phenylalanine ammonia lyase (PAL), whereas, *T. viride* resulted the highest amount of total phenol and flavonoid. *T. harzianum* resulted the highest amount of β, 1-3 glucanase and total chlorophyll content. The least amount of bio-chemicals with higher rust severity was recorded in case of Chitosan. Thus, the increments of POX activity, total phenols, total flavonoids and seed yield revealed that *T. viride* is a promising antagonistic species of fungi that may be the alternate of chemical fungicides for management of rust disease in Bangladesh.

**Keywords:** *Trichoderma*; Defensive metabolites; Faba bean; Rust disease; Control, Yield

## Introduction

It is widely agreed that one of the most significant crops on the globe is the grain legume known as the faba bean (Vicia faba L). Nutritional value of vitamins, proteins, carbohydrates and other substances has led to an increase in economic relevance around the universe (Kumar et al., 2015). It is a rich source of food and feed all over the world (Sahile et al., 2011). Sustainable nitrogen (N) inputs and crop diversification are two more ecological services provided by legumes like the faba bean (Abada et al., 2019). There was a worldwide reduction in faba bean acreage from 1962 to 1991 (FAOSTAT, 2017) and one of the reasons for its decline is the prevalence of pathogenic fungi (Usman et al., 2017). Moreover, faba-bean is susceptible to a variety of bacterial, viral, and nematode illnesses, as well as physiological problems (Stoddard et al., 2010). There is also no disease-resistant cultivars to offset those problems (Sillero et al., 2010). Fungal infections pose a major threat to many countries including Bangladesh for higher faba bean production (Abada et al., 2003) where *Uromyces viciae fabae* causing rust disease is major one (Voegele, 2006). Farmers are generally using fungicides to control this disease. Systemic fungicides provide rapid and effective control, but they are expensive and can be detrimental to the environment (Usman et al., 2017). However, indiscriminate use of fungicides resulted environmental pollution that disasters around the world. Plant pathogens also showed their resistance against those fungicides. Therefore, alternative but safe management approaches are necessary to combat such severe diseases.

Application of bioagents is a promising and environment friendly method for increasing agricultural production (Bai et al., 2008; Barakat et al., 2014; Chet, 1994; El-Mogy et al., 2018; Naseby et al., 2000; Verma et al., 2007). Biological control is a viable alternative to fungicides in the management of faba-bean rust (Abada et al., 2019) which cut down chemical pesticide use and reduce environmental hazards (Harman et al., 2004; Kubicek & Harman, 1998). *Trichoderma* spp. are using as the most promising antagonistic bioagents against many fungal pathogens (Barakat et al., 2014; El-Mogy et al., 2018; Islam et al., 2016). *Trichoderma* spp. produces many volatile secondary metabolites, including ethylene, carbon dioxide, hydrogen cyanide, aldehydes, and ketones, which are crucial in controlling many plant diseases (Barakat et al., 2014; Vinale et al., 2008; Zaher et al., 2013). Although Trichoderma use in the field was found significantly less effective (Bouhassan et al., 2004) but it is immense need to search highly effective Trichoderma spp. to control plant diseases. But no research work was done yet in Bangladesh to biocontrol of faba bean rust.

The mechanism of systemic acquired resistance utilized as a prophylactic measure which provides boost plant health and long-term protection (Kessmann 1994). Bion and chitosan are systemic acquired resistance elicitors that decrease the severity of a variety of fungal infections (Abada et al., 2019). The stimulation of the phenol pathway, hydrolytic enzymes and antimicrobial metabolites are linked to this protection, but the specific metabolites induced by bion and chitosan against faba bean rust have not yet identified in Bangladesh. Therefore, this research work was undertaken to see the status of rust of faba bean in some regions (districts) of Bangladesh, how *Trichoderma* spp. and agrochemicals affect the germination of *Uromyces viciae fabae* urediospores and to identify their antimicrobial components and enzyme activity for the control of rust disease of faba bean.

## Materials and Methods

### *Experiment 1: Status of faba bean rust incidence and severity*

Symptoms study

A typical rust symptom of bean caused by *Uromyces viciae fabae* was investigated as described during this survey (Gosaye et al., 2018). The size (µm) of the urediospore and teliospore were measured as described by (Gosaye et al., 2018).

Surveying of faba bean rust incidence and severity

A survey was done to see the status of rust incidence and severity of faba bean from six major faba bean growing areas of Bangladesh. Ten farmers plot from each area were selected and data were taken from January-March 2018.

Calculation of rust incidence and severity

Rust incidence was calculated in percentages as described by (Gosaye et al., 2018) and rust severity was calculated with the modified scale (0-6) (Godoy et al., 1997) using the following formula:

$$\text{Disease severity (\%)} = \frac{\sum(n \times v)}{6n} \times 100$$

Where:

n = Number of infected leaves in each category.

v = Numerical values of each category.

N= Total number of the infected leaves.

***Invitro assays of Uredial spores against Trichoderma* spp. *and*** agrochemicals

Experimental location

The experiment was conducted at Eco-Friendly Plant Disease Management Laboratory, Molecular Plant Pathology Laboratory, Bio-Safety Laboratory, Agronomy Farm and Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh (Latitude: 24.77 North Longitude:90.4, East Altitude: 14.00m/45.93ft) during 2019 to 2021.

*Trichoderma* spp., agrochemicals and plant variety

Three *Trichoderma* spp. *viz*. *T. harzianum* (AB935952) and *T. asperellum* (AB935955) were collected from Eco-Friendly Plant Disease Management Laboratory, Department of Plant Pathology, Bangladesh Agricultural University (BAU), Bangladesh and *T. viride* (Tv6) was collected from India. Bion and Chitosan were collected as induced biochemicals from the Department of Agricultural chemistry, BAU and Amistar Top (as positive control) was bought from commercial market, Mymensingh. A high yielding Faba bean (var. Bhograkalai) was collected from the Department of Agronomy, BAU, Mymensingh.

Preparation of Potato Dextrose Agar medium (PDA)

PDA (200 g sliced potato, 15 g agar powder, and 12 g dextrose in 1000 mL distilled water) media was prepared (Islam et al., 2016) and poured in 500ml glass bottles and sterilized in an autoclave at $121^0$C, 15 PSI for 15 min. The media was acidified with 0.2% lactic acid per 500 ml medium to avoid the contamination of bacteria.

Culturing of Trichoderma spp.

Trichoderma spp. collected from Eco-friendly disease management lab were cultured in acidified PDA medium. Mycelial disc (5 mm in diameter) of each Trichoderma were taken from actively growing Trichoderma spp. and placed on Petridishes (90 cm dia.). Then the plates were put in a growth chamber with a 12h/12h cycle (light/dark) for 7 days at 25-28°C and thus pure culture was preserved for conidial suspension (Shaiesta et al., 2012). It was then cultured on the same medium for multiplication through incubation at room temperature. The fully grown *Trichoderma* in slants was preserved in the refrigerator at 4°C for further work.

Preparation of culture filtrate of Trichoderma spp.

Liquid growth media was prepared from the 10 days old pure culture of *Trichoderma* spp**.** The filtrate was centrifuged at 4000 rpm for 30 min, then filtered again using Whatman paper into another Erlenmeyer. The obtained filtrate was prepared by passing through Millipore filter membrane 0.2 µm (Nurbailis et al., 2019).

Collection of Urediospore

Urediospore of *Uromyces viciae fabae* from the infected faba bean plants was collected by brush and prepared 106 spore/ml for inoculation (Abada et al., 2019).

Effect of the Trichoderma spp. and agrochemicals on the germination of the Urediospores *in vitro*

Flasks (250 ml.) containing broth medium was inoculated with 7 days old culture of Trichoderma spp. Inoculated flasks were incubated on a rotary shaker at 2000 rpm for 2 days at 25±1°C. The culture filtrate was sterilized using a 0.25µm syringe filter. Trichoderma spp. were grown on broth for 10 days. The culture filtrate was sterilized using a 0.25µm syringe filter. The concentrations of 0, 20, 40 and 60% from the culture filtrate of each of the Trichoderma were prepared. The concentrations of 0, 20, 40 and 60 mM of the agrochemicals, i.e. Bion (benzothiadiazole; BTH) and chitosan were prepared depending on their molecular weight. The urediospores of Uromyces viciae-fabae were brushed from freshly rust infected faba bean leaves by using sterilized camel brush and added to each concentration of the above mentioned bioagents and the agrochemicals (Abada et al., 2019). One ml. of urediospore suspension (106 urediospore/ ml water) was placed on sterilized Petri-dishes. Also, a spore suspension put in distilled sterilized water only was prepared as control. Preparations were incubated in darkness at 25±1°C for 48h (Abada et al., 2019). Three Petri-dishes for each treatment were used as replicates. The germinated urediospores were counted in a total of 100 urediospores (Hemacytometer) and the averages of the percentages of germination were calculated (Sharma et al., 2019) as follows:

$$\text{Spore germination (\%)} = \frac{\text{Number of urediospores germinated per microscopic field}}{\text{Total number of urediospores per microscopic field}} \times 100$$

***Pathogenicity tests of Uromyces viciae fabae against Trichoderma* spp*. and agrochemicals under Green house***

Pot soil preparation and raising of faba bean seedlings

The soil of the experiment was collected from an Agronomy farm field and then sundried, ground and screened through 10 mesh sieves to remove large particles and debris. The soil was dripped with 4% formalin solution @ 200ml/cft soil and covered with polythene sheets for 2-3 days. Then the soil was uncovered and kept for 4 days to release the gas formalin. Inorganic fertilizers Triple Super Phosphate (TSP) 15g, Muriate of potash (MP) 15g, and Gypsum 10g were applied to the pot soil as recommended by the Bangladesh Agriculture Research Council (BARC, 2018). Five faba-bean seeds were sown in pots (30 cm x 25 cm) on 20th December 2019 and thinning was done at 15 days after sowing (DAS) to maintain optimum plant population and growth (Paul, 2020). The emerged seedlings were thinned into three plants in each pot three weeks after sowing.

Preparation of Tricho-suspension for spray

Seven days old culture of Trichoderma was mixed with 800 ml sterile water. Then 2.5 ml Tween-20 was added for uniform mixing of Trichoderma spores in the suspension. After proper mixing, the volume was made up to 1000 ml. The number of spores per ml suspension was counted by Haemocytometer. The spore concentration of Trichoderma spp. per ml suspension was adjusted with 108 CFU (Nahar et al., 2010).

Spraying of Tricho-suspension and agrochemicals solution on faba bean plants

Faba bean plants at 60 days old were sprayed with the three *Trichoderma* spp. (@$10^8$ CFU/ml) and two induced chemicals (@60 mM) as well as the fungicide Amistar top (@60mM=1.8ml/L) 72 hrs before artificial inoculation with *U. viciae-fabae* urediospores suspension ($10^6$ spore/ml. water). Control plants were sprayed with urediospores suspension of the causal fungus only. A total of seven (7) treatments *viz*. $T_1$= *T. harzianum (*$10^8$ cfu/ml), $T_2$= *T. asperellum* ($10^8$ cfu/ml), $T_3$= *T. viride* ($10^8$ cfu/ml), $T_4$= Bion (60mM), $T_5$= Kitosan (60 mM), $T_5$= Kitosan (60 mM), $T_6$= Amistar Top (60 mM) and $T_7$= Control were used in this experiment with thrice replications in CRD design. All pots were covered with polyethylene bags for 48 h in a moist chamber at 24±2°C in a greenhouse. *Trichoderma*, and agrochemicals were re-sprayed on the grown plants at the same previous rates two weeks after the first spray. The treated plants were examined for disease severity 10 days after each spray and the averages were recorded as mentioned before. The seedlings were properly watered whenever necessary. The infected faba-bean plants were carefully examined to assess the severity as described earlier.

Data collection

Data were recorded on % disease severity, plant height (cm), fresh weight (g/plant), no. of pods/plant and seed yield (g/plant) in this greenhouse experiment.

***Experiment 2: Field experiment***

Land preparation and spraying of effective treatments on faba bean plants

The faba bean field was prepared during 20 October of each field experiment and experimental land was opened with a power tiller (Paul et al., 2020). Ploughing and cross ploughing were done with country plough followed by laddering. Land preparation was completed on 5 November and was ready for sowing seeds. Inorganic fertilizers Triple Super Phosphate (TSP) 60 kg, Muriate of potash (MP) 50kg, and Gypsum 45 kg were applied (Paul et al., 2020). The plot size was prepared 3x4 sq.m. Plant x Row distance was maintained with 30x 30 cm in RCBD design. Field experiments were carried out in Agronomy field where severe infection by faba bean rust is common. The field was prepared for sowing faba bean during November 2020 and 2021. A lot of faba-bean seeds were soaked in water before sowing.

The grown plants from seeds were sprayed with three Trichoderma spp. (108 spore suspension/ ml water), Bion was sprayed @ 60mM in comparison with the fungicide Amistar Top (60 mM) (positive control) at the first appearance of the disease symptoms. The spray with the tested Trichoderma bioagents, Bion and the fungicide was carried out three times with ten days interval from onset of the disease. The treated plants were examined for disease severity 10 days after each treatment using the devised scale (Godoy et al., 1997) and the averages were recorded.

Intercultural operation, harvesting and data recording

Intercultural operation, weeding and staking were done when necessary. Also, the dry pods of each treatment were harvested, threshed, weighted and the average of the seeds weight (kg/ha) was recorded. Data were recorded on % disease severity, plant height (cm), fresh weight (g/plant), dry weight (g/plant), no. of pods/plant and seeds yield (g/plant) in this greenhouse experiment (Abada et al., 2019).

Sample collection for biochemical analysis

Samples were collected after one month from the first spray (70 DAS) with different treatments and control plants for enzymes assay. Plant leaves were carefully collected with sterilized scissors. One plant was sampled from each replication of the treatment separately and were maintained separately for biochemical analysis. Leaves from control

(both naturally infected and uninfected) treatment were also maintained for this study. Fresh leaves were washed in running tap water and homogenized with liquid $N_2$ in a pre-chilled mortar and pestle. The homogenized leaf tissues were stored in deep freezer (−70°C) until used for biochemical analysis.

Assay of Peroxidase (POX)

POX activity was assayed as per the procedure of Hammerschmidt et al., 1982. Faba bean leaf samples (1 g) were homogenized in 2 mL of 0.1M phosphate buffer, pH 7.0 at 4°C. The homogenate was centrifuged at 16000g at 4°C for 15min and the supernatant was used as enzyme source. The reaction mixture consisted of 1.5mL of 0.05mL pyrogallol, 0.5 mL of enzyme extract and 0.5 mL of 1% $H_2O_2$. The reaction mixture was incubated at room T (28±2°C). The changes in absorbance at 420 nM were recorded at 30 s intervals for 3 min. The enzyme activity was expressed as Unit per gram fresh weight per minute (U $g^{-1}$FW $min^{-1}$).

Assay of Polyphenol Oxidase (PPO)

PPO activity was determined as per the procedure of Mayer et al., 1965 with slight modifications. In brief, leaf samples (1 g) were homogenized in 2 mL of 0.1 M sodium phosphate buffer (pH 6.8) and centrifuged at 10 000 g for 15 min at 4°C. The supernatant was used as the enzyme source. The reaction mixture consisted of 200 µL of the enzyme extract and 1.5 mL of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction, 200µL of 0.01M catechol was added, changes in absorbance recorded at 495nM and the activity was expressed as Unit cat. $g^{-1}$ FW $min^{-1}$.

Estimation of Phenylalanine Ammonia-Lyase (PAL) activity

Leaf samples (1 g) were homogenized in 3 mL of ice cold 0.1 M sodium borate buffer, pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinyl pyrrolidone. The extract was filtered through cheese cloth and the filtrate was centrifuged at 16000g for 15 min. The supernatant was used as enzyme source. PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nM (Dickerson et al., 1984). A sample containing 0.4 mL of enzyme extract was incubated with.5mL of 0.1M borate buffer, pH 8.8 and 0.5 mL of 12 m M L-phenylalanine in the same buffer for 30 min at 30°C. The amount of trans-cinnamic acid synthesized was calculated using its extinction coefficient and the enzyme activity is expressed as mg t-cinnamic acid formed $g^{-1}$ FW 60 $min^{-1}$.

Assay of β-1,3-glucanase

The enzyme solution (100 µL) was mixed with 200 µL of 0.2 % (w/v) laminarin dissolved in 0.1 M sodium phosphate buffer (pH 6.0) and incubated at 30°C for 30 min. The reaction was terminated by adding dinitro-salicylic acid solution and boiling the reaction mixture for 5 min. The absorbance at 540 nm was measured and the unit was defined as the amount of the enzyme that released reducing sugar equivalent to 1 µg glucose per min under the above conditions (Sun et al., 2006). β-1,3-glucanase was expressed as mM glucose equivalent released $g^{-1}$ FW 60 $min^{-1}$.

Estimation of Total Phenol

The total phenolic content of different extracts of faba bean leaves was determined using Folin– Ciocalteau method (Türkoğlu et al., 2007); briefly, 1 mL of extract in a volumetric flask was diluted with distilled water to 46 m L. 1 mL of Folin-Ciocalteu reagent was added and the contents of the flask were mixed thoroughly. After 3 min, 3 mL of $Na_2CO_3$ (2%) was added, then mixture was allowed to stand for 2 h with intermittent shaking. The absorbance of each mixture was measured at 760 nm. The concentration of total phenolic content was measured by plotting the calibration curve of a Gallic acid standard. Total phenol was expressed as mg gallic acid equivalent (GAE) $g^{-1}$ FW.

246 Determination of total Flavonoid content

247 The total flavonoid content of different extracts of faba bean leaves was determined (Türkoğlu et al., 2007) as follows:
248 1 mL of extract was diluted with 4.3 mL of 80 % aqueous ethanol containing 0.1 mL of 10 % aluminum nitrate and
249 0.1 mL of 1 M aqueous potassium acetate. After 40 min at room temperature, the absorbance was determined
250 spectrophotometrically at 415 nm. The total flavonoid content was measured by plotting the calibration curve of a
251 quercetin standard and expressed as mg of quercetin equivalents per gram (mg QE/g) $g^{-1}$ FW.

252 Estimation of Total Chlorophyll

253 Total Chlorophyll was estimated following the methods (Procter, 1981) with the following formula:

254 Total Chlorophyll (mg/g) = 8.02 x OD at 663 nm + 20.20 x OD at 645 nm

255

256 Statistical Analysis of the Data

257 All statistical analyses were done by 'R' program following CRD design for *in vitro* and green house experiment and
258 RCBD design for field experiment and LSD test was used to determine whether there was a significant difference in
259 treatment means at the $P \leq 0.05$ level.

260

## Results

### Symptoms of faba bean rust disease

Infected faba bean leaves, leaflets, stems, and pods showed rust symptoms as pustules filled with *Uromyces vicia-fabae* spores (urediospore) (Fig. 1a-1c). The rust pustules were initially light brown which later turns dark brown (Fig. 1c). Under microscope the rust spores were observed and confirmed with the presence of urediospore (size was about 18-30 × 16-25 µm) and teliospores (size was about 23-40 ×18-26 µm). The uredia were round, yellowish brown in color, and had double layers (Fig. 1d, 1e), whereas the teliospores were elongated brown in color with elongated stalk (Fig. 1f).

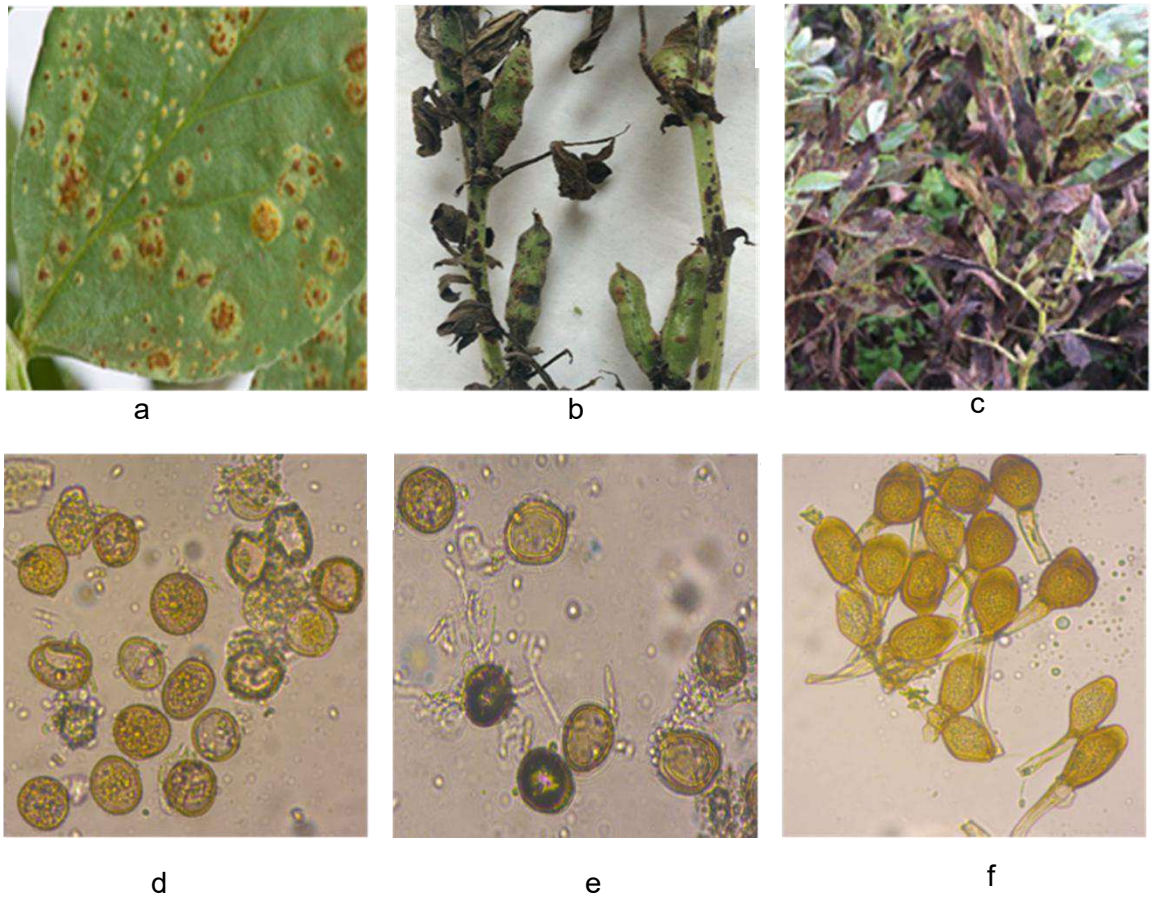


**Fig.1** Disease symptoms of faba bean rust caused by *Uromyces vicia-fabae* and its spores in different developmental stages. (a) Rusty pustule on infected leaves, (b) stems and pods, and (c) severe infected plants. (d) Non-germinated urediospore, (e) germinated urediospore, and (f) teliospore of *Uromyces viciae fabae*.

Rust disease scenario in Bangladesh

The incidence of faba bean rust was surveyed in five major faba bean cultivation districts of Bangladesh–Rangpur, Jamalpur, Mymensingh, Gazipur, and Faridpur–during January, February, and March of the 2018-2019 cropping year (Fig. 2). The highest disease incidence (DI%) was recorded in Mymensingh district followed by Faridpur, Gazipur, Jamalpur, and Rangpur. January had the lowest DI (12.5-19.2%) and March had the highest DI (32.8-68.5%) across all five locations..

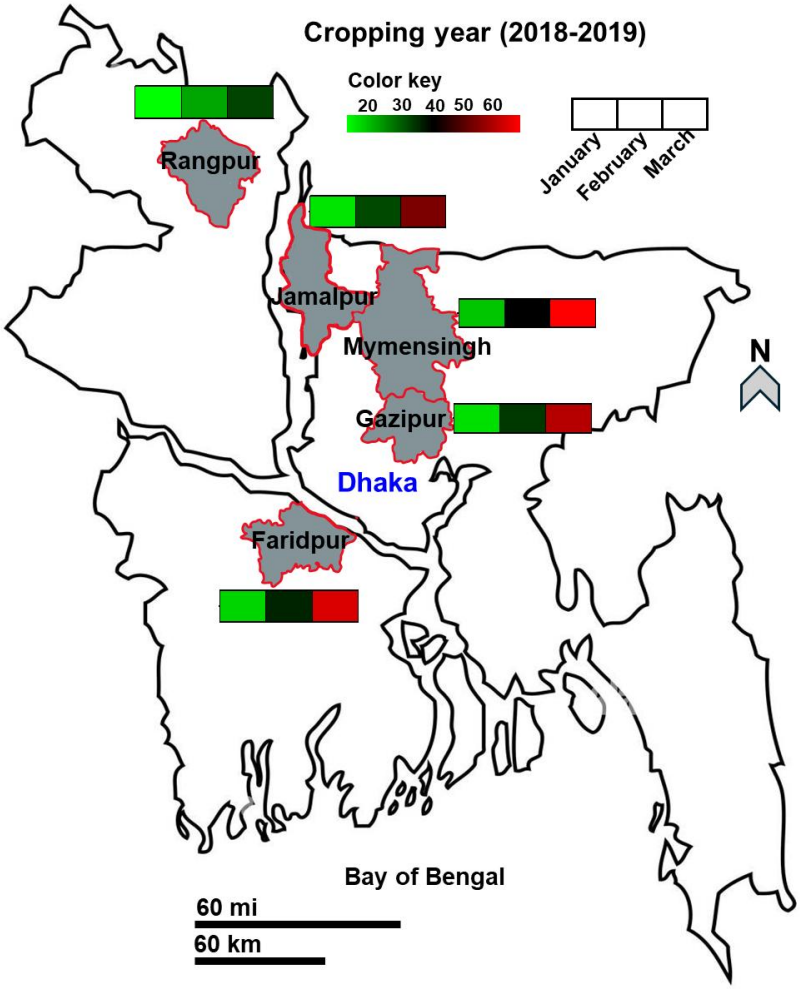


**Fig. 2** Disease incidence of faba bean rust caused by *Uromyces viciae fabae* in five major faba bean growing districts of Bangladesh during the cropping year 2018-2019. Ten farmer plots from each area were selected and data were taken from January-March 2018.

*Trichoderma spp.* and agrochemicals inhibit the germination of *U. vicia-fabae* urediospore *in vitro*

Three antagonistic bioagents, *T. harzianum*, *T. asperellum*, and *T. viride*, alongside two induced agrochemicals :Bion and Kitosan, were evaluated for their inhibitory effects on *U. vicia-fabae* urediospore germination under *in vitro* conditions (Fig. 3). Amistar Top, a well-known fungicide served as a positive control, while sterile water was used as negative control. When compared with the negative control, the results showed that all of the tested bioagents and agrochemicals substantially reduced urediospore germination. Furthermore, it was observed that as the concentration of the assessed treatments increased, the percentage of spore germination gradually decreased. Positive control Amistar Top exhibited the highest germination inhibition (by 22.00, 2.00, and 0.00%) across all three tested concentrations (20, 40, and 60 mM, respectively) in comparison with the other treatments at their respective doses. In the assessment of the five bioagents and agrochemicals, Bion demonstrated the most noteworthy germination inhibition at the 20, 40, and 60 mM concentrations resulting in inhibition rates of 68.00, 35.80, and 0.00%, respectively. In parallel, *T. viride* exhibited comparable inhibition rates of 74.00, 35.40, and 0.00% across different concentrations of culture filtrate (by 20, 40, and 60%, respectively). Moderate levels of germination inhibition were observed for *T. harzianum* (by 82.20, 46.60, and 5.50%) and *T. asperellum* (by 80.50, 48.30, and 9.40%) across varying doses of culture filtrate (by 20, 40, and 60%, respectively). Kitosan had the lowest germination inhibition (by 85.00, 65.00, and 18.00%) of the five bioagents and agrochemicals at 20, 40, and 60 mM concentrations, respectively.

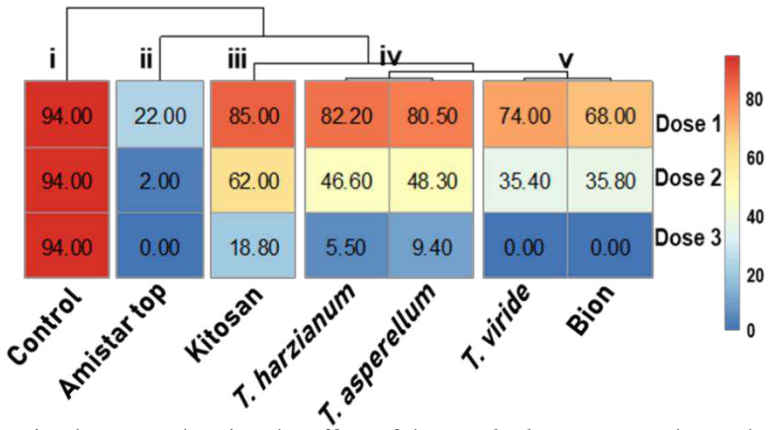


**Fig. 3** Hierarchical clustering heatmap showing the effect of the *Trichoderma* spp. and agrochemicals on the *in vitro* germination of *Uromyces viciae fabae* urediospores. Three different *Trichoderma* viz., *T. harzianum*, *T. asperellum*, and *T. viride* and two induced chemicals viz., Chitosan and Bion were used in the experiment. Sterile distilled water was used as negative control, while Amistar Top was used as positive control. Data was recorded 48 h after innoculation. Dose 1: 20 % *Trichoderma* culture (10 days old) filtrate or 20 mM I agrochemicals; Dose 2: 40 % *Trichoderma* culture (10 days old) filtrate or 40 mM agrochemicals; 60 % *Trichoderma* culture (10 days old) filtrate or 60 mM agrochemicals.

355 *Trichoderma spp.* and agrochemicals spraying reduce faba bean rust severity and help to maintain agronomic
356 parameters under greenhouse conditions

357 Inoculation of 40 days old faba bean plants with *U. vicia-fabae* urediospore resulted in 22% disease severity under
358 greenhouse conditions (Fig. 4a and S1). Hopefully, seven days post-spraying of three tested bioagents and two induced
359 agrochemicals along with positive control fungicide Amistar Top significantly reduced the disease severity compared
360 with the non-sprayed negative control plants. Specifically, rust severity was significantly lower in 'Amis. Top', 'Tvir',
361 'Bion', 'Thar', 'Tasp', and 'Kitosan' plants by 78.18, 63.63, 62.73, 60.91, 58.18, and 43.18%, respectively, compared
362 with 'Cont' plants (Fig. 4a). Among the agronomic parameters, plant height (by 90.99, 84.68, 83.78, 78.38, 68.47, and
363 45.50%) was significantly higher in 'Tvir', 'Thar', 'Amis. Top', 'Tasp', 'Bion', and 'Kitosan' plants, respectively,
364 compared with 'Cont' plants (Fig. 4b). Shoot weight (DW) (by 202.44, 197.56, 153.66, 124.39, and 114.63%) was
365 substantially greater in 'Tvir', 'Amis. Top', 'Thar', 'Tasp', and 'Bion' plants compared with the 'Cont' plants (Fig.
366 4c). However, shoot DW was comparable between 'Kitosan' and 'Cont' plants. In case of pod number (by 66.67,
367 60.00, 60.00, 53.33, 46.67, and 20.00%) and seed weight (by 67.66, 60.96, 58.08, 42.04, 39.88, and 27.66%), 'Amis.
368 Top', 'Tvir', 'Bion', 'Thar', 'Tasp', and 'Kitosan' plants, respectively, showed significant improvement compared
369 with the corresponding 'Cont' plants (Fig. 4d, 4e). Overall, the clustering heatmap constructed based on the fold-
370 change values of the aforementioned parameters clearly pointed out that Amistar Top and *T. viride* were most effective
371 in reducing rust severity while maintaining the agronomic performance under greenhouse conditions, followed by
372 Bion, *T. harzianum*, *T. asperellum*, and Kitosan (Fig. 4f).

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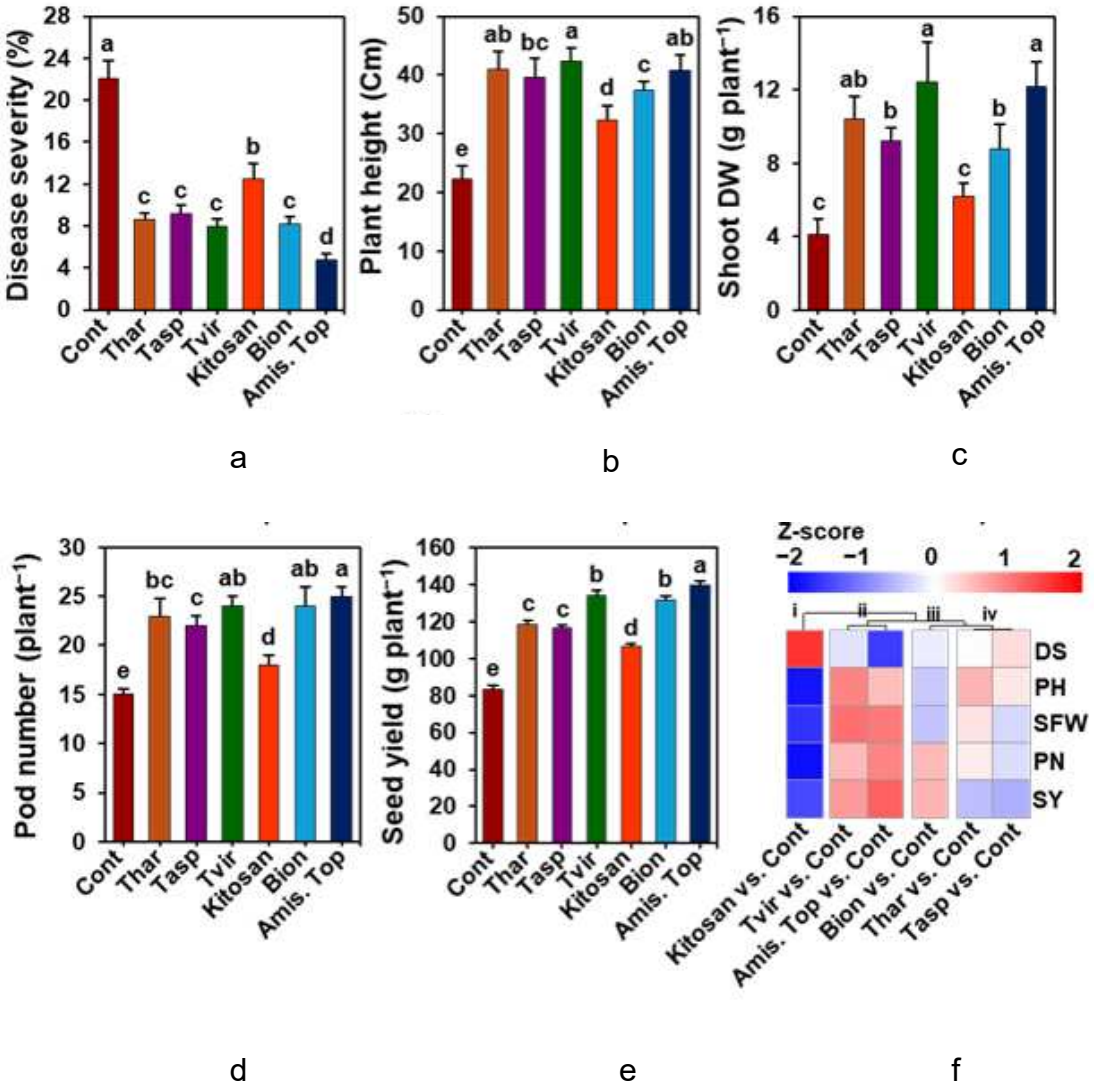
Disease severity (%)
Plant height (Cm)
Shoot DW (g plant⁻¹)
Pod number (plant⁻¹)
Seed yield (g plant⁻¹)
Cont
Thar
Tasp
Tvir
Kitosan
Bion
Amis. Top
Z-score
−2 −1 0 1 2
i ii iii iv
DS
PH
SFW
PN
SY
Kitosan vs. Cont
Tvir vs. Cont
Amis. Top vs. Cont
Bion vs. Cont
Thar vs. Cont
Tasp vs. Cont

a b c

d e f

**Fig. 4** Effect of *Trichoderma* spp. and agrochemical spraying on the severity of faba bean rust and agronomic parameters under greenhouse conditions. Faba bean plants (40 days after sowing) were sprayed with *Trichoderma harzianum, T. asperellum, and T. viride* ($10^8$ colony-forming units $mL^{-1}$) and two induced agrochemicals viz., Chitosan and Bion (60 mM). Negative control plants did not receive any spraying, while positive control plants were sprayed with Amistar Top (60 mM). All the plants were artificially inoculated with *Uromyces viciae fabae* urediospores suspension ($10^6$ spore $mL^{-1}$ water) seven days after spraying. All spraying were repeated 14 days after 1st spray. (a) Disease severity, (b) plant height, (c) shoot dry weight (DW), (d) pod number, and (e) seed yield of faba bean plants under different treatments. (f) Hierarchical clustering heatmap showing the fold-change values of the above-mentioned parameters. The mean and standard deviations ($n = 3$) are displayed as bars. Significant changes ($p \leq 0.05$) among different treatments are denoted by different letters (a-e) atop the bars and were calculated following the least significant difference test. Here the treatments are, non-sprayed infected negative control plants (Cont); *T. harzianum* sprayed infected plants (Thar); *T. asperellum* sprayed infected plants (Tasp); *T. viride* sprayed infected plants (Tvir); Chitosan sprayed infected plants (chitosan); Bion sprayed infected plants (Bion); Amistar Top sprayed infected positive control plants (Amis. Top).

*Trichoderma* spp. and agrochemicals spraying reduce faba bean rust severity and help to maintain agronomic parameters under field conditions

Faba bean plants cultivated in fields with a high susceptibility to rust infection during the years 2019-2020 and 2020-2021 exhibited disease severity levels of 33.60 and 28.00%, respectively (Fig. 5a-i and Fig. 5b-i). Three consecutive applications (at 10-day intervals commencing from the first manifestation of the disease) of Amistar Top (positive control; by 77.98 and 76.79%), *T. viride* (by 69.64 and 70.71%), Bion (by 60.71 and 63.57%), *T. harzianum* (by 50.99 and 57.50%), and *T. asperellum* (by 49.21 and 56.43%) significantly decreased disease severity in comparison with the negative control plants during both cropping years 2019-2020 and 2020-2021, respectively (Fig. 5a-i and Fig. 5b-i). Among the agronomic traits, plant height was significantly higher in 'Tvir' (by 58.20 and 57.69%), 'Amis. Top' (by 52.17 and 54.04%), 'Bion' (by 50.20 and 50.38%), 'Thar' (by 50.99 and 48.65%), and 'Tasp' (by 49.21 and 46.54%) plants compared with the 'Cont' plants during both cropping years 2019-2020 and 2020-2021, respectively (Fig. 5a-ii and Fig. 5b-ii). Shoot DW was notably greater in 'Amis. Top' (by 107.41 and 173.33%), 'Tvir' (by 162.96 and 150.00%), 'Bion' (by 142.59 and 146.67%), 'Thar' (by 111.11 and 113.33%), and 'Tasp' (by 100.00 and 90.00%) plants compared with that of 'Cont' plants during both cropping years 2019-2020 and 2020-2021, respectively (Fig. 5a-iii and Fig. 5b-iii). In the case of pod numbers, 'Amis. Top' (by 48.07 and 47.37%), 'Tvir' (by 47.70 and 42.98%), 'Thar' (by 41.44 and 37.89%), 'Tasp' (by 38.12 and 35.79%), and 'Bion' (by 36.65 and 30.53%) plants had significantly more pods compared with the 'Cont' plants during both 2019-2020 and 2020-2021 cropping years, respectively (Fig. 5a-iv and Fig. 5b-iv). Similarly, seed yield was significantly greater in 'Amis. Top' (by 47.72 and 50.79%), 'Tvir' (by 45.03 and 46.98%), 'Thar' (by 42.12 and 42.68%), 'Tasp' (by 39.97 and 42.20%), and 'Bion' (by 37.20 and 39.07%) plants as compared with the 'Cont' plants during both 2019-2020 and 2020-2021 cropping years, respectively (Fig. 5a-v and Fig. 5b-v). Interestingly, based on overall performance, it can be suggested that Amistar Top and *T. viride* were most promising in lessening the severity of rust while preserving agronomic features under field conditions, followed by *T. harzianum*, *T. asperellum*, and Bion (Fig. 5a-vi and Fig. 5b-vi).

*Trichoderma* spp. and agrochemicals spraying modulate the biochemical responses of faba bean plants in responses to rust infection under field conditions

Rust infection resulted in notable alterations in the biochemical responses of faba bean plants under field conditions, specifically increased the activities of Peroxidase (POX) by 11.17%, Polyphenoloxidase (PPO) by 14.56%, and Phenylalanine ammonia lyase (PAL) by 7.42%, while increased the contents of total phenolics by 8.89%, total flavonoids by 65.00%, and $\beta$-1,3-glucanase by 23.38% in 'Cont-inf' plants compared with 'Cont-uninf' plants (Fig. 6a- 6f). Conversely, total chlorophylls content decreased by 16.67% in 'Cont-inf' plants relative to 'Cont-uninf' plants (Fig. 6g). However, the application of *Trichoderma* spp. and agrochemicals elicited more significant biochemical responses in faba bean plants subjected to rust infection. Significantly elevated POX activity was observed in the 'Tvir', 'Bion', 'Thar', 'Tasp', and 'Amis. Top' plants (by 14.83, 13.88, 7.18, 6.70, and 4.78%, respectively) in comparison with the 'Cont-inf' plants (Fig. 6a). PPO activity significantly increased in 'Bion', 'Tvir', and 'Thar' plants (by 23.75, 14.72, and 14.05%, respectively) relative to 'Cont-inf' plants, although 'Tasp' and 'Amis. Top' plants exhibited similar PPO activity to 'Cont-inf' plants (Fig. 6b). In the context of PAL, only the 'Bion' and 'Tvir' plants exhibited considerably elevated activity compared with 'Cont-inf' plants, with increases of 12.73% and 10.00%, respectively, whilst 'Thar', 'Tasp', and 'Amis. Top' plants show comparable PAL activity to 'Cont-inf' plants (Fig. 6c). Among the non-enzymatic antioxidants, total phenolics content was significantly greater in 'Tvir', 'Bion', 'Amis' Top', 'Thar', and 'Tasp' (by 58.42, 48.02, 25.50, 20.30, and 16.09%, respectively) plants as compared with 'Cont-inf' plants (Fig. 6d). On the other hand, the total flavonoid content was markedly higher in 'Tvir', 'Thar', and 'Bion' plants (by 115.15, 58.08, and 32.32%, respectively) compared with 'Cont-inf' plants; however, it was similar among 'Tasp', 'Amis. Top', and 'Cont-inf' plants (Fig. 6e). The level of $\beta$-1,3-glucanase was significantly elevated in the plants 'Thar', 'Tvir', 'Tasp', 'Bion', and 'Amis. Top' by 86.89, 72.35, 69.61, 57.33, and 48.51%, respectively, in comparison with 'Cont-inf' plants (Fig. 6f). Lastly, total chlorophylls content was considerably higher in 'Thar', 'Tasp', 'Tvir', 'Bion', and 'Amis Top' (by 134.29, 114.29, 102.86, 90.86, and 71.43%, respectively) plants compared with 'Cont-inf' plants (Fig. 6g). Overall, it was clearly evident among the bioagents and agrochemicals that *T. viride* had the most prominent impact on the biochemical responses of faba beans to combat rust infection, followed by Bion, *T. harzianum*, *T. asperellum*, and Amistar Top (Fig. 6h).

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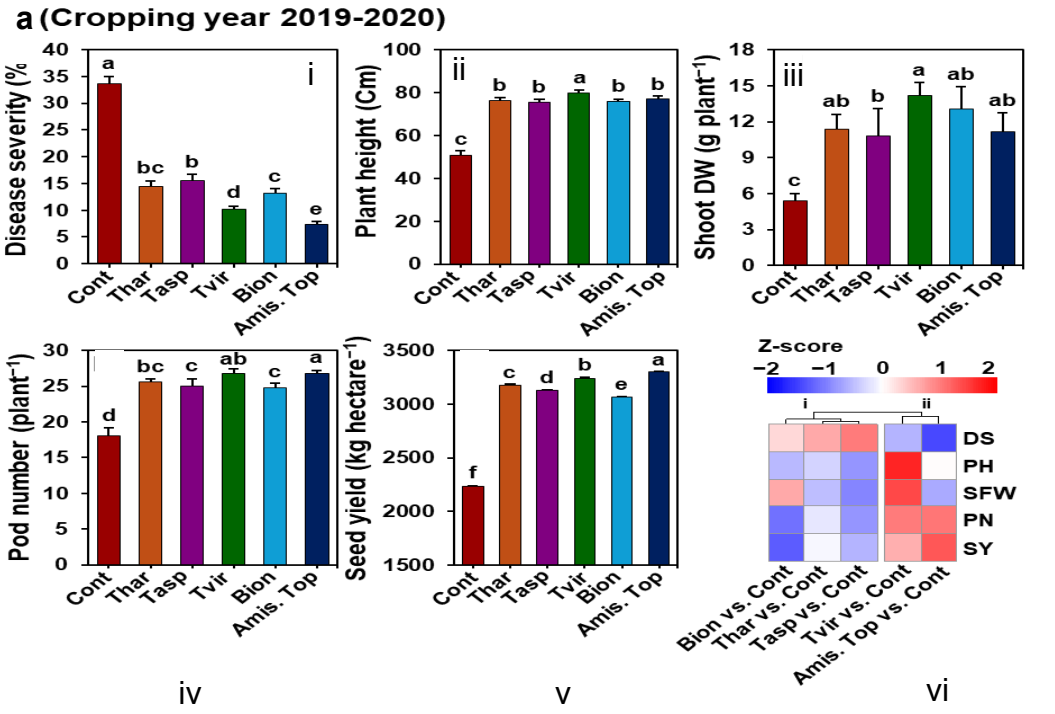
a (Cropping year 2019-2020)
Disease severity (%
Plant height (Cm)
Shoot DW (g plant⁻¹)
Pod number (plant⁻¹)
Seed yield (kg hectare⁻¹)
Cont
Thar
Tasp
Tvir
Bion
Amis. Top
i
ii
iii
iv
v
vi
Z-score
−2 −1 0 1 2
DS
PH
SFW
PN
SY
Bion vs. Cont
Thar vs. Cont
Tasp vs. Cont
Tvir vs. Cont
Amis. Top vs. Cont

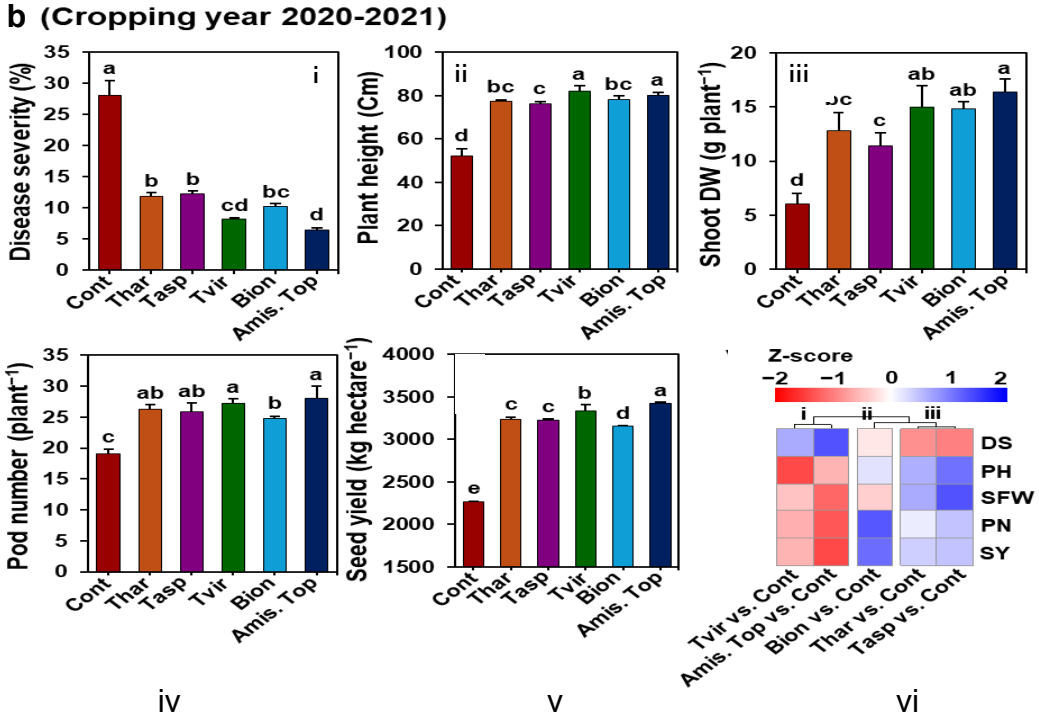
b (Cropping year 2020-2021)
Disease severity (%)
Plant height (Cm)
Shoot DW (g plant⁻¹)
Pod number (plant⁻¹)
Seed yield (kg hectare⁻¹)
Cont
Thar
Tasp
Tvir
Bion
Amis. Top
i
ii
iii
iv
v
vi
Z-score
−2 −1 0 1 2
DS
PH
SFW
PN
SY
Tvir vs. Cont
Amis. Top vs. Cont
Bion vs. Cont
Thar vs. Cont
Tasp vs. Cont

**Fig. 5** Effect of *Trichoderma* spp. and agrochemical spraying on the severity of faba bean rust and agronomic parameters grown in field prone to severe rust infection at two different cropping years. Faba bean plants were sprayed with *Trichoderma harzianum*, T. *asperellum*, and T. *viride* (108 colony-forming units mL−1) and Bion (60 mM) just after first appearance of the disease symptoms. Negative control plants did not receive any spraying, while positive control plants were sprayed with Amistar Top (60 mM). All spraying were repeated three times at 10 days interval. Field experiments were conducted at two different cropping years viz., (A) 2019-2020 and (B) 2020-2021. (a-i and b-i) Disease severity, (a-ii and b-ii) plant height, (a-iii and b-iii) shoot dry weight (DW), (a-iv and b-iv) pod number, (a-v and b-v) seed yield of faba bean plants under different treatments. (a-vi and b-vi) Hierarchical clustering heatmap showing the fold-change values of the above-mentioned parameters. The mean and standard deviations (n = 3) are displayed as bars. Significant changes ($p \leqslant 0.05$) among different treatments are denoted by different letters (a-f) atop the bars and were calculated following the least significant difference test. Here the treatments are, non-sprayed infected negative control plants (Cont); T. *harzianum* sprayed infected plants (Thar); T. *asperellum* sprayed infected plants (Tasp); T. *viride* sprayed infected plants (Tvir); Bion sprayed infected plants (Bion); Amistar Top sprayed infected positive control plants (Amis. Top).

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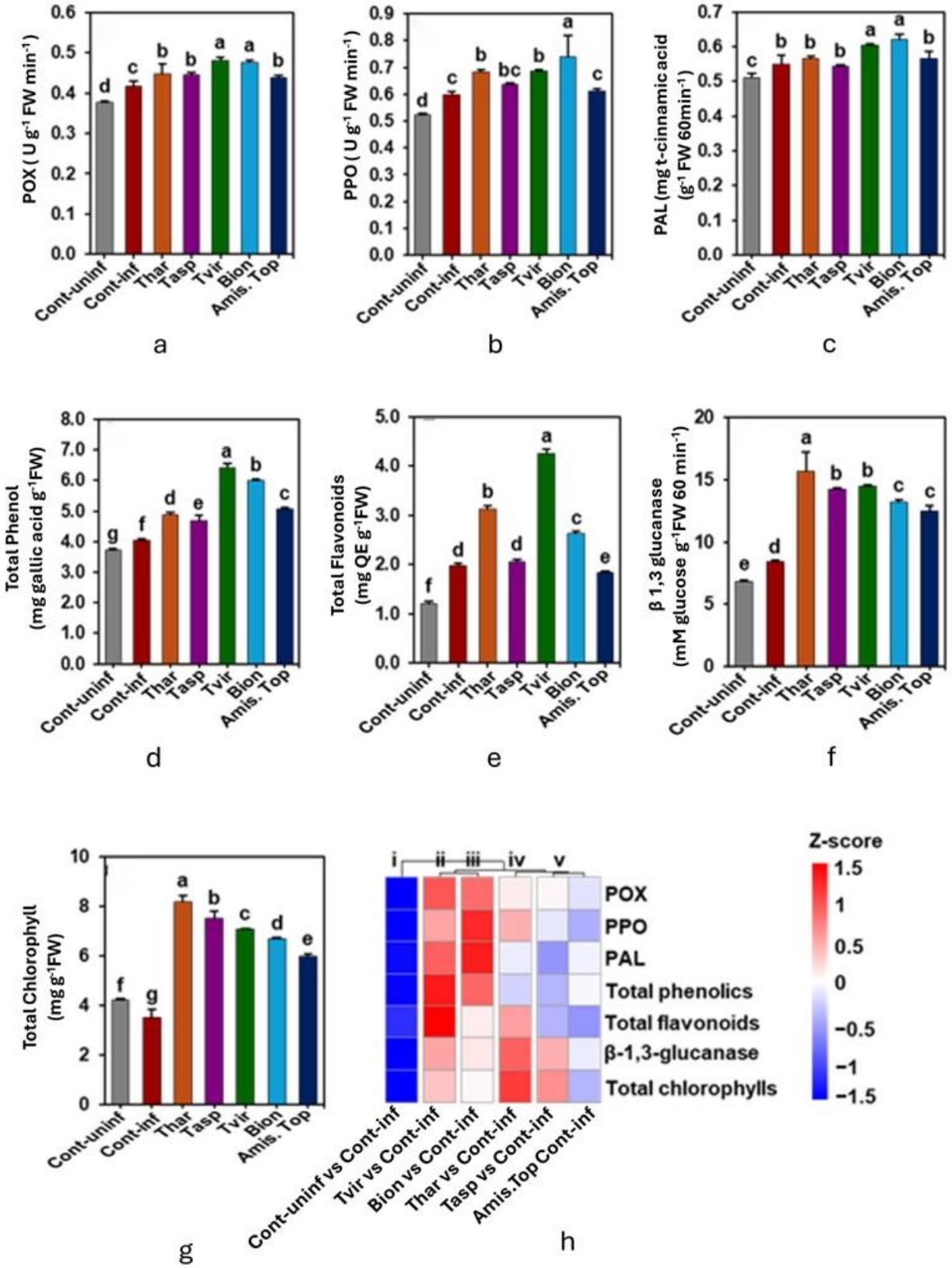
POX ( U g-1 FW min-1)
PPO ( U g-1 FW min-1)
PAL (mg t-cinnamic acid (g-1 FW 60min-1)
Total Phenol (mg gallic acid g-1FW)
Total Flavonoids (mg QE g-1FW)
β 1,3 glucanase (mM glucose g-1FW 60 min-1)
Total Chlorophyll (mg g-1FW)
Cont-uninf
Cont-inf
Thar
Tasp
Tvir
Bion
Amis. Top
a
b
c
d
e
f
g
h
i
ii
iii
iv
v
POX
PPO
PAL
Total phenolics
Total flavonoids
β-1,3-glucanase
Total chlorophylls
Cont-uninf vs Cont-inf
Tvir vs Cont-inf
Bion vs Cont-inf
Thar vs Cont-inf
Tasp vs Cont-inf
Amis.Top Cont-inf
Z-score
1.5
1
0.5
0
−0.5
−1
−1.5

**Fig. 6** Effect of *Trichoderma* spp. and agrochemical spraying on the biochemical attributes of faba bean plants grown in field prone to severe rust infection. Faba bean plants were sprayed with *Trichoderma harzianum, T. asperellum, and T. viride* ($10^8$ colony-forming units $mL^{-1}$) and Bion (60 mM) just after first appearance of the disease symptoms. Negative control plants did not receive any spraying, while positive control plants were sprayed with Amistar Top (60 mM). All spraying were repeated three times at 10 days interval and samples were collected one month (70 days after sowing) after the first spray. Enzymatic activities of (A) peroxidase (POX), (B) polyphenoloxidase (PPO), (C) phenylalanine ammonia lyase (PAL), and (D) β-1,3-glucanase in the leaves of faba bean plants under different treatments. Contents of (E) total phenolics, (F) total flavonoids, and (G) total chlorophylls in the leaves of faba bean plants under different treatments. (H) Hierarchical clustering heatmap showing the fold-change values of the above-mentioned parameters. The mean and standard deviations ($n = 3$) are displayed as bars. Significant changes ($p \leq 0.05$) among different treatments are denoted by different letters (a-g) atop the bars and were calculated following the least significant difference test. Here the treatments are, non-sprayed infected control plants (Cont-inf); non-sprayed uninfected control plants (Cont-uninf), *T. harzianum* sprayed infected plants (Thar); *T. asperellum* sprayed infected plants (Tasp); *T. viride* sprayed infected plants (Tvir); Bion sprayed infected plants (Bion); Amistar Top sprayed infected positive control plants (Amis. Top).

## Discussion

Rusty pustule with urediospores and teliospores of U. *vicia* fabae were observed on the surface of rust infected faba bean plants in all five districts of Bangladesh. The urediospores causes faba bean rust in Egypt (Abada et al., 2019) and *Uromyces viciae fabae* has also been found to cause pea rust (Prasada & Verma, 1948). Morphology of both the urediospores and teliospores isolated from faba bean rust were found almost similar with the other researcher's findings (Singh, 1973) where they reported that urediospores were round to ovate, light brown echinulate about 25-38x18-27 micron in size and teliospores were sub globose to ovate, thick walled, pedicellate with 20-30x18-26 micron (Gautam et al., 2022).

Rust incidence and severity of faba bean in Bangladesh were varied from 12.5-68.5% and 8.8-35.6%. Rust infection was found severe podding stage. Almost the similar incidence and severity was observed and revealed that percent disease incidence was varied from 23.6-78.2%, disease severity varied from 4.8-37.9% in bean rust (Gosaye et al., 2018). They also reported bean rust rust is highly severe at podding stage.

The inhibitory effect of Trichoderma spp. against uredial spores was in the range of 48.6-65.1% urediospores germination (Fig. 3). Among the three *Trichoderma* spp., *T. viride* gave the highest inhibition, where the urediospores failed to germinate at the concentration of 60% culture filtrate (Tab. 1). *Trichoderma* spp. and Bion resulted in the maximum inhibition to the germinated urediospores of *Uromyces vicia fabae*. Such inhibition may be due to the release of secondary metabolites and cell wall degrading enzymes by T. *viride* into the medium (Abd-El-Khair et al., 2010; Barakat et al., 2014; Dubos & Bulit, 1981; Zaher et al., 2013).

Non-volatile secondary metabolites of *Trichoderma* species were also found effective in suppressing the germination of urediospores of *Uromyces* and *T. viride* exhibited the highest spore inhibition followed by *T. harzianum* after 48 hrs. of confrontation. Volatile and non-volatile chemical substances synthesized by *Trichoderma* spp. are able to limit and even stop the urediospores germination (Abd-El-Khair et al., 2010).

In green house experiment, *T. viride* was found to be the most potential bioagents in reducing the rust infection and increasing plant height (cm), fresh weight (g), dry weight, no. of pods /plant and seed yield/ plant as compared with the other two *Trichoderma* spp. and the control treatment. The severity of the rust disease after inoculation with *Trichoderma* spp. was in the range of 8.0-9.2% where bion and chitosan were in the range of 8.2-12.5% (Tab. 3). *Trichoderma spp.* and bion exhibited significantly almost similar rust disease severity under green house condition. This may be due to their inducing effect on less uredial germination (Zimand et al., 1996). Researchers also found that spraying with suspension of urediopsres on faba bean plants scored a remarkable depression in rust disease severity comparing with unsprayed one (Abada et al., 2019). This result is also partially supported when they sprayed faba bean plants with suspension of unviable heated spores of *U. vicia fabae* (Abd-El-Khair et al., 2010; El-Gammal, 2005).

It is found that there is large variety of volatile secondary metabolites produced by *Trichoderma* spp. such as ethylene, carbon dioxide, hydrogen cyanide, aldehydes and ketones, which play an important role in managing the plant pathogens (Barakat et al., 2014; Prasad & Kumar, 2011; Tomasz et al., 2020; Vey et al., 2001; Zaher et al., 2013).

Among the three *Trichoderma* spp.*, T. viride* resulted the higher yield parameters followed by Bion. This may be *Trichoderma* induce the growth hormones which increase the fresh weight of faba bean and helps in increasing photosynthetic dry matter (Abd-El-Khair et al., 2019). The fungicide Amistar Top application resulted in the lowest disease severity with the highest values of number of pods (25) and their seeds yield (140g/plant). Topas, a fungicide also gave the lowest rust infection with highest yield in faba bean (Abada et al., 2019).

Plants sprayed with Amistar Top and *T. viride* resulted the lowest rust infection produced the highest plant height(cm), fresh weight (g), dry weight (g), no. of pods/plant and seed yield (kg/ha) compared with non-treated treatment during both cropping seasons. Spraying of the other two *Trichoderma* spp. also reduced rust disease severity and increased faba bean yield. Results of those parameters followed a similar trend in the year of 2020-2021. However, lower % disease severity and higher agronomic parameters were recorded in 2020-2021 cropping season. Meanwhile, the fungicide Amistar Top was found the superior treatment followed by *T. viride* for reducing the rust disease severity and increasing the produced seed yield.

The percent rust disease severity was recorded higher at 2019-2020 cropping season (where Dec.2019-Mar.2020 avg. temperature was 20.8$^0$C and 77.8 %rh) than 2020-2021 cropping season (Dec.2020-Mar.2021 avg. temperature was 22$^0$C and 77.3 % RH). This may be due to the variation of weather factors like temperature ($^0$C) and % relative humidity (%rh). It was observed that 2019-2020 cropping season had the lower temperature with higher relative humidity which enhance the rust pathogen more aggressive than 2020-2021 cropping season. This is an agreement with the results (Joseph & Hering, 1997) who reported rust pustules appear on the pods can reduce seed weight. Severe infection may cause premature defoliation, resulting in reduced seed size. Urediospores of U. viciae fabae germinate well in a temperature that ranges 5-26℃, with fastest germination at 20℃. Uromyces fabae from lentil was found that ideal temperatures for germination of uredospores is 16-22° C, while germination doesn't happen at 28-29° C. The teliospores of lentil rust can sprout at 12-22° C (Prasada & Verma, 1948).

In both the cropping season, field experimental data revealed that spraying of *Trichoderma spp*. (@$10^8$ cfu/ml) on faba-bean plants significantly reduced the rust disease severity with increasing of seed production (kg/ha) as compared with the untreated treatment. Although, Amistar Top resulted significantly the lowest rust disease severity and increased the highest seed yield production compare to spraying of *T.viride, T. harzianum , T. asperellum* and Bion; but there was no significant difference in seed yield production in between Amistar Top and *T. viride* treatments (Tab.4). Trichoderma spp. significantly reduced the rust disease severity may be either by directly inhibition of mycelial growth and sporulation of the rust pathogen with antagonistic mechanisms and enzyme production or by enhancing plant defensive mechanisms, nutrient competition and promoting plant growth (Abd-El-Khair et al., 2010; Bouhassan et al., 2004; Chet et al., 1997; Muthukumar et al., 2011; Nur et al., 2020). *Trichoderma* spp. colonize root surfaces and penetrate the epidermis that induce localized or systemic resistance responses that protect plants from the pathogenic fungi (Harman et al., 2004). It was observed that *Trichoderma* spp. increased the agronomic parameters of faba bean under both green house and field experiments. It was also reported that *Trichoderma* spp. and thiophanate-methyl significantly improve the vegetative growth parameters of bean plants viz. root length (cm), shoot length (cm), number of leaves/plants, plant fresh weight (g), plant dry weight (g), dry bean growth and yield components (Abd-El-Khair et al., 2019). Growth promotion effect is one of the mechanisms of *Trichoderma* spp. exerted for the control of phytopathogenic diseases (Benítez et al., 2004; Gajera et al., 2013; Sharma et al., 2012). *Trichoderma* spp. also produce phytohormones that promote growth characteristics of the plants and increased bean yield (Alice et al.,2016; Hexon et al., 2009). *Trichoderma* spp. reduce the rust disease by accumulating phytoalexines, lignins and induce enhance activities of chitinase and β-glucanase and such induced resistance is persistent but pathogen non-specific (Dean & Kuc, 1985; Doubrava, 1988; Kloepper et al., 2004; Metranx & Boller, 1986). T. *viride* produces antifungal metabolites which reduced seedling mortality against *Sclerotium rolfsii* (Manjula et al., 2004; Voegele, 2006). Trichoderma is capable of promoting the growth and yield of crops, such as cereals (Mahato et al., 2018; Poveda et al., 2019).

Plants treated with the *Trichoderma* spp, Bion, chitosan and the fungicide showed an increase in PAL, POX, PPO activity and β-1,3 glucanase as compared with control. *T. viride* contained a higher amount of total phenol, flavonoid and β-1,3 glucanase (Tab.6). POX and PPO are responsible for oxidization of phenolics, produce free radicals and H2O2 which is toxic to many pathogens (Pena and Kuc 1992). PPO is also involved in terminal oxidation of diseased plant tissue and thus attributed in disease resistance (Kosuge, 1996). PAL works as key enzyme in phenylpropanoid and flavonoid pathways (Morkunas & Gemerek, 2007) and involved in more than one function in plants (Scott-Carig et al., 1995).

Bion, a chemical inducer was found to reduce the rust disease incidence and severity suggesting that it helps the plant in acquired resistance against rust pathogen by improving the activity of anionic peroxidases, promoted by H2O2 accumulation which led to cell wall strengthening (Iriti & Faoro, 2003). In addition, plants sprayed with Bion recorded the highest activity of the three enzymes (POX, PPO and PAL) followed by *Trichoderma* spp. It has been found that the reduction of rust disease severity was ascribed to the increased levels of PAL, POX and PPO enzymes (Tab. 4). Peroxidase enzyme oxidizes the phenols to more fungal toxic compounds such as quinines, which inhibit both spore germination and fungal growth. Also, POX was found to participate in the synthesis of lignin (Farkas & Kiraaly, 2008; Scott-Carig et al., 1995;). POX is known to be involved in the oxidation of polymerization of hydroxycinnamyl alcohols to yield lignin (Vance et al., 1980). Increase in POX activity enhances lignification in response to chocolate

spot infection which may restrict the fungal penetration (Ride, 1983; Tarred et al., 1993). Trichoderma spp. produced highest amount of β-1,3 glucanase in compared to bion. β-1,3 glucanase has been shown to be capable of attacking cell wall of fungal pathogens, these enzymes have been proposed as direct defense enzymes of plants (Ahmed, 2015).

However, among the *Trichoderma* spp, *T. viride* yielded the highest amount of total phenol and flavonoid. These results could be discussed considering the findings who cleared those phenols are essential for biosynthesis of lignin, which is considered an important structural component of plant cell walls and thus inhibit fungal infection (Hahlbrock & Scheel, 1989). Another research explained that the first step of the defense mechanisms in plants involves a rapid accumulation of phenols at the infection site, which restricts or slows the growth of the pathogen because of its action as antioxidant, antimicrobial and photoreceptor (Lamba et al., 2008). Moreover, the participation of an endogenous supply of phenolic compounds in the plant disease resistance is dependent upon active phenol oxidase system (Melo et al., 2006). As for changes in chlorophyll, phenols and flavonoid contents in treated faba bean plants with the tested bio-inducers, all bio-inducer increased chlorophyll content in treated faba bean plants as compared with control treatment (Ahmed, 2015). Application of *T. harzianum* as foliar treatment significantly reduced the severity of chocolate spot disease as compared with untreated infected plants and also, markedly increased total chlorophyll content in treated infected plants as compared with untreated (El-Rahman et al., 2014).

From the successive two years field experiment, it is revealed that Amistar top is the chemical fungicide which can reduce the rust disease severity almost four times higher and resulted higher seed yield of faba bean over *Trichoderma spp*. but the seed yield was not statistically different with the result of *T. viride* treatment. Amistar top is a synthetic chemical while *T. viride* is a good adaptation to our local environmental conditions which can induce antimicrobial enzymes or proteins and thus, reduce the rust of faba bean disease by inhibiting uredial germination of *U. vicia fabae*.

**Conclusion**

The data obtained from this study revealed that there was a promising antagonistic species of Trichoderma spp. specially *T. viride* which could be exploited (@ $10^8$ CFU/ml) for the control of faba bean rust in Bangladesh. However, further screening can be done in this regard to get more potential *Trichoderma spp*. and uncover the underlying mechanisms.

**Acknowledgement:** Authors gratefully acknowledge the help of Dr. S. Nakkeeran, Dept. of Plant Pathology, TNAU, India for providing *T. viride* (Tv6) and Mohammad Golam Mostofa, Department of Chemistry, State University of New York College of Environmental Science and Forestry, NY, USA for his continuous support in this manuscript.

**Funding statement:** This project was funded by the Ministry of Science and Technology (MoST Government of the People's Republic of Bangladesh (Grant No. 221132) and BAURES, BAU (Grant No.2022/72/BAU).

**Author's contribution:** Conceptualization and methodology: Mohammad Delwar Hossain; Experimentation and formal analysis: Faria Noshin; Data analysis and Figures in R' programme: Zaid Bin Hossain; Edited Table and Figures: Mohammad Delwar Hossain; Writing, review and editing: Mohammad Delwar Hossain, Tawseef Al Aff, Sadia Afrin Shila and Mona H. Soliman; Supervision: Mohammad Delwar Hossain and; Project administration: Mohammad Delwar Hossain. All authors have read and approved the final version of the manuscript.

**Conflicts of Interest:** The authors declare that they have no conflicts of interest to report regarding the present study.

## Declaration of competing interest

The authors have no competing interest in declaring for the submission of this work. This includes, but is not limited to, financial or personal relationships which could negatively influence, or bias, this work.

## Data availability

Data will be made available on request.

## Supplementary data

Supplementary data to this article can be found online

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