POTENTIAL OF ENTOMOPATHOGENIC FUNGAL CULTURE FILTRATE *Nomuraea rileyi* (FARLOW) SAMSON (HYPOCREALES: CLAVICIPITACEAE) IN BENGKULU, INDONESIA AGAINST CORN PEST *Spodoptera frugiperda* (J. E. SMITH) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Fall Armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is an important maize crop pest that can cause farmers severe losses if not controlled. Biopesticide of entomopathogenic fungi are an environmentally friendly alternative control measure that can reduce farmers' dependence on chemical insecticides. Although there are several entomopathogenic fungi based insecticides such as entomopathogenic fungi conidia, they have been found to have some weaknesses. They are more stable in nature compared to synthetic insecticides and therefore require a longer time to cause pest mortality, there is a need to formulate other alternatives with the use of entomopathogenic fungi biopesticide culture filtrate. Therefore, this study aimed to evaluate the potential of Bengkulu entomopathogenic fungus *Nomuraea rileyi* culture filtrate isolate as a control agent against corn pest *S. frugiperda.* The bioactivity testing was carried out by spraying the second instar larvae of *S. frugiperda* with *N. rileyi* culture filtrate at concentrations (%): 1.0, 0.5, 0.75, 0.25, 0.125, 0.625, 0.05, 0.005 and control (sterile water). Results showed that the *N. rileyi* culture filtrate was able to cause mortality of second instar *S. frugiperda* larvae, with the mortality rate increasing with increasing culture filtrate concentration (i.e. 0.625% - 1.0%). The highest mortality was recorded at a concentration of 1% reaching 100% on day 3, while at concentration of 0.05 and 0.005%, the mortality of *S. frugiperda* larvae was under 10%. The culture filtrate of *N. rileyi* also caused mortality above 50% at concentrations of 0.50% and 0.75%. As such we suggest that the biological insecticide developed from entomopathogenic fungi *N. rileyi* culture filtrate had the potential to be used as an effective biological control agent (biolarvicide) against *S. frugiferda*.

Keywords: Biopesticides, entomopathogenic fungi, *Spodoptera frugiperda,* culture filtrate, mortality

ABSTRAK

Ulat Ratus, *Spodoptera frugiperda* adalah perosak jagung yang penting dan boleh menyebabkan kerugian besar kepada petani jika tidak dikawal. Biopestisid kulat entomopatogenik adalah alternatif untuk pengendalian perosak yang lebih mesra alam, sekaligus mengurangkan kebergantungan petani terhadap racun serangga kimia. Walaupun

terdapat banyak racun biologi berasaskan kulat konodia namun penggunaannya adalah terhad kerana terdapat beberapa kelemahan. Kulat entomopatogen yang ada dilaporkan kurang stabil daripada racun serangga sintetik dan memerlukan masa yang lebih lama untuk membunuh perosak. Oleh itu, terdapat keperluan untuk mencari alternatif penggunaan kultur filtrat biopestisid kulat entomopatogen yang berkesan. Kajian ini dijalankan adalah untuk menilai potensi metabolit kulat entomopatogen Bengkulu *Nomuraea rileyi* sebagai agen kawalan untuk perosak jagung, *S. frugiperda.* Ujian bioaktiviti dilakukan dengan mengawal larva instar kedua *S. frugiperda* pada kepekatan (%): 1.0, 0.5, 0.75, 0.25, 0.125, 0.625, 0.05, 0.005 dan kawalan (air suling). Hasil kajian menunjukkan kultur filtrat *N. rileyi* menyebabkan kematian larva instar kedua *S. frugiperda* dengan kadar kematian meningkat seiring dengan peningkatan kepekatan kultur filtrat (iaitu 0.625,% - 1.0%). Kematian tertinggi direkodkan pada kepekatan 1% mencapai 100% pada hari ke-3, sementara pada kepekatan 0.05 dan 0.005%, kematian larva *S. frugiperda* berada di bawah 10%. Kultur filtrat *N. rileyi* juga menyebabkan kematian melebihi 50% pada kepekatan 0.50% dan 0.75%. Insektisid biologi yang dikembangkan dari filtrat kulat entomopatogen *N. rileyi* berpotensi digunakan sebagai agen kawalan biologi yang berkesan (biolarvisida) ke atas *S. frugiferda*.

Kata kunci: Biopestisid, kulat entomopatogen, *Spodoptera frugiperda*, kultur filtrat, mortaliti

INTRODUCTION

Corn is one of the most important carbohydrate producing food crops in the world, apart from wheat and rice. In Indonesia, corn is the second main food commodity after rice, apart from direct consumption, corn is also the main raw material for the feed and food industry, and now corn is one of the bioenergy raw materials that has been developed in several countries (Sulaiman et al. 2018). Bengkulu Province is one of the contributors to domestic maize supply; corn production in Bengkulu only reaches 52.785 tons per year,-hence it is insufficient to meet demand (Badan Pusat Statistik Province Bengkulu 2016). One of the causes of low corn production is pest attack.

Spodoptera frugiperda (J.E.Smith) (Lepidoptera: Noctuidae) is an important maize crop pest in Indonesia. This pest is widely spread in various regions in Indonesia including West Pasaman (West Sumatra), West Java (Bandung and Garut), Lampung, and Bengkulu (Ginting et al. 2020a, 2020b, 2021; Maharani 2019; Ministry of Agriculture 2019; Trisyono et al. 2019). According to (Trisyono et al. 2019) the losses due to the attack of this pest on maize crop in Indonesia at the early stage (approximately 2 weeks old) were 100% infested plants, while the older maize had less damage. *S. frugiperda* infestation in 26.4 - 55.9% of corn can cause decrease yield by 11.57% (Baudron et al. 2019). According to Chimweta et al. (2019), the rate of damage by *S. frugiperda* to leaves, silk and corn cobs range from 25-50%, and can result in a reduction of yield by 58%.

Biopesticide such as entomopathogenic fungi are environmental friendly pest control alternative measures that have been used, for example *Beauveria bassiana, Metarhizium anisopliae, Nomuraea rileyi, Verticillium lecani* have been used to control pests *Riptortus linearis* (Herlinda 2010; Prayogo 2013). The results of field surveys conducted in Merigi, Kepahiang, and Seluma districts showed that the entomopathogenic fungus *N. rileyi* is naturally able to infect *S. frugiperda* larvae in the field (Ginting et al. 2020a). According to Mallapur et al. (2018), *N. rileyi* is able to control *S. frugiperda*, and reduce maize leaf damage by 62.50-73.05%. *N. rileyi* is also capable of causing epizootics in various insects of the order Lepidoptera such as *Heliothis zea*, *Plathypena scabra, Bombyx mori, Pseudoplusia includens*

and *Anticarsia gemmatalis* and Coleoptera (Moanaro 2017). *Beauveria bassiana, M. anisopliae,* and *N. rileyi* with conidia application are able to cause second instar *S. frugiperda* larvae mortality, neonates and egg mortality (Akutse 2019; Cruz-Avalos et al. 2019). Also, *B. bassiana* and *M. anisopliae* with endophytic mechanisms in maize can cause 100% mortality of second instar larvae of *S. frugiperda* and 75% -87% mortality of the fourth instar larvae of *S. frugiperda* (Ramos et al. 2020).

The potential of entomopathogenic fungi in controlling *S. frugiperda* imago using three isolates of *B. bassiana* (Balsamo-Crivelli) Vuillemin and six isolates of *M. anisopales* tested by applying synthetic sex pheromones and entomopathogenic fungi, showed that all isolates are pathogenic (33.3-100%) against imago (Gutiérrez-Cárdenas et al. 2019). Biopesticides applied in conventional form or conidia suspension take a longer time (i.e. 3-14 days) to cause mortality of insect pests (Gutiérrez-Cárdenas et al. 2019). Furthermore, the entomopathogenic fungi are very sensitive to changes in environmental conditions, making it difficult to produce them as stable biopesticides in nature (Bharani & Namasivayam 2017).

Therefore, it is necessary to develop other alternatives such as culture filtrate. Entomopathogenic fungi produce a filtrate that is toxic potential as an active mycoinsecticide (Gustianingtyas et al. 2020). According to Namasivayam & Bharani (2014), similar to entomopathogenic and conidia fungi biomass, the entomopathogenic fungi metabolites are capable of killing insect pests. The *N. rileyi* metabolite can cause 100% and 65% mortality of the second instar and third instar of *S. litura* respectively. Therefore, this study is aimed to develop and evaluate the potential of Bengkulu entomopathogenic fungus *Nomuraea rileyi* culture filtrate isolated as a control agent against corn pest *S. frugiperda.*

METERIALS AND METHODS

Sampling Sites and Species Identification

Nomuraea rileyi isolates were obtained from infected *S. frugiperda* larvae collected from the maize fields in Merigi sub-district, Kepahiang district, Bengkulu province, while *S. frugiperda* larvae used for bioassays were obtained from Kepahiang, and kept in the laboratory (Dutta et. al. 2014; Trisyono et al. 2019).

Isolation and Identification of Entomopathogenic Fungi

The isolation was conducted at the Plant Protection Laboratory, Faculty of Agriculture, Bengkulu University, Indonesia. The infected larvae collected were surface sterilized by immersing them in 0.5% sodium hypochlorite for two minutes, and then rinsed with sterile water. The sterilized specimens of pathogen infected larvae were transferred to a petri dish containing Saborauds Maltose Agar Yeast (SMAY) media for culturing. The cultures were incubated at room temperature ±26° C for a week. The emerging colonies were further purified by repeated subcultures on SMAY media. The isolates that appeared on SMAY media were identified microscopically and pure cultures were stored in an incubator (Namasivayam et al. 2014b).

Preparation of the Entomopathogenic Fungi Culture Inoculum

The conidia obtained from the media were harvested after 3 weeks by mixing the fungal conidia from the media with sterile water and 0.02% Tween-80 to produce a conidia suspension. The number of conidia obtained in the suspension was counted with a Neubauer hemocytometer using the serial dilution method (Gustianingtyas et al. 2020)

Preparation of *S. frugiperda* Test Insects

The propagation of *S. frugiperda* larvae was carried out by collecting larvae from the maize field, and then rearing them in plastic containers (6.5 cm wide and 4.5 cm high). In each container contain, one larva was reared and fed on young corn until it turned into a pupa. Each pupa was then transferred to a new plastic container (18 cm in wide and 30 cm high) containing a sheet of cloth, sterile sawdust (2 cm thick), and covered with 100 mesh gauze. The pupa was given 10% honey liquid feed and maintained in the container until it turned into an imago and laid eggs. The sheet of cloth that has been laid with eggs was taken and placed into a plastic box (30 cm long, 20 cm wide and 5 cm high) covered with filter paper. Both the eggs and pupae were disinfected in their respective containers using hydrogen peroxide (H_2O_2) (3% for eggs and 5% for pupae) for 5 minutes to avoid death or damage due to contaminant microorganisms.

Extraction of Entomopathogenic Fungus Bioinsecticide Culture Filtrate

Sabouraud Maltose Yeast Extract Broth (SMYB) (4% maltose, 1% peptone, 0.5% yeast extract) was used for the extraction of crude metabolites. SMYB media was prepared in one liter of water and sterilized by autoclaving. After sterilization, 10 ml of conidia suspension (1.0 x 10^8 conidia / ml) was inoculated and incubated at 25^{o}C for 7 days under continuous titling/ vibrating at 150 rpm. Following the incubation period, the conidia suspension was filtered with gauze, and the filtrate collected was extracted with ethyl acetate and concentrated using a rotary evaporator. The concentrated extract obtained was stored in a vial bottle and used for further research.

Bioassay of Bioinsecticide Activity against *Spodoptera frugiferda*

Bioinsecticidal metabolite activity was tested against 2nd instar *S. frugiferda* larvae under in vitro conditions. During testing, 6 larvae were put in a plastic container (34 mm by 21 mm) closed with a lid and aerated for the larvae and kept at room temperature ±26^{o}C in an incubator. The larvae were sprayed with treatment concentrations (%); 1.0, 0.5, 0.75, 0.25, 0.125, 0.625, 0.05, 0.005, and control (sterile water). The bioinsecticidal activity was carried out by calculating the cumulative mortality, lethal time ($LT_{25,50,75}$), and lethal concentration ($LC_{25,50,75}$). For each treatment, five replications were performed. The mortality was calculated daily for 7 days after inoculation (Namasivayam et al. 2014a).

Data Analysis

The mortality data was processed using SPSS software (16.0) to determine the variance of each treatment, and analyse for significant differences between treatments. It was continued with the (Duncan's Multiple Range Tests (DMRT) 5% test, to find significant differences between concentrations. Analysis of toxicity parameters was performed using the SPSS 16.0 program to determine the value of lethal concentration (LC) and Lethal Time (LT) (Finney 1971).

RESULTS

Characteristics of Bengkulu *N. rileyi* Isolate

The results of Bengkulu *N. rileyi* metabolite isolate, based on culture characteristics on SMAY media, showed mycelia green tosca. The microscopic observation of the fungus with lactophenol blue showed that *N. rileyi* morphologically had insulated, transparent, and branched hyphae. Conidiophores were branched into 2-6 phialides which are subglobose or short, transparent cylinders. Conidia appeared chained, elliptical, and transparent.

The Insecticidal Activity of The Culture Filtrate of *N. rileyi*

The fungal conidia and mycelia were separated from the filtrate through two stages of filtration and rotary to produce toxic fungal culture filtrate. The results of testing the insecticidal activity of the culture filtrate of *N. rileyi* showed that the culture filtrate had a larvicidal effect on second instar larvae of *S. frugiferda* (Table 1). The insecticidal activity of the *N. rileyi* culture filtrate against *S. frugiferda* showed that different concentrations of the metabolite had a different effect on second instar larvae.

Table 1. Mean mortality of the second instar of *S. frugiferda* larvae at various culture filtrate concentrations

Culture filtrate Concentrations (%)	**Mortality of second instar *S. frugiferda* larvae (%) (±SE)**		
	24 (Hours)	**48 (Hours)**	**72 (Hours)**
Control	0±0.00a	0±0.00a	0±0.00a
0.005	0±0.00a	0±0.00a	0±0.00a
0.050	0±0.00ab	3±0.20a	7±0.24a
0.625	3±0.20b	3±0.20a	13±0.20a
0.125	7±0.24c	7±0.24a	40±0.24ab
0.250	7±0.24c	10±0.24a	47±0.20ab
0.500	13±0.20d	17±0.00b	60±0.24bc
0.750	20±0.20e	33±0.00c	77±0.24cd
1.000	23±0.24f	80±0.20d	100±0.00e

*The number followed by the same letter in the same column is not significantly different according to Duncan's test at 5% significance level.

The mortality of *S. frugiferda* larvae at each concentration occurred starting on the first day except at concentrations of 0.05 and 0.005%. The larval mortality increased with increasing concentration (i.e. 0,625% - 1,0%), and the highest mortality at a concentration of 1% resulted in 100% on day 3. At metabolite concentrations 0.05 and 0.005% the mortality of larvae was under 10%. The culture filtrate of *N. rileyi* was able to cause mortality above 50%. Based on the results of the probit analysis on the first, second, and third day of observation, the lethal concentration ($LC_{25,50.75}$) was as presented in (Table 2).

Table 2. Value of Lethal concentration of second instar *S. frugiferda* larvae

Days	**Lethal concentration (LC) of second instar *S. frugiferda***		
	25%	**50%**	**75%**
1	0.96	1.15	1.34
2	0.82	0.86	0.94
3	0.67	0.77	0.87

The ability of *N. rileyi* metabolites to cause mortality against 2nd instar larvae of *S. frugiferda* show that lethal time decreased with increasing concentration (Table 3).

Table 3. Lethal Time value of second instar *S. frugiferda* larvae at various concentrations

Culture Filtrate (%)	**Lethal Time (LT) value of second instar *S. frugiferda* larvae**		
	25 (%)	**50 (%)**	**75 (%)**
Control	0.00	0.00	0.00
0.005	0.00	0.00	0.00
0.050	7.94	14.09	24.84
0.625	5.89	11.18	21.25
0.125	3.01	5.06	8.48
0.250	2.51	4.47	7.98
0.500	2.29	3.28	4.70
0.750	2.31	2.63	3.00
1.000	1.55	1.76	2.10

DISCUSSION

The results showed that the *Nomurea rileyi* isolated from infected larvae in field corn was identified based on culture characteristics on SMAY media, and microscopic observations of fungi with lactophenol blue showed that morphologically Bengkulu *N. rileyi* isolate had tosca green mycelia, insulated, transparent, hyphae and branched conidiophores with 2-6 phialides formed near the septa. The phialides were subglobose or short cylinders, transparent and smooth. The conidia appeared chained, highly elliptical, and transparent. The observed characteristics of these entomopathogenic fungi are consistent with the results of research by Dutta et al. (2014).

Insecticidal activity trials showed that the culture filtrate of *N. rileyi* had a larvicidal effect on second instar larvae of *S. frugiferda* (Table 1). Additionally the results showed that *N. rileyi* culture filtrate of different concentrations had a different effect on second instar larvae of *S. frugiferda,* with larval mortality increasing with increasing concentration. The results obtained correspond with the findings by Namasivayam & Bharani (2014), which showed that the *N. rileyi* metabolite with a concentration of 1 mg/ml caused 100% mortality of *S. litura* second instar within 1.61 days, and 65% mortality in the third instar within 2.17 days.

Like the entomopathogenic fungi and conidia fungi biomass which are conventionally used as entomopathogenic biopesticides to caused mortality to insect pests, the culture filtrate they produce also have the same function (Namasivayam et al. 2014b). There are various kinds of culture filtrate produced by entomopathogenic fungi to cause mortality in insects. These culture filtrate serve as toxins, e.g. beauverucine (cyclodepsipeptide) produced by *Beauveria* (Onofre et al. 2002), and diketopiperazines of *N. rileyi* (Marcinkevicius et al. 2017).

According to Pinnamaneni et al. (2010) *B. bassiana* filtrate culture is toxic. As the fungus produces the chitinolytic enzyme eksochitinase that degrades the insects' cuticle upon entering the body. In addition, during incubation in liquid media the fungus can produce protease enzymes (Qazi & Khachatourians 2008). The activity of extracellular enzymes and protease enzymes is able to dissolve the insect integument and cause death (Khachatourians et al. 2007; Mancillas-Paredes et al. 2019). Among the entomopathogenic fungi, *N. rileyi* is widely used in the agricultural sector as a biopesticide agent and is capable of producing various enzymes including proteases and chitinases. Diketopiperazine extracted from *N. rileyi*

supernatant has a preventive effect on oviposition of *Ceratitis capitata* (55.86%), as a repellent to *Tribolium castaneum*, and imago malformations (40%) (Marcinkevicius et al. 2017).

The results also showed that the lethal time decreased with increasing culture filtrate concentration (Table 3). In contrast to the working mechanism of the fungal conidia, the fungal culture filtrate directly causes mortality of the host insect due to the toxins produced by the fungus in liquid media, thus the time needed to cause mortality of the host insect was shorter. Soesanto et al. (2019) explains that entomopathogenic fungi *B. bassiana* contains secondary metabolites that produce toxins e.g. destruksin and eprapeptin (Zibaee et al. 2009), which can weaken the immune system of the host insect (Zibaee et al. 2011). *Beauveria bassiana* also produces protease enzyme that causes mortality of the host insects by dissolving their body proteins (Mancillas-Paredes et al. 2019).

Entomopathogenic fungi cultured in liquid media can produce conidia in the form of blastospores which can produce toxins (Mascarin et al. 2015; Mascarin et al. 2016). In a study by El-Husseini (2019), the death of *S. litura* larvae by *M. anisopliae* conidia began to occur on the fourth day after treatment, whereas with *M. anisopliae* filtrate culture, the mortality of *S. litura* occurred on the third day after application. This difference in lethal time is due to the different action of the fungal conidia from that of the culture filtrate.

El-Ghany (2015) states that the entomopathogenic fungal conidia cause mortality of the host insect starting with the fungal conidia attaching to the host insect's cuticle (Augustyniuk-Kram & Kram 2012), then, at high humidity, the conidia begin to germinate in the host cuticle (El-Ghany 2015). The conidia then germinate forming a sprout tube and continue to grow into the cuticle (Fernandes et al. 2007). Infection occurs when a sprout tube is able to penetrate the cuticle of an insect and the ability to infect it is a determining factor in the virulence of the fungus (Altre & Vandenberg 2001). After the sprout tube penetrates the cuticle and reaches the hemocoel, it produces specific infectious hyphae originating from appressoria (El-Ghany 2015). Furthermore, hyphae spread to the hemolymph and develop to produce blastospores that produce toxins, such as destruksin by *M. anisopliae* which caused mortality the host insect (Mancillas-Paredes et al. 2019).

The death of these host insects is not only caused by the toxin but also due to mechanical damage due to the penetration of the fungus into the insect's body (El-Ghany 2015). After the host insect dies, it enters a saprophytic phase which is influenced by favorable environmental conditions (Peña-Peña et al. 2015). In the dead insect's body, the fungus forms mycelia and hyphae which continue to grow to cover the body of the host insect, then the hyphae form conidiogens and conidia cells which are produced by utilizing nutrients from the host insect (El-Ghany 2015).

CONCLUSIONS

The results showed that the *N. rileyi* culture filtrate was able to cause mortality of second instar *S. frugiperda* larvae, with the mortality rate increasing with culture filtrate concentration (i.e. 0.625% - 1.0%). he highest mortality was recorded at a concentration of 1% reaching 100% on day 3, while at a concentration of 0.05 and 0.005%, the mortality of *S. frugiperda* larvae was under 10%. The culture filtrate of *N. rileyi* also caused mortality above 50% at concentrations of 0.50% and 0.75%. The biological insecticide developed from entomopathogenic fungi *N. rileyi* culture filtrate had the potential to be used as an effective biological control agent (biolarvicide) against *S. frugiferda*.

ACKNOWLEDGEMENTS

The author would like to acknowledge to the research grant for Unggulan PNBP UNIB in 2020 with Contract Number 1995/UN30.15/PG/2020 and the Department of Plant Protection, Faculty of Agriculture, Bengkulu University for providing necessary facilities for this research.

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