Virulence of *Beauveria bassiana* from Different Carbohydrate Sources Against *Riptortus linearis* L. (Hemiptera: Alydidae)

Muhammad Agung Permadi*, Rafiqah Amanda Lubis, Amir Mahmud, Qorry Hilmiyah Harahap, Haryogi Setiawan Sitanggang

Fakultas Pertanian, Universitas Muhammadiyah Tapanuli Selatan, Jalan Raja Inal Siregar, Padangsidimpuan, North Sumatra 22716, Indonesia

* muhammad.agungp@um-tapsel.ac.id

**Abstract.** *Riptortus linearis* is a cosmopolitan pest that is widespread throughout the world. These pests are also polyphagous which means this pest has many hosts. The potential of *R. linearis* as a pest needs to be monitored because it is an important pod sucking pest. The entomopathogenic fungi are one of the biological control agents that are often used to control plant pests. One of the entomopathogenic fungi that are often used to control insect pests is *Beauveria bassiana*. Media that contains all the essential nutrients needed fungi for fungal culture in the laboratory. The source of nutrition is a determining factor for the growth and virulence of entomopathogenic fungi because the rate of germination, growth, and sporulation are an indicator of the level of virulence. The research was conducted at the Laboratory of Agrotechnology, Universitas Muhammadiyah Tapanuli Selatan. This research used a Completely Randomized Design, 5 treatments (5 growth media for *B. bassiana*). The results showed that *B. bassiana* grown in the various media had no significant effect on the mortality of *R. linearis* imago. The lowest LT50 *R. linearis* was caused by *B. bassiana* infection grown on rice media.

1. **Introduction**

*Riptortus linearis* (F) (Hemiptera: Alydidae) is a cosmopolitan pest that is widespread throughout the world. These pests are also polyphagous which means this pest has many hosts. The potential of *R. linearis* as a pest needs to be monitored because it is an important pod sucking pest and spreads across locations and growing seasons, its destructive power is higher than other pod destroying pests, thus indicating a lower economic threshold level. Suction pods can cause seed yield losses both quantitatively and qualitatively (reduce seed quality). The yield loss due to pod sucking pests was up to 80% [1]. The way to damage pod-sucking pests is by sticking the stylet on the surface of the pods and soybeans, causing the soybean seeds to become deflated [2].

Farmer still rely on synthetic insecticides to control pests. Excessive and unwise application of synthetic insecticides will cause unwanted negative effects such as target pest resistance, resurgence, the killing of natural enemies and other useful insects, and environmental pollution. Therefore, an alternative pest control that is efficient, effective, and friendly to the environment is needed by using a biological control agent.
Entomopathogenic fungi are one of the biological control agents that are often used to control plant pests. Entomopathogenic fungi infect by penetrating the cuticle of the host insect, in contrast to bacteria and viruses which must be eaten by the host insect [3]. One of the entomopathogenic fungi that are often used to control insect pests is *Beauveria bassiana*. Several researchers have tested the effectiveness of this fungus against several types of pests including *Spodoptera litura* (Lepidoptera: Noctuidae) [4], mungbean insect pests [5], *Frankliniella occidentalis* (Thysanoptera: Thripidae) [6], *Diaphorina citri* (Hemiptera: Liviidae) [7], *Nezara viridula* (Hemiptera: Pentatomidae) [8].

Media that contains all the essential nutrients needed fungi for fungal culture in the laboratory. One of the agar media that is often used in the propagation of entomopathogenic fungi in the laboratory is PDA (potato dextrose agar). Several researchers have researched fungal growing media such as cassava starch [9], potato and palmirah tuber [10], and several different carbohydrate sources [11]. The source of nutrition is a determining factor for the growth and virulence of entomopathogenic fungi because the rate of germination, growth, and sporulation is an indicator of the level of virulence.

2. **Materials and methods**

The research was conducted at the Laboratory of Agrotechnology, Universitas Muhammadiyah Tapanuli Selatan. This research used a completely randomized design, 5 treatments (5 growth media for *B. bassiana*), and 3 replications. The data obtained were processed with variance and continued with the Duncan test at a 5% significance level.

2.1 **R. linearis Rearing**

Nymphs and imago were caught from asparagus bean fields at Padangsidimpuan City. The nymph and imago groups are put into a cage. Groups of imago, nymphs, and eggs are reared in the laboratory. The food that is given during the maintenance period is asparagus beans. The asparagus was washed with clean water before placing it in the cage.

2.2 **Propagation of Fungi and Preparation of Fungi Suspension**

The composition of PDA was potato 400 g, dextrose 15 g, chloramphenicol 1 g, agar 15 g, and 1 l distilled water. Cassava agar media and sweet potato agar media has made the same as the manufacture of PDA. Rice media and corn media are made by washing each rice and corn thoroughly, steaming half-cooked, and then cooled in a plastic tray. Media as much as + 50 g is put into clear plastic bags heat-resistant HDPE (high-density polyethylene), sterilized in an autoclave (for 35 minutes with a temperature of 121°C and a pressure of 1 atm). After cooled, each media was inoculated with *B. bassiana*. Inoculation was carried out by spraying 1 ml of fungal conidia suspension with a conidia density of $10^6$ conidia/ml into the media bag. All stages were carried out under sterile conditions in laminar airflow. The culture was incubated for 21 days. Each *B. bassiana* from each media was made into a suspension. The conidia density of each suspension was calculated with a Neubauer-improved hemocytometer. This study used a conidia density of $10^8$ conidia/ml.

2.3 **Inoculation of Entomopathogenic fungi on R. linearis**

The application was carried out by spraying the fungal conidia suspension of *B. bassiana* $10^8$ conidia/ml on *R. linearis* imago. Each treatment unit consisted of 11 adult insects sprayed with 2 ml of conidia suspension.

2.4 **Observation**

The observation parameter was the number of *R. linearis* imago that died after *B. bassiana* application. Observations were made from 1 day after application to 7 days after application.
3. Result and discussion

3.1 Mortality of *R. linearis*

The research showed that *B. bassiana* grown in various media had no significant effect on mortality of *R. linearis* imago. The research results can be seen in the following table.

| *B. bassiana* growth media      | Imago mortality (%) |
|---------------------------------|----------------------|
| Sweet potato agar media         | 87.87 A              |
| Rice media                      | 84.84 A              |
| Cassava agar media              | 81.81 A              |
| Corn media                      | 78.78 A              |
| PDA                             | 78.78 A              |

Note: Numbers followed by the same letters in the same column show no significant difference based on Duncan (5%).

The Source of nutrients contained in the growth media is a determining factor for the growth and virulence of entomopathogenic fungi. Because of the rate of germination, growth, and sporulation is an indicator of the level of virulence. [12] stated that the type of fungus, the origin of the isolate, genetic differences, the growth medium influenced the entomopathogenic in infecting insects. However, it is different from this study, where the growth media did not affect the fungus *B. bassiana* in infecting *R. linearis* imago. All the growth media tested in this study were able to provide the nutrients needed for the growth of *B. bassiana*, so that there was no significant difference in *R. linearis* mortality caused by *B. bassiana* from different growth media.

The infection mechanism of entomopathogenic fungi begins with the attachment of the fungal conidia to the insect cuticle [3]. Then the spores germinate and penetrate the insect's body. In the next stage, the fungus grows and develops into the insect's blood (hemolymph). The fungus will accelerate reproduction by separating the hypha body to fight insect resistance. At the same time, the antibiotic toxins produced by the fungus both weaken and rapidly kill insects. Furthermore, the hyphae will grow and fill the entire insect body. When the fungus begins to develop, the insect shows symptoms of illness, such as uncoordinated movement and consequently the insect's death.

At the beginning of the death of the test insects, there was no sign of mycelium growing on the test insects' organs. The new fungal mycelium appeared for the first time after the test insects died. Mycelia begin to grow and are found in the articulation organs, especially the legs, then the oral organs then develop in the thoracic part. The articulation organs, including the joints of the legs, thoracic, mouth, and abdominal joints are very flexible areas so that they are easier to penetrate by fungal conidia.

| *B. bassiana* growth media      | LT$_{50}$ imago (*R. linearis*) (days) |
|---------------------------------|---------------------------------------|
| Sweet potato agar media         | 4.66                                  |
| Rice media                      | 4.46                                  |
| Cassava agar media              | 4.54                                  |
| Corn media                      | 4.71                                  |
| PDA                             | 5.00                                  |

The mortality of *R. linearis* imago caused by fungal infection takes time because the fungus requires several stages of the process to infect and kill insects, namely conidia attachment to the insect's body, germination, penetration, invasion, and colonization in the hemocoel, tissues, and organs. The time for each of these stages varies depending on the type of fungus, host, and environment. The lowest LT$_{50}$ of
Imago *R. linearis* was caused by infection with *B. bassiana* grown on rice media. The lower the LT$_{50}$ imago *R. linearis*, the better the results in controlling these insects. This means that *B. bassiana* grown in rice media has a faster rate of infecting and killing *R. linearis* imago compared to *B. bassiana* grown in other test media.

4. Conclusions

The results showed that *B. bassiana* grown in the various media tested had no significant effect on mortality of *R. linearis* imago. The lowest LT$_{50}$ of imago *R. linearis* was caused by infection with *B. bassiana* grown on rice media.

References

[1] M. S. Y. I. Bayu, "Tingkat seranganberbagaihamapolong pada plasma nutfahkedelai," in Pros Sem NasMasyBiodivIndon., Vol. 1, No. 4, pp. 878-883, 2015.

[2] M.M. Rahman, U.T. Lim, "Evaluation of mature soybean pods as a food source for two pod-sucking bugs, *Riptortus pedestris* (Hemiptera: Alydidae) and *Halyomorpha halys* (Hemiptera: Pentatomidae)," in PLoS ONE., vol. 12, no. 4, pp. 1-16, 2017.

[3] D. Rai, V. Updhyay, P. Mehra, M. Rana, A.K. Pandey, “Potential of entomo-pathogenic fungi as biopesticides,” in Ind. J. Sci. Res. and Tech., vol. 2, no. 5, pp. 7-13, 2014.

[4] D. N. Erawati, I. Wardati, and S. Humaida, “Potential of *Beauveria bassiana* Lowland Isolates against *Spodoptera litura* in Tobacco Plant,” in IOP Conf. Series: Earth and Environmental Science, 2018, pp. 1–7.

[5] M. S. Y. I. Bayu and Y. Prayogo, “Field efficacy of entomopathogenic fungi *Beauveria bassiana* (Balsamo.) for the management of mungbean insect pests,” in IOP Conf. Series: Earth and Environmental Science, 2018, pp. 1–9.

[6] Y. Gao et al., “Potential use of the fungus *Beauveria bassiana* against the western flower thrips *Frankliniellaoccidentalis* without reducing the effectiveness of its natural predator *Orius sauteri* (Hemiptera: Anthocoridae),” Biocontrol Sci. Technol., vol. 22, no. 7, p. 803_812, 2012.

[7] M. A. Permadi, R. Anwar, and T. Santoso, “Pemanfaatancendawan *Beauveria bassiana* (Bals.) Vuill. sebagai Miko-Insektisida terhadap kutu loncat jeruk *Diaphorina citri* Kuw. (Hemiptera: Liviidae),” BioLink J. Biol. Lingkungan, Ind. Kesehat., vol. 4, no. 1, pp. 82–88, 2017.

[8] M. A. Permadi, R. A. Lubis, L. S. Siregar, “Virulensi beberapa isolat cendawan entomopatogen terhadap nimfa kepik hijau *Nezara viridula* Linn.(Hemiptera: Pentatomidae),” in Jurnal Agrohita, vol. 2 no. 2, pp. 52-60, 2018.

[9] C. K. Kwoseh, M. Asomani-Darko, and K. Adubofour, “Cassava starch-agar blend as alternative gelling agent for mycological culture media,” Bots. J. Agric. Appl. Sci., vol. 8, no. 1, pp. 8–15, 2012.

[10] S. Martyniuk and J. Oron, “Use of Potato Extract Broth for Culturing Root-Nodule Bacteria,” Polish J. Microbiol., vol. 60, no. 4, pp. 323–327, 2011.

[11] M. A. Permadi, Mukhlis, B. S. Samosir, D. Y. Siregar, M. Wayni, ". Physiology Characterization of Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae* on Different Carbohydrate Sources," in J. Phys.: Conf. Ser. 1477 072007.

[12] Y. Prayogo, “Efikasi cendawan entomopatogen *Beauveria bassiana* Bals. Terhadap kepik hijau (*Nezara viridula* L.),” in Jurnal HPT Tropika, vol. 13, no. 1, pp. 75-86, 2013.

Acknowledgment

Thanks to Universitas Muhammadiyah Tapanuli Selatan for providing research funding through the APB Internal Grants UM-Tapsel 2019 so that this research can be carried out.