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A comparison of synthetic fungicide and *Trichoderma* spp. applications against clubroot disease on cabbage

Abstract. Clubroot is one of the important diseases affecting members of the Cruciferae family. This disease is caused by soil-borne pathogen, called *Plasmodiophora brassicae.* The pathogen produces motile spores called zoospores. This pathogen results in a drop of cruciferous plant productivity. The objectives of this research were to identify specific *Trichoderma* species in three districts in Sumatra Utara and to evaluate the potency of *T. harzianum* (both local species from Berastagi and species developed by Indonesian Vegetable Research Institute (IVEGRI)) to control the disease and then comparing them with the application of synthetic fungicide. The research was conducted in a greenhouse of Research Installation and Application of Agricultural Technology (IP2TP), Berastagi, North Sumatera from April–September 2018, using Completely Randomized Design (CRD) with eight treatments: C1 (control-without *Trichoderma*), C2 (*T. harzianum* IVEGRI obtained from corn substrate 2 g/polybag), C3 (*T. harzianum* IVEGRI obtained from rice substrate 2 g/polybag), C4 (*T. harzianum* IVEGRI obtained from corn substrate 4 g/polybag, C5 (*T. harzianum* IVEGRI obtained from rice substrate 4 g/polybag), C6 (local *T. harzianum* obtained from corn substrate 2 g/polybag), C7 (local *T. harzianum* obtained from corn substrate 4 g/polybag) and C8 (synthetic fungicide Nebijin). Each treatment contained 10 polybags of plants. These treatments were replicated four times. The results exhibited there were 3 *Trichoderma* species found in Berastagi: *T. harzianum, T. viride* and *T. koningii.* Also, 4 g of local *T. harzianum* (corn substrate) has better performance (0% disease incidence and 0% disease severity) compared to other treatments.

Keywords: antagonist, biological control, disease severity, pathogen

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Introduction

Cabbage (*Brassica oleraceae* var. *capitata* L.) is one of exported commodities which has a good prospect to be widely cultivated in Indonesia as the demand of this leafy crop is increasing. Statistics Indonesia (2017) informed that cabbage production in Indonesia fluctuated in 2012–2016. In 2012, the production was 22.56 tons ha^{-1}, increased to 22.69 tons ha^{-1} in 2013 and 22.75 tons ha^{-1} in 2014. However, the production was decreased to 22.33 tons ha^{-1} in 2015 and 21.94 tons ha^{-1} in 2016, with the decrease percentage reaching up to 5.80%. One obstacle that led to this decline is the presence of clubroot disease caused by the *Plasmodiophora brassicae* Wor. Clubroot is an important soil-borne disease of cruciferous crops throughout the world (Gossen et al., 2013; Päsold et al., 2013). It has been reported to cause high yield loss between 50–100%. Infection 10–20% causing loss 25% (Rennie et al., 2011). In Indonesia, estimation of crop failure in cabbage reached by 88.60% and 5.42%–64.81% to choy sum (Darmiati & Sudarma, 2017).

Infected plants show several symptoms where the main symptom is swollen and distorted roots (Gahatraj et al., 2019). This hypertrophy on roots causes malfunction of the xylem and causes plants to have difficulty absorbing and transporting water and nutrients properly. As a result, plants are stunted, wilt easily, and may have yellowing leaves, leading to premature death (Deora et al., 2013). Soils contaminated by this fungus have always been an obstacle for cabbage plantations due to their high resistance to environmental changes in the soil.

Farmers have been implementing several efforts to control this disease by applying resistant plant varieties, crop rotation, soil solarization, adjusting soil pH, planting trap crops, and the use of synthetic fungicides (Hwang et al., 2012). In Karo Regency, North Sumatra, 90% of cabbage farmers have been applying synthetic fungicides to control this pathogen. However, these chemicals did not significantly control the disease due to the resting spore of *P. brassicae* having a great ability to survive without a host in soil. The intensive use of chemicals exhibited undesirable effects such as water, air, soil pollution, and health problems to humans and animals.

Hasyim et al. (2015) stated that using natural enemies or antagonistic organisms can reduce the negative impacts caused by the continuous use of chemicals. Several antagonistic fungi, such as *T. harzianum*, *Aspergillus niger*, and *Gliocladium*; antagonistic bacteria *Bacillus subtilis, B. polymyxa, B. thuringiensis, B. pantotkenticus, Burkholderia cepacia*, and *Pseudomonas fluorescens* (Soenartiningsih et al., 2014; Sun et al., 2017; Antastia et al., 2019) have been assessed for their biocontrol activities against harmful pathogens. *Trichoderma* spp. cause growth inhibition by microparasitism, antibiosis and competition activities and attack the pathogens by stealing the nutrition from the pathogens (Wahyuno et al., 2009; Ha, 2010). The application of biological agents in controlling plant diseases is more effective due to their specific activity. It means that this agent reveals better results in its area of origin. Therefore, it is essential to evaluate specific *Trichoderma* species in Berastagi, North Sumatra which can control clubroot disease for having higher and safer cabbage production.

The objectives of this research were to identify specific *Trichoderma* species in three districts in Sumatra Utara and to evaluate the potency *T. harzianum* (both local species from Berastagi and species developed by IVEGRI), to control the disease and then compare them with the application of synthetic fungicide.

Materials and Methods

The research was conducted in the laboratory and greenhouse in IP2TP, Berastagi, North Sumatera from April–September 2018. The research used cabbage cv. Grand 11 and *Trichoderma* isolates.

A bamboo was cut at both ends. This bamboo was used for *Trichoderma* isolation. The hole inside bamboo was cleaned with running water. Fermented rice was placed into the hole as a medium, and then the hole was closed tightly using plastic wrap. This treated bamboo was put into soil (7–10 cm in depth) for 7–10 days. After 7-10 days, the bamboo was brought to the laboratory to be examined and observed. The fungus growing inside the bamboo was assessed by characterizing its morphology, such as colony color and shape. Those fungi possessing similarities with *Trichoderma* species were then cultured in PDA media in Petri dishes. When *Trichoderma* species were identified, they were re-cultured in Sabouraud Dextrose Agar (SDA) and Sabouraud Yeast Maltose (SYM).

The screened *Trichoderma* species were

propagated in the laboratory on Sabouraud Dextrose Agar (SDA) with conidial density 10^7 conidia/ml and then were transferred into a glass jar containing 50 ml of Sabouraud Yeast Maltose (SYM) with the *Trichoderma* on the tip of the glass jar (Misrah & Khan, 2015). The jar was then covered with aluminum foil and was centrifuged at 150 rpm for 9 hours and incubated for 3 days. After that, the fungi were propagated in rice and cracked corn media. The rice and corn had to be washed and boiled for 10 minutes. Each 250 g of the media was placed into a plastic bag. The bambooed pipe was installed on the tip of the plastic bag, and the pipe was covered with sterile cotton, and autoclaved for 1 hour at 121^0C. The substrates were then removed and cooled for ±12 hours. Propagated *Trichoderma* were put into 3-day-old SYM, and poured into plastic bags containing corn substrate. These bags were incubated for 14 days and were observed every two days. After 14 days, the culture resulting from each substrate was harvested, ground, and saved in the refrigerator to be used in the research (Mishra & Khan, 2015).

The inoculum of *P. brassicae* was obtained from infected cabbage. The infected roots were blended into suspension. The suspensions with conidial density 10^7 were then applied to the sterilized soil (Mishra & Khan). These experiments were divided into two stages, where at first stage, all isolates of *Trichoderma* species from Berastagi were examined by applying them into infected soil (sterilized soil treated with *P. brassicae* suspensions) to find the best *Trichoderma* species. After the best species found, we continued to the second stage where the best local *Trichoderma* species (*T. harzianum*) is needed to be re-examined and to be compared with *T. harzianum* obtained from IVEGRI and synthetic fungicide of Nebijin by applying it to infected topsoil and manure (4:1) and was uniformly applied into the media twice, in the seedling and the replanting cabbage and then covered it with soil (±1 cm). The experiments were arranged using Completely Randomized Design (CRD) with 8 treatments: C1 (control-without *Trichoderma*), C2 (*T. harzianum* IVEGRI obtained from corn substrate 2 g/polybag), C3 (*T. harzianum* IVEGRI obtained from rice substrate 2 g/polybag), C4 (*T. harzianum* IVEGRI obtained from corn substrate 4 g/polybag), C5 (*T. harzianum* IVEGRI obtained from rice substrate 4 g/polybag), C6 (local *T. harzianum* obtained from corn substrate 2 g/polybag), C7 (local *T. harzianum* obtained from corn substrate 4 g/polybag) and C8 (synthetic fungicide Nebijin 2 gr per experiment), with four replications. Each treatment consists of 10 plant polybags (size 2.5 kg). The total was 320 test plants.

Parameters observed where Disease Incidence was calculated using Townsend and Heuberger Index (Yudiarti, 2007):

$$DSI = \frac{a}{b} x\ 100\%$$

Where: DI = Disease Incidence, a = number of plants infested and b = total number of plants observed.

The severity of clubroot was calculated using the Townsend and Heuberger Index (Yudiarti, 2007) as follows:

$$DSI = \frac{\sum(n\ x\ v)}{Z\ x\ N}\ x\ 100\%$$

Where: DSI = Disease Severity Index, n = number of infected plants at score v, v = disease score, Z = maximum disease score and N =total number of plants observed.

The disease severity was also assessed by visually estimating the degree of gall development on the lateral and main root system, by harvesting some plants examples, using 0-3 disease score given by Kuginuki et al. (1999), where score 0 = no symptoms of galling, score 1 = small galls on <1/3 of roots, score 2 = moderate/ medium galls on 1/3-2/3 of roots and score 3 = severe/ large galls on >2/3 of roots. Analysis of Variance (F Test) was performed, and means were separated using Least Significant Difference (LSD) at probability level 5%.

Results and Discussions

Characteristics of *Trichoderma* spp. The results given in Table 1 indicated different characteristics of *Trichoderma* species observed. The results in Table 1 revealed that there were 7 isolates (D1, D2, Be.1, Be.2, Be.3, M1, and M2) of *Trichoderma* found in three different districts. These isolates were inoculated in PDA media and were propagated for 7 days. These colonies expressed similar colors at 2 and 7 days after application (white and dark green). They were also uniform in round shape (Gupta & Sharma, 2013; Kusmawanto et al., 2022; Agnihotri et al., 2023) and the development of hyphae was slow. Previous research provided findings that *Trichoderma* colony exhibited different colors

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during their development, starting with white color, light green, and dark green on the last day of observation (day 7th) (Syahputra et al., 2017; Nichols et al., 2018; Sanna et al., 2022; Yadav et al., 2022). The microscopic observation was carried out to examine the size and shape of its conidia, conidiophore, phialides, and hyphae, according to the identification book by Watanabe (2018).

Results in Table 2 clearly showed that the three species possessed different conidiophores and phialides, and only two species revealed the similar shape of conidia, *T. harzianum* and *T. koningii*. This finding is in line with the research of Gusnawaty et al. (2014), where species *T. harzianum* has erect and branched conidiophore, short and thick phialides.

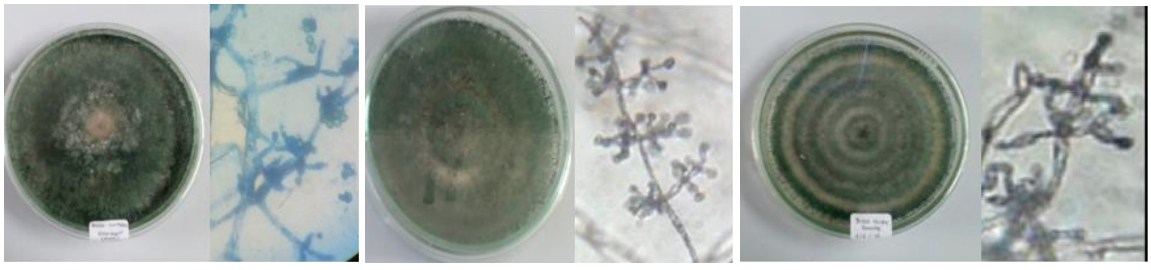
The color is green at 7 days of development (Figure 1a). *T. koningii* expressed erect and branched conidiophore, tapering phiallides, with a thinner conidial wall (Figure 1b). *T. viride* demonstrated branched conidiophore, short and thick vertical phialides with the spore balls form at the tip of it, and the color is green in culture (Figure 1c).

Table 1. Colony development of different *Trichoderma* isolates from three districts

Location (district)	Isolate Codes	Color of the colony after being planted into PDA (days)			Colony shape
		1	4	7	
Dolat Rayat					
	D1	White	Light green	Dark green	Round
	D2	White	Light green	Dark green	Round
Berastagi					
	Be.1	White	Light green	Dark green	Round
	Be.2	White	Whitish light green	Dark green	Round
	Be.3	White	Light green	Dark green	Round
Merek					
	M1	White	Whitish light green	Dark green	Round
	M2	White	Light green	Dark green	Round

Table 2. Microscopic observation of *Trichoderma* isolates from three districts

No	Species	Isolate	Shape		
			Conidiophore	Phialides	Conidia
1.	*T. harzianum*	D1, Be.1	Erect, branched	Short and thick	Ovate
2.	*T. koningii*	D2, M2, Be.3	Erect, branched	Tapering towards apex	Ovate
3.	*T. viride*	M1, Be.2	Branched	Short, thick, vertically arranged	Ovate



a b c

Figure 1. Macroscopic and microscopic appearance of three *Trichoderma* isolates, namely (a) *T. harzianum*, (b) *T. koningii*, (c) *T. viride*

Table 3. The effect of different *Trichoderma* isolates application on the clubroot disease incidence and severity

Treatment	Disease incidence (%)	Disease severity (%)

Jo = Control	100 d	100 d
J1 = *T. harzianum* from Dolat Rayat	30.50 b	10.30 ab
J2 = *T. koningii* from Dolat Rayat	45.55 bc	28.55 bc
J3 = *T. harzianum* from Berastagi	0.00 a	0.00 a
J4 = *T. koningii* from Berastagi	47.00 bc	37.10 bc
J5 = *T. viride* from Berastagi	40.50 bc	25.10 b
J6 = *Trichoderma koningii* from Merek	75.00 c	45.50 c
J7 = *Trichoderma viride* from Merek	57.50 bc	38.25bc
Coefficient of variance	12.42	10.89

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %.

Table 4. The effect of different *Trichoderma* isolates and synthetic fungicide application on the clubroot disease incidence from 1-10 weeks after application

Treatment	Disease Incidence (%)									
	1 WAA	2 WAA	3 WAA	4 WAA	5 WAA	6 WAA	7 WAA	8 WAA	9 WAA	10 WAA
C1 (control, without *Trichoderma*)	0 a	0 a	0 a	10 b	20 b	30 c	40 d	50d	70 f	90 e
C2 (*T. harzianum* IVEGRI obtained from corn substrate 2 g/polybag)	0 a	0 a	0 a	0 a	0 a	10 b	20 c	30 b	40 c	40 c
C3 (*T. harzianum* IVEGRI obtained from rice substrate 2 g/polybag)	0 a	0 a	0 a	0 a	0 a	10 b	20 c	30 b	50 d	60 d
C4 (*T. harzianum* IVEGRI obtained from corn substrate 4 g/polybag)	0 a	0 a	0 a	0 a	0a	0 a	10 b	20 b	20 b	30 ab
C5 (*T. harzianum* IVEGRI obtained from rice substrate 4 g/polybag)	0 a	0 a	0 a	0 a	0a	0 a	10 b	20 b	30 b	40 c
C6 (*T. harzianum* Berastagi obtained from corn substrate 2 g/polybag)	0 a	0 a	0 a	0 a	0 a	0 a	10 b	20 b	30 b	30 b
C7 (*T. harzianum* Berastagi obtained from corn substrate 4 g/polybag)	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 o	0 a	0 a
C8 (synthetic fungicide Nebijin)	0 a	0 a	0 a	0 a	10 b	10 b	20 c	30 c	40 c	40 c
Coefficient of Variance (%)	**0**	**0**	**0**	11.77	15.22	16.06	17.07	19.55	22.02	25.78

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %

Trichoderma spp. have been proven to control soil-borne fungal pathogens. The results of ANOVA indicated that *T. harzianum* significantly reduced clubroot disease compared to other *Trichoderma* species (Table 3).

From the results given in Table 3, it indicated that *T. harzianum* obtained from the Berastagi District exhibited the best result (0% disease incidence and 0% disease severity), followed by species *T. harzianum* obtained from Dolat Raya (30.50% disease incidence and 10.30% disease severity). In comparison, the highest disease incidence and severity occurred in plants treated with *T. koningii* from the Merek District (75.00% and 45.50%). It can be assured that specific *Trichoderma* obtained from its origin place demonstrated better development and ability due to their adaptation to the place, resulting in rapid control of *P. brassicae.*

T. harzianum species also grew faster than the other two species, enabling it to degrade the cell wall of the host-pathogen faster. Chamzurni et al. (2013) reported that *T. harzianum* performed better than *T. koningii* in controlling *Rhizoctonia solani* in chilli plant as 75% of chilli seeds possessed better development. The excellent

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ability of local *T. harzianum* obtained from Berastagi in controlling clubroot disease (Table 3) has allowed this antagonist to be compared with *T. harzianum* developed by IVEGRI in all experiments and also to be compared with the use of synthetic fungicide (Table 4)

The results described that the application of *T. harzianum* both from Berastagi and IVEGRI significantly suppressed the growth of clubroot disease in cabbage plants. The application of the antagonist gave significant results on clubroot disease control. The lowest disease incidence has been found in plants treated with 4 g local *T. harzianum* obtained from corn substrate (C7), resulting in 0% intensity, followed by the application of 4 g *T. harzianum* IVEGRI obtained from corn substrate (C4) with disease intensity of 30%. The highest intensity was exhibited by control (C1, without *Trichoderma*) with 90% damage at 10 weeks after application (WAA), followed by the application of 2 g *T. harzianum* IVEGRI obtained from rice substrate (C3) (60%). These findings indicated that substrates play an important role in antagonist ability to suppress the pathogen development, resulting in low severity (Soenartiningsih et al., 2014).

In addition, the ability of antagonists in suppressing the pathogen growth was influenced by nutrient sufficiency provided from the planting media such as carbon, nitrogen, carbohydrate, and glucose (Yulia et al., 2017), which enables them to compete for the growth space, inhibiting pathogen from colonizing and slowly dying (Amaria et al., 2013). Fast colonization by antagonists has disabled pathogens to grow and widened the colonization.

The application of antagonist *Trichoderma* spp. has influenced the performance and also the appearance of its roots (Figure 2).

a **b**

Figure 2. The comparison between (a) untreated plant and (b) treated ones. The used Trichoderma was *T. harzianum* from Berastagi, Sumatra Utara

Table 5. The effect of different *Trichoderma* isolates and synthetic fungicide application on the clubroot disease severity and root fresh weight

Treatment	Disease Severity (%)	Root fresh weight (g)
C1 (control, without *Trichoderma*)	85.65 e	43.48 e
C2 (*T. harzianum* IVEGRI obtained from corn substrate 2 g/polybag)	37.77 c	29.61 b
C3 (*T. harzianum* IVEGRI obtained from rice substrate 2 g/polybag)	79.11c	37.49 d
C4 (*T. harzianum* IVEGRI obtained from corn substrate 4 g/polybag)	28.52 b	19.70 b
C5 (*T. harzianum* IVEGRI obtained from rice substrate 4 g/polybag)	39.11	32.55 c
C6 (*T. harzianum* Berastagi obtained from corn substrate 2 g/polybag)	29.02 b	20.02 b
C7 (*T. harzianum* Berastagi obtained from corn substrate 4 g/polybag)	0.00 a	7.90 a
C8 (synthetic fungicide Nebijin)	45.19 d	35.2 c
Coefficient of variance	**15.85**	**23.82**

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %

The application of 4 g local *Trichoderma* obtained corn substrate caused no attack to the plants (0%) with the lowest root weight (7.90 g), followed by the application of 4 g *Trichoderma*

obtained from corn substrate (28.52 % and 19.70g). The untreated plants have possessed the highest disease severity (85.65%), which resulted in the highest root weight (63.48g) (Table 5). It indicated that the attack of *P. brassicae* has a positive correlation to root weight. Not only suppressed the growth of *P. brassicae,* these beneficial fungi also have reduced the diseases caused by not only bacterial and fungal pathogens, for instance *Ralstonia solanacearum* in tomato and brinjal (Guo et al, 2021; Qulsum et al., 2023), *Erwinia carotovora* in potato and cabbage (Le et al., 2020; Sulaiman et al., 2020), *Phythopthora* spp. in pepper and chestnut (Timila & Manandhar, 2020; Frascella et al., 2022), *Fusarium* spp. in asparagus, cereal, tomato and chili (Anjum et al., 2020; Hasan et al., 2020; Modrzweska et al., 2022; Brizuela et al., 2023), but also nematodes (Ibrahim et al., 2020; Javeed et al., 2021; Yan et al., 2021; Nafady et al., 2022, Kassam et al., 2023). Dwiastuti (2016) reported that higher disease severity cause d higher swollen roots, where these swollen roots contributed to higher weight of roots. Trichoderma suppress the invasion of pathogen by releasing enzymes β-1,3 glucanase, chitinase, and cellulase to inhibit cell wall permeability of pathogen's, causing pathogen mortality.

Conclusion

There were three species of *Trichoderma* spp. found in three different districts in Berastagi Regency, namely, *T. harzianum, T. viride* and *T.koningii.* The application of 4 g local (Berastagi) *Trichoderma* obtained from corn substrate has significantly suppressed clubroot disease incidence and severity in cabbage plants by 0%.

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