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Formulation of Indigenous *Trichoderma harzianum* Th-B18 on the Growth and Yield of Shallot (*Allium ascalonicum* L.) in Pb-Contaminated Media

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ABSTRACT

This study was conducted to test several indigenous *Trichoderma harzianum* Th-B18 formulations to determine their effects on the growth and yield of shallots on Pb-contaminated land, in an effort to increase shallot productivity. This study used a Completely Randomized Design (CRD) with a single factor with four levels. The treatment of *T. harzianum* Th-B18 formulations were tested on shallots planted in polybags contaminated with lead, namely control (without treatment; indigenous *T. harzianum* formulation in the form of *Breynia androgyna* (sweet leaf) pellets, dose 3 g.polybag^{-1}; indigenous *T. harzianum* Th-B18 formulation in the form of dry corn rice, dose 3 g.polybag^{-1}; commercial *Trichoderma* formulation in the form of solution, 10 mL.polybag^{-1}, all of which were applied 0 days after planting. The treatment of the indigenous *Trichoderma harzianum* Th-B18 fungi formulation had a significantly different effect on the leaf length variable. In the observation of leaf length 14 and 63 days after planting, the formulation of *T. harzianum* Th-B18 sweet leaf pellets 3 g. polybag^{-1} (+ 10 grains)showed the longest leaf length and was significantly different from the treatment without *T. harzianum* Th-B18, formulation of *T. harzianum* Th-B18 solid dry corn 3 g.polybag^{-1} and liquid formulation of *T. harzianum* commercial product 100 mL.polybag^{-1}. The best formulation of indigenous *T. harzianum* Th-B18 fungus on the growth and yield of shallots on Pb-contaminated land was the formulation of *T. harzianum* Th-B18 sweet leaf (*Sauropus androgynous*) pellets 3 g.polybag^{-1}.

INTRODUCTION

Shallots are widely used as a spice for seasoning, both for household cooking, restaurants, and food industry ingredients. In addition, shallots can be used as traditional medicinal ingredients (Dinata et al. 2021).

This decline in shallot production can be caused by several factors, including pest and disease attacks, unpredictable weather changes, and crop failure (Dewa & Pamungkas 2023). Efforts to increase the productivity of shallot plants include the use of stimulators for plant growth.

One of the functional microorganisms that can be used as a stimulator, biological soil fertilizer, and biofungicide is *Trichoderma* sp. *Trichoderma* sp. can be used as a decomposing organism and as a biological agent. In addition, *Trichoderma* sp. impede the growth and spread of fungal toxins that cause disease in plants, such as *Monilifome*, *Sclerotium rolfsii*, and *Sclerotium rilfisi*. When used as a biological fertilizer and biological agent, *Trichoderma* sp. is effective in preventing stem rot, root rot that causes wilting of plants, and white root fungus disease in rubber plants (East Kalimantan Provincial Plantation Service 2017).

The use of microbial inoculants in *Trichoderma*-based products has been successful in relation to plant diseases, plant growth, decomposition processes,

bioremediation, and the production of secondary metabolites in agroecosystems. This surprising finding has great benefits for the agricultural industry in implementing environmentally friendly agricultural practices (Zin & Badaluddin 2020). *Trichoderma* sp., which is used as a biocontrol agent, can help reduce expenditure on pesticides and fertilizer costs, increase plant productivity, and increase income compared to the use of synthetic fertilizers or pesticides. The use of *Trichoderma* sp. is also beneficial for maintaining soil health. Several studies have shown that *Trichoderma* sp. can suppress the growth of plant pathogenic fungi, such as *Pythium arrhenomanes*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Alternaria tenuis,* and *Botrytis cinerea*. Several studies have shown that the use of *Trichoderma* sp. can improve plant health by creating a good environment for plants and producing secondary metabolites in large quantities.

Vinale et al. (2013) showed that the application of *Trichoderma harzianum* N47 increased root length, the number of root nodules, and the number of lateral roots in pea plants. This is in line with the research of Cai et al. (2013), who showed that the application of the *Trichoderma harzianum* SQR-T037 strain helped root growth, increased root length and the number of root tips, and encouraged the growth of tomato seedlings. In addition, *T. harzianum* SQR-T037 releases a secondary metabolite called harzianolide. Harzianolide significantly induced the growth of tomato seedlings planted in both hydroponic and soil systems.

Contamination of the heavy metal Pb has occurred in water areas (Rachmadiarti et al. 2024), land areas in the form of rice fields in mining areas (Xiong et al. 2024), and other agricultural lands. Using pesticides above the recommended dosage can pollute soil and plants. The accumulation of metals in soil reduces crop yields, soil quality and fertility, soil microbial activity, and the entry of toxins into the food chain (Atafar et al. 2010). Other research results show that phosphate fertilizers in the neutral soil category contain 5-156 $mg.L^{-1}$ of the heavy metal lead (Pb). Continuous use of high-dose fertilizers can increase lead levels in the soil, thereby increasing plant absorption of lead. Lead, a heavy metal, is also found in pesticides. Pesticide raw materials extracted from petroleum can contain heavy metals, such as lead (Hartini 2011).

Heavy metal contamination of Pb in agricultural land can be caused by pesticides or pollution discharge from motor vehicles, especially on land near highways. Liquid and solid pesticides contain Pb, including Antracol 70 WP, Dithane M 45 80 WP, Furadan 3G, Goal 240 EC, and Bulldog 25 EC (Hartini 2010). In Herawaty and Nadhira (2009), 68.70% of farmers used two types of pesticides each time they sprayed, only 9.1% used one pesticide, but some others (9.1%) even used three types. In addition, most (44.4%) farmers used doses exceeding the recommendations, and some (12.1%) even used 2 times the recommended dose. Meanwhile, 36.4% of the farmers followed the recommended dose. This treatment was caused by the assumption among them that the recommended dose was not effective in controlling Plant Pest Organisms (OPT).

The use of fertilizers can also contribute to heavy metal pollution. N, P, and K fertilizers are generally used to support plant growth and development. These contain several compounds, such as Cd and Pb. The levels of heavy metals will increase with the continuous use of fertilizers. Moreover, metals such as Cd and Pb do not have physiological activity or function. In addition, the use of phosphate fertilizers increases the levels of Cd and other toxic elements, such as F, Hg, and Pb, in the soil (Raven et al. 1998).

Efforts that can be made to overcome excessive contamination of the soil due to pollution by toxic heavy metals, such as arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), and mercury (Hg), can be overcome by implementing a bioremediation system. Bioremediation is the use of organisms, including plants and microorganisms, to address soil pollution containing heavy metals such as mercury, lead, arsenic, and cadmium (Leyval et al. 2002).

Agrochemicals, such as fertilizers and pesticides, cause heavy metal pollution, which can reduce the quality of natural resources and soil productivity. The range of total heavy metal content of soil Pb in Brebes Regency, namely in Wanasari District, is 17.10-20.68 ppm, and in Larangan District is 21.06-26.31 ppm, while the Pb content in shallot bulbs ranges from 1.54-3.43 ppm and 1.47-2.53 ppm, respectively, for Wanasari and Larangan Regencies (Hajoeningtijas et al. 2018). The threshold set by the Ministry of Health for soil is 12.75 ppm (Soil Research Institute 2002). According to the criteria of the Directorate General of Drug and Food Research, Ministry of Health, the standard threshold value of heavy metal Pb in bulbs is 0.2 ppm.

The results of the study by Hajoeningtijas et al. (2018) showed that in shallot land contaminated with Pb, there were several types of indigenous non-symbiotic fungi found in Wanasari and Larangan Districts, namely *Penicillium oxalicum, Trichoderma cf. harzianum* Th-B18*, Aspergillus nigri, Penicillium dangeardii, Penicillium citrinum, Penicillium* sp.*, Aspergillus terreus,* and *Dichotomomyces cejpii*. According to Malgorzata et al. (2014), *Trichoderma* sp. is one of the microorganisms that is most resistant to chemicals and natural or synthetic toxins and is able to reduce them. The use of indigenous non-symbiotic fungi,

such as *Trichoderma harzianum,* in contaminated soil can be an effective bioremediation agent. Therefore, this study was conducted to test several indigenous *Trichoderma harzianum* formulations to determine their effect on the growth and yield of shallots on land contaminated with Pb, in an effort to increase shallot productivity.

MATERIALS AND METHODS

Experimental Design

This study used a single-factor Completely Randomized Design (CRD) with four levels. The four levels were the control and three different T. *harzianum* Th-B18 formulation treatments. Each treatment was performed eight times. A total of 32 experimental units were used. Each experimental unit was a polybag measuring 20 cm in diameter and 20 cm in height, containing one shallot plant (Table 1).

Rejuvenation and Formulation of *Trichoderma harzianum* Th-B18

Multiplication of *Trichoderma harzianum* Th-B18 fungal isolates (collection of the Basic Agrotechnology Laboratory of FPP UMP) (Hajoeningtijas et al. 2018), which had the best resistance to lead-contaminated media (Hajoeningtijas et al. 2020). The isolates were rejuvenated by growing them on PDA media for 4 days.

Media F1 (indigenous *T. harzianum* Th-B18 formulation in pellet form) was obtained by filtering commercial katuk (*Sauropus androgynous*) powder using a 60 mesh sieve. *T. harzianum* Th-B18 inoculation media was obtained by mixing bran and commercial sweet leaf powder with a composition of 31:10.5. The resulting media mixture was moistened with 15 mL of molasses and 42 mL of sterile distilled water to form the katuk leaf pellets. The formed media mixture was then sterilized in an autoclave for 30 min at 121ºC. The sterile mixed medium was then inoculated with rejuvenated *T. harzianum* Th-B18 as much as one ose needle and stirred evenly with a spatula. The inoculated mixed media were then incubated for one month in a sterile closed room. After one month, the mixed media were checked to determine the growth of *T. harzianum* (Fazil et al. 2018). After the mixed media was relatively dry, the mixed media was then put into a pellet molding machine (Soekarno et al. 2014).

Table 1: *Tharzianum Th-B18* formulation treatments tested on shallot plants planted in polybags contaminated with lead.

Treatment	Description
F_0	Control (no treatment), onion plants grown on lead-contaminated soil
F_1	Formulation of indigenous *T. harzianum* Th-B18 in the form of *Breynia androgyna* (sweet leaf) pellets, dose 3 g.polybag^{-1}, applied at 0 days after planting (dap).
F_2	Formulation of indigenous *T. harzianum* Th-B18 in the form of dry corn rice, dose 3 g.polybag^{-1}, applied at 0 dap.
F_3	Formulation of *T. harzianum* in the form of a solution, 10 mL.polybag^{-1}, applied at 0 dap.

Media F2 (indigenous *T. harzianum* Th-B18 formulation in the form of corn rice) was prepared by washing and cleaning 100 g of corn rice. The clean corn rice was then placed in a plastic bag and sterilized. Sterile corn rice was cooled first and then inoculated with the selected *T. harzianum* Th-B18 isolate as much as one sterile needle. Thereafter, the corn rice medium was incubated for 14 days (Fazil et al. 2018). Media F3 (liquid formulation) is a commercial product of *Tricho plus AP*® (with the active ingredient *Trichoderma harzianum* Th-B18 30×10^6 cfu/gram).

Planting Media

Planting media were obtained from the land around the greenhouse with an ultisol soil type. The soil obtained was mashed and sterilized in an oven at 80 °C for 8 h. Each polybag was filled with 8 kg of soil without basic fertilizer as a planting medium. Polybags that were ready to plant were then applied with $Pb(NO_3)_2$ at a concentration of 0.3 g per polybag, applied 2 days before planting, and mixed evenly. Each polybag was marked according to the treatment (Hajoeningtijas et al. 2022a). The F1 and F2 formula treatments were given as much as 3 g.polybag^{-1} by immersing it in the planting medium to a depth of ± 3 cm. The F3 treatment was administered as much as 10 mL.polybag^{-1} in the root area, while F0 (control) was without treatment. All treatments were applied once at the time of planting or 0 dap.

Planting

The planting material was healthy, intact, and qualified shallot bulbs with a diameter of 1.5-1.8 cm. The dry outer skin layer was removed, and the tip of the bulb was cut to about one-third of the size of the bulb with a sharp, sterile knife to ensure uniform growth (Jumini et al. 2010). The cut marks were allowed to dry before planting in polybags. The bulbs were placed in the middle of the polybag and buried in the soil to a depth of ⅔ the size of the bulb.

Fertilization was performed according to the recommended fertilizer dosage (Anonymous 2021). The recommended fertilization for shallots is urea, KCl, and SP36 fertilizers at doses of 300 kg.ha^{-1}, 300 kg.ha^{-1}, and 200 kg.ha^{-1}, respectively, given in three stages (Table 2). Fertilizer was given by burying it in the soil to a depth of 2 cm.

Maintenance included replanting a maximum of 14 days after planting (dap). After the plants are 60 days old, the

Table 2: Package for administering urea, KCl, and SP36 fertilizers in this experiment.

Stage	dap	Dose [kg.ha^{-1}]	Dose [g.polybag^{-1}]
1	14	SP36 (200)	SP36 (0,58)
2	21	KCl (150) Urea (150)	KCl (0,4) Urea (0,4)
3	42	KCl (150) Urea (150)	KCl (0,4) Urea (0,4)

watering frequency needs to be reduced so that the bulbs can have the desired bright red color (Hasan & Ruswadi 2016). Manual weeding and pest and disease control use biological pesticides. Shallots are harvested at the age of 70 days with signs such as 60% softening of the stem neck, the plant lying down, and yellowing of the leaves.

Observation Variables

Observations were made on the total leaf length and number of leaves per clump at time intervals of 7, 14, 21, 28, 35, 42, 49, 56, and 63 days after planting (Yuliantika et al. 2019). Root length was measured after the plant roots were cleaned using the nailed wooden board grid method with a grid size of 1 cm, and then calculated using the following formula:

Root length (R) = number of intersections x 3.93 (Gliessman 2006)

Wet plant weight (g), dry plant weight (g), number of tubers (fruit), tuber diameter (cm), wet tuber weight (g), and dry tuber weight (g) were measured at harvest time. Dry plant weight and dry onion tuber weight were measured after the shallot bulbs were dried in an oven at 80ºC for 48 h. A planting media test was also carried out to determine the presence of *Trichoderma harzianum* fungi until the end of the planting period. Analysis of Total Pb Content in Planting Media (mg.L^{-1}) using Atomic Absorption Spectrophotometry (AAS).

Data Analysis

The research results are presented in the form of average values ± standard deviations, both in the form of tables and graphs. The statistical analysis used was one-way analysis of variance and Tukey's honest significant difference test at a significance level of 0.05. However, before the analysis of variance was carried out, the basic assumptions of the test were first fulfilled, especially the distribution of data (normality) and homogeneity of variance between groups. The Liliefors and Bartlett tests at a significance level of 0.05 were used to identify the two basic assumption tests. All statistical tests were performed using Costat 6.311 software (https://cohortsoftware.com/).

Bartlett's test showed that the dependent variable of leaf length from 7 hst - 63 dap had the same variance for the four different treatment groups with consecutive statistical values on 7 dap ($\chi 2 = 5.67$; db = 3; p = 0.13), on 14 dap ($\chi 2 = 5.67$; db = 3; p = 0.13), … on 63 dap ($\chi 2 = 2.32$; db = 3; p = 0.51). One-way ANOVA showed that there was no significant effect of T. harzianum formulation on leaf length at 7 dap ($F_{3,28} = 2.776$; p = 0.06) with an effect size of $\omega 2 = 0.14$, which states that the medium effect size or 18% of the variance can be explained.

RESULTS

Table 3 shows that *T. harzianum* Th-B18 treatment significantly affected shallot leaf length in Pb-contaminated soil, especially from 14 days after planting to 63 days after planting. For example, the F1 treatment (katuk leaf pellet formulation) produced longer leaf lengths than the control (F0), with a consistent significant difference up to 63 days after planting. However, other variables such as leaf number, root length, wet and dry plant weight, and tuber yield (number, diameter, wet and dry weight) had no effect at all observation points. Thus, although *T. harzianum* Th-B18 effectively increased plant vegetative growth, its effect on plant yield, such as tuber number and weight, remained insignificant in Pb-contaminated soil conditions.

Based on Table 4, the results of the variance analysis showed that *Trichoderma harzianum* treatment had a significant effect on leaf length at 14, 21, 28, 35, 49, 56, and 63 hst. However, the treatment showed no significant effect on other variables (Tables 5 and 6).

Table 4 presents the results of further tests for variables that showed significant effects. At leaf lengths of 14 and 63 hst, the longest leaves were obtained in the F1 (indigenous *T. harzianum* Th-B18 formulation in pellet form) treatment, which was significantly different from the F0, F2, and F3 treatments. The leaf lengths at 21, 28, and 35 days were the shortest in the F0 treatment. The F3 treatment had the longest leaves at 49 and 56 hst, measuring 26.87 cm and 25.53 cm, respectively, and these values were significantly different from those of the other treatments.

Table 7 presents the results of the analysis of the total Pb content of the planting media 63 days after the shallots were harvested. At the beginning of planting, the Pb content was conditioned to be contaminated by adding 0.3 g per polybag (24.75 mg.L^{-1}) for all treatments. After being used for shallot cultivation, it showed a decrease in all treatments, namely, an average of 31.11 mg.L^{-1} (F0), 38.06 mg.L^{-1} (F1), 26.11 mg.L^{-1} (F2), and 40.11 mg.L^{-1} (F3).

The planting media test was also conducted to determine the presence of microscopic test results for the existence of *Trichoderma harzianum* in planting media 63 days after

Table 3: Anova results of growth respnse and yield of shallots in Pb-contaminated soil media with T. *harzianum* Th-B18 formulation treatment.

SK	db	Response Variable								
		PD 7	PD 14	PD 21	PD 28	PD 35	PD 42	PD 49	PD 56	PD 63
F	3	7,85(0,06)^{tn}	26,29 (0,01)*	69,47 (<0,001)***	104,87 (<0,001)***	97,38 (<0,001)***	51,12(0,07) ^{tn}	61,87 (0,01)*	85,39 (0,02)*	56,49 (0,03)*
KK		14,71	13,53	11,35	11,97	9,23	18,19	14,47	16,53	14,08
		JD 7	JD 14	JD 21	JD 28	JD 35	JD 42	JD 49	JD 56	JD 63
F	3	8,33(0,34)^{tn}	23,28 (0,28)^{tn}	53,11(0,23)^{tn}	83,21(0,15) ^{tn}	83,53(0,31) ^{tn}	117,54 (0,38)^{tn}	179,04 (0,34)^{tn}	252,61 (0,31)^{tn}	353,71 (0,15)^{tn}
KK		21,96	21,88	24,55	25,24	28,17	31,15	33,12	34,66	34,07
		PA	BBT	BKT	JU	DU	BBU	BKU	-	-
F	3	138,39(0,06) ^{tn}	188,98 (0,21)^{tn}	9,06(0,10)^{tn}	12,28(0,11) ^{tn}	36,23(0,12) ^{tn}	61,51(0,28) ^{tn}	7,29(0,07) ^{tn}	-	-
KK		41,34	33,31	46,30	28,96	31,70	47,75	64,59	-	-

Table 4: Effect of T. *harzianum* Th-B18 formulation on the average length of shallot leaves (cm) in Pb-contaminated planting media every week.

Treat-ment	Plant age (dap)								
	7	14	21	28	35	42	49	56	63
F0	10,38±2,01	14,98±1,41b	16,15±1,94b	17,73±1,41b	19,52±2,14b	21,24±3,06	21,94±3,48b	22,93±4,99b	24,54±4,87b
F1	12,51±2,11	19,19±2,84a	22,34±2,11a	25,70±3,53a	27,29±2,16a	27,24±2,02	27,38±2,50a	30,18±3,18a	30,51±2,92a
F2	10,84±1,45	17,47±2,79ab	19,27±3,17a	23,05±3,01a	25,00±2,80a	24,06±7,01	28,02±3,27a	29,27±3,42a	29,28±3,32ab
F3	11,99±0,85	16,10±1,78ab	22,29±1,53a	25,08±2,54a	26,42±1,86a	25,41±4,10	26,87±5,28ab	28,53±6,11ab	29,42±4,57ab
HSD	1,72	2,34	2,32	2,80	2,32	4,56	3,86	4,69	4,10
KK	14,71	13,53	11,35	11,96	9,23	18,19	14,47	16,53	14,08

Table 5: Effect of *Trichoderma harzianum* Th-B18 formulation on the average number of red onion leaves (cm) in Pb-contaminated planting media.

Treatment	Age of plant (days)								
	7	14	21	28	35	42	49	56	63
F0	13,13± 2,42	20,00±3,78	24,63±4,50	26,00±4,14	28,63±5,50	32,88±8,49	36,50±10,72	40,63±13,23	41,25±13,32
F1	12,88±1,81	20,88±1,81	26,50±2,39	30,25±3,45	33,13±4,91	38,75±4,68	44,00±6,89	47,88±9,54	45,88±10,75
F2	11,38±2,92	18,50±4,90	24,38±8,02	26,63±8,40	29,38±9,16	33,13±11,44	37,13±13,83	40,50±15,65	41,38±15,84
F3	11,13±3,27	17,00±5,32	20,38±6,95	22,38±8,77	25,25±11,41	29,50±14,57	32,63±16,30	34,13±16,97	30,25±13,69
HSD	2,72	4,28	6,02	6,80	8,39	10,71	12,74	14,48	13,85
KK	21,95	21,88	24,55	25,24	28,16	31,15	33,12	34,66	34,06

Table 6: The effect of *Trichoderma harzianum* Th-B18 formulation on the average root length (cm), wet plant weight (g), dry plant weight (g), number of tubers (fruit), tuber diameter (cm), wet tuber weight (g), and dry tuber weight (g) of shallots in Pb-contaminated planting media at 70 days after planting.

Treatment	Response Variable						
	PA	BBT	BKT	JU	DU	BBU	BKU
F0	14,33±4,65	27,14±8,10	2,94±0,94	8,50±2,51	10,95±3,11	11,83±4,72	1,50±0,73
F1	14,52±3,33	38,28±6,62	5,45±1,03	9,63±1,85	13,94±3,28	17,48±4,10	3,35±1,16
F2	23,06±7,21	34,14±12,23	4,14±1,94	8,13±2,53	11,72±3,90	12,04±7,04	1,99±1,43
F3	15,60±10,49	30,03±14,38	4,74±3,20	6,63±2,56	15,62±5,73	15,63±9,81	3,37±2,64
Tukey (HSD)	7,15	11,05	2,05	2,43	4,24	6,96	1,69
KK (%)	41,34	33,31	46,30	28,96	31,70	47,75	64,59

Keterangan: Values are presented in mean and standard deviation ($\bar{x}$ ± SD), numbers in the same column/variable mean statistically different in Tukey's test with a significance level of 5%, KK (%): Coefficient of Diversity, PA: Root Length, BBT: Plant Wet Weight, BKT: Plant Dry Weight, JU: Number of Bulbs, DU: Bulb Diameter, BBU: Bulb Wet Weight, BKU: Bulb Dry Weight

Table 7: Results of analysis of total Pb-content of planting media (mg/L).

No.	Code	Test Result
		Lead (Pb) Total
1	F0	31.11
2	F1	38.06
3	F2	26.11
4	F3	40.11

planting (DAP). The fungus was still alive until the end of the planting media. Fig. 1 shows that 63 days after planting, the applied *T. harzianum* survived in the Pb-contaminated planting media, as indicated by its typical morphological characteristics and greenish color.

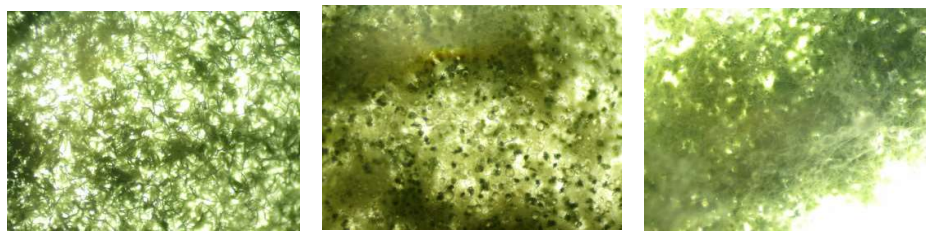
DISCUSSION

The leaf length at almost all observation ages (except observations at 7 and 42 hst) showed that the treatment without the addition of *Trichoderma harzianum* had the shortest leaf length and was significantly different from the formulations of *Trichoderma harzianum* F1, F2, and F3. This demonstrates the role of this fungus in promoting plant growth.

T. harzianum is an antagonistic fungus commonly used as a controller of soil-borne pathogens (biological agents), microorganisms that decompose (biodecomposers) organic matter, and stimulate plant growth (Hardianti et al. 2014, Hermosa et al. 2012, 2013). In addition, the biological agent *T. harzianum* has a better working mechanism than other types of *Trichoderma* sp.. According to Sriwati et al. (2014), T. harzianum can survive and control the growing space well, so it has a high level of competition if it reproduces in the same growing environment as the pathogen. The function of *T. harzianum* in plants continues to increase with increasing knowledge, in the form of a role in increasing plant growth and production, because it can play an active role in stimulating the development of plant cells as plant growth regulators (Fitri et al. 2012), as shown in the results of this study.

T. harzianum produces Indole Acetic Acid (IAA), which can help plant growth and development, and has a positive effect on leaf length in this study. Yadav et al. (2011) showed that the fungus *Aspergillus niger* produced IAA (85 $\mu g.mL^{-1}$) and *T. harzianum* (68 $\mu g.mL^{-1}$), and *Penicillium citrinum* (52 $\mu g.mL^{-1}$) at 3 days of incubation at a temperature of 30°C. IAA plays an important role in supporting plant growth because it can stimulate plant root growth, allowing plants to absorb nutrients and water more optimally. In addition, *T. harzianum* is a biological agent that can produce IAA, which plays a role in supporting vegetative growth but cannot play a direct role in plant production components. The application of *T. harzianum* fungus cannot directly affect the yield variables of potato plants because it has a primary role as a disease controller and degrades organic matter, not as a source of nutrients for plants (Hermawan et al. 2013). This shows that *T. harzianum* plays an important role in stimulating tissue formation in plant roots, thus facilitating the absorption of water and nutrients. However, plant growth and productivity are influenced by the availability of nutrients in the planting medium.

At leaf lengths of 14 and 63 hst, the longest leaves were obtained in the F1 treatment, which was significantly different from those of the F0, F2, and F3 treatments. The F1 treatment is a *T. harzianum* pellet formulation. According to research by Manoharachary et al. (2021), several *Trichoderma* strains provide increased levels of auxin-like compounds. Manoharachary et al. (2021) also explained that growth regulators, such as molecules similar to cytokinins and gibberellins, were observed in plants treated with *Trichoderma*. *Trichoderma* treatment has been shown to have a significant effect on the vegetative and generative components and several components of soybean plant yield (Sutrisno et al. 2022). In addition, the administration of *Trichoderma* sp. has a significant effect on the height and number of leaves in tomato plants (Rizal et al. 2019). These findings align with the results of the study by Pradana et al. (2021), which examined the in vitro growth of Cavendish



(F1) (F2) (F3)

Fig. 1: Microscopic test results for the existence of *Trichoderma harzianum* in planting media 63 days after planting (40x) (F1 = *T. harzianum* formulation in pellet form; F2 = *T. harzianum* formulation in the form of corn rice; F3 = liquid formulation is one of the commercial products of Tricho plus AP).

bananas. In this study, the observed effect of adding *Trichoderma harzianum* to the shallot planting medium on leaf length is likely influenced by Pb stress, which can hinder plant growth, even though the plants were grown under controlled environmental conditions.

This occurs because, according to Gupta et al. (2014), under optimal conditions for plant growth, the administration of *Trichoderma* provides less benefit to plant growth. Gupta et al. (2014) also reported that the administration of *Trichoderma* had a better growth effect on low-vigor sweet corn plants compared to high-vigor sweet corn plants that were treated. The results of a study by Uddin et al. (2015) showed that the addition of *Trichoderma* did not always significantly increase the growth of tomato plants under optimal conditions. Yedidia et al. (2001) tested the effect of *Trichoderma* on the growth of cucumber plants under optimal nutritional conditions and showed that the effect of *Trichoderma* on the growth of cucumber plants was not significant.

Trichoderma is a species-specific microorganism; therefore, each *Trichoderma* species has a different effect on different plant species. For example, Gupta et al. (2014) tested various hybrid strains and found that some gave a positive growth response to T22, some did not respond, and some gave a negative response. *Trichoderma* isolates from the rhizosphere of zoysia grass have been shown to stimulate the growth of wheat and soybean plants. *Trichoderma* is a species-specific fungus; therefore, it is necessary to pay attention to the initial population of *Trichoderma*, so that it is in accordance with the population that is considered capable of influencing plant growth. In addition, *Trichoderma* must be added to plants that are suitable for *Trichoderma* growth in the rhizosphere. In this study, the *Trichoderma harzianum* used was a pure culture isolated from Pb-contaminated shallot fields from several sub-districts in Brebes Regency, and tested on shallot plants treated with Pb stress, so it was assumed to be a species that was suitable based on location specifics (indigenous).

The environmental conditions of *T. harzianum* growth greatly determine its effectiveness and efficiency in promoting the growth of shallots. The availability of food sources for *T. harzianum* in plant media greatly determines its effectiveness in increasing plant growth. The application of *T. harzianum* is carried out on a planting medium with a mixture of soil and rice husk charcoal, so that the food source comes from rice husk charcoal, which takes a long time to be broken down into organic molecules that are easily absorbed. According to Baihaqi et al. (2013), food sources, rainfall, and insufficient air humidity affect the growing space for the growth of *Trichoderma* sp. and also affect the efficiency of *Trichoderma* sp. applications, which have an impact on planting. Shoresh & Harman (2008) stated that secondary metabolites that function to increase plant root growth can be produced by *T. harzianum*, thereby increasing the plant's ability to absorb nutrients from the soil.

Plant dry weight reflects the accumulation or net absorption of photosynthetic assimilation obtained during plant growth and development. The increase in fresh weight and plant growth can significantly affect the dry weight of the plant. In this study, the results showed no significant effect for either variable. This is suspected in the treatment without *T. harzianum*, where the nutrient needs were met by the mixture of soil and rice husk charcoal used. Rice husk charcoal in the planting medium is thought to overcome Pb stress conditions through the binding mechanism of the organic material, so that the Pb content in the medium does not affect the growth of shallots at F0.

The treatment did not show a significant effect on other variables (Tables 5 and 6), including tuber diameter, tuber wet weight, and tuber dry weight. It is suspected that T. harzianum treatment only affects the vegetative phase of the plant, including auxin production. In the next phase, in the form of photosynthate accumulation in the tuber, the effect of Pb began to be observed. According to Gothberg (2008), high Pb content in plant tissue causes a decrease in leaf chlorophyll levels, thereby disrupting the photosynthesis process, which then results in reduced production from a plant. Based on Table 7, the Pb content in the growing medium is suspected to be due to the addition of Pb(NO3)2, as well as Pb already present in the medium, originating from residues of organic chemical fertilizers and pesticides after being used for shallot cultivation.

Treatment F2 (indigenous *T. harzianum* Th-B18 formulation in the form of corn rice) resulted in the lowest total Pb content in the medium. This is in line with the research of Natalia et al. (2014), who found that the spore density of the corn flour mixture had the highest spore density compared to the treatment of adding molasses and white glutinous rice flour. So that corn flour is quite effective to be used as an organic material mixed with *Trichoderma* spp. The success of a microorganism as a bioremediation agent is influenced not only by environmental factors and the number of spores but also by its germination power (spore viability) and virulence.

In addition, the carrier media must increase effectiveness and shelf life, be compatible with the environment, not cause phytotoxicity in plants, and be cheap and easy to obtain (Jeyarajan & Nekkeeran 2000). Talc, compost, and molasses carrier materials can increase the growth capacity of *Trichoderma* spp. (Amaria et al. 2016). It is suspected

Table 8: Results of analysis of heavy metal content Pb in roots, leaves and bulbs of shallots with humic-fulvic acid dosage treatment and non-symbiotic fungi in planting media contaminated with heavy metal Pb.

No.	Code	Pb Content [ppm]			
		Roots	Leaves	Bulbs	Decrease accumulation Pb Bulbs [%]
1	K0F4	7,815	8,021	1,850	50,282
2	K1F4	92,922	5,239	2,747	26,176
3	K2F4	5,060	6,159	9,509	-155,550
4	K3F4	28,098	5,100	5,801	-55,899

Note: threshold value 0.2 ppm (Directorate General of POM, Ministry of Health)

K0 – K4 = humic-fulvic acid dose; F4 = *T. harzianum* Th-B18

(Hajoeningtijas et al. 2022a, 2022b)

that the high carbohydrate content in corn flour and talc, among others, supports the effectiveness of *Trichoderma* sp. as a bioremediation agent. The F0 treatment (without *T. harzianum*) decreased at the end of shallot cultivation, allegedly due to the ability of rice husk charcoal in the medium to bind the heavy metal Pb. In the study by Hajoeningtijas et al. (2022b), the highest Pb accumulation was found in the roots, followed by bulbs and shallot leaves, with approximately the same average.

In previous studies, the comparison of Pb content between roots and leaves in each treatment from the highest to the lowest Pb content was root organs> leaves. The high Pb content in the roots is because Pb can replace other ions from the root exchange site and is strongly bound to the root space. To regulate the toxic Pb material entering their bodies, plants regulate it by relocalizing the Pb metal in the roots, so that it is found that the Pb content in the roots is often higher than in other plant organs (Table 8).

The absorption rate and bioaccumulation coefficient depend not only on the level of heavy metal soil contamination but also on the plant-microbe interactions in the soil. These results indicate that *Trichoderma harzianum* can reduce the adverse effects of Pb by accumulating heavy metals and making them unavailable to roots (Zhang et al. 2018). Symbiosis with fungi under abiotic stress, such as heavy metal exposure, is known to be an effective habit of plants (Millar & Bennett 2016). Other studies have shown that heavy metal absorption and toxicity are effectively attenuated in Arabidopsis thaliana by the inactivation of heavy metals by *Trichoderma* asperellum (Kuffner et al. 2008).

While in the treatment F1 (sweet leaf pellet formulation of indigenous of *T. harzianum* Th-B18 3 g (+ 10 grains). polybag^{-1}, F2 (solid dry corn formulation of indigenous of *T. harzianum* Th-B18 3 g.polybag^{-1}), F3 (liquid formulation of commercial products of *T. harzianum* 100 mL.polybag^{-1}), the presence of *T. harzianum* itself has several mechanisms to overcome the high content of Pb contamination in the planting medium. The fungus develops various defense mechanisms to minimize heavy metal toxicity, including the release of extracellular molecules (organic acids) produced by fungi and the release of heavy metals by intracellular molecules (phytochelin and metallothionein) (Baldrian 2003).

In another study, it was revealed that the tolerance of *Trichoderma* asperellum to Pb^{2+} was the result of various reactions. Pb^{2+} can increase the synthesis of polysaccharides, proteins, thiol compounds and oxalic acid. In the early stages of Pb^{2+} stress, *Trichoderma* asperellum can quickly initiate an extracellular emergency mechanism, synthesize oxalic acid in mycelia, and excrete it extracellularly to eliminate free Pb^{2+} and reduce its toxicity of Pb^{2+} to cells. By transporting Pb^{2+} into cells, it can increase the synthesis of polysaccharides, proteins, and thiol compounds to absorb and convert Pb^{2+} and reduce damage to cells (Sun et al. 2020).

The mechanism by which *Trichoderma* helps plants tolerate heavy metal stress is by increasing root biomass production, protecting hyperaccumulation of toxins in plant tissues to avoid oxidative damage to plants, and increasing the availability and efficiency of nutrients. This fungus can also decompose toxic contaminants. *Trichoderma* is effective because it has an extracellular enzyme system that catalyzes reactions that degrade aromatic toxic compounds (Hasan & Ruswadi 2016). The use of indigenous non-symbiotic fungi, such as *Trichoderma harzianum* Th-B18, can be an effective bioremediation agent in contaminated soil.

CONCLUSIONS

The treatment of the indigenous *Trichoderma harzianum* fungi formulation had a significantly different effect on the leaf length variable. In the observation of leaf length 14 and 63 days after planting, the formulation of *T. harzianum* Th-B18 sweet leaf pellets 3 g.(+ 10 grains) polybag^{-1} showed the longest leaf length and was significantly different from the treatment without *T. harzianum*, formulation of *T. harzianum* Th-B18 solid dry corn 3 g.polybag^{-1}, and liquid formulation of *T. harzianum* commercial product 100 mL.polybag^{-1}. The best formulation of indigenous *Trichoderma harzianum* fungi on the growth and yield of shallots on Pb-contaminated land was the formulation of *T. harzianum* Th-B18 sweet leaf pellets 3 g (+ 10 grains).polybag^{-1}.

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