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Study on Copper Tolerance of *Trichoderma*

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ABSTRACT: The present study was envisaged to know the tolerance potential of *Trichoderma* sp. to copper compounds *in vitro* for possible incorporation in the integrated disease management strategy at field level. The study was carried out during 2020-2021 at SASRD, NU, Medziphema Campus. Copper tolerance ability of *Trichoderma asperellum* was tested by growing it in media containing two copper compounds namely copper oxychloride and copper sulfate separately each with concentrations of 250, 500, 750 and 1000ppm. The growth test indicated that *T. asperellum* was most inhibited at 1000ppm concentration of copper sulfate and copper oxychloride, the colony diameter were 18.17 and 5.67mm respectively relative to 61.83mm in control. Among all the treatments, the highest mycelial growth was recorded in treatment receiving copper oxychloride at 250ppm concentration with 85.67mm diameter of *Trichoderma asperellum* which is statistically at par with growth in control plate, whereas the treatment receiving copper oxychloride at 1000ppm recorded the lowest mycelial growth of 13.33mm. In the treatment containing copper oxychloride at 250ppm, *Trichoderma asperellum* inhibited mycelial growth of *Fusarium oxysporum* f.sp. *pisi* to 24.17 mm.

Keywords: *Trichoderma asperellum,* copper oxychloride, copper sulfate, *Fusarium oxysporum* f.sp. *pisi*, Compatibility, Tolerance.

INTRODUCTION

One of the most important biocontrol agents is *Trichoderma* spp. the most frequently isolated and studied soil fungi and present in plant root atmosphere (Harman *et al*., 2004). *Trichoderma* sp. belongs to phylum Ascomycota, order Hypocreales and family Hypocreaceae (Chaverri and Samuel, 2004). Many advantages can be gained by using Trichoderma in agriculture; it can colonise roots and rhizosphere of plants, mechanisms in managing plant pathogens like mycoparasitism, antibiosis and induction of systemic resistance in plants, encouraging plant development, triggers development of roots (Harman *et al*., 2004). The interaction between *Trichoderma* and plants is restricted to the first epidermal cell layer of the root bark. Plant benefit from this symbiotic relationship because it protects them from diseases, also encourage plant and root growth by increasing nutrient availability (Hermosa *et al*., 2012).

The species under the genus *Trichoderma* are well characterized by broad adoption to stress conditions caused by heavy metals like cadmium, copper, mercury, zinc and lead (Williams *et al*., 2003; Maldaner *et al*., 2020; Mohammadian *et al*., 2017). Among environmental stresses in soil most important are the presence of heavy metals and various chemicals, fluctuation of temperature or water potential (Kredics *et al*., 2001). *T. asperellum* tolerated the CuOH concentration up to 500 ppm and toxicity symptoms appeared beyond concentration of 500 ppm (Singh *et al*., 2018). High metal absorption ability was seen *Trichoderma* and in other filamentous fungi such as *Penicillium*, and *Aspergillus* species, especially for Cu and Co (Dusengemungu *et al*., 2020). The copper tolerance of *Trichoderma* sp. has been studied (Kredics *et al*., 2001; Anand *et al*., 2006). The selection of copper tolerant *Trichoderma* may be a better option in plant disease management as copper fungicides are often used in field condition for management of plant diseases. In this backdrop, the present work was carried

out to study the copper tolerance ability in *Trichoderma asperellum* and efficacy of *Trichoderma asperellum* against plant pathogenic fungi in presence of copper compound.

MATERIALS AND METHODS

The present study was carried out in the laboratory of the Department of Plant Pathology, SASRD, Nagaland University, Medziphema Campus, Nagaland, located at an altitude of 310 m above mean sea level with the following geographical coordinates: 25° 45´ 45´´N;93° 51´45´´E. The parental strains of *T. asperellum* and pathogen *Fusarium oxysporum* f. sp. *Pisi* were obtained from the stock culture of Department of Plant Pathology, SASRD, NU, Medziphema and maintained on PDA slants at 4°C in refrigerator.

Preparation of stock solution of copper oxychloride and copper sulfate. To prepare stock solution of copper oxychloride five grams of copper oxychloride 50% WP was added to 500 ml conical flask containing 100 ml distilled water and the volume was made up to 250 ml to get 10000 ppm stock solution. For preparation of stock solution of copper sulfate two and half grams of copper sulfate was added to 500 ml conical flask containing 100 ml distilled water, volume made up to 250 ml to get 10000 ppm stock solution of 250 ml copper sulfate.

Preparation of poisoned media with different concentrations of copper oxychloride and copper sulfate

To prepare PDA media containing 250, 500, 750 and 1000 ppm concentrations of copper oxychloride and copper sulfate, 100 ml conical flasks containing media were added with 2.5, 5, 7.5 and 10 ml of 10000 ppm stock solution of copper oxychloride and copper sulfate respectively, volume was made up to 100 ml with PDA media.

In vitro* study of copper tolerance ability in *Trichoderma asperellum

Copper tolerance ability of *Trichoderma asperellum* was tested by adopting poisoned food technique (Sinclair and Dhingra 1995). Appropriate dilutions of 250 ppm, 500 ppm, 700 ppm and 1000 ppm were prepared from stock solution of 10000 ppm of copper compounds (copper sulfate and copper oxychloride). Twenty ml of copper compounds-amended medium (250 ppm, 500 ppm, 700 ppm and 1000 ppm) were poured in each sterilized Petri plates (90mm diameter). Streptomycin was added to the poisoned medium at the time of pouring to prevent bacterial contamination. Suitable checks were maintained without addition of copper compounds. From seven-days old culture of *Trichoderma asperellum* a uniform disc of 5mm was cut with cork borer and inoculated aseptically and placed on to the center of Petri plate. These plates containing PDA amended with different concentration of copper compounds were kept upside down for better contact of pathogen to the media. The plates were incubated at 25±2°C in BOD incubator. The colony diameter (mm) of the *Trichoderma asperellum* was determined by measuring the average radial growth on the 4th day after inoculation, when the control plates were full. Average radial growth was measured by using a measuring scale from the Petri plated lower view. Based on the observations recorded, percent inhibition of the *Trichoderma asperellum* was calculated using formulae given by Vincent (1947).

Efficacy of *Trichoderma asperellum* against plant pathogens in presence of copper compound. Efficacy of *Trichoderma asperellum* was tested against *Fusarium oxysporum* f.sp. *pisi* under *in vitro* condition by adoption of dual culture technique (Morton and Stroube 1955). Twenty milliliter copper compound amended sterilized PDA was aseptically poured in sterilized Petri plates and allowed to solidify. Mycelial discs (5mm) taken from the actively growing colonies of the test pathogen and *T. asperellum* (7 days old culture) were placed simultaneously on the PDA plates opposite to each other, 1 cm apart from the periphery. Three replications were maintained for each treatment. The inoculated Petri plates were incubated at 27±2°C. And the plates without copper compounds act as control. First observation was taken just after contact of pathogen and antagonist and radial growth of the test pathogen was measured. Based on the observations recorded, percent inhibition was calculated using formulae given by Vincent (1947).

$$\% I = ((C-T)/C)*100$$

Where, I= Percent inhibition of pathogens by antagonist, C= Radial growth in control (mm), T= Radial growth in the treatment (mm).

Statistical analysis and interpretation. The Fisher's method of analysis of variance was used to analyse the data. The significance of variance among the data was calculated out by calculating the F value and comparing it with the tabulated value of F (Snedocor and Cochran, 1967). The treatment means were also compared among themselves by calculating Critical difference. Critical difference (CD) was calculated for comparison in those cases where "F" test was significant at 5 per cent level of significance as given by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

By using poison food technique, tolerance ability of *Trichoderma asperellum* was evaluated against copper compounds. The data presented in Table 1 shows mycelia growth measured after 24 hours, 48 hours, 72 hours and 96 hours of inoculation. The copper compounds significantly inhibited the mycelia growth of *T. asperellum.* At 24 hours after inoculation, highest mycelial growth was recorded in T_1: Copper oxychloride 250ppm 19.00 mm which is statistically on par with T_5: Copper sulfate 250ppm 18.33 mm. At 48hours, the highest mycelial growth was recorded in T_1: Copper oxychloride 250ppm 55.33 mm and found

superior over other treatments. Least growth was noticed in T_4: Copper oxychloride 1000ppm 0.00mm. At 72hours, highest mycelia growth was recorded in T_1: Copper oxychloride 250ppm 69.67mm and followed by T_5: Copper sulfate 250 ppm 63.33mm, the least growth was noticed in T_4: Copper oxychloride 1000ppm 9.33 mm. At 96hours, T_1: Copper oxychloride 250ppm recorded highest growth of 85.67mm which is statistically at par with T_0: Control 90.00 mm. The treatment T_4: Copper oxychloride 1000 ppm recorded least mycelia growth of 13.33 mm. Findings of present investigation revealed that *T. asperellum* can grow in presence of copper compounds at 250 ppm and at increasing concentration of copper compounds, radial growth of mycelia was decreased. The findings are in agreement with several earlier reports. Chahdi *et al.* (2019) who reported that the mycelia growth of *T. asperellum* was 31.1 mm in medium amended with copper sulfate at 1000 mg L^{-1} in contrast to 68.1 mm in control. Maheshwary *et al.* (2020) carried out compatibility test of some fungicides with *T. asperellum* and reported that *T. asperellum* showed compatibility with copper hydroxide and copper oxychloride.

Singh *et al.* (2018) reported that *T. asperellum* (MH593785) tolerated the CuOH concentration up to 500ppm and beyond 500 ppm copper toxicity symptoms appeared, the mycelial growth inhibition percent were observed as 54.125% and 65.83% at 750ppm and 1000 ppm respectively by using poison food technique. Maldaner *et al.* (2020) also evaluated isolates of *Trichoderma* against copper ($CuSO_4$) and reported that some isolates showing reduced mycelial growth above 100 mg L^{-1} concentrations. Anand *et al.* (2006) and Ting and Choong (2009) reported that the mechanism of tolerance in *Trichoderma* isolate depends on bioaccumulation and binding of copper on cell wall surface. Tolerant isolates of *T. asperellum* deposit the metal on the cell wall, whereas, the metal causes destruction of mycelia in sensitive isolates. Copper enters the cell wall, causing increase in vacuole size and shrinking of the cytoplasmic material, resulting in destruction of cell was reported by Ladi *et al.* (2020).

Efficacy of *T. asperellum* against *F. o.*f. sp. *pisi* in presence of copper compounds by using dual culture technique is shown in Table 2. In T_1: Copper oxychloride 250ppm *F. o*. f.sp. *pisi* recorded mycelia growth of 24.17 mm in presence of *T. asperellum* which is statistically on par with T_0: Control (25.33 mm), followed by T_5: Copper sulfate 250ppm (23.83mm). The antagonistic activity of *T. asperellum* towards the pathogen *F. o*. f.sp. *pisi* was influenced by increasing concentrations of copper compounds. Copper compounds may have increased the inhibitory effect of volatile compounds generated by *T. asperellum* through an antibiosis mechanism (Chahdi *et al.*, 2019). The results of the present investigation are in accordance with Chahdi *et al.* (2019) who reported that *T. asperellum* inhibited the growth of *V. dahlia* in presence of copper sulfate. Kapoor (2008) evaluated the effect of volatile compounds of *Trichoderma* on the growth of *Sclerotium rolfsii* and *Fusarium oxysporum* f.sp. *pisi* and found inhibitory to both these pathogens. *Trichoderma viride* showed maximum inhibition on radial growth of *Fusarium oxysporium* f.sp. *capsici* (76.74±0.4) in dual culture assay was revealed by Aswini *et al.* (2016).

Table1: *In vitro* evaluation of copper compounds on mycelia growth of *T. asperellum*.

Treatment	Mycelial growth (mm)				Mean
	24hrs	48hrs	72hrs	96hrs	
T_0 (Control): Medium without copper compound	27.97 (22.00)	49.79 (58.33)	61.34 (77.00)	71.57 (90.00)	51.84 (61.83)
T_1: Copper oxychloride 250ppm	25.84 (19.00)	48.06 (55.33)	56.58 (69.67)	67.75 (85.67)	49.26 (57.42)
T_2: Copper oxychloride 500ppm	21.42 (13.33)	29.56 (24.33)	39.82 (41.00)	49.79 (58.33)	35.82 (34.25)
T_3: Copper oxychloride 750ppm	18.12 (9.67)	22.25 (14.33)	29.56 (24.33)	36.27 (35.00)	27.15 (20.83)
T_4: Copper oxychloride 1000ppm	0.00 (0.00)	0.00 (0.00)	17.79 (9.33)	21.42 (13.33)	13.77 (5.67)
T_5: Copper sulfate 250ppm	25.35 (18.33)	42.51 (45.67)	52.73 (63.33)	64.65 (81.67)	46.29 (52.25)
T_6: Copper sulfate 500ppm	23.32 (15.67)	36.89 (36.00)	47.87 (55.00)	59.34 (74.00)	42.23 (45.17)
T_7: Copper sulfate 750ppm	19.97 (11.67)	28.20 (22.33)	38.65 (39.00)	48.83 (56.67)	34.71 (32.42)
T_8: Copper sulfate 1000ppm	0.00 (0.00)	22.79 (15.00)	27.27 (21.00)	37.26 (36.67)	25.23 (18.17)
SEm±	0.71	1.14	1.24	1.88	_
CD (p=0.05)	2.11	3.82	3.68	5.57	_

Note: Figures in the parentheses are original values which are subjected to arc sine transformation.

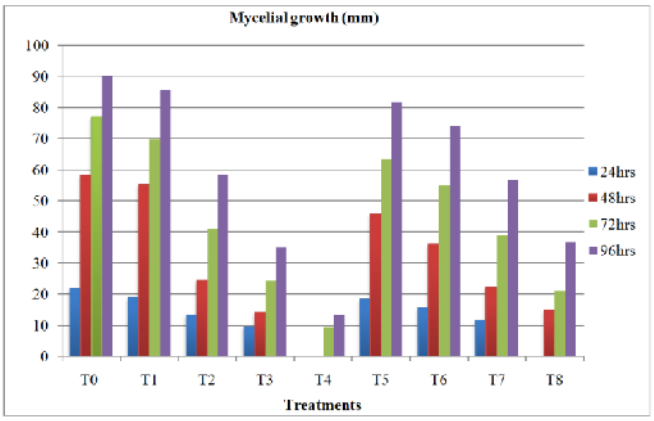

Fig. 1. *In vitro* evaluation of copper compounds on mycelia growth of *T. asperellum.*

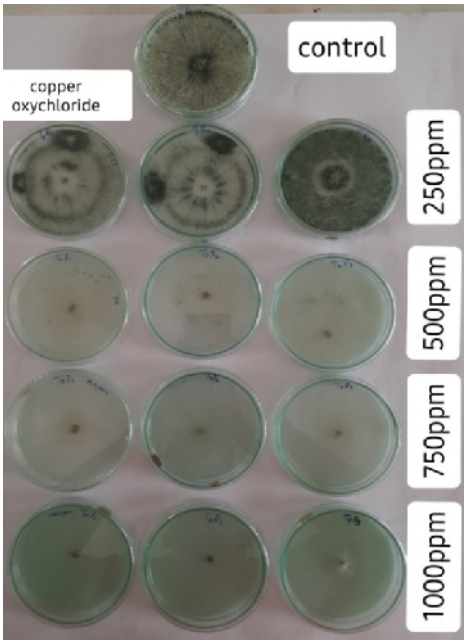

Plate 1: *In vitro* evaluation of copper compounds on mycelia growth of *T. asperellum* at 96 hours after inoculation.

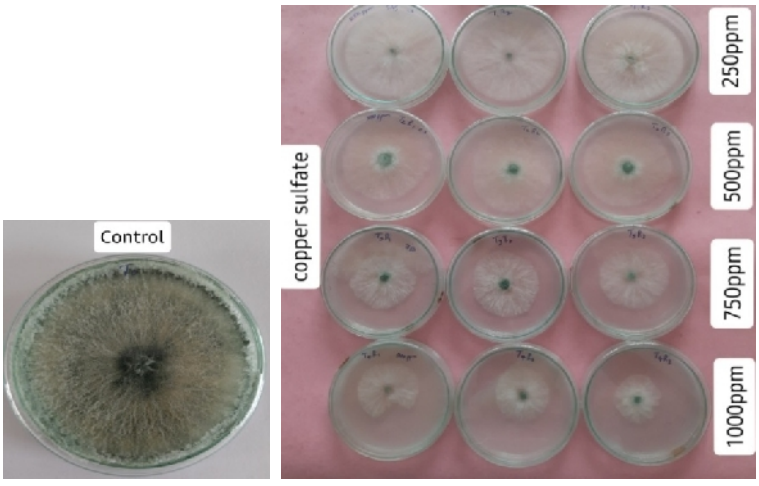

Plate 2: *In vitro* evaluation of copper compounds on mycelia growth of *T. asperellum* at 96 hours after inoculation.

Table 2*: In vitro* evaluation of copper compounds and *T. asperellum* on mycelial growth of *F. o.* f.sp. *pisi*

Treatment	Mycelial growth of pathogen (mm)
T_0 (Control): Medium without copper compound	25.33
T_1: Copper oxychloride 250ppm	24.17
T_2: Copper oxychloride 500ppm	23.33
T_3: Copper oxychloride 750ppm	15.67
T_4: Copper oxychloride 1000ppm	13.33
T_5: Copper sulfate 250ppm	23.83
T_6: Copper sulfate 500ppm	19.33
T_7: Copper sulfate 750ppm	13.67
T_8: Copper sulfate 1000ppm	12.67
SEm±	0.45
CD (p=0.05)	1.34

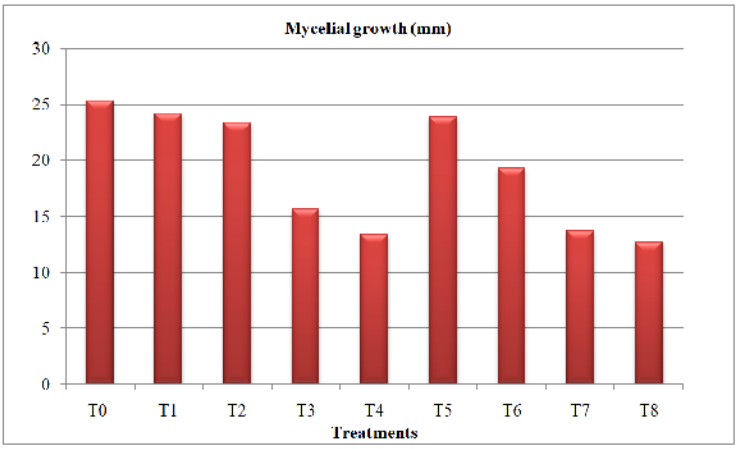

Fig. 2. *In vitro* evaluation of copper compounds and *T. asperellum* on mycelial growth of *F. o.* f.sp. *pisi.*

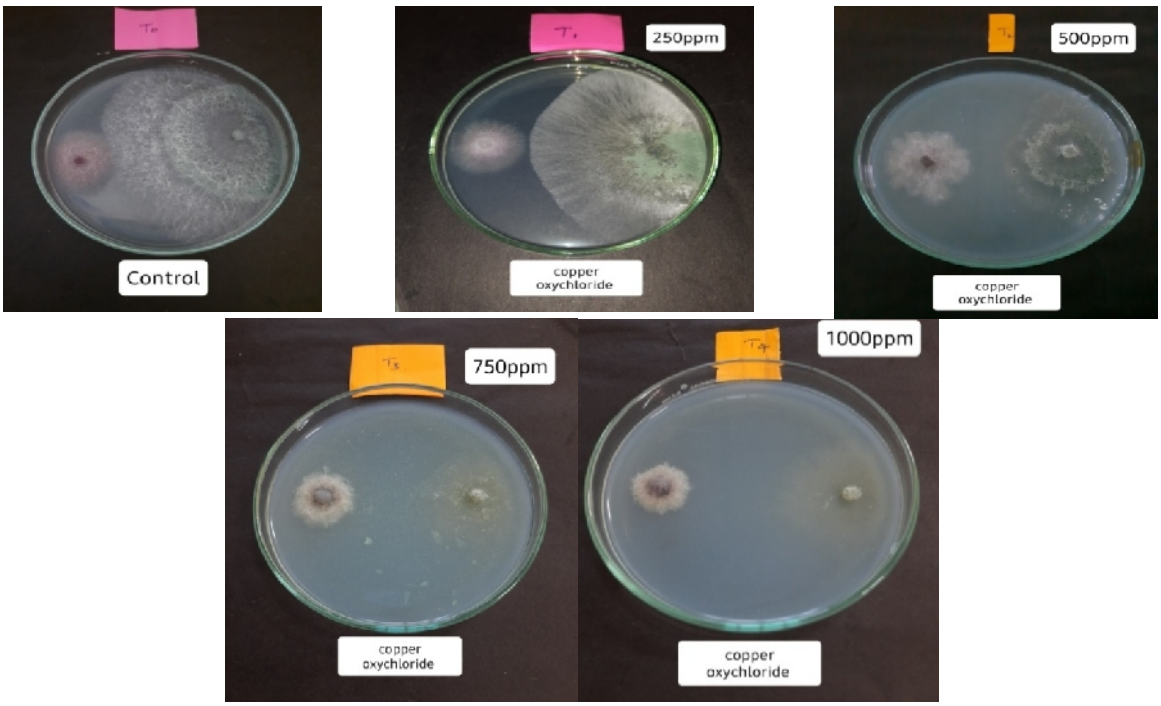

Plate 3: *In vitro* evaluation of copper compounds and *T. asperellum* on mycelial growth of *F. o.* f.sp. *pisi* after 72 hours of incubation.

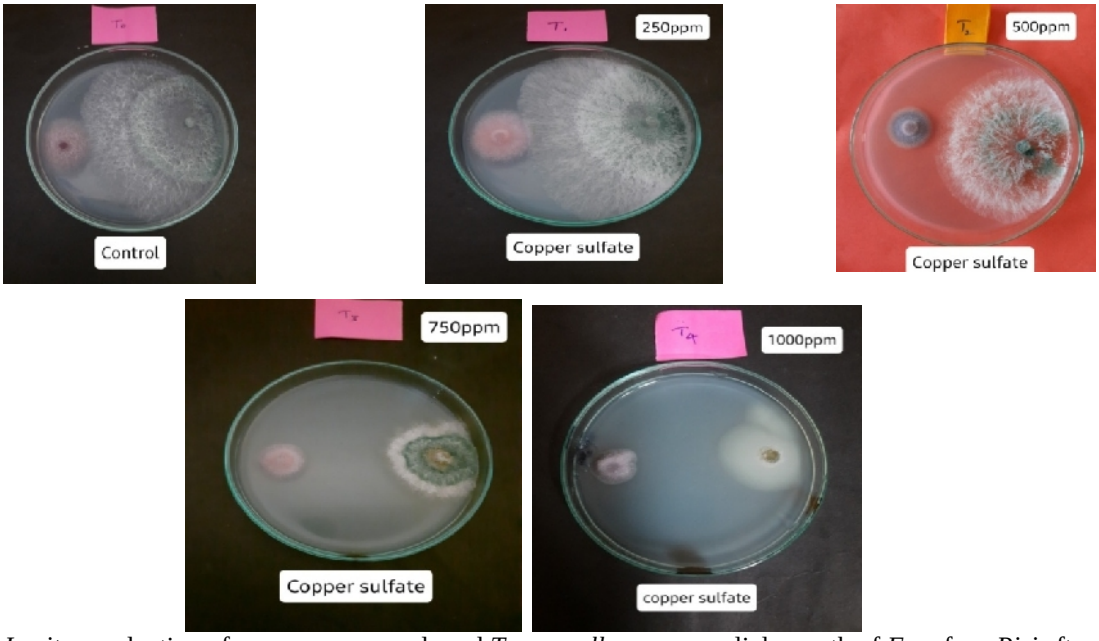

Plate 4: *In vitro* evaluation of copper compounds and *T. asperellum* on mycelial growth of *F. o.* f.sp. *Pisi* after 72 hours of incubation.

CONCLUSIONS

Based on results obtained in the present investigation, it shows that *T. asperellum* can grow at 250 ppm concentration of copper oxychloride and copper sulfate and it can inhibit the growth of pathogen also compared to other treatments. This result leads to the conclusion that *T. asperellum* may be used with copper compounds at low concentration to control plant pathogens in the integrated disease management programme after a through field study on the same.

FUTURE SCOPE

Biological control is a great tool in plant disease management because it is beneficial to both plant and environment. It includes biocontrol agents which controls pathogens growth and reduces its effect on plant. Ultimately, further studies on combining *Trichoderma* sp. with copper compounds would reveal more information to control plant pathogens in the integrated disease management programme.

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Conflict of Interest. None.

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