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Effect of Application of UV Irradiated *Beauveria bassiana* and *Metarhizium anisopliae* on Larval Weight and Mortality of *Spodoptera litura*

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ABSTRACT
Entomopathogenic fungi have been widely used to control insect pests. The objective of this experiment was to find out the insecticidal activity of filtrate of entomopathogenic fungal cultures exposed to ultra violet (UV) C against the larvae of *Spodoptera litura.* The fungi used were *Beauveria bassiana* and *Metarhizium anisopliae* and their liquid cultures exposed to UV C (5, 10, 15, 20, and 30 watts) for 6 hours. The results showed that the larva mortality caused by *B. bassiana* culture filtrate without irradiation was the highest (97.3%) and significantly different from those caused by *M. anisopliae* culture filtrate (96.0%). However, the mortality caused by *B. bassiana* culture filtrate irradiated with UV C decreased significantly compared to the mortality caused by *M. anisopliae* culture filtrate irradiated by UV C. LT_{50} of the filtrate of *M. anisopliae* culture irradiated with UV C was 10.51 days and was significantly shorter than those of *B. bassiana* (18.09 days). Thus, the *M. anisopliae* was more resistant to irradiation compared to *B. bassiana.*

Keywords: *Beauveria bassiana, insect pests, LT_{50},* Metarhizium anisopliae, *mortality*

1. INTRODUCTION

Freshwater swamps in Indonesia are about 9.2 Mha [1] and the land is flooded more than 6 months every year [2]. Such condition makes the soil can be planted only with specific crops that adapt to the wet conditions [3, 4]. In the dry season, farmers generally cultivate rice [5], while others cultivate several vegetables, such as cucumber, bitter-melon, yard long beans, ridge gourd [3], and chilli [6].

Chilli is a dominant vegetable crop cultivated in freshwater swamp. The main problem on chilli crop is pest attack such as *Spodoptera litura* [7], thrips [6, 8], and fruit flies [9]. An approach to reduce the population and pest attacks environmentally friendly and to produce healthy chili products is by utilizing a bio-control agents [10], *i.e* the use of entomopathogenic fungi. The entomopathogenic fungi have been known to be effective to various species of insect pests, such as *Beauveria bassiana* [11, 12] and *Metarhizium anisopliae* [13, 14]. *B. bassiana* culture filtrate can cause mortality up to 100% of *Spodoptera litura* larvae [11]. The effectiveness of culture filtrate is influenced by many environmental factors, hence further study needs to be conducted. The previous studies showed that light and sunlight, temperature, and humidity can affect the effectiveness of the fungi filtrate [15, 16].

Blazing sunlight can kill the fungi [17]. Short waves produced by ultraviolet (UV) radiation have been proven to reduce the viability of the entomopathogenic fungi conidia [18] and even kill the fungi [15]. The UV-B radiation at 6153.3 $mW \cdot m^{-2}$ exposed for 5 minutes could decrease the germination of *B. bassiana* and *M. anisopliae* conidia from 94% to 52% and 96% to 54%, respectively [17]. Radiation of UV-B at 978 $mW \cdot m^{-2}$ could cause several isolates of *B. bassiana* to be tolerant [19]. Thus, the tolerant isolates will be superior and can be developed and applied to the field. The aim of this research was to study the effect of an insecticidal activity of filtrate of entomopathogenic fungal cultures irradiated with ultra violet (UV) C on the larvae of *S. litura*.

1.1. Materials and Methods

This study was conducted at the Entomology Laboratory, Department of Pests and Plant Diseases, Faculty of Agriculture, Sriwijaya University from May to November 2018. The average temperature during the bioassay was 29.78ºC and relative humidity was 82.72%.
The isolates used in this study were explored by Safitri *et al*. (2018), namely *B. bassiana* with BSwTd2 code obtained from oil palm peat soil in Talang Dabok and *M. anisopliae*

coded MKbTp2 obtained from the highland cabbage soil in Talang Patai. Each isolate was treated with UV C irradiation with wavelengths ranged from 200-280 nm. Factorial Randomized Block Design with the first factor of 2 species of the fungi and the second factor of irradiation intensity was used. The mortality and weight data were presented in Tables 1-3.

1.1.1. Test insect preparation

The test insect in this experiment was *S. litura*. The larvae and eggs were collected from the synthetic pesticides-free chili crops in the experimental field of the Faculty of Agriculture, Sriwijaya University.The larvae were fed with chili leaves in a plastic cage (30 cm high x 25 cm in diameter) covered with gauze and the feed was replaced daily. When approaching the pupae stage, the last instar larvae were placed into a plastic cage containing 3 cm thick sterilized soil. Then, the pupae were transferred into an insect cage. The eggs of *S. litura* were collected by placing chilli plant. The eggs laid on the chilli leaves were transferred into the plastic cage which already provided with fresh chili leaves for feeding the newly hatched larvae. The mass rearing was carried out until getting a second generation of larvae. The third generation of the second 1-day-old instar was used as test insects in this experiment.

1.1.2. Preparation of the entomopathogenic fungi and production of culture filtrate

Sabouraud Dextrose Agar (SDA) medium enriched with *Tenebrio molitor* flour was used to increase fitness of *B. bassiana* and *M. anisopliae* isolates Herlinda method [10]. As many as 16.2 g of SDA medium was added with 250 ml of distilled water, then mixed with 1 g of *T. molitor* flour which had been sterilized at 100° C for 4 hours. Each culture isolate of 1 x 1 cm^2 of the 21 days-old SDA medium (Figure 1) was grown in SDB (Sabouraud Dextrose Broth) medium. The SDB medium was prepared in advance with as many as 30 g added 1000 ml distilled water. Then, the liquid culture (culture broth) fungi was incubated for 6 weeks (Figure 2). The culture broth for each isolate of the SDB medium was then filtered to separate the culture or supernatant filtrate from the pellets (hyphae, mycelia, and conidia/spore) through two stages using the Cheong method (2015). A total of 100 ml culture broth on SDB was filtered into the erlenmeyer flask (500 ml volume) using Whatman No. 42 filter paper covered with 1 cm thick cotton to produce ± 70 ml of crude culture filtrate. Then, the crude filtrate culture was filtered using a syringe filter (0.45 µm-25 mm). The filtering with a syringe filter was carried out by means of 1 ml of crude culture filtrate drawn using a hypodermic needle (volume 6 ml). The needle was removed and the base of the needle was attached to a syringe filter. Then, the needle was refitted to the hypodermic needle and the 1 ml of the crude filtrate was filtered using a syringe filter to obtain culture filtrate (Figure 3).

The culture filtrates were poured into Petri dish (9 cm in diameter). Then, each isolate was illuminated for 6 hours using UV C at 0, 5, 10, 15, 20, and 30 watts (= 0, 5000, 10000, 15000, 20000, and 30000 $mW.m^{-2}$), and control without the fungi (distilled water). The distance between the light source and the Petri dish was 12.5 cm (Figure 4).

1.1.3. Bioassays for assessing insecticidal activity of the culture filtrates

The irradiated culture filtrates were tested for their insecticidal activity against the second instar of *S. litura* larvae. As many as 5 pieces of chili leaves were dipped with the pure culture filtrate and then air-dried at room temperature. The air-dried chili leaves were put into a plastic cylinder whose top was covered with gauze (9.5 cm in diameter and 15.5 cm high), after that the 25 unfed larvae for 24 hours were introduced into the plastic cylinder. After 6 hours, the 25 larvae were transferred to another plastic cylinder containing 10 pieces of fresh leaves. Chilli leaves were replaced daily. The dead larvae were recorded and the larvae body was weighed every day for 13 days.

1.1.4. Data analysis

The larva mortality and weight data were analysed using analysis of variance (ANOVA) and presented in Tables 1-3. The Least Significant Difference (LSD) Test was employed to test for significant differences between treatments (isolates) at P = 0.05. LT_{50} values were calculated by using probit analysis. All data were analysed using SAS University Edition software 2.7 9.4 M5.

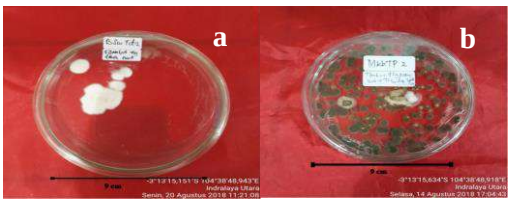

Figure 1 Agar culture of *Beauveria bassiana* (a) and *Metarhizium anisopliae* (b) in the SDA medium

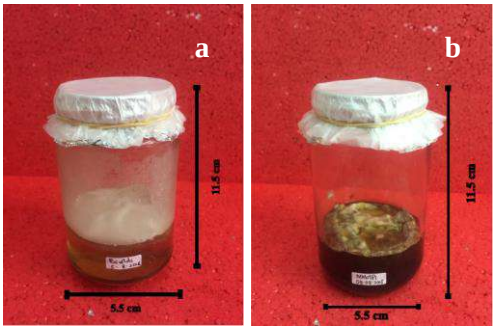

Figure 2 Broth culture of *Beauveria bassiana* (a) and *Metarhizium anisopliae* (b) in the SDB medium

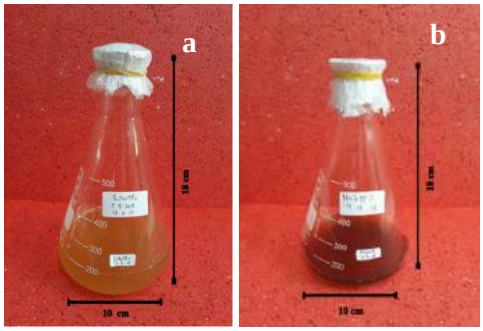

Figure 3 Culture filtrate of *Beauveria bassiana* (a) and *Metarhizium anisopliae* (b)

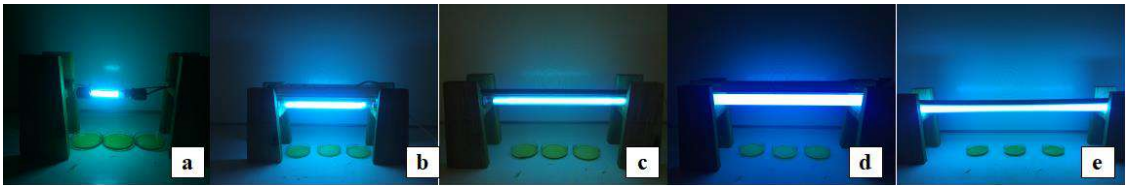

Figure 4 The radiation treatment 5 watts (a), 10 watts (b) , 15 watts (c), 20 watts (d), and 30 watts (e)

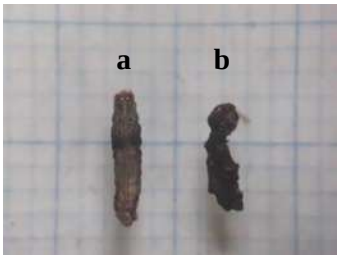

Figure 5 The healthy larvae of *Spodoptera litura* (a) and the dead one (b) caused by *Beauveria bassiana* culture filtrate (b)

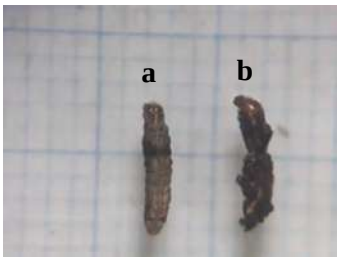

Figure 6 The healthy larvae of *Spodoptera litura* (a) and the dead one (b) caused by *Metarhizium anisopliae* culture filtrate

1.2. Our Contribution

This paper presents new information that the culture filtrate of *M. anisopliae* still caused the high mortality of *S. litura* larvae at the UV C irradiation of up to 30 watts for 6 hours. *M. anisopliae* could survive and was more tolerant of high irradiation intensity.

1.3. Paper Structure

The rest of the paper is organized as follows. Section 2 presents symptoms of *S. litura* larvae fed with the fungal culture-filtrate. Section 3 presents data of the effect of fungi on larvae weight. Section 4 shows the mortality of larvae and the time needed by 50% of dead larvae (LT_{50}) caused by fungal culture filtrate. Finally, Section 5 concludes the paper and presents direction for future research.

2. RESULTS AND DISCUSSION

The *S. litura* larvae fed with the culture-filtrate treated leaves showed similar symptoms. At a day after feeding, the movement of larvae was slower than larvae fed on the untreated leaves. The feeding activity of the larvae kept declining and the body began to shrink and dull. Two days later, the larvae bodies got shrivelled, wrinkled, hard, dry, increasingly dull and black, odourless and eventually died (Figures 5 and 6). Before the larvae died, they secreted green liquid. Such larvae then grown in SDA media, after 5 to 7 days there were found no hyphae, mycelia, or conidia of the fungal. Therefore, the death of the larvae did not cause by fungi.

The data of the effect of fungi on larvae weight showed that the weight was higher on that of the *M. anisopliae* treatment than the other treatments (Table 1). However, these data were higher because they were related to the initial weights of the larvae used in the *M. anisopliae* treatment, hence the data did not reflect the influence of fungal. The data showed that the older the larvae, the higher the weight were. Yet, after 11 days of application the larva weight began to decrease.

The intensity of irradiation (UV C) to the culture filtrate of fungi was significantly affected the larvae weight 13 days after fed with the culture-filtrate treated leaves. The culture filtrate without irradiation treatment (0 watt) caused the larvae weight to drop significantly compared to the control treatment (Table 2). The culture filtrate was illuminated with irradiation intensities from 5 to 30 watt resulting in the larval weight which was not significantly different from the control using the distilled water. However, there were no significant interactions found between the fungus species and irradiation intensity.

The fungi species significantly affected the mortality of larvae and the time needed by 50% of dead larvae (LT_{50}). The larvae mortality caused by the culture filtrate of *M. anisopliae* was significantly higher than that of *B. bassiana*. The LT_{50} caused by the culture filtrate of *M. anisopliae* were significantly shorter than that of *B. bassiana*. Thus, the culture filtrate of *M. anisopliae* was more effective in killing the *S. litura* larvae.

The intensity of the culture filtrate of the fungi irradiation significantly affected the mortality and LT_{50} larvae of *S. litura*. The fungal filtrate exposed to 0 watt irradiation intensity caused the highest larva mortality (96.66%) and was significant when compared to other treatments. In addition, LT_{50} was the shortest treatment (7.62 days) and was no significantly different from the other treatments.

Fungi species and radiation intensity significantly affected the mortality and LT_{50} larvae of *S. litura* (Table 3). *M. anisopliae* filtrate tended to be more tolerant of the intensity of irradiation when compared to *B. bassiana*, for example when exposed to 30 watts, the larvae mortality by *M. anisopliae* was 9.33%, whereas that by *B. bassiana* was only 4%. Likewise, the LT_{50} was affected by the species of fungi and irradiation intensity. For example, in 30-watt irradiation intensity, the LT_{50} larvae caused by *M. anisopliae* were shorter (17.67 days) than those caused by *B. bassiana* (42.66 days).

The *S. litura* larvae feeding on leaves applied to the culture filtrates of *B. bassiana* and *M. anisopliae* showed that the symptoms of the body got shrunken, contracted, dried, and odourless. According to Ayudya et al. (2019), the insect died due to the toxic compounds contained in the culture of filtrate fungi, not due to its conidia infection. The insects died due to the conidia fungi generally showed shrivelled and hard symptoms, and from the body of the host insects grew mycelia, hyphae, and conidia fungus on the surface of the insect integument [12], whereas in this study there were no mycelia, hyphae, and conidia fungus growing in the body of *S. litura*. Consequently, the *S. litura* larvae died due to the toxic compounds contained in the culture filtrate fungus.

The intensity of the culture filtrate irradiation significantly affected the larvae weight. The irradiation intensity of 0 watt caused the larvae weight to drop significantly due to the fact that the culture filtrate did not change so that it remained effective in reducing the larvae weight. However, if the culture filtrate was illuminated with an intensity of 5 to 30 watts, the larva weight was higher than that of 0 watt intensity. This higher larval weight indicated that the culture filtrate began to decrease in effectiveness. The culture filtrate was less able to reduce appetite of the larvae and they remained healthy with normal weight like those of treated with distilled water (control).

Although the irradiation of 5 to 30 watts against the culture filtrate of the fungi began to decrease the effectiveness of the fungi, the *M. anisopliae* was more tolerant of the UV C irradiation than *B. bassiana*. It is new information that the culture filtrate of *M. anisopliae* still caused the high mortality of *S. litura* larvae despite the UV C irradiation of up to 30 watts for 6 hours; the mortality still reached 9.33%. *M. anisopliae* could survive and was more tolerant of high irradiation intensity because this fungus had a darker pigment which was more resistant to UV light than

of the white fungus such as *B. bassiana* [20]. However, in the application of the entomopathogenic fungi in the field, it is still necessary to consider that the application should be carried out in the morning or evening when the sun does not shine brightly. The main problem in the utilization of the fungi is because of the low tolerance to sunlight.

Table 1 The effect of species fungi on larvae weight of *Spodoptera litura* larvae

Species of fungi	Larvae weight (g/larvae) after fungal filtrate UV treatment						
	1 day	3 days	5 days	7 days	9 days	11 days	13 days
Beauveria bassiana	0.30^{a}	0.58	1.08^{a}	1.64^{a}	4.42	3.14	3.01
Metarhizium anisopliae	0.38^{b}	0.59	1.19^{b}	1.81^{b}	4.31	3.28	3.53
ANOVA F-value	34.56*	0.27^{ns}	8.75*	5.61*	0.52^{ns}	0.28^{ns}	2.69^{ns}
P value (0.05)	0.001	0.61	0.01	0.02	0.47	0.60	0.11
LSD test	0.01	-	0.02	0.03	-	-	-

Note: * = significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to LSD test, ns = not significantly different

Table 2 The effect of the intensity of UV irradiation for fungal filtrate on larvae weight of *Spodoptera litura* larvae

Intensity of UV irradiation	Larvae weight (g/larvae) after UV fungal filtrate treatment						
	1 day	3 days	5 days	7 days	9 days	11 days	13 days
Control (aquadest)	0.33	0.61	1.11	1.66	4.51	3.24	3.59^{ab}
0 watt	0.34	0.54	1.08	1.70	4.76	3.34	1.39^{a}
5 watts	0.33	0.56	1.24	1.78	4.58	3.36	3.38^{ab}
10 watts	0.33	0.57	1.13	1.66	3.92	3.08	3.37^{ab}
15 watts	0.36	0.55	1.18	1.83	4.40	3.18	3.82^{ab}
20 watts	0.35	0.64	1.10	1.72	4.23	2.92	3.75^{ab}
30 watts	0.36	0.62	1.11	1.72	4.16	3.31	3.60^{ab}
ANOVA F-value	0.58^{ns}	0.78^{ns}	1.44^{ns}	0.42^{ns}	0.61^{ns}	0.20^{ns}	4.07*
P value (0.05)	0.74	0.59	0.23	0.853	0.72	0.973	0.01
LSD test	-	-	-	-	-	-	0.49

Note: * = significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to LSD test, ns = not significantly different

Table 3 The effect of fungal species and the intensity of UV irradiation for fungal filtrate on mortality and LT_{50} of *Spodoptera litura* larvae

Species of fungi x intensity of irradiation	Mortality (%)	LT_{50} (days)
Control (aquadest)	0^{a}	-
B. bassiana x 0 watts	97.33 ± 2.67^{hi}	6.41 ± 0.24^{ab}
B. bassiana x 5 watts	54.66 ± 1.33^{f}	12.16 ± 0.31^{bc}
B. bassiana x 10 watts	32.00 ± 2.31^{d}	16.95 ± 1.50^{bcd}
B. bassiana x 15 watts	30.66 ± 4.81^{d}	20.59 ± 3.35^{cd}
B. bassiana x 20 watts	12.00 ± 2.31^{c}	27.85 ± 16.37^{d}

Table 3 (continuation)

B. bassiana x 30 watts	4.00±2.31[b]	42.66±29.80[e]
Control (aquadest)	0[a]	-
M. anisopliae x 0 watts	96.66±4.00[h]	7.62±0.20[ab]
M. anisopliae x 5 watts	68.00±4.00[g]	9.24±0.34[abc]
M. anisopliae x 10 watts	50.67±35.3[ef]	10.98±0.32[bc]
M. anisopliae x 15 watts	38.66±5.81[de]	12.51±1.68[bc]
M. anisopliae x 20 watts	26.60±2.67[d]	15.58±1.40[bc]
M. anisopliae x 30 watts	9.33±2,67[c]	17.67±1.71[bcd]
ANOVA F-value	1.248*	4.300*
P value (0.05)	0.00	0.00
LSD test	5.25	11.56

Note: * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to LSD test, ns = not significantly different

3. CONCLUSION

Metarhizium anisopliae is more tolerant of irradiation compared to *B. bassiana*. This implies that *M. anisopliae* has more potential to survive in agroecosystems with relatively more intense sunlight such as in the tropical lowlands such as freshwater swamps.

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Peter Hendriks is a senior publishing professional with broad experience in both professional and scientific publishing. He obtained an MBA from the University of Groningen after which he joined Wolters Kluwer in 1987 as a Management Trainee. In 1991 he became Publisher and later Business Unit Director at Kluwer Professional Netherlands, and in 1999 he joined Kluwer Academic Publishers, initially as Vice President U.S., and was later appointed as CEO & President in 2001. Kluwer Academic Publishers was then bought by private equity investors and merged with Springer in 2003 where Peter became a member of the Springer Executive Board in different roles for the next 13 years. He left what had by then become Springer Nature in 2016 after which he took up a number of supervisory and advisory board positions in different companies (including Atlantis Press). From April 2017 to December 2018, he served as CEO of

Press). From April 2017 to December 2018, he served as CEO Dutch educational publisher Malmberg which belongs to th Sanoma media group. Since then he has become Partner & C.E.O at 227 Search and continues to act as Advisory Board Member for several companies.

Charles Chui

Charles Chui, Ph.D. Wisconsin-Madison, is Research Professor of Mathematics at Hong Kong Baptist University and Consulting Professor of Statistics at Stanford University. He is also Curators' Professor Emeritus of the University of Missouri and Distinguished Professor Emeritus of Texas A&M University, where he had joint appointments in four departments and two colleges, namely the Department of Mathematics and Department of Statistics (College of Science) and the Department of Electrical Engineering and Department of Computer Science (College of Engineering). His current research interest is in Computational and Applied Mathematics with a focus on real-world data processing, visualization, and understanding for big data areas such as blind source decomposition and feature extractions of time series, medical images, surveillance videos and high-dimensional complex data on unknown manifolds of much lower dimensions. After spending over two decades of dedicated research in Function Theory, Approximation Theory, Harmonic Analysis and Computational Mathematics, he turned his attention to the applications of mathematics, particularly in solving real-world problems, first by working on medical imaging in collaboration with a team of radiologists and physicists in MD Anderson Cancer Center in Houston, followed by founding his first company in Silicon Valley in California based on his expertise in image compression and manipulation.

Frank van Harmelen

Frank van Harmelen is a Professor in Knowledge Representation & Reasoning in the AI department (Faculty of Science) at the Vrije Universiteit Amsterdam. After studying mathematics and computer science in Amsterdam, he moved to the Department of AI in Edinburgh, where he was awarded a PhD in 1989 for his research on meta-level reasoning. While in Edinburgh, he worked with Dr. Peter Jackson on Socrates, a logic-based toolkit for expert systems, and with Prof. Alan Bundy on proof planning for inductive theorem proving. After his PhD research, he moved back to Amsterdam where he worked from 1990 to 1995 in the SWI Department under Prof. Wielinga. He was involved in the REFLECT project on the use of reflection in expert systems, and in the KADS project, where he contributed to the development of the $(ML)^2$ language for formally specifying Knowledge-Based Systems. In 1995 he joined the AI research group at the Vrije Universiteit Amsterdam, where he was appointed Professor in 2002, and is currently leading the Knowledge Representation & Reasoning Group.

Chongfu Huang

Chongfu Huang is a full Professor at Beijing Normal University and President of the Society for Risk Analysis - China. He received his B.A.Sc. in Mathematics from Yunnan University, Kunming, China; his M.A.Sc. in Earthquake Engineering from the Institute of Engineering Mechanics, Harbin, China; and his Ph.D. in Applied Mathematics from Beijing Normal University. He worked at the Chinese University of Hong Kong as a Research Associate, and at Tokyo University of Science as an Associate Professor in 1996. As a visiting Professor, he worked

at the University of Ghent in Belgium in 1997 and at the University Nebraska in Omaha in 2000. From 2000 to 2001,

was a Mercator Professor and worked at the University of Dortmund in Germany. As a visiting Professor, he worked again at Tokyo University of Science and at the University of Ghent in Belgium in 2004 and 2006 respectively.

Jie Lu

Jie Lu is Professor and Head of the School of Software in the Faculty of Engineering and Information Technology, as well as Director of the Decision Systems and e-Service Intelligence Research Laboratory in the Centre for Quantum Computation & Intelligent Systems, at the University of Technology Sydney (UTS) in Australia. She received her PhD from Curtin University of Technology in Western Australia in 2000. Her main research interests lie in the area of computational intelligence systems, decision support systems, uncertain information processing, recommender systems and e-Government and e-Service intelligence. She has published five research books and 300 articles in academic journals, including IEEE Transactions on Fuzzy Systems, DSS and Information Systems, and various conference proceedings, and has won five Australian Research Council (ARC) discovery grants, an Australian Learning & Teaching Council grant and 10 other research and industry linkage grants. She also received the first UTS Research Excellence Medal for Teaching and Research Integration in 2010.

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Zeger Karssen

Zeger Karssen is the founder and President of the Atlantis Press group. He holds degrees in both Philosophy and Artificial Intelligence from the University of Amsterdam and since then has gained extensive experience in the fields of scientific research, publishing and internet technology. Earlier occupations include work as a Senior Researcher in Artificial Intelligence for the University of Amsterdam and for a private lab in Paris. Since 2000, he worked as a Publisher for industry-leading scientific publishing house Elsevier where he managed a portfolio of 15 internationally renowned journals in the field of Artificial Intelligence and launched four new journals as well as two major book series. Zeger founded Atlantis Press in 2006 and manages the company from its offices in Paris and Amsterdam. Within the company he acts as Publishing Director for all product portfolios as well as Publisher in the field of Artificial Intelligence. Besides this, Zeger has also worked as advisor for the European Commission and is currently Associate Professor

for Digital Publishing at the Free University of Brussels (UL

Remco de Boer

Remco de Boer is Chief Executive Officer (CEO) of the Atlantis Press group. He holds degrees in Artificial Intelligence & Computer Science (both cum laude - with distinction) from the University of Amsterdam. Before joining Atlantis Press, he spent 16 years in various roles across publishing, media, consulting and financial services, of which 9 years in Asia (Hong Kong & Indonesia) and the Middle East (Dubai). In academic publishing, he held positions at Elsevier/RELX as Editorial Office Manager in Health Sciences, Publisher for a portfolio in mathematics, Business Analyst for Science & Technology Books and most recently as Executive Publisher heading Elsevier's International Journals & Partnerschip Publishing division for Europe, Middle East & Africa. In between, he worked as Senior Strategy & Management Consultant for different industries and as Chief Strategy Officer for IBG Consulting Group in Hong Kong. He also spent 3 years in banking as International Manager for HSBC and Regional Head of Product Management for HSBC's Payments & Cash Management division in Middle East & North Africa based in Dubai. Remco joined Atlantis Press in January 2018 as CEO of Atlantis Press International, the AP subsidiary which is focused on journals, publishing services and consultancy services. He was subsequently promoted to Group CEO in May 2020.

Ran Dang

Ran Dang is Managing Director of Atlantis Press China. She is responsible for strategic and day-to-day management of acquisitions and publishing activities in Greater China, with

particular focus on development of the proceedings and jou portfolios, as well as on providing publishing services to societies and institutions. Ran holds a Bachelor's degree in Animal Science from Henan Agricultural University (Zhengzhou, China), a Master's degree in Biological Technologies and Engineering from Ningbo University (Ningbo, China) and a Master's degree in Biochemistry and Molecular Biology from Zhejiang University (Hangzhou, China). Prior to joining Atlantis Press she gained 6 years of experience in academic publishing, including Senior Managing Editor & Section Leader at MDPI, Publishing Support Manager at Elsevier and Project/General Manager for MLS Journals. Ran joined Atlantis Press as Junior Publisher & Promotion Manager in September 2018, aiming to develop the AP proceedings series in China and to play a local supporting role for the expansion of the AP footprint in China across all product portfolios. She was promoted in May 2019 to manage the new Atlantis Press China office.

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IT & PROJECTS TEAM

With the increasing importance of data and technology in the STM publishing industry in particular, Atlantis Press over the years gradually transformed into an IT-driven publisher with a highly skilled team of IT and product specialists. Please meet our IT & projects team below.

Gerard van Helden

Gerard van Helden is the Head of IT Development for Atlantis Press responsible for planning and implementation of the IT development and maintenance strategy as well as managing the wider team of IT developers spread across multiple locations (soon to be expanded to Chennai, India). He has been working as an IT developer and manager since 2000 mainly developing web applications, a ticketing system for football clubs and cultural/theatrical organizations, and generally managing IT teams for several start-up as well as scale-up companies. Gerard's core expertise is full-stack developer with a strong focus on agile software solutions. Prior to joining Atlantis Press he was senior full-stack developer (Java, Python, TypeScript, ReactJSX) for the Dutch government. He subsequently joined the Atlantis Press IT team in September 2018 and was promoted to Head of IT Development in March

2018 and was promoted to Head of IT Development in March 2020.

Martijn Vogten

Martijn Vogten is a cloud specialist and Senior Software Engineer in the Atlantis Press IT & projects team responsible for system architecture design and 24/7 IT support. After obtaining a Master's degree in Applied Physics at Delft University in The Netherlands, Martijn started his IT career at one of the leading Dutch software companies where he developed web applications with Java and JavaScript. Since 2005 he has been active as an independent software engineer and systems architect for a number of leading brands as well as a web application startup. His core expertise is in the design of highly available and highly scalable systems. Martijn joined the Atlantis Press IT team in early 2017 and works from Delft in The Netherlands.

Debora Woinke

Debora Woinke is Product Manager in the Atlantis Press IT & projects team responsible for project management, testing, roll-out and commer-cialization of new AP products, technical features and processes. After obtaining a degree in Art History, Debora graduated in Sciences and Technologies of Information and Communication at the Free University of Brussels (ULB) in Belgium. Since then she worked as freelance translator, copy editor and IT advisor, and in 2017 founded a small publishing company specialized in art books. Debora joined Atlantis Press in 2016 where she initially managed the production of proceedings and books and was also responsible for indexation, archiving and setting up communication strategies for new

publishing projects. She was promoted to Junior Publisher
the Atlantis Press proprietary journals portfolio in early 201

and moved into her current role as Product Manager in late 2019 based in Brussels, Belgium.

Inge Balqis

Inge Balqis is Project Assistant in the Atlantis Press IT & projects team responsible for providing administrative support to ongoing projects. After attending international schools in Dubai (Star International School) and Indonesia (Canggu Community School), she obtained her International Baccalaureate (IB) from Amsterdam International Community School in The Netherlands in June 2019. She currently studies at Erasmus University Rotterdam where she attends a Bachelor's program in Liberal Arts & Sciences with a specialization in the Life Sciences. Inge joined Atlantis Press in her current role as Project Assistant in January 2020 and is based in Rotterdam, The Netherlands.

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Effect of Application of UV Irradiated Beauveria bassiana and Metarhizium anisopliae on Larval Weight and Mortality of Spodoptera litura

By Arum Setiawan

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Advances in Biological Sciences Research, volume 8

International Conference and the 10th Congress of the Entomological Society of Indonesia (ICCESI 2019)

Effect of Application of UV Irradiated *Beauveria bassiana* and *Metarhizium anisopliae* on Larval Weight and Mortality of *Spodoptera litura*

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ABSTRACT

Entomopathogenic fungi have been widely used to control insect pests. The objective of this experiment was to find out the insecticidal activity of filtrate of entomopathogenic fungal cultures exposed to ultra violet (UV) C against the larvae of *Spodoptera litura*. The fungi used were *Beauveria bassiana* and *Metarhizium anisopliae* and their liquid cultures exposed to UV C (5, 10, 15, 20, and 30 watts) for 6 hours. The results showed that the larva mortality caused by *B. bassiana* culture filtrate without irradiation was the highest (97.3%) and significantly different from those caused by *M. anisopliae* culture filtrate (96.0%). However, the mortality caused by *B. bassiana* culture filtrate irradiated with UV C decreased significantly compared to the mortality caused by *M. anisopliae* culture filtrate irradiated by UV C. LT_{50} of the filtrate of *M. anisopliae* culture irradiated with UV C was 10.51 days and was significantly shorter than those of *B. bassiana* (18.09 days). Thus, the *M. anisopliae* was more resistant to irradiation compared to *B. bassiana*.

Keywords: *Beauveria bassiana, insect pests, LT_{50},* Metarhizium anisopliae, *mortality*

1. INTRODUCTION

Freshwater swamps in Indonesia are about 9.2 Mha [1] and the land is flooded more than 6 months every year [2]. Such condition makes the soil can be planted only with specific crops that adapt to the wet conditions [3, 4]. In the dry season, farmers generally cultivate rice [5], while others cultivate several vegetables, such as cucumber, bitter-melon, yard long beans, ridge gourd [3], and chilli [6].

Chilli is a dominant vegetable crop cultivated in freshwater swamp. The main problem on chilli crop is pest attack such as *Spodoptera litura* [7], thrips [6, 8], and fruit flies [9]. An approach to reduce the population and pest attacks environmentally friendly and to produce healthy chili products is by utilizing a bio-control agents [10], *i.e* the use of entomopathogenic fungi. The entomopathogenic fungi have been known to be effective to various species of insect pests, such as *Beauveria bassiana* [11, 12] and *Metarhizium anisopliae* [13, 14]. *B. bassiana* culture filtrate can cause mortality up to 100% of *Spodoptera litura* larvae [11]. The effectiveness of culture filtrate is influenced by many environmental factors, hence further study needs to be conducted. The previous studies showed that light and sunlight, temperature, and humidity can affect the effectiveness of the fungi filtrate [15, 16].

Blazing sunlight can kill the fungi [17]. Short waves produced by ultraviolet (UV) radiation have been proven to reduce the viability of the entomopathogenic fungi conidia [18] and even kill the fungi [15]. The UV-B radiation at 6153.3 $mW \cdot m^{-2}$ exposed for 5 minutes could decrease the germination of *B. bassiana* and *M. anisopliae* conidia from 94% to 52% and 96% to 54%, respectively [17]. Radiation of UV-B at 978 $mW \cdot m^{-2}$ could cause several isolates of *B. bassiana* to be tolerant [19]. Thus, the tolerant isolates will be superior and can be developed and applied to the field. The aim of this research was to study the effect of an insecticidal activity of filtrate of entomopathogenic fungal cultures irradiated with ultra violet (UV) C on the larvae of *S. litura*.

1.1. Materials and Methods

This study was conducted at the Entomology Laboratory, Department of Pests and Plant Diseases, Faculty of Agriculture, Sriwijaya University from May to November 2018. The average temperature during the bioassay was 29.78°C and relative humidity was 82.72%. The isolates used in this study were explored by Safitri *et al.* (2018), namely *B. bassiana* with BSwTd2 code obtained from oil palm peat soil in Talang Dabok and *M. anisopliae*

coded MKbTp2 obtained from the highland cabbage soil in Talang Patai. Each isolate was treated with UV C irradiation with wavelengths ranged from 200-280 nm. Factorial Randomized Block Design with the first factor of 2 species of the fungi and the second factor of irradiation intensity was used. The mortality and weight data were presented in Tables 1-3.

1.1.1. Test insect preparation

The test insect in this experiment was *S. litura*. The larvae and eggs were collected from the synthetic pesticides-free chili crops in the experimental field of the Faculty of Agriculture, Sriwijaya University.The larvae were fed with chili leaves in a plastic cage (30 cm high x 25 cm in diameter) covered with gauze and the feed was replaced daily. When approaching the pupae stage, the last instar larvae were placed into a plastic cage containing 3 cm thick sterilized soil. Then, the pupae were transferred into an insect cage. The eggs of *S. litura* were collected by placing chilli plant. The eggs laid on the chilli leaves were transferred into the plastic cage which already provided with fresh chili leaves for feeding the newly hatched larvae. The mass rearing was carried out until getting a second generation of larvae. The third generation of the second 1-day-old instar was used as test insects in this experiment.

1.1.2. Preparation of the entomopathogenic fungi and production of culture filtrate

Sabouraud Dextrose Agar (SDA) medium enriched with *Tenebrio molitor* flour was used to increase fitness of *B. bassiana* and *M. anisopliae* isolates Herlinda method [10]. As many as 16.2 g of SDA medium was added with 250 ml of distilled water, then mixed with 1 g of *T. molitor* flour which had been sterilized at 100° C for 4 hours. Each culture isolate of 1 x 1 cm^2 of the 21 days-old SDA medium (Figure 1) was grown in SDB (Sabouraud Dextrose Broth) medium. The SDB medium was prepared in advance with as many as 30 g added 1000 ml distilled water. Then, the liquid culture (culture broth) fungi was incubated for 6 weeks (Figure 2). The culture broth for each isolate of the SDB medium was then filtered to separate the culture or supernatant filtrate from the pellets (hyphae, mycelia, and conidia/spore) through two stages using the Cheong method (2015). A total of 100 ml culture broth on SDB was filtered into the erlenmeyer flask (500 ml volume) using Whatman No. 42 filter paper covered with 1 cm thick cotton to produce ± 70 ml of crude culture filtrate. Then, the crude filtrate culture was filtered using a syringe filter (0.45 μm-25 mm). The filtering with a syringe filter was carried out by means of 1 ml of crude culture filtrate drawn using a hypodermic needle (volume 6 ml). The needle was removed and the base of the needle was attached to a syringe filter. Then, the needle was refitted to the hypodermic needle and the 1 ml of the crude filtrate was filtered using a syringe filter to obtain culture filtrate (Figure 3).

The culture filtrates were poured into Petri dish (9 cm in diameter). Then, each isolate was illuminated for 6 hours using UV C at 0, 5, 10, 15, 20, and 30 watts (= 0, 5000, 10000, 15000, 20000, and 30000 $mW.m^{-2}$), and control without the fungi (distilled water). The distance between the light source and the Petri dish was 12.5 cm (Figure 4).

1.1.3. Bioassays for assessing insecticidal activity of the culture filtrates

The irradiated culture filtrates were tested for their insecticidal activity against the second instar of *S. litura* larvae. As many as 5 pieces of chili leaves were dipped with the pure culture filtrate and then air-dried at room temperature. The air-dried chili leaves were put into a plastic cylinder whose top was covered with gauze (9.5 cm in diameter and 15.5 cm high), after that the 25 unfed larvae for 24 hours were introduced into the plastic cylinder. After 6 hours, the 25 larvae were transferred to another plastic cylinder containing 10 pieces of fresh leaves. Chilli leaves were replaced daily. The dead larvae were recorded and the larvae body was weighed every day for 13 days.

1.1.4. Data analysis

The larva mortality and weight data were analysed using analysis of variance (ANOVA) and presented in Tables 1-3. The Least Significant Difference (LSD) Test was employed to test for significant differences between treatments (isolates) at P = 0.05. LT_{50} values were calculated by using probit analysis. All data were analysed using SAS University Edition software 2.7 9.4 M5.

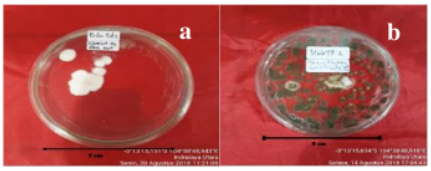

Figure 1 Agar culture of *Beauveria bassiana* (a) and *Metarhizium anisopliae* (b) in the SDA medium

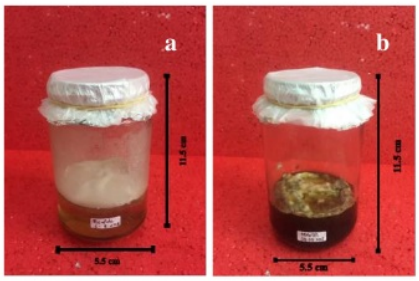

Figure 2 Broth culture of *Beauveria bassiana* (a) and *Metarhizium anisopliae* (b) in the SDB medium

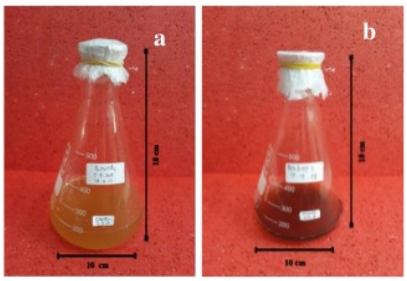

Figure 3 Culture filtrate of *Beauveria bassiana* (a) and *Metarhizium anisopliae* (b)

Figure 4 The radiation treatment 5 watts (a), 10 watts (b) , 15 watts (c), 20 watts (d), and 30 watts (e)

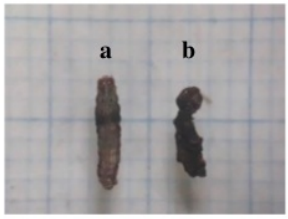

Figure 5 The healthy larvae of *Spodoptera litura* (a) and the dead one (b) caused by *Beauveria bassiana* culture filtrate (b)

Figure 6 The healthy larvae of *Spodoptera litura* (a) and the dead one (b) caused by *Metarhizium anisopliae* culture filtrate

1.2. Our Contribution

This paper presents new information that the culture filtrate of *M. anisopliae* still caused the high mortality of *S. litura* larvae at the UV C irradiation of up to 30 watts for 6 hours. *M. anisopliae* could survive and was more tolerant of high irradiation intensity.

1.3. Paper Structure

The rest of the paper is organized as follows. Section 2 presents symptoms of *S. litura* larvae fed with the fungal culture-filtrate. Section 3 presents data of the effect of fungi on larvae weight. Section 4 shows the mortality of larvae and the time needed by 50% of dead larvae (LT_{50}) caused by fungal culture filtrate. Finally, Section 5 concludes the paper and presents direction for future research.

2. RESULTS AND DISCUSSION

The *S. litura* larvae fed with the culture-filtrate treated leaves showed similar symptoms. At a day after feeding, the movement of larvae was slower than larvae fed on the untreated leaves. The feeding activity of the larvae kept declining and the body began to shrink and dull. Two days later, the larvae bodies got shrivelled, wrinkled, hard, dry, increasingly dull and black, odourless and eventually died (Figures 5 and 6). Before the larvae died, they secreted green liquid. Such larvae then grown in SDA media, after 5 to 7 days there were found no hyphae, mycelia, or conidia of the fungal. Therefore, the death of the larvae did not cause by fungi.

The data of the effect of fungi on larvae weight showed that the weight was higher on that of the *M. anisopliae* treatment than the other treatments (Table 1). However, these data were higher because they were related to the initial weights of the larvae used in the *M. anisopliae* treatment, hence the data did not reflect the influence of fungal. The data showed that the older the larvae, the higher the weight were. Yet, after 11 days of application the larva weight began to decrease.

The intensity of irradiation (UV C) to the culture filtrate of fungi was significantly affected the larvae weight 13 days after fed with the culture-filtrate treated leaves. The culture filtrate without irradiation treatment (0 watt) caused the larvae weight to drop significantly compared to the control treatment (Table 2). The culture filtrate was illuminated with irradiation intensities from 5 to 30 watt resulting in the larval weight which was not significantly different from the control using the distilled water. However, there were no significant interactions found between the fungus species and irradiation intensity.

The fungi species significantly affected the mortality of larvae and the time needed by 50% of dead larvae (LT_{50}). The larvae mortality caused by the culture filtrate of *M. anisopliae* was significantly higher than that of *B. bassiana*. The LT_{50} caused by the culture filtrate of *M. anisopliae* were significantly shorter than that of *B. bassiana*. Thus, the culture filtrate of *M. anisopliae* was more effective in killing the *S. litura* larvae.

The intensity of the culture filtrate of the fungi irradiation significantly affected the mortality and LT_{50} larvae of *S. litura*. The fungal filtrate exposed to 0 watt irradiation intensity caused the highest larva mortality (96.66%) and was significant when compared to other treatments. In addition, LT_{50} was the shortest treatment (7.62 days) and was no significantly different from the other treatments.

Fungi species and radiation intensity significantly affected the mortality and LT_{50} larvae of *S. litura* (Table 3). *M. anisopliae* filtrate tended to be more tolerant of the intensity of irradiation when compared to *B. bassiana*, for example when exposed to 30 watts, the larvae mortality by *M. anisopliae* was 9.33%, whereas that by *B. bassiana* was only 4%. Likewise, the LT_{50} was affected by the species of fungi and irradiation intensity. For example, in 30-watt irradiation intensity, the LT_{50} larvae caused by *M. anisopliae* were shorter (17.67 days) than those caused by *B. bassiana* (42.66 days).

The *S. litura* larvae feeding on leaves applied to the culture filtrates of *B. bassiana* and *M. anisopliae* showed that the symptoms of the body got shrunken, contracted, dried, and odourless. According to Ayudya et al. (2019), the insect died due to the toxic compounds contained in the culture of filtrate fungi, not due to its conidia infection. The insects died due to the conidia fungi generally showed shrivelled and hard symptoms, and from the body of the host insects grew mycelia, hyphae, and conidia fungus on the surface of the insect integument [12], whereas in this study there were no mycelia, hyphae, and conidia fungus growing in the body of *S. litura*. Consequently, the *S. litura* larvae died due to the toxic compounds contained in the culture filtrate fungus.

The intensity of the culture filtrate irradiation significantly affected the larvae weight. The irradiation intensity of 0 watt caused the larvae weight to drop significantly due to the fact that the culture filtrate did not change so that it remained effective in reducing the larvae weight. However, if the culture filtrate was illuminated with an intensity of 5 to 30 watts, the larva weight was higher than that of 0 watt intensity. This higher larval weight indicated that the culture filtrate began to decrease in effectiveness. The culture filtrate was less able to reduce appetite of the larvae and they remained healthy with normal weight like those of treated with distilled water (control).

Although the irradiation of 5 to 30 watts against the culture filtrate of the fungi began to decrease the effectiveness of the fungi, the *M. anisopliae* was more tolerant of the UV C irradiation than *B. bassiana*. It is new information that the culture filtrate of *M. anisopliae* still caused the high mortality of *S. litura* larvae despite the UV C irradiation of up to 30 watts for 6 hours; the mortality still reached 9.33%. *M. anisopliae* could survive and was more tolerant of high irradiation intensity because this fungus had a darker pigment which was more resistant to UV light than

of the white fungus such as *B. bassiana* [20]. However, in the application of the entomopathogenic fungi in the field, it is still necessary to consider that the application should be carried out in the morning or evening when the sun does not shine brightly. The main problem in the utilization of the fungi is because of the low tolerance to sunlight.

Table 1 The effect of species fungi on larvae weight of *Spodoptera litura* larvae

Species of fungi	Larvae weight (g/larvae) after fungal filtrate UV treatment						
	1 day	3 days	5 days	7 days	9 days	11 days	13 days
Beauveria bassiana	0.30[a]	0.58	1.08[a]	1.64[a]	4.42	3.14	3.01
Metarhizium anisopliae	0.38[b]	0.59	1.19[b]	1.81[b]	4.31	3.28	3.53
ANOVA F-value	34.56*	0.27[ns]	8.75*	5.61*	0.52[ns]	0.28[ns]	2.69[ns]
P value (0.05)	0.001	0.61	0.01	0.02	0.47	0.60	0.11
LSD test	0.01	-	0.02	0.03	-	-	-

Note: * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to LSD test, ns = not significantly different

Table 2 The effect of the intensity of UV irradiation for fungal filtrate on larvae weight of *Spodoptera litura* larvae

Intensity of UV irradiation	Larvae weight (g/larvae) after UV fungal filtrate treatment						
	1 day	3 days	5 days	7 days	9 days	11 days	13 days
Control (aquadest)	0.33	0.61	1.11	1.66	4.51	3.24	3.59[ab]
0 watt	0.34	0.54	1.08	1.70	4.76	3.34	1.39[a]
5 watts	0.33	0.56	1.24	1.78	4.58	3.36	3.38[ab]
10 watts	0.33	0.57	1.13	1.66	3.92	3.08	3.37[ab]
15 watts	0.36	0.55	1.18	1.83	4.40	3.18	3.82[ab]
20 watts	0.35	0.64	1.10	1.72	4.23	2.92	3.75[ab]
30 watts	0.36	0.62	1.11	1.72	4.16	3.31	3.60[ab]
ANOVA F-value	0.58[ns]	0.78[ns]	1.44[ns]	0.42[ns]	0.61[ns]	0.20[ns]	4.07*
P value (0.05)	0.74	0.59	0.23	0.853	0.72	0.973	0.01
LSD test	-	-	-	-	-	-	0.49

Note: * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to LSD test, ns = not significantly different

Table 3 The effect of fungal species and the intensity of UV irradiation for fungal filtrate on mortality and LT_{50} of *Spodoptera litura* larvae

Species of fungi x intensity of irradiation	Mortality (%)	LT_{50} (days)
Control (aquadest)	0[a]	-
B. bassiana x 0 watts	97.33±2.67[hi]	6.41±0.24[ab]
B. bassiana x 5 watts	54.66±1.33[f]	12.16±0.31[bc]
B. bassiana x 10 watts	32.00±2.31[d]	16.95±1.50[bcd]
B. bassiana x 15 watts	30.66±4.81[d]	20.59±3.35[cd]
B. bassiana x 20 watts	12.00±2.31[c]	27.85±16.37[d]

Table 3 (continuation)

B. bassiana x 30 watts	4.00±2.31[b]	42.66±29.80[e]
Control (aquadest)	0[a]	-
M. anisopliae x 0 watts	96.66±4.00[h]	7.62±0.20[ab]
M. anisopliae x 5 watts	68.00±4.00[g]	9.24±0.34[abc]
M. anisopliae x 10 watts	50.67±35.3[ef]	10.98±0.32[bc]
M. anisopliae x 15 watts	38.66±5.81[de]	12.51±1.68[bc]
M. anisopliae x 20 watts	26.60±2.67[d]	15.58±1.40[bc]
M. anisopliae x 30 watts	9.33±2,67[c]	17.67±1.71[bcd]
ANOVA F-value	1.248*	4.300*
P value (0.05)	0.00	0.00
LSD test	5.25	11.56

Note: * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to LSD test, ns = not significantly different

3. CONCLUSION

Metarhizium anisopliae is more tolerant of irradiation compared to *B. bassiana*. This implies that *M. anisopliae* has more potential to survive in agroecosystems with relatively more intense sunlight such as in the tropical lowlands such as freshwater swamps.

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Judul Karya Ilmiah : Effect of Application of UV Irradiated Beauveria bassiana and Metarhizium anisopliae on Larval Weight and Mortality of Spodoptera litura

Jumlah Penulis : Siti Herlinda, Sangkut Sri Oktareni, Suparman, Erise Anggraini, Elfita, **Arum Setiawan**, Marieska Verawaty, Hasbi, Benyamin Lakitan

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Jumlah Penulis : S Herlinda, A J Fajriah, Suparman, E Anggraini, Elfita, **A Setiawan**, M Verawaty, Hasbi and Arsi

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☐ Prosiding Forum Ilmiah Nasional
☐ Makalah tidak disajikan dalam seminar/symposium.lokakarya, tetapi dimuat dalam prosiding internasional
☐ Makalah tidak disajikan dalam seminar/symposium.lokakarya, tetapi dimuat dalam prosiding nasional
☐ Makalah disajikan dalam seminar internasional (Tetapi tidak dimuat dalam prosiding)
☐ Makalah disajikan dalam seminar nasional (Tetapi tidak dimuat dalam prosiding)

I. Hasil Penilaian Validasi:

No.	ASPEK	URAIAN/KOMENTAR PENILAIAN
1	Indikasi Plagiasi	3 %
2	Linieritas	V

II. Hasil Penilaian *Peer Review*:

Komponen Yang Dinilai	Nilai Maksimal Jurnal Ilmiah (Isikan di kolom yang sesuai)						Nilai Akhir Yang DIperoleh
	Prosiding Forum Ilmiah Internasional (Maks. 30)	Prosiding Forum Ilmiah Nasional (Maks. 10)	Makalah TIdak diseminarkan tetapi dimuat dalam prosiding internasional (Maks. 10)	Makalah TIdak diseminarkan tetapi dimuat dalam prosiding nasional (Maks. 5)	Makalah disajikan dalam seminar internasional (Tetapi tidak dimuat dalam prosiding) (Maks. 5)	Makalah disajikan dalam seminar nasional (Tetapi tidak dimuat dalam prosiding) (Maks. 3)	
Kelengkapan dan Kesesuaian unsur isi paper (10 %)	3						3
Ruang Lingkup dan kedalaman pembahasan (30 %)	9						9
Kecukupan dan Kemutahiran data/Informasi dan metodologi (30 %)	9						8
Kelengkapan unsur dan Kualitas penerbit / prosiding (30 %)	9						8
Total = (100 %)	30						28
Kontribusi Pengusul (Penulis Pertama/Anggota Utama)	IOP Conference Series: Earth and Environmental Science, Southeast Asia Plant Protection Conference 2019 (**Scopus**). Volume: 468(1): halaman1-8 Penulis ke 7 dari 9 penulis. Nilai maksimal 93,3%. Nilai Pengusul: (0,4 x 0,93 x 30)/8: 1,4						

KOMENTAR/ULASAN PEER REVIEW	
- Kelengkapan dan Kesesuaian Unsur	Format lengkap, ada abstrak hingga referensi.
- Ruang Lingkup dan Kedalaman Pembahasan	Masih dalam lingkup bidang ilmu. Pembahasan cukup mendetail dan jelas.
- Kecukupan & Kemutakhiran Data & Metodologi	Data cukup banyak dan jelas. Metode yang digunakan sudah umum.
- Kelengkapan Unsur & Kualitas Penerbit	ISBN/ISSN 1755-1315 Penerbit Jur. Perlindungan Tanaman Fak. Pertanian IPB. Berkualitas.

Yogyakarta, 6 Juli 2020

Penilai 2

tanda tangan .....

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Jabatan/Pangkat : Guru Besar/ Pembina Utama Madya/IVd