Prepublished copy

Nature Environment & Pollution Technology

This is a peer-reviewed prepublished version of the paper

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

Oetami Dwi Hajoeningtijas[1],† , Teguh Pribadi[1], Hamami Alfasani Dewanto[1], Noor Faizul Hadry Nordin[2] and Raidar Sagita[1]

[1] Agrotechnology Department, Agriculture and Fishery Faculty, Universitas Muhammadiyah Purwokerto, Jl. K. H. Ahmad Dahlan PO BOX 202, Banyumas, Central Jawa, Indonesia 53182
[2] The Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia
†Corresponding author: oetamidwihajoeningtyas@ump.ac.id

Key Words	Indigenous, Pb, Shallot, Trichoderma harzianum Th-B18
DOI	https://doi.org/10.46488/NEPT.2025.v24i04.D1763 (DOI will be active only after the final publication of the paper)
Citation for the Paper	Hajoeningtijas, O. D., Pribadi, T., Dewanto, H. A., Nordin, N. F. H. and Sagita, R., 2025. Formulation of Indigenous Trichoderma Harzianum Th-B18 on the Growth and Yield of Shallot (Allium Ascalonicum L.) in Pb Contaminated Media. Nature Environment and Pollution Technology, 24(4), p. D1763. https://doi.org/10.46488/NEPT.2025.v24i04.D1763

ABSTRACT

The 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 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 indigenous *Trichoderma harzianum* Th-B18 fungi formulation gave 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* 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 is 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 medicine ingredients (Dinata 2021).

This decline in shallot production can be caused by several factors, including pest and disease attacks, unpredictable weather changes, and crop failure factors (Dewa, 2023). Efforts that can be made to increase the productivity of shallot plants are to utilize 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 biological agent. In addition, *Trichoderma* sp. impede the growth and spread of fungal toxins that cause disease in plants produced by *Monilifome, Sclerotium rolfsii* and *Sclerotium rilfisi*. When used as a biological fertilizer and biological agent, *Trichoderma* sp. really 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 inoculant in *Trichoderma*-based products shows its success in relation to plant diseases, plant growth, decomposition processes, and bioremediation, as well as the production of secondary metabolites in agroecosystems. This surprising finding brings great benefits to the agricultural industry to implement environmentally friendly agricultural practices (Zin and Badaluddin, 2020). *Trichoderma* sp. which is used as a biocontrol agent or biological control can help reduce expenditure on pesticide and fertilizer costs, increase plant productivity and rising income compared to the use of synthetic fertilizers or pesticides. The use of *Trichoderma* sp. is also very good 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. In line with the research of Cai et al., (2013) which 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, the *T. harzianum* SQR-T037 strain releases a secondary metabolite called harzianolide. Harzianolide

significantly induces the growth of tomato seedlings both planted with hydroponic and soil systems.

Contamination of the heavy metal Pb 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 land. Using pesticides above the recommended dosage can pollute the soil and plants. Accumulation of metals in the 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 of the heavy metal lead (Pb). Continuous use of high-dose fertilizers can increase lead levels in the soil, thereby increasing the absorption of lead by plants. Heavy metal lead is also found in pesticide ingredients. Pesticide raw materials extracted from petroleum can contain lead heavy metals (Hartini, 2011).

Heavy metal contamination of Pb in agricultural land can be caused by pesticides used or from pollution discharge from motor vehicles, especially in land near highways. Liquid and solid pesticides do contain Pb, including Antracol 70 WP, Dhitane M 45 80 WP, Furadan 3G, Goal 240 EC, Bulldog 25 EC (Hartini, 2010). In Herawati's study (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 of pesticides. In addition, most (44.4%) of farmers used doses exceeding the recommendations, some of them (12.1%) even used 2 times the recommended dose. Meanwhile, 36.4% of 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 be a factor in heavy metal pollution. N, P, and K fertilizers are generally used to support plant growth. In them, there are several compounds with contents such as Cd and Pb. The levels of heavy metals will of course increase with the continuous use of fertilizers. Moreover, metals such as Cd and Pb do not have physiological activity/function. In addition, the use of phosphate fertilizers also increases the levels of Cd and other elements such as F, Hg, and Pb which are toxic to 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 include arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), mercury (Hg); can be overcome by implementing a bioremediation system. Bioremediation is the use of organisms, including plants and microorganisms, as a solution to the problem of 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., 2017). The threshold set by the Ministry of Health is 12.75 ppm for soil (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 in bioremediation. 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.

RESEARCH METHOD

Experimental design

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

Table 1. *T. harzianum* 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 day 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 solution, 10 ml polybag^{-1}, applied at 0 dap

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 have the best resistance to lead-contaminated media (Hajoeningtijas et al., 2019). Rejuvenation of the isolates was carried out by growing the purified isolates on PDA media and incubating them for 4 days.

Media F1 (indigenous *T. harzianum* Th-B18 formulation in pellet form) was obtained by filtering commercial katuk (*Sauropus androgynous*) powder with 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 katuk leaf pellets. The formed media mixture was then sterilized in an autoclave for 30 minutes at a temperature of 121ºC. The sterile mixed media 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 was then incubated for one month in a sterile closed room. After one month, the mixed media was 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).

Media F2 (indigenous *T. harzianum* Th-B18 formulation in the form of corn rice) began with washing and cleaning 100 g of corn rice. The clean corn rice was then put into plastic and sterilized. The sterile corn rice was cooled first and then inoculated with the selected *T. harzianum* Th-B18 isolate as much as one ose needle. After that, the corn rice media was incubated for 14 days (Fazil et al., 2018). While media F3 (liquid formulation) is one of the commercial products of *Tricho plus AP*® (with the active ingredient *Trichoderma harzianum* Th-B18 30 x 10^6 cfu/gram).

Planting media

Planting media was obtained from land around the greenhouse with ultisol soil type. The soil obtained was mashed and sterilized with an oven at 80ºC for 8 hours. 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 $Pb(NO_3)_2$ as much as 0.3 g per polybag applied 2 days before planting and mixed evenly. Each polybag was marked according to the treatment given (Hajoeningtijas *et al.*, 2022). The F1 and F2 formula treatments were given as much as 3 grams

of polybag^{-1} by immersing it in the planting medium to a depth of ± 3 cm. The F3 treatment was given as much as 10 ml of polybag^{-1} in the root area while F0 (control) was without treatment. The application of all treatments was carried out 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 about a third of the size of the bulb with a sharp, sterile knife so that growth is uniform (Jumini et al., 2010). The cut marks are allowed to dry before being planted 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 carried out according to the recommended fertilizer dosage (Balitbangtan, 2021). The recommended shallot fertilization is urea, KCl, and SP36 fertilizers with doses of 300 kg ha^{-1}, 300 kg ha^{-1}, and 200 kg ha^{-1} respectively given in three stages (Table 2). Fertilizer is given by burying the fertilizer in the soil to a depth of 2 cm.

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)	KCl (0,4)
		Urea (150)	Urea (0,4)
3	42	KCl (150)	KCl (0,4)
		Urea (150)	Urea (0,4)

Maintenance includes replanting a maximum of 14 days after planting (dap). Watering after the plants are 60 days old, the frequency needs to be reduced so that the bulbs can have the desired bright red color (Hasan and Ruswadi, 2016). Manual weeding, while pest and disease control uses biological pesticides. Shallots are harvested at the age of 70 days with signs such as the stem neck has softened by 60%, the plant is lying down, and the leaves are also yellowing.

Observation variables

Observations were made on the total leaf length and number of leaves per clump with 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 first, using the nailed wooden board grid method with a grid size of 1 cm, then calculated using the 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), dry tuber weight (g) were carried out at harvest time. Dry plant weight and

dry onion tuber weight were observed after the shallot bulbs were dried using an oven at a temperature of 80º C for 48 hours. A planting media test was also carried out to determine the presence of *Trichoderma harzianum* fungi still alive until the end in the planting media. Analysis of Total Pb Content in Planting Media (mg/l) using the Atomic Absorption Spectrophotometry (AAS).

Data analysis

The research results obtained 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 is carried out, the basic assumptions of the test are first fulfilled, especially the distribution of data (normality) and homogeneity of variance between groups. The Liliefors test and the Bartlett test at a significance level of 0.05 are used to identify the two basic assumption tests. All statistical tests applied here are assisted by Costat 6.311 software (https://cohortsoftware.com/).

Bartlett's test showed that the dependent variable of leaf length from 7 hst - 63 hst had the same variance for the four different treatment groups with consecutive statistical values PD7 HST ($\chi2 = 5.67$; db = 3; p = 0.13), PD14 HST ($\chi2 = 5.67$; db = 3; p = 0.13), … PD63 HST ($\chi2 = 2.32$; db = 3; p = 0.51). One-way ANOVA showed that there was no significant effect of T. harzianum formulation on leaf length at 7 hst ($F3,28 = 2.776$; p = 0.06) with an effect size of $\omega2 = 0.14$ which states that the medium effect size or 18% of the variance can be explained.

RESULTS AND DISCUSSION

Result

Table 3. ANOVA Results of Growth Response and Yield of Shallots in Pb-Contaminated Soil Media with T. *harzianum* 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	-	-

Perlakuan	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 4. The effect of *Trichoderma harzianum* formulation on the average length of red onion leaves (cm) in Pb-contaminated planting media every week.

Table 5. The effect of Trichoderma harzianum formulation on the average number of red onion leaves (cm) in Pb-contaminated planting media.

Perlakuan	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 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), 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

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

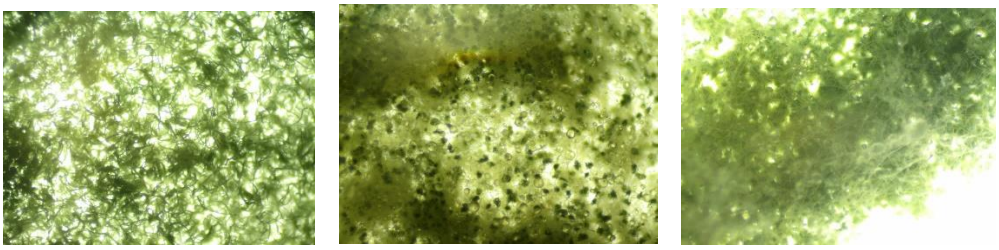
Table 4 also presents the results of further tests for variables that showed a significant effect. At leaf lengths of 14 hst and 63 hst, the longest leaves were obtained in the F1 (indigenous *T. harzianum* Th-B18 formulation in pellet form) treatment, which was significantly different from the F0, F2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

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

Table 7. presents the results of the analysis of the total Pb content of the planting media at 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) for all treatments. After being used for shallot cultivation, it showed a decrease in all treatments, namely an average of 31.11 mg/l (F0), 38.06 mg/l (F1), 26.11 mg/l (F2), and 40.11 mg/l (F3).



(F1) (F2) (F3)

Figure 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)

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 planting (dap) fungus that was still alive until the end of the planting media. Based on Figure 1, it shows that 63 days after planting, the applied *T. harzianum* was able to survive in the Pb-contaminated planting media, which is indicated by typical morphological characteristics, greenish in color.

Discussion

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

T. harzianum is one type of antagonistic fungus that is commonly applied as a controller of soil-borne pathogens (biological agents), microorganisms that decompose (biodecomposer) organic matter and stimulate plant growth (Hardianti et al., 2014; Hermosa et al., 2012; Hermosa et al., 2013). In addition, the biological agent type *T. harzianum* has a better working mechanism compared to other types of *Trichoderma* sp.. According to Sriwati et al., (2014) T. harzianum is able to survive and control the growing space well so that 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 along 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 also shown in the results of this study.

T. harzianum produces Indole Acetic Acid (IAA) which can help plant growth and development so that it has a positive effect on leaf length in this study. Research by Yadav et al. (2011) showed that the fungus *Aspergillus niger* produced IAA of (85 ug mL) and *T. harzianum* (68 ug mL-1-1) and *Penicillium citrinum* (52 ug mL) at 3 days of incubation at a temperature of 30 °C. IAA has an important role in supporting plant growth because it can stimulate plant root growth so that plants can 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 degrading organic matter, not as a source of nutrients for plants Hermawan et al., (2013). This shows that *T. harzianum* has an important role in stimulating tissue formation in plant roots, thus facilitating the absorption of water and nutrients, but the increase in plant growth and productivity is influenced by the availability of nutrients in the planting medium.

At leaf lengths of 14 hst and 63 hst, the longest leaves were obtained in the F1 treatment, which was significantly different from 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 very significant effect on the vegetative, generative components and several components of soybean plant yields Sutrisno et al., (2022). In addition, the administration of *Trichoderma* sp has a very significant effect on the growth of height and number of leaves in

tomato plants Rizal et al., (2019). This is in accordance with the results of the study by Pradana et al. (2021) on the growth of Cavendish bananas from in vitro results, in this study the real effect of the addition of *Trichoderma harzianum* to the shallot planting medium on the leaf length variable is thought to be due to Pb stress which can disrupt plant growth and although during the study the plants were cultivated in controlled environmental conditions.

This happens because according to Gupta et al., (2014) in optimal conditions for plant growth, the administration of Trichoderma provides less benefit for 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 the study by Uddin et al., (2015) showed that the addition of Trichoderma did not always significantly increase the growth of tomato plants in optimal conditions. Research by Yedidia et al., (2001) tested the effect of Trichoderma on the growth of cucumber plants under optimal nutritional conditions showing that the effect of Trichoderma on the growth of cucumber plants was not significant.

Trichoderma is one of the species-specific microorganisms so each Trichoderma species will have a different effect on different plant species, for example in the research of Gupta et al., (2014) various hybrid strains were tested and it was 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 was shown to stimulate the growth of wheat and soybean plants. Trichoderma is a species-specific fungus so 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, the plants to which Trichoderma will be added must be plants that are indeed suitable, so that Trichoderma can grow in the rhizosphere of the plant. 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 the effectiveness and efficiency of T. harzianum in helping the growth of shallots. The availability of food sources for *T. harzianum* in the plant media greatly determines the effectiveness of *T. harzianum* 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 will also affect the efficiency of *Trichoderma* sp. applications which have an impact on planting. Shoresh and Harman (2008) stated that secondary metabolite materials 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 is a reflection of the accumulation or net absorption of photosynthetic assimilation obtained during plant growth and development. The increase in fresh weight and plant growth can have a significant effect on the dry weight of the plant. In this study, for both variables, the results showed no significant effect. This is suspected in the treatment without *T. harzianum*, the nutrient needs have been met by the mixture of soil and rice husk charcoal used. Rice husk charcoal in the planting medium is thought to be able to overcome Pb stress conditions through the binding mechanism by the organic material, so that the Pb content in the medium does not affect the growth of shallots at F0.

Meanwhile, in other variables (Tables 5, 6), the treatment did not show a significant effect, including on tuber diameter, tuber wet weight, and tuber dry weight. It is suspected that the T. harzianum treatment only has an effect on the vegetative phase of the plant, including by producing auxin. In the next phase, in the form of photosynthate accumulation in the tuber, the effect of Pb has begun to be seen. According to Gothberg (2008), high Pb content in plant tissue causes a decrease in leaf chlorophyll levels so that the photosynthesis process is disrupted, which then results in reduced production from a plant.

Table 7. presents the results of the analysis of the total Pb content of the planting media at the age of 63 days after the shallots were harvested. At the beginning of planting, the Pb content to be contaminated was carried out by adding 0.3 g per polybag (24.75 mg/l) for all treatments. The Pb content in the planting media is suspected, apart from the addition of $Pb(NO_3)_2$, there is also Pb that is already contained in the media used which comes from residues of organic chemical fertilizers and pesticides. After being used for shallot cultivation. The planting media showed changes in all treatments, namely an average of 31.11 mg/l (F0), 38.06 mg/l (F1), 26.11 mg/l (F2) and 40.11 mg/l (F3).

Treatment F2 (indigenous T. harzianum Th-B18 formulation in the form of corn rice) gave the lowest total Pb content of the media. This is in line with the research of Natalia et al., (2014), the calculation of 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. It is suspected that 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 requirements for the carrier media are that it can increase effectiveness and shelf life, is compatible with the environment, does not cause phytotoxicity in plants, and the carrier material is cheap and easy to obtain (Jeyarajan & Nekkeeran, 2000). Talc, compost, and molasses carrier materials can increase the growth capacity of Trichoderma sp. (Amaria et al., 2016). It is suspected 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 media to bind the heavy metal Pb. In the study of Hajoeningtijas et al. (2022) the highest Pb accumulation was found in the roots, followed by bulbs and shallot leaves with approximately the same average.

Table 8. Results of Analysis of Heavy Metal Content Pb in Roots, Leaves and Bulbs Red Onion 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., 2022)

In previous studies, the comparison of Pb content between roots and leaves in each treatment from the highest to the lowest Pb content is root organs> leaves. The high Pb content in the roots is because the Pb metal is able to replace other ions from the root exchange side 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 how plant-microbe interactions work in the soil. These results indicate that Trichoderma harzianum can reduce the adverse effects of Pb, including by accumulating heavy metals and making them unavailable to roots (Zhang et al.,

2018). Symbiosis with fungi under abiotic stress such as heavy metals is known to be an effective habit of plants (Millar and Bennett, 2016). Other studies have shown that through the influence of heavy metal inactivation by Trichoderma asperellum inoculation, heavy metal absorption and toxicity are effectively attenuated in Arabidopsis thaliana (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), F2 (solid dry corn formulation of indigenous of *T. harzianum* Th-B18 3 g / polybag), F3 (liquid formulation of commercial products of *T. harzianum* 100 ml / polybag), the presence of *T. harzianum* itself has several mechanisms to overcome the high content of Pb contamination in the planting medium. The fungus develop various defense mechanisms to minimize heavy metal toxicity, including release by extracellular molecules (organic acids) produced by fungi and 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 Pb2+ is the result of various reactions. Pb2+ can increase the synthesis of polysaccharides, proteins, thiol compounds, and oxalic acid. In the early stages of Pb2+ stress, Trichoderma asperellum can quickly initiate an extracellular emergency mechanism, synthesize oxalic acid in mycelia and excrete it extracellularly to eliminate free Pb2+ and reduce the toxicity of Pb2+ to cells. By transporting Pb2+ into cells, it can increase the synthesis of polysaccharides, proteins, thiol compounds to absorb and convert Pb2+ and reduce damage to cells (Sun et al., 2020).

The mechanism applied by Trichoderma in helping plant tolerance to heavy metal stress is through 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 also has the ability to decompose toxic contaminants. Trichoderma is effective because it has an extracellular enzyme system that catalyzes reactions that can degrade aromatic toxic compounds (Hasan and Ruswadi, 2016). The use of indigenous non symbiotic fungi such as *Trichoderma harzianum* Th-B18 in contaminated soil can be an effective bioremediation agent in bioremediation.

CONCLUTION

The treatment of indigenous *Trichoderma harzianum* fungi formulation gave 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 is the formulation of *T. harzianum* Th-B18 sweet leaf pellets 3 g (+ 10 grains) polybag^{-1}.

ACKNOWLEDGMENTS

We would like to thank Universitas Muhammadiyah Purwokerto, as part of this work funded.

REFERENCES

Amaria, W., F. Soesanthy, Y. Ferry, 2016, The Effectiveness Of Biofungicide Trichoderma Sp.With Three Kinds Of Carrier On White Root Disease Rigidoporus Microporus, J. Tidp 3(1), 37–44 Maret, 2016

Atafar, Z., Mesdaghinia, A., Nouri, J., Homaee, M., Yunesian, M., Ahmadimoghaddam, M., & Mahvi, A. H. 2010. Effect of Fertilizer Application on Soil Heavy Metal Concentration. *Environmental Monitoring and Assessment*, *160*(1–4), 83–89. https://doi.org/10.1007/s10661-008-0659-x

Baihaqi, A., M. Nawawi, dan A.L. Abadi. 2013. Teknik Aplikasi *Trichoderma* sp. terhadap Pertumbuhan dan Hasil Tanaman Kentang (*Solanum tuberosum* L.). *J. Produksi Tanaman* 1:30-39.

Baldrian, P. 2003. Interactions of heavy metals with white-rot fungi. *Enzyme and Microbial Technology*, 32(1), 78-91.

Cai, F., Yu, G., Wang, P., Wei, Z., Fu, L., Shen, Q., & Chen, W. 2013. Harzianolide, A Novel Plant Growth Regulator and Systemic Resistance Elicitor from *Trichoderma harzianum*. *Plant Physiology and Biochemistry*, *73*, 106-113.

Dewa, N. N., & Pamungkas, D. P. 2023. Analisis Segmentasi Citra Daun Bawang Merah Menggunakan Metode Deteksi Tepi Prewitt. *JISCOM*. 1(2): 31-37

Dinata, G. F., Aini, L. Q., & Abadi, A. L. 2021. Pengaruh Pemberian Plant Growth-Promoting Bacteria Indigenous terhadap Pertumbuhan Tanaman Bawang Merah (*Allium ascalonicum*). In *Agropross: National Conference Proceedings of Agriculture* (pp. 283-288).

East Kalimantan Provincial Plantation Service. 2017. Manfaat *Trichoderma* sp. dan Cara Pembiakannya. *Artikel Perkebunan*. https://disbun.kaltimprov.go.id/artikel/manfaat-trichoderma-sp-cara-pembiakkannya. Diakses pada 14 Oktober 2023.

Fazil, M., Sriwati, R., & Chamzurni, T. 2018. Aplikasi Beberapa Bentuk Formulasi *Trichoderma* spp dalam Mengendalikan Penyakit Layu Fusarium pada Tanaman Tomat. *Jurnal Ilmiah Mahasiswa Pertanian Unsyiah*, *3*(2), 217–229. https://doi.org/https://doi.org/10.17969/jimfp.v3i2.7478

Fitria, E., E. Kesumawaty, B. Basyah, Asis, 2012, Peran *Trichoderma harzianum* sebagai Penghasil Zat Pengatur Tumbuh terhadap Pertumbuhan dan Produktivitas Varietas Cabai (*Capsicum annuum* L.), *J. Agron. Indonesia*, April 2021, 49(1):45-52, DOI: https://dx.doi.org/10.24831/jai.v49i1.34341, SSN 2085-2916 e-ISSN 2337-3652, Tersedia daring http://jai.ipb.ac.id.

Gliessman, S. R. 2006. *Field and Laboratory Investigations in Agroecology (Taylor & Francis Group (ed.)).* CRC Press.

Gothberg, A. 2008. Metal Fate and Sensitivity in The Aquatic Tropical Vegetable Ipomea Aquatica. Departement of Applied Enviromental Science. Stockholm University. 39 p.

Gupta, V. K., Schmoll, M., Herrera-Estrella, A., Upadhyay, R. S., Druzhinina, I., & Tuohy, M. G. (Eds.). 2014. *Biotechnology and Biology of Trichoderma*. ELSEVIER.

Hajoeningtijas, O.D., I. Mansur, N. Ekowati, Tamad, I. M. Wibowo. 2018. Uji Isolat Fungi Non – Simbiosis Indigenus Lahan Bawang Merah Tercemar Logam Berat pada Variasi Konsentrasi Pb, *Laporan Hasil Penelitian* (belum dipublikasikan).

Hajoeningtijas, O.D., I. Mansur, N. Ekowati and Tamad, 2020, Uji Spesies Fungi Non-Simbiosis Indigenous Dan Asam Humat-Fulvat Pada Bawang Merah Dengan Media Tanam Tercemar Pb, *Laporan Hasil Penelitian* (belum dipublikasikan).

Hajoeningtijas, O.D., A. M. Purnawanto, M.R. Inayah, 2022, Respon Pertumbuhan dan Hasil Bawang Merah (*Allium ascalonicum* L.) pada Media Tanam Tercemar Pb dengan Perlakuan *Dichotomomyces cejpii* dan Asam Humat-Fulvat, *Laporan Hasil Penelitian* (belum dipublikasikan).

Hajoeningtijas, O.D., I. Mansur, N. Ekowati and Tamad, 2022, The Indigenous Arbuscular Mycorrhizal Fungi Consortium in Shallot Cultivation with Lead-Polluted Media, *Nature Environment and Pollution Technology*, pp. 53-59 Vol. 21 No. 1, https://doi.org/10.46488/NEPT.2022.v21i01.006

Hardianti, A.R., Y.S. Rahayu, M.T. Asri. 2014. Efektivitas Waktu Pemberian *Trichoderma Harzianum* dalam Mengatasi Serangan Layu *Fusarium* pada Tanaman Tomat Varietas Ratna. *J. Lentera Bio*. 3:21-25.

Hartini, E. 2011. Kadar Plumbum (Pb) dalam Umbi Bawang Merah di Kecamatan Kersana Kabupaten Brebes. *Jurnal Visikes*, *10*(1), 69–75.

Hasan, M., Ruswadi, R. 2016. Pengaruh Jenis Pupuk Kandang terhadap Pertumbuhan dan Produksi Bawang Merah (Allium ascalonicum L.) di Serang, Banten. *Jurnal Ilmiah Respati*, *7*(2), 642–649. https://doi.org/10.52643/jir.v7i2.18

Herawaty A, Nadhira. 2009. *Kajian Penggunaan Pestisida oleh Petani Pemakai serta Informasi dari Berbagai Stakeholder Terkait di Kabupaten Karo Sumatera Utara*. Diakses di www.info.stppmedan.ac.id/pdf/jurnalhera1.pdf pada tanggal 13 Mei 2018.

Hermawan, R., M.D. Maghfoer, T. Wardiati. 2013. Aplikasi *Trichoderma harzianum* terhadap Hasil Tiga Varietas Kentang di Dataran Medium. *J. Produksi tanaman* 1:464-470.

Hermosa, R., A.V.I. Chet, E. Monte. 2012. Plant Beneficial Effects of *Trichoderma* and of Its Genes. J. Microbiology 158:17-25.

Hermosa, R., M.B. Rubio, R.E. Cardoza, C. Nicolas, E. Monte, S. Guteirrez. 2013. The Contribution of Trichoderma to Balancing the Cost of Glant Growth and Defense. *J. Microbiology* 16:69-80.

Jeyarajan, R., Nakkeeran, S. 2000. Exploitation of Microorganisms and Viruses as Biocontrol Agents for Crop Disease Management. in Biocontrol Potential and Their Exploitation in Sustainable Agriculture (Pp. 95– 116). Upadhyay Et Al. (Ed.), Kluwer Academic/ Plenum Publishers, Usa. Retrieved From Http://Dx.Doi. Org/10.1007/978-1-4615-4209-4_8.

Jumini, J., Sufyati, Y., & Fajri, N. 2010. Pengaruh Pemotongan Umbi Bibit dan Jenis Pupuk Organik terhadap Pertumbuhan dan Hasil Bawang Merah. *Jurnal Floratek*, *5*(2), 164–171.

Kuffner, M., Puschenreiter, M., Wieshammer, G., Gorfer, M and Sessitch, A. 2008. Rhizosphere Bacteria Affect Growth and Metal Upatake of heavy metal Accumulating Willows. *Plant Soil*, 304:35-44

Leyval, C., Joner, E.J., Val, C. and Haselwandter, K. 2002. Potential of Arbuscular Mycorrhizal Fungi for Bioremediation. In: Gianinazzi, S., Schuepp, H., Barea, J.M. and

Haselwandler, K. (eds), *Mycorrhizal Technology in Agriculture*, Burkhiluser Verlag, Switzerland, pp. 175-186

Malgorzata J. Kacprzak, Karolina Rosikon, Krzysztof Fijalkowski, and Anna Grobelak, 2014, The Effect of Trichoderma on Heavy Metal Mobility and Uptake by *Miscanthus giganteus*, *Salix* sp., *Phalaris arundinacea*, and *Panicum virgatum*, Institute of Environmental Engineering, Czestochowa University of Technology, Ulica Brzeznicka 60a, 42-200 Czestochowa, Poland, Hindawi Publishing Corporation *Applied and Environmental Soil Science* Volume 2014, Article ID 506142, 10 pages http://dx.doi.org/10.1155/2014/506142.

Manoharachary, C., Singh, H. B., & Varma, A. (Eds.). 2021. *Trichoderma:Agricultural Applications and Beyond* (*Soil Biology*, Vol. 61). Springer.

Millar, N. S., A. E. Bennett, 2016, Stressed Out Symbiotes: Hypotheses for the Influence of Abiotic Stress on Arbuscular Mycorrhizal Fungi, Oecologia (2016) 182:625–641 DOI 10.1007/S00442-016-3673-7

Natalia, A. G., T. N. Aeny, Jokoprasetyo, 2014, Uji Keefektifan Trichoderma spp. dengan Bahan Campuran yang Berbeda dalam Menghambat Pertumbuhan *Sclerotium rolfsii* Penyebab Penyakit Rebah Kecambah pada Kacang Tanah, *J. Agrotek Tropika*. Issn 2337-4993 Vol. 2, No. 3: 408 – 413, September 2014.

Pradana, K.W., R. Dwiyani, G. N. A. S. Wirya, 2021, Pengaruh Penambahan *Trichoderma* Spp. pada Media Tanam Terhadap Pertumbuhan Bibit Pisang (*Musa acuminata*) Cavendish Hasil Kultur Jaringan, *Agro Bali : Agricultural Journal*, Vol. 7 No. 1: 205-211, March 2024, e-ISSN 2655-853X, https://doi.org/10.37637/ab.v7i1.1568.

Rachmadiarti, F., Winarsih, H. Fitrihidajati, T. Purnomo, S. Kuntjoro, F. A. Nafidiastri, R. Yolanda, R. Ambarwati, D. Anggorowati, W. Budijastuti, U. Faizah, D. Putriarti, N. F. Rosyidah, 2024, Bioaccumulation of Lead (Pb) and Cadmium (Cd) in *Padina Australis* Hauck at Palang Beach, Tuban, East Java, Indonesia, *Nature Environment and Pollution Technology*, 2024 pp. 1507-1516 Vol. 23 No. 3, https://doi.org/10.46488/NEPT.2024.v23i03.020

Raven, P. H., L. R. Berg, and G. B. Johnson, 1998. *Environment*, Saunders College Publishing, New York, NY, USA, 2nd edition

Rizal, S., Novianti, D., & Septian, M. 2019. Pengaruh Jamur *Trichoderma* sp https://doi.org/10.37637/ab.v7i1.1568 terhadap Pertumbuhan Tanaman Tomat (*Solanum lycopersicum* L.) Syamsul. *Indobiosains*, *1*(1), 1044–1052. https://doi.org/10.31851/indobiosains.v1i 1.2297

Shoresh, M., G.E. Harman. 2008. The Relationship Between Increased Growth and Resistance Induced in Plants by Root Colonizing Microbes. Plant Signaling Behav. 3:737-739.

Sriwati, R., T. Chamzurni, L. Kemalasari. 2014. Kemampuan Bertahan Hidup *Trichoderma harzianum* dan *Trichoderma virens* Setelah Ditumbuhkan Bersama dengan Jamur Patogen Tular Tanah Secara In Vitro. *J. Floratek* 9:14-21.

Soekarno, B. P. W., Surono, S., & Susanti, S. 2014. Formula Pelet Berbahan Aktif *Trichoderma s*p. dan Aplikasinya terhadap Penyakit Rebah Kecambah pada Tanaman Mentimun. *Jurnal Fitopatologi Indonesia*, *10*(5), 153.

Soil Research Institute, 2002, Penelitian Inventarisasi dan Pengendalian Dampak Lingkungan, Laporan Akhir, *Bagian Proyek Penelitian dan Pengembangan Kesuburan Tanah dan Iklim*, Bogor. Hal:1-5

Sun, H., L. Wu, Y. Hao, C. Liu, L. Pan, Z. Zhu, 2020, Tolerance Mechanism of Trichoderma Asperellum To Pb2+: Response Changes of Related Active Ingredients Under Pb2+ Stress, *Rsc Adv*., 2020, 10, 5202, Doi: 10.1039/C9ra10517d Rsc.Li/Rsc-Advances, This Journal Is © The Royal Society Of Chemistry 2020

Sutrisno, D. K., Hartatik, S., & Dewanti, P. 2022. Peranan Trichoderma terhadap Pertumbuhan dan Hasil Tanaman Kedelai (Glycine Max) pada Kondisi Cekaman Kekeringan. *Jurnal Agrinika: Jurnal Agroteknologi Dan Agribisnis*, *6*(1), 76–86. https://doi.org/10.30737/agrinika.v6i1.23 39

Uddin, A. F. M. J., Hussain, M. S., Rahman, S. S., Ahmad, H., & Roni, M. Z. K. 2015. Effect of Trichoderma Concentrations on Growth and Yield of Tomato. *Bangladesh Res. Pub. J, 11*(3), 228–232. http://www.bdresearchpublications.com/admin/journal/upload/1410085/1410085. pdf.

Vinale, F., Nigro, M., Sivasithamparam, K., Flematti, G., Ghisalberti, E. L., Ruocco, M., ... & Lorito, M. 2013. Harzianic Acid: a Novel Siderophore from *Trichoderma harzianum*. *FEMS microbiology letters*, *347*(2), 123-129.

Xiong, M., G. Q. Dai, R. G. Sun, Z. Zhao, 2024, Passivation Effect of Corn Vinasse Biochar on Heavy Metal Lead in Paddy Soil of Pb-Zn Mining Area, *Nature Environment and Pollution Technology*, 2024 pp. 419-426 Vol. 23, No. 1, https://doi.org/10.46488/NEPT.2024.v23i01.036

Yadav, J., J.P. Verma, K.N. Tiwari. 2011. Plant Growth Promoting Activities of Fungi and Their Effect on Chickpea Plant Growth. *Asian J. Biol. Sci*. 4:291299.

Yedidia, I., Srivastva, A. K., Kapulnik, Y., & Chet, I. 2001. Effect of *Trichoderma harzianum* on Microelement Concentrations and Increased Growth of Cucumber Plants. *Plant and Soil*, *235*(2), 235–242. https://doi.org/10.1023/A:1011990013955

Yuliantika, Z., Nizar, A., & Saikhu, M. 2019. Pengaruh Pertumbuhan dan Produksi Bawang Merah (*Allium ascalonicum* L.) terhadap Pemberian Kompos Limbah Rumput Laut (*Gracilaria* sp.). *Jurnal Teknologi Pertanian Andalas*, *23*(2), 172–178.

Nur A. Zin, Noor A. Badaluddin. 2020. Biological Function of Trichoderma spp. for Agriculture Applications. *Annals of Agricultural Sciences* (65) 2, December 2020, Pages 168-178.

Zhang, X., Li, X., Yang, H., & Cui, Z. 2018. Biochemical Mechanism of Phytoremediation Process of Lead and Cadmium Pollution with *Mucor circinelloides* and *Trichoderma asperellum*. *Ecotoxicology and Environmental Safety, 157, 21–28.* doi:10.1016/j.ecoenv.2018.03.047