

Insecticidal activity of entomopathogenic fungi and neem-based biopesticide (*Azadirachta indica*) on *Spodoptera litura*

Ade Aliyya¹, Apriwi Zulfitri^{1*}, Umi Zakiah², Arief Heru Prianto¹, Titik Kartika¹, Nova Hariani², Demi Zulfiana¹, and Ikhsan Guswenrivo^{1*}

¹Research Center for Biomaterials, Indonesian Institute of Sciences, Indonesia

²Faculty of Mathematics and Natural Sciences, Mulawarman University, Indonesia

Abstract. Chemical pesticide has been used extensively to control *Spodoptera litura* that attack economically important crops. The use of synthetic pesticides with high intensity is detrimental to the quality of life. The objective of this study was to evaluate the insecticidal activity of entomopathogenic fungi and neem-based biopesticide on *S. litura* using spraying and forced feeding method. The treatments were spore solution of *Trichoderma viride*, *Metarhizium* sp. T4.B23, *Metarhizium* sp. B2.2 applied at 10⁸ spores/mL, biopesticide formulation containing neem extract (Agr1) at 10% (v/v), and water as control. Each treatment was sprayed 0.3 mL directly onto larvae of *S. litura* while in forced feeding method, the larvae were fed on 1 gr of treated water spinach. Entomopathogenic fungal spore solution did not show efficacy on *S. litura* larvae in both methods, while Agr1 caused 90 and 40% mortality in spraying and forced feeding method, respectively. Furthermore, the efficacy of Agr1 was compared to Agr2 that contains neem extract and wood vinegar. Agr2 caused 100% mortality when sprayed onto *S. litura* larvae, and more than 90% of larvae were died when forced to feed treated water spinach. Agr2 has better potential than Agr1 to control *S. litura*.

1 Introduction

Spodoptera litura Fabricius (Lepidoptera: Noctuidae) in the larva phase is polyphagous, a pest to 15 species of the vegetable plant [1] both in the vegetative and generative stages, thus causing damage to leaves and fruits. Moreover, an outbreak of *S. litura* will cause host plant and yield losses [2]. *S. litura* is also one of the important pests to chili (*Capsicum annum* L.) and has become one of the limit factors of chili cultivation [3].

The less eco-friendly chemical synthetic pesticide has been used to control *S. litura* in agriculture. The loss of untargeted insect such as insect pests' natural enemies and insect pest resistance to one or more pesticides are increasingly urgent worldwide problems due to the overtime application of synthetic pesticides. The natural enemy has a vital role in controlling pests as a biological agent. One of the promising options of biological agents for controlling

* Corresponding author: apriwi07@yahoo.com

S. litura is entomopathogenic fungi, such as *Metarhizium* sp., because of their high reproduction capacity, short life cycle, spore-forming ability under the bad condition to sporulation and growth, target-selective, and low probability to cause resistance [4]. Entomopathogenic fungi can infect insects and arthropods directly, and most of these fungi are not dangerous to plants and are relatively not poisonous to other animals [5].

Another potential insect pest control agent to be used as a biopesticide is *Azadirachta indica* seed extract. Neem-based products including traditional preparations and formulations, have been used extensively to control of insect pests attacking crops [6]. Formulation of *A. indica* seeds has been reported showing antifeedant [7,8], fecundity suppression [9], growth inhibition [10], ovicidal and larvicidal activity [11] and repellence [8] against many different insects at low dosages. *A. indica* molecules weaken the cuticle defense system of young instars causing easy penetration of pathogenic organisms [12]. *A. indica* containing limonoids as active pesticide ingredients. The major limonoid compound found are azadirachtin salanin, meliantriol, and nimbin with azadirachtin accounted for the most active and abundant phytochemical content of *A. indica* [13]. Azadirachtin isomer A is the most plentiful and biologically active in azadirachtin, which has shown repellent, antifeedant, and insecticidal activity against pests [13]. This study aimed to evaluate the insecticidal activity of fungal spore suspension and *A. indica* seed extract against *S. litura*.

2 Materials and methods

The research was carried out from December 2020 to March 2021 in the green house of Zoologi division, Research Center for Biology and Laboratory of Bioproduct Formulation of iLaB, Research Center for Biomaterials, LIPI.

2.1 Fungal culture

Isolate of *Trichoderma viride*, *Metarhizium* sp. T4.B23, and *Metarhizium* sp. B2.2 was sub cultured on potato dextrose agar (PDA) and then incubated in a room at $27 \pm 1^\circ\text{C}$ for 21 days. Then, 21 days, the sub cultured isolate was inoculated to autoclaved parboiled rice media and incubated for 14 - 21 days until spores emerged. The spores were then harvested using a sieve, and the density was calculated per 1 gram of pure spores.

2.2 Bioassay of entomopathogenic fungi and biopesticide formulation containing neem extract (Agr1) against *S. litura* using spraying and forced feeding method

Agr1 is a biopesticide formulation containing *A. indica* seed extract at 40 EC. The bioassay was conducted to evaluate the toxicity of both agents against the 2nd – 3rd instar of *S. litura* in a controlled environment. Agr1 formulation concentration used in this bioassay was 5% (v/v) in dH₂O, while the spore density of the fungal applied was 10⁸ spores/mL.

For the spraying method, as much as 0.3 mL of each treatment was directly sprayed to *S. litura* body, while in the forced feeding method, 5 grams of water spinach leaf were sprayed with 1mL of each treatment, respectively. After sprayed, the larvae were put in a disposable petri dish and given water spinach as food regularly. dH₂O was used as a control, and each treatment used 33 larvae.

In the forced feeding method, every 5 grams of water spinach leaves was sprayed using 1 mL treatment solution, and each *S. litura* was fed with 1 gram sprayed leaves without prior starvation. Control leaf was sprayed using dH₂O. The larvae were fed with untreated water spinach after all treated leaves were consumed by the larvae. The mortality observation for

both methods was conducted at 2 hours, 4 hours, and 6 six hours after treatment and every 24 hours for seven days. The treatments were incubated at 26-28 °C.

2.3 Bioassay of Agr1 and Agr2 against *S. litura* using spraying and forced feeding method

Agr2 is an improvement of Agr1 with the addition of pyroligneous acid as known as wood vinegar. The concentration used in this assay was 10% (v/v) for both formulations. The method, observation, replication, and incubation condition were conducted in the same manner as the previous bioassay.

3 Result

S. litura was sprayed using treatment solution. After spraying, *S. litura* was observed during 168 hours to observe the insecticidal effect of treatment solution, especially the effect of spores that came into the *S. litura* body through their trachea and other pores. Observations were made to provide sufficient time for spores of *T. viride*, *Metarhizium* sp. T4.B23, and *Metarhizium* sp. B2.2 attaches to insect cuticle, germinates, penetrates body cavity, and grows into mycelium. After the mycelium growth, thin-walled blastospores were formed, circulated within the insect body, and eventually forming lethal mycelial biomass. This mycelium grows back through the cuticle, covers the body surface, and develops a large number of distributive conidia.

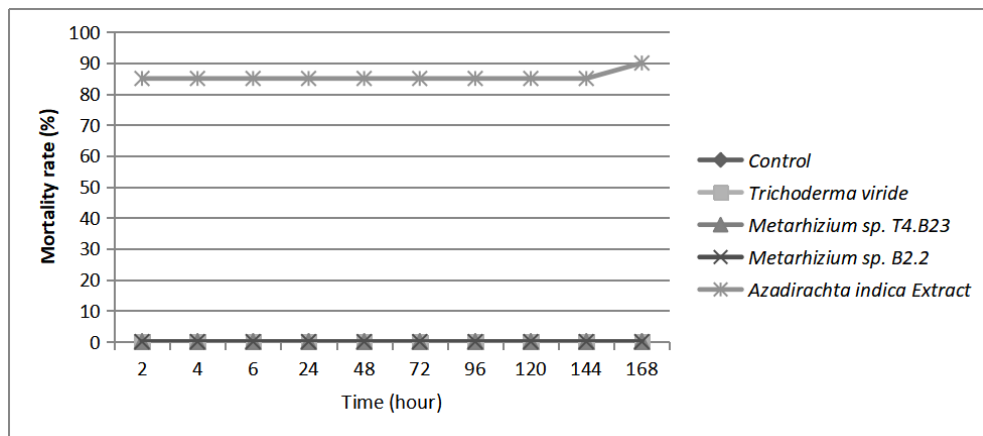


Fig.1 Mortality percentages of *S. litura* after sprayed using dH₂O, spore suspension of *T. viride*, *Metarhizium* sp. T4.B23, *Metarhizium* sp. B2.2, and *A. indica* seed extract

A. indica seed extract had the highest mortality rate (Fig. 1). Spore suspension of *T. viride*, *Metarhizium* sp. T4.B23, and *Metarhizium* sp. B2.2 did not cause death in *S. litura*, possibly caused by fungal spores that were unable to infect and colonize *S. litura*. *S. litura* was also treated with once sprayed leaves. In forced feeding method, leaves sprayed with dH₂O, spore's suspension of *T. viride*, *Metarhizium* sp. T4.B23, *Metarhizium* sp. B2.2, and *A. indica* seed extract are used as feed to *S. litura*, respectively. Untreated leaves were given to the larvae after all treated leaves were consumed was done to observe the treatment solution's effect, to the digestion system of *S. litura* after consumed.

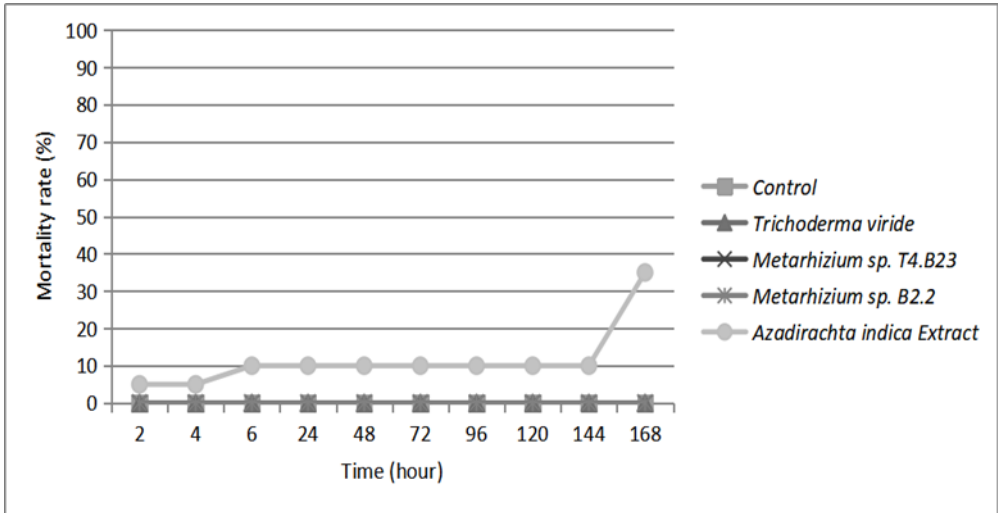


Fig. 2 Mortality percentages of *S. litura* after fed with leaves sprayed using dH₂O, spore suspension of *T. viride*, *Metarhizium sp. T4.B23*, *Metarhizium sp. B2.2*, and *A. indica* seed extract

Death only occurred after *S. litura* was force-fed with leaves sprayed with *A. indica* extract (Fig. 2). Forced feeding of *S. litura* by spraying the leaves using *A. indica* extract did not directly cause death. The death of *S. litura* occurred after several days after treatment. *S. litura* that was fed with leaves sprayed using spore suspension did not show any insecticidal activity during observation time. During the observation, the behavior of *S. litura* also did not change. It indicated that the spore of *T. viride*, *Metarhizium sp. T4.B23*, and *Metarhizium sp. B2.2* cannot infect *S. litura* through the digestion system.

The insecticidal activity of Agr1 was then compared to Agr 2. Agr 2 is an improvement of Agr1 with the addition of wood vinegar. Both formulations were sprayed directly to the larvae three times at 0 hour, 24 hours, and 48 hours. Repeated spraying was conducted to observe the effect of accumulated *A. indica* extract on the *S. litura* body surface.

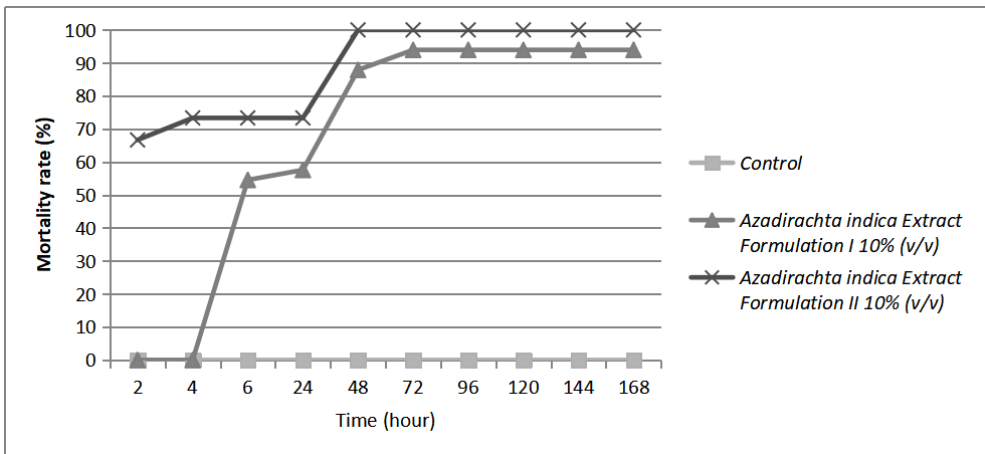


Fig. 3. Mortality percentages of *S. litura* after sprayed using dH₂O, Agr1 (Formulation I) and Agr2 (Formulation II)

Agr1 and Agr2 caused mortality to *S. litura* and repeated spraying on the *S. litura* body surface may be increasing the mortality rate. Wood vinegar in formulation II increased the mortality rate and shortened the time for *S. litura* to die.

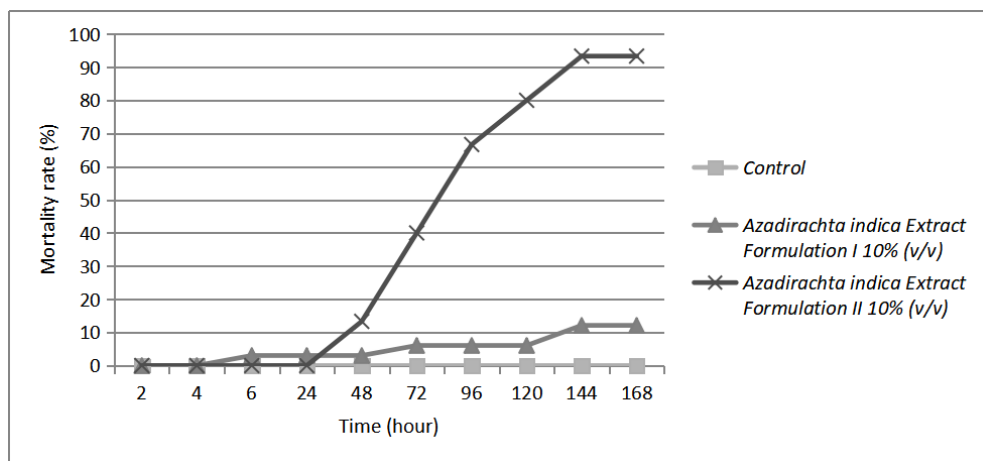


Fig. 4 Mortality percentages of *S. litura* after fed with leaves that sprayed using dH₂O, Formulation I of *A. indica* extract, and Formulation II of *A. indica* seed extract

Meanwhile in force feeding method, the mortality rate caused by Agr2 was higher than that of Agr 1. Mortality rate for the two formulations was increased during the observation. However, the mortality rate of Agr1 was not as significant as Agr2. The mortality rate of Agr1 only 12% at the end of observation and doesn't increase much during the observation. The mortality rate of larvae fed with Agr2 treated leaves was kept increasing during observation and reached 93% after 144 hours of observation.

4. Discussion

The decreased infection and penetrating ability of *Metarhizium* sp. T4.B23 and *Metarhizium* sp. B2.2 is may be caused by reduced insect cuticle-degraded enzyme production due to the prolonged storage of the isolate at the artificial media, such as PDA. Decreasing insect cuticle-degraded enzyme production also can be caused by recultured the isolate at artificial media too many times without reinfection to the insect. Nutrition of artificial media to storage and recultured will affected the virulence of produced spore [14]. Fungal infection is also affected by temperature and water activity because each fungus has its optimal temperature and water activity to sporulation and growth. *Metarhizium* sp. optimal for increased temperature higher than 30 °C in the environment with water activity between 0.94 - 0.995 [15].

T. viride, which cannot infect *S. litura*, is a soil fungus, not an entomopathogen but *T. viride* has the potential as biocontrol because of antagonism and parasitism against other fungi [16]. *T. viride* produces the chitinolytic and glucanolytic enzyme, but *T. viride* enzyme structure not available to the degraded exoskeleton of the insect. Chitinase of *T. viride* has a different protein structure to the chitinase of entomopathogenic [17].

Azadirachta indica extract that directly sprayed to *S. litura* body caused *S. litura* mortality because of the extract containing azadirachtin. Azadirachtin came into the *S. litura* body through the cuticle, spiracle, or other sensory organ and then paralyzed neuron of the insect body [18]. Azadirachtin neurotoxin was targeted the neuroendocrine system. The

effect of azadirachtin caused the color of *S. litura* larva to become darker and then died [19]. Azadirachtin and other active compounds, such as meliantriol, salanin, and nimbin, can affect the feeding ability, growing of *S. litura* body, reproduction ability, molting process, disturbing hybridization and sexual communication, decreasing of egg hatching ability, and disturbing chitin forming [20].

Azadirachtin and other active compounds entering the digestion system through the sprayed leaves that fed to the *S. litura*. Azadirachtin and other active compound reach the breathing-trachea system, entering blood circulation and the neuro system. Azadirachtin disturbing electron transfer on the mitochondria, which will disturb the cardiovascular system, breathing system, and neuro system, and make the *S. litura* died. Disturbed neuro systems also will be affected by the hormone, such as exdison [21].

Adding wood vinegar to the formulation showed an increase to the *S. litura* mortality rate. Wood vinegar as an additional component has the potential to increase the activity of the active compound of *A. indica* extract because the acetic acid in wood vinegar can help penetrate the toxin into the larvae body more effective because acetic acid will interfere with cuticle permeability [22]. Further research is needed in various aspects including direct application of *Agr2* to plants in the field to ensure the formula does not affect plant health and also to find an efficient dosage and method of application.

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