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Compatibility Test of Four *Trichoderma* spp. Isolates on Several Synthetic Pesticides

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ABSTRACT

This research aimed to study the compatibility of some *Trichoderma* spp. isolates on some synthetic chemical pesticides carried out at the Laboratory of Plant Protection, Faculty of Agriculture, Jenderal Soedirman University from April up to July 2014. Trichoderma isolates were derived from rhizosphere exploration on ginger, banana, pineapple and shallot. The synthetic pesticides used were mancozeb and propineb (fungicides), oxytetracycline and streptomycin sulfate (agrimycin, bactericides), carbofuran (nematicide), and deltamethrin and prefenophos (insecticides: synthetic pyrethroids and chiral organophosphates, respectively). The compatibility test used food poisoning method in a completely randomized design with three replicates. Variables observed were discolouration, sporulation, colony diameter, conidia density, and fungal growth at pesticides treatment. The data were analyzed by F test at 5 % significant level and continued by Duncan Multiple Range Test (DMRT) when there was a significant difference. The result of the research showed that the most significant decreasing of *Trichoderma* spp. was found on mancozeb for shallot, ginger, and banana isolates, and propineb for pineapple isolate, respectively, 89.4, 97.7, 93.3, and 95.2 %. This result was in line with colour, sporulation, and inhibition level observation.

INTRODUCTION

Trichoderma spp. are soil-borne fungus to control biologically of plant pathogens especially resulated by soil-borne pathogens given much attention and play an important role in integrated diseases management (IDM). Genus *Trichoderma* is the most characteristic beneficial isolated soil fungi because of its competence to guard plants and maintain pathogen community under various soil circumstances. This antagonist has been either everywhere studied or marketable as biopesticides, biofertilizers, and soil amendments (Harman, 2006; Howell, 2003). *Trichoderma* spp. also provides abundant biologically active suspension, including enzymes to degrade pathogen cell wall and more secondary metabolites (Vinale et al., 2008).

Trichoderma spp. have been isolated and screened from several rhizospheres, that was ginger (Soesanto et al., 2005), shallot (Santoso, Soesanto, & Haryanto, 2007), banana (Haryono, Prihatiningsih, Wardhana, & Soesanto, 2009), and pineapple (L. Soesanto collection) rhizospheres. These isolates have been tested to suppress some soil-borne plant pathogens. *Trichoderma* spp. was used to manage *Fusarium oxysporum* (Probowo, Prihatiningsih, & Soesanto, 2006; Santoso, Soesanto, & Haryanto, 2007; Soesanto et al., 2005; Wardhana, Soesanto, & Utami, 2009), *Phytophthora* spp. (Karthikeyan, Kumar, & Kumar, 2003), *Sclerotium rolfsii* (Elad, Barak, & Chet, 1984; Henis, Adams, Lewis, & Papavizas, 1983; Jegathambigai, Wilson Wijeratnam, & Wijesundera, 2010), *Rhizoctonia solani* (Erper et al., 2013), and *Botrytis cinerea* (Barakat, Abada, Abou-Zeid, & El-

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Gammal, 2014; Zimand, Elad, & Chet, 1996). Study of inhibition mechanisms on Trichoderma spp. have been done, such as on competition ability (Bailey et al., 2008; Howell, 2003), antibiosis (Bailey et al., 2008; Benítez, Rincón, Limón, & Codón, 2004), mycoparasitism (Bailey et al., 2008; Reithner, Ibarra-Laclette, Mach, & Herrera-Estrella, 2011), enzyme activities (Benhamou & Chet, 1997; Haggag & Abo-Sedera, 2005), plant growth promoting fungi (Benítez, Rincón, Limón, & Codón, 2004), and organic waste decomposer (Haggag & Abo-Sedera, 2005; Soytong & Quyet, 2013).

Synthetic pesticides are highly used in almost all agricultural systems especially IDM system to prevent and to control plant diseases. Pesticides are easily found in the market and practically applied in the field so that the establishment of pesticides in Indonesia market is highly increasing (Direktorat Pupuk dan Pestisida, 2016). The pesticides are tremendously and commonly utilized to improve plant productivity by combating plant pests and diseases (Aktar, Sengupta, & Chowdhury, 2009; Cooper & Dobson, 2007). The other benefits of pesticides are for protection of crop losses, control of vector disease, and quality of food. Besides their advantages, pesticides also have numerous disadvantages such as adverse impact to human health, to food, on environment, on water and soil contamination, and to non-target organisms (Aktar, Sengupta, & Chowdhury, 2009; Chowdhury, Pradhan, Saha, & Sanyal, 2008; Sengupta, Aktar, Alam, & Chowdhury, 2010).

Integrated disease management combines biological, physical, cultural, and chemical component strategies applied to all plants and crops and many plant pathogens including fungi, bacteria, viruses, and nematodes. The pathogens can cause extremely harmful to crops from small to significant losses primarily in the tropical developing country. Application of single IDM component to the plant pathogens resulted in an insignificant result. The application of IDM has been proved to be more effective in the way of holistic manner than the use of single IDM component strategy (El Khoury & Makkouk, 2010). As a component of IDM, the antagonist *Trichoderma* spp. should be compatible with other IDM components in the agricultural system. The compatibility of *Trichoderma* spp. with the other components is needed to improve the performance of the plant disease management, so that the disease could be prevented and controlled, and the agricultural product could be saved and increased (Paret, Dufault, Momol, Marois, & Olson, 2015). The role of synthetic chemical pesticides in the agricultural system is still needed in the disease management, and the compatibility of both components need to be studied. The compatibility of *Trichoderma* spp. with the other components is required.

Trichoderma spp. has wide variability in controlling plant pathogens included its compatibility with chemical pesticides. This variability is caused by many influencing factors (Gómez, Chet, & Herrera-Estrella, 1997), especially the genetics factor. Exploration of *Trichoderma* spp. indicated some potential isolates that could control plant pathogens (Haryono, Prihatiningsih, Wardhana, & Soesanto, 2009; Santoso, Soesanto, & Haryanto, 2007; Soesanto et al., 2005). However, the compatibility of *Trichoderma* spp. isolates with general pesticides are not known yet. Therefore, this research was carried out to determine the compatibility of selected *Trichoderma* spp. isolates with commonly applied synthetic chemical pesticides including fungicides, insecticides, bactericide, and nematicides.

MATERIALS AND METHODS

The research was conducted at the Laboratory of Plant Protection, Agricultural Faculty, Jenderal Soedirman University, Purwokerto, from April to July 2014. Four *Trichoderma* spp. isolates and six pesticides used arranged split-plot design with three replicates.

Trichoderma spp. Isolates Preparation

Trichoderma spp. were prepared by plating on PDA (Tuite, 1969), then incubated for 3 days at room temperature (26 ± 1°C) daylight. The *Trichoderma* isolates were derived from rhizosphere exploration on ginger (Soesanto et al., 2005), banana (Haryono, Prihatiningsih, Wardhana, & Soesanto, 2009), pineapple (L. Soesanto collection), and shallot (Santoso, Soesanto, & Haryanto, 2007). The isolates were identified as *T. harzianum* Rifai for ginger, banana, and shallot isolates, while the pineapple one had not been identified.

Synthetic Pesticides Preparation

The synthetic pesticides were mancozeb and propineb (fungicides), oxytetracycline and streptomycin sulfate (agrimycin, bactericides), carbofuran (nematicide), and deltamethrin and prefenophos (insecticides: synthetic pyrethroids and chiral organophosphates, respectively). The choice of the pesticides was based on the frequency of their usage in Indonesia. All pesticides were prepared by mixing with sterile water according to label recommended.

Compatibility Test *In Vitro*

The compatibility test was conducted with food poisoning method (Khan & Shahzad, 2007) in completely randomized design with three replicates, by adding 1 droplet of the pesticides in Petri dish mixed with PDA just before plating. The Petri dish was homogenized and after solid, each *Trichoderma* spp. isolates were inoculated 5 mm discs of a seven-day-old culture of *Trichoderma* spp. isolates. After that, they were punched by sterilized cork borer and put a single disc in each Petri dish containing PDA and pesticide with the help of inoculating needle under an aseptic condition or PDA without pesticides as a control. Then, they were incubated at room temperature (26 ± 1°C) for 5 days or at least the growth of control reached the edge of Petri dish. Each treatment was repeated four times.

Observation and Measurements

Variables observed were discolouration, sporulation, colony diameter, conidia density, fungal growth at pesticides treatment compared to control with the formula.

$$I = 100(C-T)/C$$

Where: I = inhibition percentage; C = *Trichoderma* spp. colony diameter at control; T = *Trichoderma* spp. colony diameter at pesticides treatment (Gowdar, Babu, Nargund, & Krishnappa, 2006). The mycelial dry weight of the antagonist was measured based on Lilly & Barnett (1951) and Sutton & Starzyk (1972).

Data Analysis

The data were analyzed by F test at 5 % significant level and continued by Duncan Multiple Range Test (DMRT) when there was a significant difference.

RESULTS AND DISCUSSION

Growth Inhibition

Some pesticides significantly affected colony discolouration (Tabel 1). The colony of all *Trichoderma* spp. isolates had discolouration from green to white after growing on PDA supplemented by mancozeb, propineb, and prefenophos, while on PDA supplemented by carbofuran, oxytetracycline and streptomycine sulphate (agrimycin), and deltamethrin, the colony was still green in colour as the control. Moreover, the spotted isolate of Trichoderma spp. which change colour to white, i.e., in the treatment of mancozeb, propineb, and prefenophos, is considered low, whereas green colony isolates produce more conidium. (Table 1 and Table 2).

Table 1. Discolouration of treated all *Trichoderma* spp. colony after three days incubation

Treatments	Colour
Control	Green
Carbofuran	Green
Mancozeb	White
Propineb	White
Oxytetracycline and streptomycine sulphate	Green
Deltamethrin	Green
Prefenophos	White

At Fig. 1, some pesticides resulted in higher growth inhibition of all *Trichoderma* spp., especially mancozeb, propineb, and prefenophos, although among isolates of *Trichoderma* spp. showed indifferent growth inhibition at similar pesticide. This result is in line with colony discolouration (Table 1). The highest growth inhibition was found at prefenophos in all *Trichoderma* spp. isolates by a range of 74.10 - 80.38 %, while mancozeb and propineb inhibited the growth as a range of 29.81 - 43.18 % and 27.27 - 33.21 %, respectively. The lowest growth inhibition was showed by bactericide (oxytetracycline and streptomycin sulphate or agrimycin), i.e., in a range of 0.38 - 4.55 %. Based on kinds of *Trichoderma* spp. isolate, the ginger isolate was the most sensitive isolate on prefenophos compared to other isolates, while different growth inhibition between prefenophos and mancozeb and propineb as high as 47.4 and 65.7 %, respectively.

Antagonists against plant pathogenic fungi have been applied to control plant pathogens, and 90 % of the applications have been done with various fungus Trichoderma strains (Benítez, Rincón, Limón, & Codón, 2004). The influence of fungicides toward the advantageous action of microbes is significant to understand due to its value of danger analogous by synthetic fungicide applied in agriculture. Maximized crop productiveness and economic profit will be achieved with the utilise of the products to suppress the pathogens well, but sustaining beneficial organisms (Yang, Hamel, Vujanovic, & Gan, 2011). Research of the antagonist *Trichoderma* spp. on pesticides have escorted to a better comprehension of compatibility instrument to gain better-integrated application in an agricultural system, especially in IDM. Modes of action from fungicide have never been successfully demonstrated, and the auxiliary effects of the synthetic fungicides are not sufficiently perceived. Hence, the synthetic fungicide used may have contradictive impacts which are obscure to forecast (Lo, 2010). If the formulations made by the recommended dose of insecticides with the bio-control agent *Trichoderma* spp. and used for the management of various plant pests, they will show a promising effect than the chemicals alone. It costs effectively and also environment-friendly (Mahfut, Joko, & Daryono, 2016; Singh, Srivastava, Shrivastava, & Singh, 2012).

Table 2. The effect of synthetic pesticides on colony diameter, conidia density, and mycelial dry weight of *Trichoderma* spp. isolates

Treatments	Colony diameter (mm)	Conidia density (x10^6 conidia ml^{-1})	Mycelial dry weight (mg)
F cal.T	159.57 **	64.31 **	17.76 **
F tab 5 %	2.78	2.78	2.78
Trichoderma shallot	41.3 b	93.7 a	0.0203 b
Trichoderma ginger	65.3 a	86.4 b	0.0193 b
Trichoderma banana	64.3 a	60.1 d	0.0194 b
Trichoderma pineapple	64.9 a	80.9 c	0.0261 a
F cal.F	305.87 **	450.06 **	73.67 **
F tab 5 %	2.27	2.27	2.27
Control	80.0 a	123.0 b	0.0305 a
Carbofuran	72.6 b	102.0 d	0.0268 b
Mancozeb	48.8 d	14.0 f	0.0118 c
Propineb	56.2 c	14.8 f	0.0138 c
Agrimicyn	78.4 a	134.3 a	0.0258 b
Deltamethrin	58.4 c	112.5 c	0.0298 a
Prefenofos	18.3 e	61.5 e	0.0108 c
F cal. TXF	5.28 **	36.12 **	19.68 **
F tab 5%	1.80	1.80	1.80
Shallot, Control	55.3 ef	160.0 b	0.031 c-e
Shallot, Carbofuran	51.0 fg	139.0 c	0.026 d-g
Shallot, Mancozeb	32.0 h	17.0 l	0.015 j-n
Shallot, Propineb	37.7 h	32.0 k	0.009 n-p
Shallot, Agrimicyn	54.3 ef	147.0 bc	0.029 c-f
Shallot, Deltamethrin	44.7 g	110.0 e	0.025 e-h
Shallot, Prefenofos	14.3 j	51.0 j	0.007 op
Ginger, Control	88.3 a	133.0 cd	0.020 g-j
Ginger, Carbofuran	83.3 abc	106.0 ef	0.016 j-m
Ginger, Mancozeb	51.0 fg	3.0 l	0.009 n-p
Ginger, Propineb	64.0 d	12.0 l	0.009 n-p
Ginger, Agrimicyn	88.7 a	160.0 b	0.019 h-k
Ginger, Deltamethrin	64.3 d	174.0 a	0.055 a
Ginger, Prefenofos	17.3 ij	17.0 l	0.007 op
Banana, Control	88.0 a	75.0 hi	0.032 cd
Banana, Carbofuran	80.0 bc	67.0 i	0.032 cd
Banana, Mancozeb	50.0 fg	5.0 l	0.012 l-p
Banana, Propineb	64.0 d	9.0 l	0.013 k-o
Banana, Agrimicyn	84.0 ab	92.0 fg	0.023 f-i
Banana, Deltamethrin	61.3 de	84.0 gh	0.018 i-l
Banana, Prefenofos	22.7 i	89.0 gh	0.006 p
Pineapple, Control	88.3 a	124.0 d	0.039 b
Pineapple, Carbofuran	76.0 c	96.0 efg	0.033 c
Pineapple, Mancozeb	62.0 de	31.0 k	0.011 m-p
Pineapple, Propineb	59.0 de	6.0 l	0.024 f-i
Pineapple, Agrimicyn	86.7 ab	138.0 cd	0.032 cd
Pineapple, Deltamethrin	63.3 d	82.0 gh	0.021 g-j
Pineapple, Prefenofos	18.7 ij	89.0 gh	0.023 f-i

Remarks: Numbers accompanied by the same letter at the same column are not significantly different at DMRT α %5

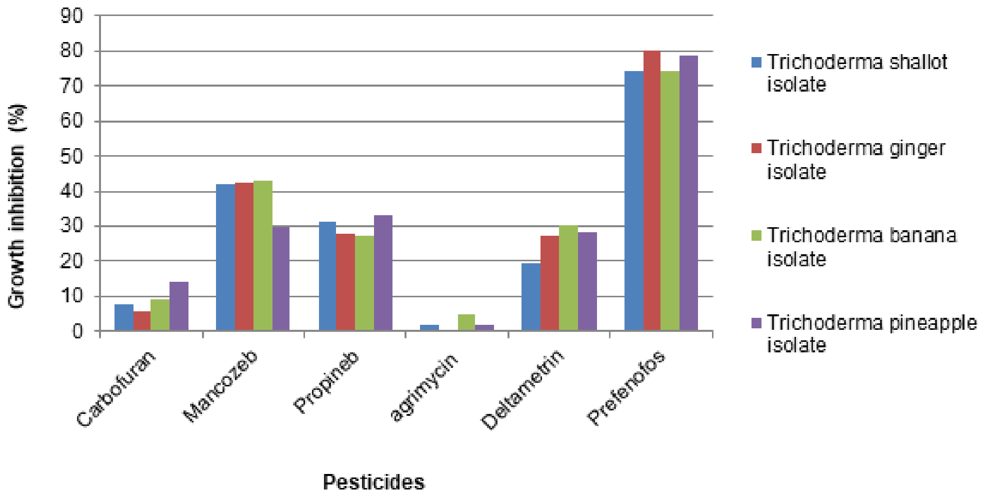

Fig. 1. Growth inhibition of *Trichoderma* spp. isolates due to pesticides

The growth and development of *Trichoderma* spp. isolates were influenced by given pesticides (Table 2). All isolates were affected by the pesticides in colony diameter, conidia density, and mycelial dry weight. Colony diameter of *Trichoderma* spp. shallot isolate was smaller than other isolates and in line with its mycelial dry weight. However, the highest conidia density was found in the isolate (93.7×10^6 conidia ml^{-1}) and differ significantly with other isolates ($p < 0.01$). *Trichoderma* spp. pineapple isolate gave the highest colony diameter, and this was in line with its mycelial dry weight.

Based on the results, almost all chemical synthetic pesticides indicated a negative effect on colony diameter of all *Trichoderma* spp. isolates, which resulted in decreasing mycelial dry weight and conidia density. Prefenophos could decrease the highest colony diameter but not in conidia density and mycelial dry weight as high as 77.1, 50.0, and 64.6 %, respectively. The highest decreasing conidia density of *Trichoderma* spp. isolates were found in mancozeb and propineb, i.e. 88.6 and 88.0 %, respectively; while bactericide (oxytetracycline and streptomycine sulfate or agrimycin) seems to stimulate increasing conidia density only 8.4 %.

Deltamethrin is a synthetic pyrethroid insecticide widely used on fruits and vegetables to control the household, industrial, and veterinary pests. Deltamethrin is highly toxic to some aquatic organisms, such as Nile tilapia (*Oreochromis niloticus* L.) fingerlings (Yildirim et al., 2006), freshwater mussel (Köprücü & Seker, 2008), and *Daphnia magna* (Day & Maguire, 1990). Oda & El-Maddawy (2012) point out that deltamethrin has danger effects for male reproductive systems and the protective effect of vitamin E and selenium combination on deltamethrin induced the deleterious effects of deltamethrin on male potency. However, deltamethrin has less effect on conidia density of *Trichoderma* spp. isolates.

Mancozeb and propineb inhibit conidia density of all *Trichoderma* spp. isolates wherein the highest inhibition happened. This result is contrast to Bagwan's (2010) research which stated Trichoderma applied for seed coating or irrigation would be harmonious with several synthetic or non-synthetic chemical, such as synthetic fungicides (thiram, copper oxychloride, mancozeb); pesticides; herbicides; and botanical pesticides (neem oil, neem leaves extract, wild sorghum leaves extract, neem cake, castor cake and mustard cake extracts) for the IDM of soil-borne pathogens on peanut. Another activity of mancozeb fungicide affecting metabolism of target cells, can also influence bacteria included in both cycle of soil C and N (Černohlávková, Jarkovský, & Hofman, 2009; Cycoń, Piotrowska-Seget, & Kozdrój, 2010). These fungicides performance are widely applied in agronomic system because of the wide spectrum activity of plant pathogen control, but the synthetic fungicides may have side effects on other microbes due to their various sites impacts of biochemistry (Dwimartina, Arwiyanto, & Joko, 2017; Yang, Hamel, Vujanovic, & Gan, 2011).

Table 2 showed significant interaction between *Trichoderma* spp. isolates and pesticides for all parameters. Based on the isolates, ginger, banana, and pineapple isolates showed the highest colony diameter grown on media supplemented by carbofuran and agrimycin, and in line with mycelial dry weight and conidia density. The lowest inhibition of colony diameter and mycelial dry weight of these isolates was indicated on agrimycin treatment compared to other pesticides, but on ginger isolate, this bactericide could stimulate colony diameter growth though only 0.5 %. The lowest colony diameter of all *Trichoderma* spp. Isolates were found at prefenophos, i.e., 74.1, 80.4, 74.2, and 78.8 %, respectively.

The combination between fungicide tolerant biological control agents and reduced levels of fungicide IDM strategies would increase the level of plant pathogens suppression identical to that obtained by the synthetic fungicides with full dosage (Monte, 2001). The effect of integration of *Trichoderma* with fungicides was reported by Sharma, Singh, & Sugha (1992) in controlling *Sclerotinia sclerotiorum*. When biocontrol agents *Trichoderma harzianum* and *Aspergillus niger* were incorporated with two synthetic fungicides, Foltaf 80W (Captafol 80 %) and Blue Copper-50, for protection from pigeon-pea wilt. The combination of biological agents and the fungicides was more suppressed the disease effectively than only the fungicides were used (Bhatnagar, 1995). Biopesticides can degrade more quickly than synthetic chemical pesticides and can supplement the synthetic pesticides used in integrated pest management (IPM) programs, which offer potentially higher crop yield and can reduce the use of conventional pesticides (Thakore, 2006). *Trichoderma* spp. are a soil microorganism. The soil microbial populations can influence plant growth and production in agricultural systems so that understanding the effects of synthetic fungicides on soil microbes can be important manner (Joko et al., 2012).

There were some isolates resulted in the highest conidia density and mycelial dry weight but gave the lowest colony diameter, such as shallot isolate on deltamethrin, ginger isolates on deltamethrin, and banana isolates on deltamethrin and prefenophos. In all isolates, all pesticides decreased conidia density compared to control except ginger, banana, and pineapple isolates showed increasing the density because of agrimycin, i.e., 16.9, 18.5, and 10.2 %, respectively, and deltamethrin and prefenophos could increase banana isolate conidia density, i.e., 10.7 and 15.7 %, respectively. The greatest decrease was found on mancozeb for shallot, ginger, and banana isolates, and propineb for pineapple isolate respectively 89.4, 97.7, 93.3, and 95.2 %. This result was in line with colour, sporulation, and inhibition level observation (Table 1 and Fig. 1).

CONCLUSION

Mancozeb for shallot, ginger, and banana isolates, and propineb for pineapple isolate decreased the growth of *Trichoderma* spp., respectively, 89.4, 97.7, 93.3, and 95.2 %. This result was in line with colour, sporulation, and inhibition level observation.

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