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Raw Secondary Metabolites of *Trichoderma harzianum* T10 in Tapioca Flour Towards Cucumber Damping-off

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Abstract. *Trichoderma harzianum* is effective for controlling soil-borne pathogenic fungi and producing secondary metabolites. When applied in the field, the raw secondary metabolites are quickly decreased directly by sunlight. This research aimed to obtain the most effective concentration of tapioca flour in development of raw secondary metabolites of *Trichoderma harzianum* T10, its effect on damping-off and growth of cucumber. This research was carried out at the screen house and the Plant Protection Laboratory, Faculty of Agriculture, Jenderal Soedirman University. The study was conducted in two stages, i.e., *in vitro* and *in planta*. The *in vitro* stage used completely randomized design with five repetitions and five treatments consisted of *T. harzianum* T10 in Potato Dextrose Broth, and in 0.5; 1; 1.5; and 2% of tapioca flour media. In *in planta*, randomized block design was used with five repetitions and six treatments consisted of control, *T. harzianum* T10 in PDB, and in 0.5; 1; 1.5, and 2% of tapioca flour media. Variables observed were density of conidia, disease incubation period, disease incidence, AUDPC, maximum growth potential, germination ability, plant height, canopy fresh weight, root length, and fresh root weight. Result of the research showed that the highest conidial density (1.23×10^7 conidia mL^{-1}) of *T. harzianum* T10 was found in 2% tapioca flour with an increase of 63.28% compared to the PDB. The tapioca flour of 1 and 2%, and PDB could suppress the disease incidence by 81.82%. The lowest AUDPC was at 2% tapioca flour. The raw secondary metabolites could not delay the incubation period significantly and increase cucumber plant growth. Tapioca flour with the right concentration to produce high conidia density and high raw secondary metabolites. The benefits are to find other cheaper ingredients in promoting antagonistic fungal growth and the use of antagonistic fungal bioactive compounds to control plant pathogen

Key words: Cucumber; Damping-Off, Raw Secondary Metabolites; Tapioca Flour; *Trichoderma harzianum* T10

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INTRODUCTION

Trichoderma sp. is considered as potential antagonistic fungus that is often used for biological control (Munir et al., 2013; Ghazanfar et al., 2018). *Trichoderma* sp. works against fungal phytopathogens either indirectly by competing for nutrients and space, modifying environmental conditions, promoting plant growth and plant defensive and antibiosis mechanisms; or directly through mechanisms such as mycoparasitism. One spesies of *Trichoderma* spp. is *T. harzianum* Rifai (Chaverri et al, 2015). *T. harzianum* has been isolated from various plant rhizospheres and tested for its effect on several diseases. For example, *T. harzianum* T10 isolated from ginger rhizosphere against Fusarium wilt (Soesanto et al., 2013) and Phytophthora wilt (Soesanto et al., 2019). *Trichodema* sp. has the ability as a mycoparasite (Bouziane et al., 2016) and antibiotics (Vinale et al., 2014), as well as producing extracellular compounds that can be toxic to pathogens (Al-Taweil et al., 2009) and enzymes that can degrade pathogen cell walls (Bae et al., 2017).

T. harzianum is effective for controlling soil-borne pathogenic fungi (Munir et al., 2013; Ghazanfar et al., 2018). One of the soil-borne fungi is *Pythium* spp. which with their wide distribution and host range causing damping-off to cucumber (Rostami et al., 2015; Lamichhane et al., 2017). Efforts to control damping-off that have been carried out, including using soluble silicon (Si) (Fayadh & Aledani, 2011; Yassin et al., 2016), cultivars resistance (Rostami et al., 2015), and biological control (Soekarno et al., 2014; Aljarah, 2017). Synthetic chemical pesticides have been used as well, but this approach is likely to be limited due to negative impacts on human health and the environment (Keswani et al., 2019). The secondary metabolites produced by agriculturally important microorganisms have an important role in improving the quality of the crops. *T. harzianum* as a biological control agent can produce secondary metabolites (Mukherjee et al., 2012; Vinale et al., 2012; 2014).

Microbes, included biological agents, when applied in the field are quickly decreased in population directly by sunlight (Rashid et al., 2016) and by chemical substances (Li et al., 2017; Muturi et al.,

2017) so as to limit their ability. One strategy to avoid the degradation is the use of raw secondary metabolites in liquid formula (Pathma et al., 2011; Mutawila et al., 2015). Several studies have utilized carriers for liquid formulation of *Trichoderma* sp. biofungicides, such as talc (Sriram et al., 2011; Mukherjee et al., 2013; Patel and Patel, 2014), compost (Panahian et al., 2012; Damiri et al., 2014), and molasses (Rahnama, 2012). However, the use of tapioca flour for liquid formulations is still limited for biological control.

This study aimed to determine the most effective concentration of tapioca flour in development of raw secondary metabolites of *T. harzianum* T10, the effect of *T. harzianum* T10 in the formula on damping off and on growth of cucumbers. The benefits are to find other cheaper ingredients in promoting antagonistic fungal growth and the use of antagonistic fungal bioactive compounds to control plant pathogen

METHODS

Preparation of *Pythium* spp.

Pythium spp. were isolated from damping-off symptomatic chilli seedlings at Sikapat Gandatapa Village, Sumbang District, Banyumas Regency. The infected seedling was cut 1-2 cm, then aseptically grown on the PDA (potato dextrose agar) for identification (Patil & Rathore, 2018). The culture was purified and maintained for further studies.

Preparation of *T. harzianum* T10

T. harzianum T10 grown on PDA was propagated on cracked corn media by taking isolates using cork drill ± 5 mm in diameter. Inoculated cracked corn media with *T. harzianum* T10 was incubated for 6-14 days until the spores and mycelium grew evenly (Heydari & Pessarakli, 2010; Gusnawaty et al., 2014).

Preparation of raw secondary metabolites

Raw secondary metabolites of *T. harzianum* T10 were made in potato dextrose broth (PDB) with tapioca flour as the carrier. The liquid media of tapioca flour (5, 10, 15, and 20 g L^{-1}) was made by mixing the flour with sugar 10 g L^{-1} and heated to boiling. Each medium was filled into a sterile glass bottle for 100 mL, cooled, and added with *T. harzianum* T10 from the cracked corn media with a density of 10^6 conidia mL^{-1}. Furthermore, each treatment was shaken using a shaker with a speed of 150 rpm for 7 days at room temperature (Han et al., 2012). The mixture was centrifuged at 13000 rpm for 5 min. at room temperature. The supernatant was then transferred into sterile bottle (Figure 1) (Wu et al., 2017).

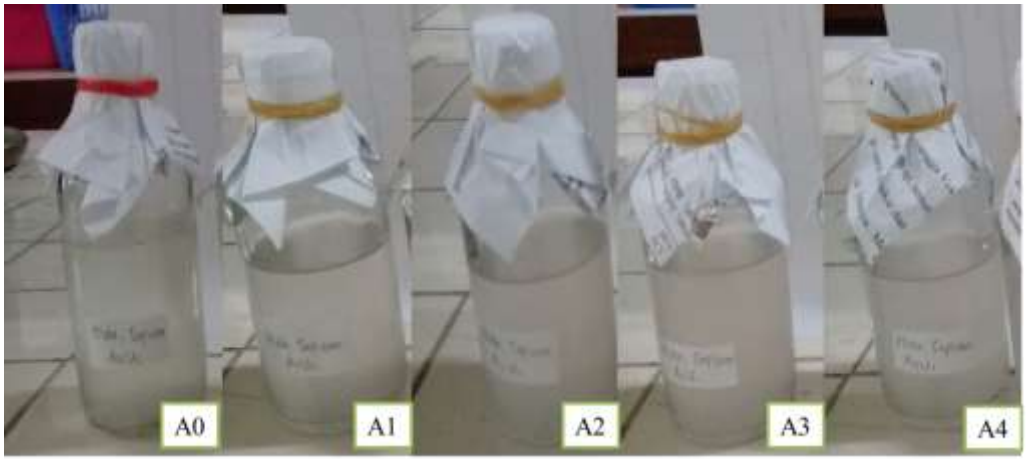

Figure 1. Raw secondary metabolites of *T. harzianum* T10 in different media. Note: A0 = PDB, A1-A4 = tapioca flour at concentration of 0.5; 1; 1.5; and 2%, respectively.

Application of raw secondary metabolites

In the planting hole, two cork drill *Pythium* spp. were placed. The planting media were composed by soils mixed with cow manure (1:1, w/w). Cucumber seeds were then placed on top and covered with thin layer of soils. The raw secondary metabolites of *T. harzianum* T10 according to the treatment were dripped on the seeds as much as 50 mL. As a control, *Pythium* spp. was inserted into the hole, covered with soil, then cucumber seeds were placed on it and covered with soil again. The experiment was carried out in the screen house.

In vitro experiment

In vitro experiments were arranged in a completely randomized design consisting of five replications and five treatments (A0 = raw secondary metabolites made in PDB, and A1-A4 = in tapioca flour of 5, 10, 15, and 20 g L^{-1}, respectively).

In planta experiment

In planta experiments were arranged in a randomized block design with five replications and six treatments (control, *T. harzianum* T10 secondary metabo-

lites in PDB, and in tapioca flour with concentrations of 5, 10, 15, and 20 g L^{-1}).

Observed variables

The conidial density was calculated by preparing a conidial suspension and by using a haemocytometer. The conidial suspension was taken as much as 1 mL using the eyedropper while stirring. The suspension onto the haemocytometer was covered with a glass cover and waited up for 1 minute until the suspension was stable. Conidial density was calculated using a microscope with a magnification of 100 x on the haemocytometer (a + b + c + d + e). The calculation was repeated four times and the density was calculated using formula as follows:

$$S = [(t \times d)/(b \times 0.25)] \times 10^6$$

Where: S = number of conidia per gram of media culture, t = the number of conidia calculated on the count media (a, b, c, d, e), d = degree of dilution, n = the number of small squares observed (i.e. 5 x 16 = 80 small squares), and 0.25 = correction factor (Akagi et al., 2015).

The disease incidence was calculated according to the formula:

$$IP = \frac{n}{N} \times 100\%$$

Note: IP = disease incidence, n = number of plants attacked, N = number of plants observed (Noordzij et al., 2010).

AUDPC (area under the disease progress curve) were calculated by the rule that approximate the area under a curve by dividing the area into a number of strips of equal width. Then, the sum of approximate area of each strip by the area of the trapezium formed will give the approximation of area under the curve. The formula used is according to Ling et al. (2017). Plant growth parameters observed included maximum growth potential, germination ability, plant height difference, canopy wet weight, root length, and root wet weight.

Data analysis

Data were analyzed by analysis of variance (ANOVA) at 5% level of significance. The significant different results between treatments were further tested using HSD at 5% significance level.

RESULT AND DISCUSSION

Conidial density

Based on Figure 2, the best concentration for growth and development of *T. harzianum* T10 was tapioca flour at 2% concentration with an increase of 63.28% compared to PDB. This was presumably because at this concentration the available nutrient content supports the growth of *T. harzianum* T10 so that the amount of conidia produced is more than other treatments.

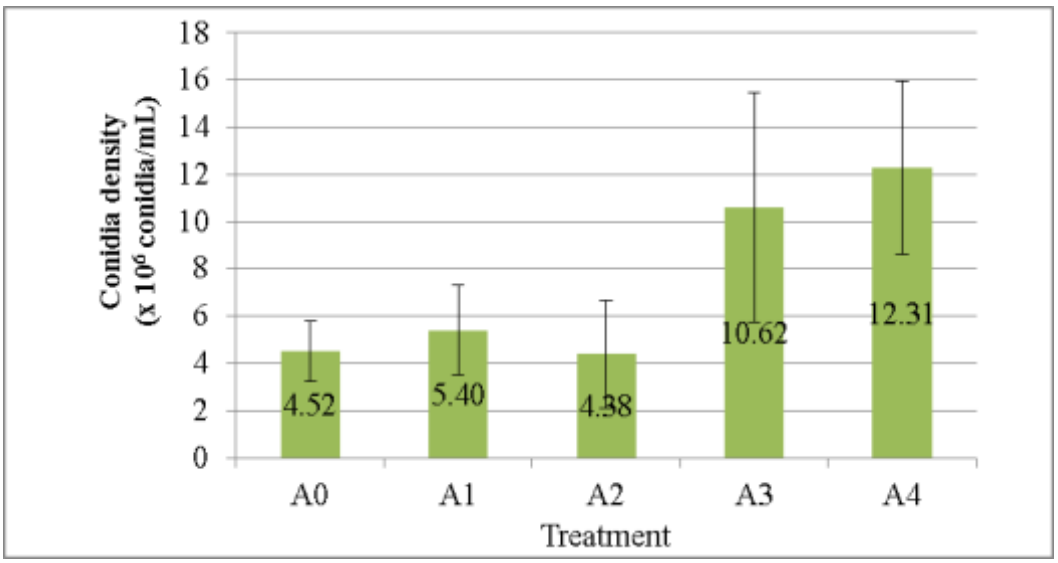

Figure 2. Density of *T. harzianum* T10 conidia. Note: A0 = PDB, A1-A4 = tapioca flour at concentration of 0.5; 1; 1.5; and 2%, respectively.

Mishra & Khan (2015) reported that differences in the number of *T. viride* conidia formed is possible closely related to the nutritional content of substrate. Borin et al. (2015) suggested that the growth of *T. reesei* and *Aspergillus niger* rely heavily on the availability of carbohydrates that is used as an energy source for growth.

One of the nutrients contained in tapioca flour (Rose Brand) is carbohydrates by 87.95% (AOAC, 1999). The carbohydrate content is quite high. Patil et al. (2018) reported that the high carbohydrate content in an ingredient can be a potential source of nutrients and carbon for the growth of the *T. harzianum* fungus. In accordance with a study by Ezeonu et al. (2016), carbohydrates, especially sugar, are mostly used by fungi on a large scale for their metabolic processes.

Incubation period

The statistical analysis results in Table 1 show that raw secondary metabolites did not significantly influence the incubation period. It means that the raw secondary metabolites produced by *T. harzianum* T10 have not been able to significantly delay the incubation period of *Pythium* spp. This is allegedly due to environmental conditions that support the development of *Pythium* spp. and the fungus could adapt to the new location immediately. In accordance to Sutton et al. (2006), the initial (primary) inoculum in root rot epidemics is chiefly zoospores produced from sporangia formed by germinating oospores, or perhaps by mycelium in plant residues, soil, hydroponic pipes and tubing, and other inoculum sources in the crop environment. This is in line with the report of Muthu (2016) that physical and nutritional factors play an important role in governing reproductive phases of *Pythium* sp.

Table 1. The effect of treatments on pathosystem component

Treatments	Incubation period (dai)	Disease incidence (%)	AUDPC
control	11.0a	55 a	327.5
T. harzianum T10 + PDB	17.2a	10 b	50
T. harzianum T10 + flour 0.5%	16.4a	20 ab	155
T. harzianum T10 + flour 1%	16.2a	20 ab	90
T. harzianum T10 + flour 1.5%	19.0a	10 b	75
T. harzianum T10 + flour 2%	19.0a	10 b	5

Note: Numbers followed by the same letter in the same column show no significant difference in HSD with an error rate of 5%. dai = days after inoculation. Data on incubation period were transformed to √x, data on disease events were transformed to arcsin √x.

The average incubation period of the disease in plants applied with the raw secondary metabolites of *T. harzianum* T10 seems to be delayed by 32.10-42.11% compared to controls. In general, the treatments tended to delay the incubation period compared to the control even though statistically the difference was not significant. *T. harzianum* secretes a well-balanced cellulolytic complex in tapioca, which efficiently hydrolyzes cellulosic substrates into monomeric glucose. Maximum cellulose activity was obtained with banana flour (168 U/ml) followed by potato and tapioca flours (Rubeena et al., 2013).

Disease incidence

The results of the statistical analysis in Table 1 show that the raw secondary metabolites had a significant influence on the incidence of the disease and was in line with the incubation period. PDB and tapioca flour concentration of 1.5 and 2% were able to reduce the incidence of disease by 81.82%. The treatment of flour concentrations of 0.5 and 1% can reduce the incidence of the disease by 63.64%.

In general, the raw secondary metabolites could reduce the disease incidence even though the results of treatment using flour concentrations of 0.5% and 1% were not significantly different compared to control (Figure 3). The raw secondary metabolites play a role in suppressing the disease incidence. *Trichoderma* spp. are well known for their ability to produce a wide range of antibiotic substances and for their ability to parasitize the other fungi. *Trichoderma* spp. produce at least three classes of compounds (peptides, proteins and low-molecular-weight compounds) that generate plant defense responses (Keswani et al., 2019). Secondary metabolites produced *in vitro* may have antimicrobial activity at high concentration so that the disease incidence will be decreased (Köhl et al., 2019).

Figure 3. Growth of cucumber after application of the treatments.

AUDPC

The AUDPCs were calculated numerically using the trapezoidal rule and were plotted for successive assessment dates. Based on Figure 4, the highest AUDPC value occurs in control crop and this is in line with the disease incidence in Table 1. This condition is due to the absence of secondary metabolites resulted in the greater attack of *Pythium* spp. than of in the treatment. Plants classified as experiencing disease in this study are exposed to fall and only develop symptoms. According to Gilbert & Parker (2010), in control plants, the average plants experienced the highest disease incidence. The highest disease incidence in control is caused by activity of the pathogens which are more quickly enter and infect plant tissue, and the absence of a mechanism of plant resistance to pathogen infection. This is because of pathogen recognition to crop structure in a several defense responses to inhibit pathogen invasion. One of the first structural barriers that fungal pathogens have to break and enter to their hosts is cell walls. The cell walls are mainly composed of carbohydrates (Rodriguez-Moreno et al. 2017).

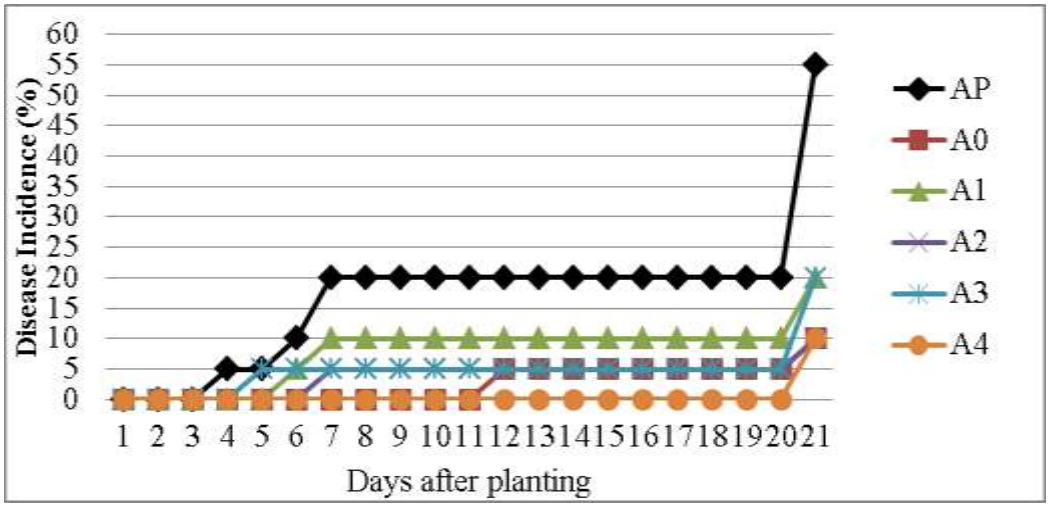

Figure 4. AUDPC value of cucumber seedling damping off. Note: AP = Control, A0 = PDB, A1-A4 = tapioca flour at concentration of 0.5; 1; 1.5; and 2%, respectively.

Based on Figure 4, an increase of *Pythium* spp. infection was seen on the last day of observation. Suffert & Guibert (2007) stated that the *Pythium* spp. can also attack after sprouts appear on the ground surface. In older plants, this pathogen can cause injury to the stem. Part of the wound infected with *Pythium* spp. turns pale and runny causing significant damage to yields.

In general, the raw secondary metabolites of *T. harzianum* can suppress disease progression well. This condition is seen in AUDPC values that because of the raw secondary metabolites, the plants are less likely to develop disease than controls. Mukherjee et al. (2012) and Vinale et al. (2014) stated that *T. harzianum* produces antibiotics that can reduce the growth of phytopathogenic fungus such as *Phytium*

sp. Antibiotic compounds produced by *T. harzianum* are trichorzins and harzianins (Marik et al., 2019). Both types of compounds are secondary metabolites that are thought to inhibit the growth of pathogenic fungi (Khan et al., 2020).

The lowest AUDPC value was found at 2% tapioca flour concentration. This is assumed because the 2% concentration is the highest flour concentration which produces the highest conidia density so that the metabolite compounds produced are thought to be more than the flour concentration lower than the 2%. Hanudin et al. (2013) explained that the lower the AUDPC value, the more effective the treatment is in controlling plant pathogens.

Growth components

The results of the statistical analysis in Table 2 and Figure 2 show that the raw secondary metabolites did not significantly influence the growth components including maximum growth potential, germination ability, plant height, canopy wet weight, root length, and root wet weight of cucumber. The maximum growth potential of the control group was 95%, while it was more than 95% in the treatment with raw secondary metabolites. The germination ability of control was 95%, while it was more than 85% in the treatment with secondary metabolites.

Table 2. The effect of treatment on growth component

Treatments	MG (%)	GA (%)	PH (cm)	RL (cm)	CW (g)	RW (g)
Control	95a	95a	37.27a	29.12a	19.81a	1.77a
T. harzianum T10 + PDB	100a	95a	34.90a	36.75a	18.57a	1.78a
T. harzianum T10 + flour 0.5%	100a	90a	38.37a	27.85a	20.40a	1.83a
T. harzianum T10 + flour 1%	100a	100a	38.53a	31.23a	16.14a	2.15a
T. harzianum T10 + flour 1.5%	95a	85a	32.00a	27.48a	18.81a	1.65a
T. harzianum T10 + flour 2%	95a	90a	37.45a	29.12a	19.81a	1.71a

Note: Numbers followed by the same letters in the same column show no significantly different results in the 5% HSD after being transformed to √x. MG = maximum growth potential, GA = germination ability, PH = plant height, CW = canopy wet weight, RL = root length, RW = root wet weight.

The percentage of germination consisting of maximum growth potential and germination ability in the experiments is quite high. This is presumably because the seeds planted have a good vigor. Good vigor is supported good aeration in control and treatment plants so that the raw secondary metabolites do not have a significant effect. These results are in line with the study by Finch-Savage & Bassel (2016) that showed that seeds are able to grow normally, even though the natural conditions are not optimum are called seeds that have a good vigor.

Statistical analysis (Table 2) shows that the raw secondary metabolites do not significantly influence the plant height. This is presumably because organic fertilizer given at the beginning of planting is directly responded by the plant as a source of nutrition to stimulate its growth. These results are in line with the research of Shofiyani & Budi (2014), that explained that the provision of organic fertilizer at the beginning of planting had a direct influence on the availability of nutrients needed by plants during the study so that the role of biological agents had no significant effect.

Cow manure provides essential macro- and micronutrients that are necessary for plant growth. The provision of cow manure can increase the availability of nutrients and absorption of N elements that are needed in the vegetative growth of plants (Sriyanto et al. 2015). Furthermore, Tola et al. (2007) explained that cow manure contains a number of nutrients and organic matter that can improve the physical, chemical, and biological soil properties. Rifqifauzi (2014) stated that an appropriate level of aeration and nutrient availability is able to support the development of rooting. The development of a good root system determines the vegetative growth of plants which ultimately determines the reproductive phase and yield of plants.

The optimum temperature is thought to support photosynthesis. Cucumber is a thermophilic and frost-susceptible crop, growing best at a temperature above 20°C (Singh et al. 2017). The daily temperature observed during the study (28.5°C) is included in the optimum temperature category.

The novelty of this research is the use of antagonistic fungi in terms of raw secondary metabolites or bioactive compounds and not conidia based. In addition, the discovery of tapioca flour with the right concentration could be used to produce high conidia density and high raw secondary metabolites or bioactive compound.

The benefits of the results are to find other cheaper ingredients in promoting antagonistic fungal growth and the use of antagonistic fungal bioactive compounds to control plant pathogen.

CONCLUSION

In conclusion, the most effective tapioca flour concentration for *T. harzianum* growth was 2%, indicated by the highest conidial density (1.23×10^7 conidia mL^{-1}) with an increase of 63.28% compared to PDB. Tapioca flour concentration of 1%, 2% and PDB could reduce the incidence of disease by 81.82%. The lowest AUDPC value was found in flour concentrations of 2% and the raw secondary metabolite of *T. harzianum* was not been able to significantly delay the disease incubation period. The raw secondary metabolites of *T. harzianum* was not been able to increase the cucumber growth.

REFERENCES

Akagi, A., Jiang, C., & Takatsuji, H. (2015). *Magnaporthe oryzae* inoculation of rice seedlings by spraying with a spore suspension. *Bio-protocol*, *5*(11), e1486.

Aljarah, N. 2017. The activity of *Metarhizium* sp. to control *Pythium aphanidermatum* causal agent of cucumber damping off under greenhouse conditions. *International Journal of Science and Research* (IJSR) *6*(8): 1098-1101.

Al-Taweil, H.I., Osman, M.B., Aidil, A.H., & Wan-Yussof, W.M. (2009). Optimizing of *Trichoderma viride* cultivation in submerged state fermentation. *Am. J. Appl. Sci. 6*: 1277-1281.

Bae, S.-J., Park, Y.-H., Bae, H.-J., Jeon, J., & Bae, H. (2017). Molecular identification, enzyme assay, and metabolic profiling of *Trichoderma* spp. *Journal of Microbiology and Biotechnology 27*(6): 1157-1162.

Borin, G.P., Camila Cristina Sanchez, C.C., de Souza, A.P., de Santana, E.S., de Souza, A.T., Leme, A.F.P., Squina, F.M., Buckeridge, M., Goldman, G.H., & de Castro Oliveira, J.V. (2015). Comparative secretome analysis of *Trichoderma reesei* and *Aspergillus niger* during growth on sugarcane biomass. *PLoS ONE 10*(6): e0129275.

Bouziane, Z., Dehimet, L., & Chaouch, N.K. (2016). Inhibitory activity of *Trichoderma viride* against *Phytophthora infestans* that affects the Spunta potato (*Solanum tuberosum* L.) variety. *African Journal of Microbiology Research 10*(29): 1121-1127.

Chaverri, P., Branco-Rocha, F., Jaklitsch, W., Gazis, R., Degenkolb, T., & Samuels, G.J. (2015). Systematics of the *Trichoderma harzianum* species complex and the re-identification of commercial biocontrol strains. *Mycologia* 107(3): 558–590.

Damiri, N., Mulawarman, & Mutiara, M. (2014). Effect of temperature and storage on effectiveness of *Trichoderma viride* as biocontrol agents of *Rigidoporus microporus*, pathogen of white root on rubber. *AGRIVITA 36* (2): 169–173.

Ezeonu, C.S., Umaru, I.J., Sindama, A., & Onwurah, I.N.E. (2016). Estimation of total carbohydrate and sugar contents of fungi treated rice husks. *FUW Trends in Science & Technology Journal 1*(1): 45-49.

Fayadh, M.A. & Aledani, M.A. (2011). Effect of some microelements and biological control agents in control of tomato seedling damping-off caused by *Rhizoctonia solani* Kuhn. *Basra J. Agric. Sci*. *24*(1): 53-68.

Finch-Savage, W.E. & Bassel, G.W. (2016). Seed vigour and crop establishment: extending performance beyond adaptation. *Journal of Experimental Botany 67*(3): 567–591. DOI:10.1093/jxb/erv490.

Ghazanfar, M.U., Raza, M., Raza, W., & Qamar, M.I. (2018). Trichoderma as potential biocontrol agent, its exploitation in agriculture: A review. *Plant Protection*, 02(03): 109-135.

Gilbert, G.S. & Parker, I.M. (2010). Rapid evolution in a plant-pathogen interaction and the consequences for introduced host species. *Evol. Appl. 3*(2): 144–156.

Gusnawaty, H.S., Taufik, M., Syair, & Esmin. (2014). Efektifitas *Trichoderma* indigenus hasil perbanyakan pada berbagai media dalam mengendalikan penyakit layu Fusarium dan meningkatkan pertumbuhan serta produksi tanaman tomat (*Lycopersicum esculentum). Jurnal Agriplus 24* (2): 99-110.

Han, J.S., Cheng, J.H., Yoon, T.M., Song, J., Rajkarnikar, A., Kim, W.G., Yoo, I.D., Yang, Y.Y., & Suh, J.W. (2012). Biological control agent of common scab disease by antagonistic strain *Bacillus* sp. sunhua. *Journal of Applied Microbiology 99*: 213–221.

Hanudin, Nawangsih, A.A., Marwoto, B., & Tjahyono, B. (2013). Komposisi formula biobakterisida berbahan aktif rizobacteri untuk pengendalian penyakit busuk lunak pada anggrek *Phalaenopsis*. *Jurnal Hortikultura 23* (3): 224-254.

Heydari, A. & Pessarakli, M. (2010). A review on biological control of fungal plant pathogens using microbial antagonists. *Journal of Biological Sciences 10*(4): 273-290.

Keswani, C., Singh, H.B., Hermosa, R., García-Estrada, C., Caradus, J., He, Y.-W., Mezaache-Aichour, S., Glare, T.R., Borriss, R., Vinale, F., & Sansinenea, E. (2019). Antimicrobial secondary metabolites from agriculturally important fungi as next biocontrol agents. *Applied Microbiology and Biotechnology 103*: 9287–9303.

Khan, R.A.A., Najeeb, S., Hussain, S., Xie, B., & Li, Y. (2020). Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic fungi. *Microorganisms 8*(817).

Köhl, J., Kolnaar, R., & Ravensberg, W.J. (2019). Mode of action of microbial biological control agents against plant diseases: Relevance beyond efficacy. *Front. Plant Sci*., 19 July 2019

Lamichhane, J.R., Dürr, C., Schwanck, A.A., Robin, M.-H., Sarthou, J.-P., Cellier, V., Messéan, A., & Aubertot, J.-N. (2017). Integrated management of damping-off diseases. A review. *Agron. Sustain. Dev*. *37*(10): 1-25.

Ling, A.S.C., Kamil, M.J.A., Chong, K.P., & Ho, C.M. (2017). Assessing the cocoa genotypes for resistance to black pod using the area under the disease-progress curve (AUDPC). *Bulgarian Journal of Agricultural Science 23*(6): 972–997.

Marik, T., Tyagi, C., Balázs, D., Urbán, P., Szepesi, A., Bakacsy, L., Endre, G., Rakk, D., Szekeres, A., Andersson, M.A., Salonen, H., Druzhinina, I.S., Vágvölgyi, C., & Kredics, L. (2019). Structural diversity and bioactivities of peptaibol compounds from the Longibrachiatum clade of the filamentous fungal genus *Trichoderma*. *Front. Microbiol*. *10*: 1434.

Mishra, P.K. & Khan, F.N. (2015). Effect of different growth media and physical factors on biomass production of *Trichoderma viride*. *People's Journal of Scientific Research 8*(2): 11-16.

Mukherjee. P.K., Horwitz, B.A., & Kenerley, C.M. (2012). Secondary metabolism in *Trichoderma*– a genomic perspective. *Microbiology 158*: 35–45.

Mukherjee, P.K., Horwitz, B.A., Herrera-Estrella, A., Schmoll, M., & Kenerley, C.M.. (2013). *Trichoderma* research in the genome era. *Annual Review of Phytopathology 51*: 105–29.

Munir, S., Jamal, Q., Bano, K., Sherwani, S.K., Bokhari, T.Z., Khan, T.A., Khan, R.A., Jabbar, A., & Anees, M. (2013). Biocontrol ability of Trichoderma. *International Journal of Agriculture and Crop Sciences 6*(18): 1246-1252.

Mutawila, C., Vinale, F., Halleen, F., Lorito, M., & Mostert, L. (2015). Isolation, production and in vitro effects of the major secondary metabolite produced by *Trichoderma* species used for the control of grapevine trunk diseases. *Plant Pathology 65*(1): 104-113.

Muthu, K.A. (2016). Occurrence and distribution of indigenous isolates of *Pythium* species in northern India. *Advances in Plants & Agriculture Research 4*(4): 319–327.

Muturi, E.J., Donthu, R.K., Fields, C.J., Moise, I.K., & Kim, C.-H. (2017). Effect of pesticides on microbial communities in container aquatic habitats. *Sci Rep. 7*: 44565.

Noordzij, M., Dekker, F.W., Zoccali, C., & Jager, K.J. (2010). Measures of disease frequency: Prevalence and incidence. Nephron. Clin. Pract. *115*:c17–c.

Panahian, G., Rahnama, K., & Jafari, M. (2012). Mass production of *Trichoderma* spp. and application. *International Research Journal of Applied and Basic Sciences* 3 (2): 292–298. .

Patel, R. & Patel, D. (2014). Screening of *Trichoderma* and antagonistic analysis of a potential strain of *Trichoderma* for production of a bioformulation. *International Journal of Scientific and Research Publications 4* (10): 1–6.

Patil, A.D. & Rathore, M.S. (2018). Isolation of pythium species from damping off affected onion rhizospheric soil, using baiting technique. *Journal of Pharmacognosy and Phytochemistry 7*(4): 12-13.

Patil, V.M., Patole, K.R., Paprikar, M.S., & Rajput, J.C. (2018). Effects of glucose base formulation of *Trichoderma viride* on seed germination and seedling parameters of tomato (*Solanum lycopersicum*). *Indian Journal of Plant Sciences 7*(2): 6-11.

Pathma, J., Rahul, G.R., Kamaraj, K.R., Subashri, R., & Sakthivel, N. (2011). Secondary metabolite production by bacterial antagonists. *Journal of Biological Control 25*(3): 165-181. DOI: 10.18311/jbc/2011/3716.

Rahnama, K. (2012). Mass production of *Trichoderma* spp and application. *International Research Journal of Applied and Basic Sciences 3*(2): 292-298.

Rashid, M.I., Mujawar, L.H., Shahzad, T., Almeelbi, T., Ismail, I.M.I., & Oves, M. (2016). Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils. Microbiological Research *183*: 26-41.

Rifqifauzi, A. (2014). Pengaruh penyiraman dan dosis pemupukan terhadap pertumbuhan kangkung (*Ipomoea reptans*) pada komposisi media tanam tanah+pasir. *Agrotrop 4* (2): 104 -111.

Rodriguez-Moreno, L., Ebert, M.K., Bolton, M.D., & Thomma, B.P.H.J. (2017). Tools of the crook infection strategies of fungal plant pathogens. *The Plant Journal 93*(4): 664-674.

Rostami, F., Alaei, H., Karimi, H.R., & Abad, A.B. (2015). Controlling the root and stem rot of cucumber, caused by *Pythium aphanidermatum*, using resistance cultivars and grafting onto the cucurbit rootstocks. *Azarian Journal of Agriculture 2*(1): 19-24.

Rubeena, M., Neethu, K., Sajith, S., Sreedevi, S., Priji, P., Unni, K.N., Sarath Josh, M.K., Jisha, V.N., Pradeep, S., & Benjamin, S. (2013). Lignocellulolytic activities of a novel strain of

Trichoderma harzianum. *Advances in Bioscience and Biotechnology 4*(2). Article ID:28417,8 pages.

Shofiyani, A. & Budi, G.P. (2014). Development of *Fusarim* disease control technology with biological agent in Mas cultivar banana in land infected. *AGRITECH 16* (2): 157-173.

Singh, M.C., Singh, J.P., Pandey, S.K., Mahay, D., & Shrivastva, V. (2017). Factors affecting the performance of greenhouse cucumber cultivation-A review. *International Journal of Current Microbiology and Applied Sciences 6*(10): 2304-2323.

Soekarno, B.P.W., Surono, & Susanti. (2014). Formula pelet berbahan aktif *Trichoderma* sp. dan aplikasinya terhadap penyakit rebah kecambah pada tanaman mentimun. *Jurnal Fitopatologi Indonesia 10* (5): 153–159.

Soesanto, L., Kustam, and E. Mugiastuti. 2019. Application of Bio P60 and Bio T10 in combination against phytophthora wilt of papaya. *Biosaintifika: Journal of Biology & Biology Education 11*(3): 339-344.

Soesanto, L., Mugiastuti, E., Rahayuniati, R.F., & Dewi, R.S. (2013). Uji kesesuaian empat isolat *Trichoderma* spp. dan daya hambat in vitro terhadap beberapa patogen tanaman. *Jurnal HPT Tropika 13*(2): 117–123.

Sriram, S., Roopa, K.P., & Savitha, M.J. (2011). Extended shelf-life of liquid fermentation derived talc formulations of *Trichoderma harzianum* with the addition of glycerol in the production medium. *Crop Protection 30*: 1334-1339.

Sriyanto, Astuti, D.P., & Sujalu, A.P. (2015). Pengaruh dosis pupuk kandang sapi terhadap pertumbuhan dan hasil tanaman terung ungu dan terung hijau (*Solanum melongena* L.). *Jurnal AGRIFOR 14* (1): 39-44.

Suffert, F. & Guibert, M. (2007). The ecology of a Pythium community in relation to the epidemiology of carrot cavity spot. *Applied Soil Ecology 35*: 488-501.

Sutton, J.C., Sopher, C.R., Owen-Going, T.N., Liu, W., Grodzinski, B., Hal, J.C., & Benchimol, R.L. (2006). Etiology and epidemiology of Pythium root rot in hydroponic crops: current knowledge and perspectives. *Summa Phytopathologica 32*(4).

Tola, F. Hamzah, Dahlan, dan Kaharuddin. 2007. Pengaruh penggunaan dosis pupuk bokashi kotoran sapi terhadap pertumbuhan dan produksi tanaman jagung. *Jurnal Agrisistem 3* (1): 1-8.

Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Ruocco, M., Woo, S., & Lorito, M. (2012). Trichoderma secondary metabolites that affect plant metabolism. *Natural Product Communications 7*(11): 1545 – 1550.

Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Woo, S.L., Nigro, M., Marra, R., Lombardi, N., Pascale, A., Ruocco, M., Lanzuise, S., Manganiello, G., & Lorito, M. (2014). *Trichoderma* secondary metabolites active on plants and fungal pathogens. *The Open Mycology Journal 8*(Suppl-1, M5): 127-139.

Wu, Q., Sun, R., Ni, M., Yu, J., Li, Y., Yu, C., Dou, K., Ren, J., & Chen, J. (2017). Identification of a novel fungus, *Trichoderma asperellum* GDFS1009, and comprehensive evaluation of its biocontrol efficacy. *PLoS One 12*(6): e0179957.

Yassin, M.A., El-Samawaty, A.E., Moslem, M.A., & Sayed, R.M. (2016). Evaluation of potassium and sodium silicates against *Fusarium* spp. causing damping-off disease of cotton seedling. *Fresenius Environmental Bulletin 25*(4): 1117-1124.