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Adalah penulis pertama pada naskah yang berjudul "Effect pesticides to entomopathogen fungi from citrus orchard" yang diterbitkan dalam IOP Conf. Series: Earth and Environmental Science (2021) 803 012021 IOP Publishing doi:10.1088/1755-1315/803/1/011001.

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2.	Harwanto	Kontributor Utama, melakukan koreksi terhadap draft naskah.
3.	Unun Triasih	Kontributor Anggota, melaksanakan penelitian
4.	Dina Agustina	Kontributor Anggota, melaksanakan penelitian

Demikian surat pernyataan ini saya buat dengan sebenarnya untuk dapat digunakan sesuai dengan keperluannya.

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PREFACE

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 <p>The Electrochemical Society Advancing solid state & electrochemical science & technology 2021 Virtual Education</p> <p>Fundamentals of Electrochemistry: Basic Theory and Kinetic Methods Instructed by: Dr. James Noël Sun, Sept 19 & Mon, Sept 20 at 12h–15h ET</p> <p>Register early and save!</p>	
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PREFACE
CHAIRPERSON
SIWI GAYATRI, PH.D.

Assalamualaikum Wr. Wb.

Prof. Yos Johan Utama, Rector of Diponegoro University

Dr. Ir. Bambang Waluyo Hadi Eko Prasetyono, Dean of Faculty of Animal and Agricultural Sciences, Diponegoro University

*Distinguished guests, speakers, and all participants of the **INTERNATIONAL CONFERENCE ON REFRAMING FOOD SOVEREIGNTY AFTER COVID-19***, I am very happy to welcome you all at this conference today, 20th October 2020 in Semarang, Indonesia. The conference has been honored by the attendance of 4 keynote speakers: Vietnam, Australia, Thailand, and Indonesia.

The committee has seen a very big interest to the seminar and finally accepted 80 abstracts, in which will be presented in parallel session. The participants are from many universities, research agencies and government institutions across Indonesia. Selected papers from this conference will be published in a reputable international proceeding IOP Conference Series: Earth and Environmental Sciences (EES). Therefore, we are proud to keep the high standard for the selection of abstract and full paper. There are four main topics covered in this conference, namely: there are 4 topics discussed; (1) Animal Sciences, (2) Plant Sciences, (3) Food Sciences, and (4) Agribusiness.

On behalf of the organizing committee, I would like to express my sincerest gratitude to keynote speakers, presenters, distinguished guests, participants and also sponsors who have contributed to the success of this conference. The committees are committed to give our best to make this conference interesting and beneficial for all the participants. We are glad to accept your input to make the program better and please do not hesitate to reach us in case you need our assistance.

Last but not least, I thank all of the colleagues, organizing committee, student technical committee and all parties who have worked hard to make the **INTERNATIONAL CONFERENCE ON REFRAMING FOOD SOVEREIGNTY AFTER COVID-19** possible.

Wassalamualaikum Wr. Wb.



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ABSTRACT

COVID-19 has changed daily realities in every side of the world. But for millions of people, fears about access to food have made the crisis even worse. COVID-19 pandemic inhibits daily activity, even stops it for a moment. The effect is not only perceived on aspects of public health, environment, social issue, but also the economy. Many people are affected by the pandemic, it's even harder for vulnerable groups. The food system was broken long before coronavirus came along. The industrial and commodity-based food system has failed to adequately feed many people in this world. This isn't due to a lack of food but to the conditions of extreme inequality, and the wrong type of food being produced, traded or promoted by powerful corporate interests that control the food and agriculture sectors. COVID-19 has once again shown us just how risky it is to let corporations be in charge of feeding people. A system is needed for protecting the human rights, such as access to food and fair treatment and recognition for workers, and respects the ecological boundaries we depend on. It's called food sovereignty.

Concept of Food Sovereignty becomes more and more urgent and apparent on different levels, from sector level to global level. According to Via Campesina, food sovereignty is the right of peoples to healthy and culturally appropriate food produced through ecologically sound and sustainable methods, and their right to define their own food and agriculture systems. It puts those who produce, distribute and consume food at the heart of food systems and policies rather than the demands of markets and corporations. It defends the interests and inclusion of the next generation. It offers a strategy to resist and dismantle the current corporate trade and food regime, and directions for food, farming, pastoral and fisheries systems determined by local producers. Food sovereignty prioritizes local and national economies and markets and empowers peasant and family farmer-driven agriculture, artisanal fishing, pastoralist-led grazing, and food production, distribution and consumption based on environmental, social and economic sustainability. Food sovereignty is more human rights based than similar concepts of food security and, to a lesser extent, food justice. Food sovereignty emphasizes the reclamation of land, food, livelihoods, and identities of food insecure individuals via their direct participation in the design and implementation of food systems.

According to FAO, food sovereignty allows communities control over the way food is produced, traded and consumed. It could create a food system that is designed to help people and the environment rather than make profits for multinational corporations. The food sovereignty movement is a global alliance of farmers, growers, consumers and activists. Big business dominates our global food system. A small handful of large corporations control much of the production, processing, distribution, marketing and retailing of food. This concentration of power enables big businesses to wipe out competition and dictate tough terms to their suppliers. It forces farmers and consumers into poverty and hunger. Under this system, around a billion people are hungry and around two billion are obese or overweight. Movements of people across the world are fighting for food sovereignty.



It could only be undertaken by knowledge sharing between many disciplines, ranging from social sciences to life sciences. In parallel sessions of FAAS Conference, there are 4 topics discussed; (1) Animal Sciences, (2) Plant Sciences, (3) Food Sciences, and (4) Agribusiness. The discussion topics are expected to be able to provide input on the problem on food sovereignty.

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Peer review declaration

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Effect pesticides to entomopathogen fungi from citrus orchard in vitro

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Effect pesticides to entomopathogen fungi from citrus orchard in vitro

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Abstract. Pesticides are a group of chemicals that are intentionally applied to the environment with the aim of suppressing pests and plant diseases and protecting agricultural products. Most pesticides do not specifically target pests and diseases only during application, but also affect the products produced and human health due to the residue and the effect on non-target pests including entomopathogen. The purpose of this study was to determine the effect of pesticides applied in controlling pests and diseases of citrus plants towards the growth of entomopathogenic fungi in vitro. This test used three active ingredients of pesticides namely Mankozeb (fungicide), Profenofos and Lambda cyhalothrin (insecticides). Dosage of pesticides in the treatments were 0.25 times, 1-time, 2-times of the recommended doses and control (without pesticides). Five types of entomopathogenic fungi used were *Metarhizium anisopliae*, *Hirsutella* sp., *Beauveria bassiana*, *Paecilomyces* sp. and TB.8 (not yet identified). Each treatment with 3 replications. The size of entomopathogenic fungi showed the influence of pesticides on the growth of entomopathogenic fungi. A quarter dose of profenofos insecticide had the potential to inhibit the growth of all isolates, except *M. anisopliae*. All isolates did not show significant growth reductions after treated with various doses of Lambda-cyhalothrin insecticide. Mancozeb fungicide had a negative effect on the growth of all entomopathogenic fungi isolates. The highest spore density on the 21st days was *Paecilomyces* sp. under the Profenofos treatment. Lambda-cyhalothrin did not affect the spore productions in all entomopathogenic fungi isolates. Otherwise, spore production was not observed even at the lowest concentrations of Mancozeb treatment. Differences in the active ingredients of pesticide affected the growth and sporulation of entomopathogen.

1. Introduction

Pesticides are the groups of chemicals that are purposely applied to the environment with aim to suppress plant and animal pests and to protect agricultural and industrial products. However, the majority of pesticides tend to have a broad-spectrum effect, which don't specifically target one species or group of species only and have adverse effects due to its toxicity. These pesticides may also affect non-target organisms (plants and animals including entomopathogen fungi) that can lead to the loss of biodiversity in the environment. Furthermore, these pesticides are also hard to degrade, it persists and contaminate the environment. The development of Integrated Pest Management (IPM) strategies using beneficial control agents like entomopathogen fungi combined with chemical pesticides is useful to sustainable agriculture practice.

Entomopathogens are effective to control pest insects and the effect of this biocontrol is not inferior to its chemical counterparts. Wilcken *et al* [1] reported that the control of *T. peregrinus* nymph and



adult using microbial insecticides including entomopathogenic *Beauveria bassiana* and *Metarhizium anisopliae* was similar result with the chemical pesticides application with control efficiency more than 80 % after 21 days aerial application.

Previous studies have shown that entomopathogenic fungi are normally associated with the pest. Lima *et al* [2] reported that entomopathogenic fungi *Aschersonia cf. aleyrodis* Webber and *Aegerita webberi* Fawcett were associated with citrus blackfly in Southern Bahia. In studies conducted by Ezz [3], thirteen entomopathogen are also associated with scale insect. Despite normally found within the pests, entomopathogen can also be found in the soil of the host plant [4]. Galan-Franco *et al* [5] confirmed the presence of entomopathogen in twenty three percent of the soil samples from entomopathogenic fungi according through macroscopic and microscopic characteristics observations.

Entomopathogenic fungi plays an important role to control citrus pests and various species of this group have been used to target some key insect pests within the Integrated Pest Management system in citrus. Entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces fumosoroseus* and *Paecilomyces lilacinus* are known to control citrus mealybug (*Planococcus citri*) [6] [7]. *Diaphorina citri* can be controlled using *B. bassiana* (isolates B1) and *M. anisopliae* (isolates Ma129) [8] and formulation of *Isaria fumosorosea* [9]. *B. bassiana* also has a potential to control citrus leafminer (*Phyllocnistis citrella*) [10], *H. thompsonii* and *P. fumosoroseus* as alternative for biocontrol of *Eutetranychus orientalis* (citrus brown mite) [11].

Entomopathogen also effects to non-target organism and the effect is not deleterious to many organisms. Seiedy *et al* [12] reported that predatory *Amblyseius swirskii* is susceptible to *B. bassiana* when treated directly to the mites. The effects of entomopathogen to non-target organisms normally varies among species. *B. basiana* Bb. 5335 and *M. anisopliae* Ma 7965 are found to be an effective as a biological control agent for insect pests, but relatively safe for non-target organisms. *B. basiana* in particular is considered non-pathogenic to natural enemies dan various beneficial soil insects. *M. anisopliae* is considered as pathogenic organism to *Chrysoperla carnea* and *Dichypus tamaninii* [13].

Entomopathogens may be applied separately or combined with other entomopathogens. In study conducted by Wakil *et al* [14] the combination of entomopathogen *H. bacteriophora* and *B. bassiana* has successfully inhibit the growth of targeted insects by reducing its weight and variation during its development. The application of entomopathogens may also be combined with certain botanical insecticides and produce a synergistic effect, such as the entomopathogen *Lecanicillium lecanii* in combination with *Annona squamosa* seed powder and *Jatropha curcas* seed powder to control brown stink bug eggs [15]. Treatment of entomopathogen fungi or botanical insecticide (pyrethrum) to control aphid mortality was had significantly higher than control. In contrast, application on combination of pyrethrum and entomopathogenic fungus had additive effect to increase aphid mortality [16]. The combination of entomopathogenic fungi with sublethal concentration of insecticides may increase the ability of entomopathogenic fungi to control of pest. There for, the occurrence of insecticide resistance among target organism can be suppressed [17]. Certain pesticide can be applied with entomopathogen because it had no deleterious effects on the percentage of viable conidia, vegetative growth or conidia production [18]. The mixture application of chemical pesticides and biopesticides may reduce the quantity of chemical pesticides, which is a major cause of environmental pollution.

In vitro studies have showed that some pesticides can inhibit the sporulation and germination of entomopathogenic fungi [19]. A few papers have been published on the effects of pesticides to the growth and germination of *Hirsutella* species and other entomopathogenic fungi, specifically, *H. aphidis* (Petch) [20], *H. nodulosa* (petch) –entomopathogen of strawberry mite [21,22] and *B. bassiana* [22]. Pesticide may play an important role on the natural occurrence, infectivity and population dynamics of entomopathogenic fungi in the field. This condition may also exist in the citrus orchards. The use of pesticides can affect the entomopathogenic fungal population and growth. Furthermore, the active ingredient within the pesticides may also give different effect on the entomopathogenic fungal isolates.

The aim of this research is to know the effect of pesticide to mycelial growth and spore production of entomopathogen fungi from citrus orchard in vitro.

2. Materials and Methods

2.1 Pesticide testing of some entomopathogenic fungi

The study was conducted at Phytopathology Laboratory, Indonesian Citrus and Subtropical Fruits Research Institute, East Java, Indonesia. The in vitro compatibility study was done to evaluate the effect of chemical pesticides on the growth of entomopathogenic fungi through poisoned food technique. This experiment was carried out in Complete Randomized Design (CRD) with three replications. The pesticides used in this study were Mankozeb (fungicide), Profenofos and Lambda cyhalothrin (insecticides). The doses used in pesticides were 0.25-times recommendation dose (0.0375 g/50 mL aquadest), 1-time recommendation dose (0.15 g/50 mL aquadest), 2-times recommendation dose (0.3 g/50 mL aquadest) and control (without pesticides). Potato Dextrose Agar (PDA) medium used in this treatment was supplemented with Terramycin (0.1 mL/mL). A total of 10 mL warm sterile PDA medium was poured into a petri dish, then the given dose of insecticide was aseptically added. The mixture was stirred and poured.

Four entomopathogenic fungal isolates were used in this experiment, namely TB.8 (unidentified isolate), *Hirsutella* sp., *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces* sp. That fungal isolates were isolated from citrus pest using PDA medium, identification by microscopy observation at 400 X magnification and maintained at PDA slants.

The compatibility study was performed by inoculating the entomopathogenic fungal isolates into PDA medium containing pesticides with different concentrations. The diameter of entomopathogenic fungi in the treatment medium were measured every 2 days for 21 days. The spore density of treated isolates is measured using hemocytometer at 7, 14, and 21 days after inoculation and expressed as fungal conidia mL⁻¹. Microscopic observations were performed under a microscope with 400 X magnification. Microscopical observations of fungal spore density are then calculated using the formula:

$$C = \frac{t}{(n \times 0,25)} \times 10^6$$

Note:

C : spore density per mL of solution

t : the total number of spores in the sample box observed

N : number of sample boxes observed

0.25 : a correction factor for the use of small-scale sample boxes in a hemocytometer

2.2 Data analysis

The data were analyzed with Analysis of Variance (ANOVA) at 5% error rate. The average was tested using Duncan Multiple Range Test (DMRT) p <0.05.

3. Result and discussion

3.1. Entomopathogenic fungi development on Potato Dextrose Agar (PDA) medium containing pesticides at various concentrations

Population of four isolates of entomopathogenic fungi were found in citrus orchards. These isolates originated from citrus psyllids *Diaphorina citri*, aphids, another citrus pest and also from soil of citrus orchard. These dead psyllids were characterized as being mummified and covered to various extents by synnemata produced by the fungus. Mummified cadavers with synnemata, which serve as point

sources for new infections of the fungus in psyllids. There are the entomopathogen used on this study (Figure 1).

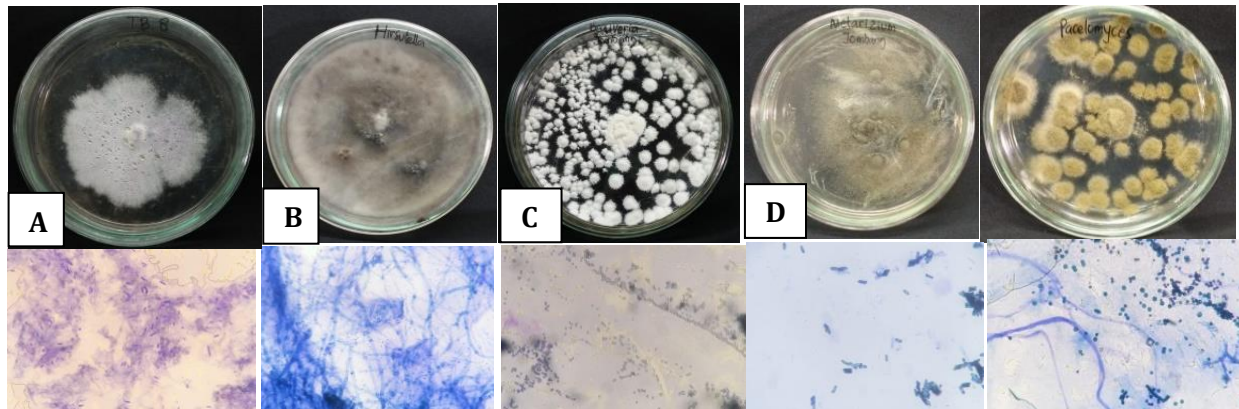


Figure 1. Isolates on Potato Dextrose Agar (PDA) and morphology of entomopathogen fungi at 400 X magnification. (A) TB 8 isolate, (B) *Hirsutella* sp., (C) *Beauveria bassiana*, (D) *Metarhizium anisopliae* and (E) *Paecilomyces* sp.

The Profenofos treatment has negatively affected the vegetative growth of several entomopathogenic fungi. *M. anisopliae*, TB 8 and *Beauveria bassiana* were among isolates that were heavily adverse. These isolates displayed significant gradual reductions in the diameter as the concentrations of the Profenofos in the medium increased (Figure 2). Among all isolate used, TB.8 was the isolate that experienced the most significant growth reduction due to this treatment, the diameter of its colony decreased from 8 mm to 1 mm. Contrary to those isolates, *Hirsutella* sp. and *Paecilomyces* sp. experienced a positive impact on its vegetative growth after the increase of Profenofos concentrations on its growth medium at 2-times recommendation dose. Similar results reported by [23] that many fungal isolates reacted differently to the presence of various chemicals in their growth medium. Some may experience a negative impact on its growth after treated with Profenofos at concentrations of 100-300 $\mu\text{g g}^{-1}$ but recovered quickly afterward, and other isolates may not even be affected at all.

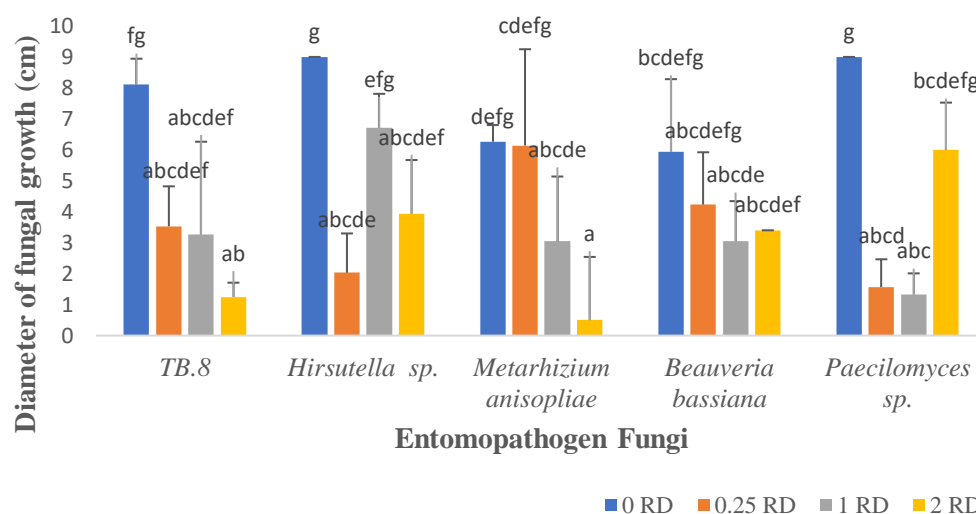


Figure 2. Vegetative growth of entomopathogen fungi on different doses of Profenofos pesticide (Mean followed by different letters indicate significant differences at DMRT $p < 0.05$)

In terms of spore density, all entomopathogenic fungal isolates used in this experiment still showed some moderate spore productions after treated with two-time concentrations of Profenofos for two weeks, except for *Hisutella* sp. (Table 1). These results may suggest that Profenofos is considered less toxic to the most entomopathogenic fungus. Therefore, applying Profenofos with suitable doses is safe for non-target organisms such as entomopathogenic fungi. Insecticide has a relatively small effect on the growth and germination of entomopathogenic fungi compared to fungicides [19]. According to [24], an insecticide is considered less toxic to most fungal isolates is because it doesn't completely kill and eliminate the ability of fungal to produce spore. These conditions allow the fungal isolates to survive, reproduce and adapt to low metabolic levels in the long run.

Table 1. The average of entomopathogenic fungi spore density by Profenofos insecticide treatment at 7, 14 and 21 days

Entomopatogen fungal isolates	Mean of spore density (10^6 spore/mL)								
	day 7			day 14			day 21		
	0.25 RD	1 RD	2 RD	0.25 RD	1 RD	2 RD	0.25 RD	1 RD	2 RD
<i>TB. 8</i>	4.9	8.07	0.5	0.73	5.15	0.77	1	6.22	0.7
<i>Hirsutella</i> sp.	0.7	1.1	0.92	0	3.47	0	0	3.75	0
<i>Beauveria bassiana</i>	0.73	0.98	0.32	2.1	2.3	3.7	2.95	2.38	3.73
<i>Metarhizium anisopliae</i>	8.02	101.22	0.97	0.3	3.23	0.37	0.4	3.83	0.52
<i>Paecilomyces</i> sp.	0.98	4.53	0.98	3.92	7.78	3.98	4.27	9.05	4.33

Note: RD = Recommendation dose

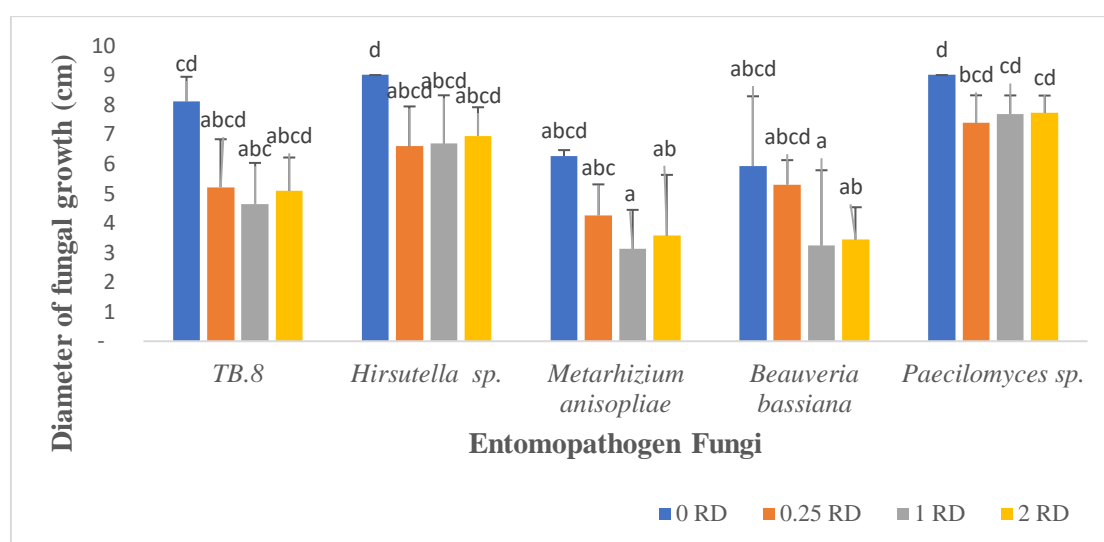


Figure 3. Vegetative growth of entomopatogenic fungi on different doses of Lambda-cyhalothrin insecticide (Mean followed by different letters indicate significant differences at DMRT $p < 0.05$)

All entomopathogenic fungi used in this experiment did not show significant growth reductions after treated with various doses of Lambda-cyhalothrin pesticide (Figure 2). In contrast to the Profenofos treatment, the vegetative growth of all entomopathogenic fungal isolates tends to decrease gradually as the concentrations of the Lambda-cyhalothrin in the medium increase, but the differences were not quite significant among concentrations. The addition of Lambda-cyhalothrin to the medium also did not affect the spore productions in all entomopathogenic fungi isolates used in this experiment, except for *Hirsutella* sp. and *B. bassiana* at 2-times recommendation dose (Table 2). These results may suggest that the Lambda-cyhalothrin have a minor toxic effect to the entomopathogenic fungus and compatible to be used in tandem with biopesticides or insecticides.

Pesticides, in general, had small impacts on the growth of fungal isolates. However, the presence of pesticides in higher concentrations may still alleviate the fungal isolates. In their research, they showed that the fungal population reduced gradually and reached a minimum of 10 kg ha⁻¹ concentration [25]. It suggested that pesticide concentration used in the field or medium has to be controlled. Therefore, it will not interfere with the growth of antagonistic or entomopathogenic fungal that benefits the environment.

Table 2. The average of entomopathogenic fungi spore density development by Lambda-cyhalothrin insecticide treatment at 7, 14 and 21 days

Entomopatogen fungal isolates	Mean of spore density (10 ⁶ spore/mL)								
	day 7			day 14			day 21		
	0.25 RD	1 RD	2 RD	0.25 RD	1 RD	2 RD	0.25 RD	1 RD	2 RD
<i>TB. 8</i>	3.95	2.1	2.63	3.03	0.7	0.42	2.9	0.92	0.42
<i>Hirsutella</i> sp.	0.32	4.2	0	0.52	0.17	0	0.7	0.35	0
<i>Beauveria bassiana</i>	1.83	0.42	3.45	0.85	0.42	3.08	3.15	0.77	0
<i>Metarhizium anisopliae</i>	7.7	0.4	5.77	0.43	0.98	1.97	1.6	1.17	2.12
<i>Paecilomyces</i> sp.	5.5	13.97	6.58	14.53	5.63	1.48	17.9	5.63	4.27

Note: RD = Recommendation dose

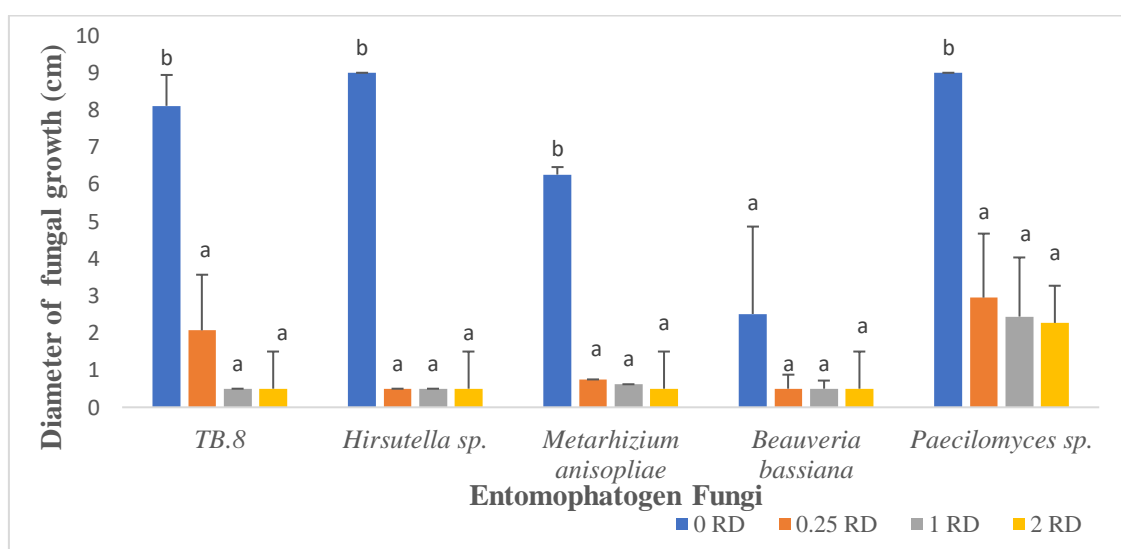


Figure 4. Vegetatif growth of entomopatogen fungi on different doses of Mancozeb fungicide (Mean followed by different letters indicate significant differences at DMRT p < 0.05)

Mancozeb displayed strong inhibition effects on the growth of all entomopathogenic fungal isolates used in this experiment (Figure 3). The diameter of fungal isolates treated with Mancozeb at those concentrations varies around 0.5-3 cm. The spore productions of these fungal isolates also were heavily affected by the additions of Mancozeb fungicide to the medium. In most entomopathogenic fungi, spore production was not observed even at the lowest concentrations of Mancozeb (Table 3). These results suggest that Mancozeb targets a wide range of fungal isolates. Therefore, using it in tandem with a fungal biological control agent may not be recommended [26]. This study showed that Mancozeb at 0.25-times, 1-time, and 2-times recommended doses are effective to inhibit the growth of various fungal isolates in the field, especially *B. bassiana*. Walia *et al* [27] also reported that the presence of Mancozeb at a minimum of 100 ppm on the medium is deleterious to fungal populations. According to [28], the effectiveness of Mancozeb is due to its ability to inhibit sporulation.

Table 3. The average of entomopathogenic fungi spore density development by Mancozeb fungicide treatment at 7, 14 and 21 days

Entomopatogen fungal isolates	Mean of spore density (10^6 spore/mL)								
	day 7			day 14			day 21		
	0.25 RD	1 RD	2 RD	0.25 RD	1 RD	2 RD	0.25 RD	1 RD	2 RD
<i>TB. 8</i>	0	0	0.47	1.03	0	0	3.88	0	0
<i>Hirsutella sp.</i>	0	0	0	0	0	0	0	0	0
<i>Beauveria bassiana</i>	0.9	0.53	1.08	2.26	0	0	0.32	0.55	0
<i>Metarhizium anisopliae</i>	0	0	0	0.37	0	0	0	0	0
<i>Paecilomyces sp.</i>	0.35	0.15	0.18	19.1	14.15	4.43	14.95	8.43	7.68

Note: RD = Recommendation dose

4. Conclusion

Pesticide plays an important role on the growth of entomopathogenic fungi isolated from citrus orchard. It affects the vegetative growth and sporulation of entomopathogenic fungi. All entomopathogenic fungi experienced the most severe impact on its growth due to the presence of Mancozeb, while the presence of Profenofos and Lambda-cyhalothrin did not adversely impact the growth of the fungi. These results suggest that the effect of pesticide on entomopathogenic fungi depended on the active ingredients and the species of target entomopathogenic fungi.

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