

# Research Journal of Pharmaceutical, Biological and Chemical Sciences

## Endophytic Fungi As Biopesticides Against Rice Black Bug On Rice Plant.

Nur Amin\*, La Daha, and dan Nurariaty Agus.

Department of Plant Pests and Diseases, Hasanuddin University, South Sulawesi Indonesia.

### ABSTRACT

The previous study have documented the presence of endophytic fungi provide a protection of the plant hosts against insect herbivore, parasitic nematodes and plant pathogens. The present study aimed to investigate endophytic fungi *Beauveria bassiana* and *Trichoderma* sp. against rice black bugs *Paraeucosmetus pallicornis* in Greenhouse. The results showed that both the endophytic fungi *Beauveria bassiana* and *Trichoderma* sp statically different on mortality of the insect to compare with the control. The endophytic fungi *Beauveria bassiana* and *Trichoderma* sp was found back in the dead of the insect rice black bugs. The endophytic fungi *Beauveria bassiana* and *Trichoderma* sp are endophytic fungi which provide potential biological control for rice black bugs.

**Keywords:** Endophytic fungi, *Beauveria bassiana*, *Trichoderma* sp., Rice black bug

\*Corresponding author

## INTRODUCTION

Indonesia is the world's third-largest rice producer and also one of the world's biggest rice consumers. The country's rice area expanded from 11.4 million ha in 1995 to 13.2 million ha in 2010, which represented 24% of the total agricultural area. Rice yield increased slightly from 4.3 t/ha in 1995 to 5 t/ha in 2010. Rice is the most important food crop in the country. Relative to other sources, the share of per capita caloric intake from rice fell a little from 50.7% (1,260 kcal per day) to 47.6% (1,259 kcal per day) in 2009, while rice accounted for 42.9% per day of per capita protein requirements in 1995, which likewise decreased slightly to 39.6% per day in 2009.

A rice black bug is only as big as a "black bean" but it is very destructive. It sucks the juice from the midrib of leaves and panicles at the milk stage. In most cases, it feeds on the basal part of the tillers most often at night. A report from the Department of Agriculture said that during the vegetative stage, plants attacked by this become stunted. The youngest leaf shoot of the tiller fold longitudinally, turns yellowish to reddish brown, and later dies.

Rice black bugs are common pests to the ears damaged, deflated even empty panicles. Described by some farmers that the rate of moderate-severe attacks can decrease the production reaches 50%. This pest damage by sucking the liquid well on young grain and the grain that is rather old. Grain attacked be deflated / dry and the visible grain leather brown spots. Former the sucking on rice usually issued some sort of liquid sugar and allegedly where the growth of fungi that cause a bitter taste in rice [15].

Endophytic fungi are quite common in nature and some of them had been shown to have adverse effects against insects. The previous study have documented the presence of endophytic fungi provide a protection of the plant hosts against insect herbivore [13; 16; 7; 17 and 18], parasitic nematodes [10; 14], and plant pathogens [9; 11 and 16]. The present study aimed to investigate endophytic fungi *Beauveria bassiana* and *Trichoderma* sp. against rice black bugs *Paraeucosmetus pallicornis* in Greenhouse.

## MATERIALS AND METHODS

### Source of Endophytic Fungi

Endophytic fungi *Beauveria bassiana* and *Trichoderma* sp. was originally isolated from healthy branches of cocoa plant of local clone of South Sulawesi M.05 [12].

### Production of Isolates of Endophytic Fungi in Powder Form

Isolates of endophytic fungi *Beauveria bassiana* and *Trichoderma* sp. which is known to be effective in the other several studies and stored in the Laboratory of Plant Pathology, first purified and propagated on PDA (Potato Dextrose Agar). The isolates of endophytic fungi *Beauveria bassiana* and *Trichoderma* sp. propagated in rice medium containing chitin (1.0 gr). The rice medium that has been soaked for 3 hours put into a flask 100 grams, and autoclaved at 121 °C for 30 minutes and after which by using a corkborer (diam. 0.5 mm), five pieces of endophytic fungi were inoculated in once the fungi started growing, the flask were shaken to assure an even fungal growth. The grown fungi then incubated at 30 °C for 48 hours. The rice medium along with the fungi then blended to produce a powder for further study.

### Investigation of Endophytic Fungi *Beauveria bassiana* dan *Trichoderma* sp. Against Rice Black bug on Rice Plain Green House

This study investigated of mortality of rice black bug after the treatment of endophytic fungi *Beauveria bassiana* and *Trichoderma* sp. Rice seeds were planted on an plastic pot of 5 kg of soil. The treatment of endophytic fungi *Beauveria bassiana* and *Trichoderma* sp. on rice plants is done by spraying directly of all part of rice plant 3 times with a dose of  $1 \times 10^8$  CPU (Colony Forming Units). At the time of the rice plant has formed the heads, then put 10 of rice black, which have been given the lid. Observation of the mortality of rice Black bug carried out for 3 days.

**Investigation of Endofitisms of Isolates *Beauveria bassiana* and *Trichoderma* sp. On Rice Black Bug**

The rice black bugs *Paraeucosmetus pallicornis* were found dead on this investigation as in point 3 above, is done re-isolation of isolates *Beauveria bassiana* and *Trichoderma* sp. This is to ensure that the mortality of rice black bugs *Paraeucosmetus pallicornis* is the result of the treatment of endophytic fungi. The rice black bugs *Paraeucosmetus pallicornis* were found dead laid out on PDA, then every day is observed whether there is a direct growth of endophytic fungi on the rice black bug. Then calculated the percentage of endofitisms contained in the rice black bug.

**Data Analysis**

Anova was also performed to determine the effects of endophytic fungi *Beauveria bassiana* and *Trichoderma* sp. to mortality of rice black bug. The percent data were arcsine-transformed before being subjected to Anova. When significant differences were detected, means were separated using Tukey's test at 5% probability level.

**RESULT AND DISCUSSION**

**Investigation of Endophytic Fungi *Beauveria bassiana* dan *Trichoderma* sp. Against Rice Black bug on Rice Plain Green House**

The results showed that both the endophytic fungi *Beauveria bassiana* and *Trichoderma* sp statistically different on mortality of the rice black bugs *Paraeucosmetus pallicornis* to compare with the control (Table 1). On the first day showed that the mortality of rice black bugs *Paraeucosmetus pallicornis* in the treatment of *Trichoderma* sp. higher compared with the treatment of *Beuveria bassiana*, although it did not show statistically significant difference.

**Tabel 1. Mean of Mortality of Rice Black Bugs *Paraeucosmetus pallicornis* After the Treatment With Endophytic fungi *Beauveria bassiana* and *Trichoderma* sp.**

| Treatment                 | Mortality of Rice Black Bug |       |       |
|---------------------------|-----------------------------|-------|-------|
|                           | 1 DAI                       | 2 DAI | 3 DAI |
| Control                   | 0 b                         | 0 b   | 10 a  |
| <i>Beauveria Bassiana</i> | 16 a                        | 32 a  | 68 c  |
| <i>Trichoderma sp.</i>    | 22 a                        | 34 a  | 58 b  |

Columns followed by different letters are significantly different from another according to Tukey's (Test (P< 0.05) n = 5.

DAI = Day After Inoculation With Endophytic Fungi Isolate

**Investigation of Endofitisms of Isolates *Beauveria bassiana* and *Trichoderma* sp. On Rice Black Bug**

Endofitisms of isolates of *Beauveria bassiana* and *Trichoderma* sp. on the rice black bugs *Paraeucosmetus pallicornis* is 80 % to compare with 0 % of control (Table 2)

**Tabel 2. Percent of Endofitisms of isolates of *Beauveria bassiana* and *Trichoderma* sp. on the rice black bugs *Paraeucosmetus pallicornis***

| Perlakuan                 | % Endofitisme |
|---------------------------|---------------|
| Control                   | 0             |
| <i>Beauveria bassiana</i> | 80            |
| <i>Trichoderma sp.</i>    | 80            |

The results showed that both the endophytic fungi *Beauveria bassiana* and *Trichoderma* sp statistically different on mortality of the rice black bugs *Paraeucosmetus pallicornis* to compare with the control (Table 1). On the first day showed that the mortality of rice black bugs *Paraeucosmetus pallicornis* in the treatment of *Trichoderma* sp. higher compared with the treatment of *Beuveria bassiana*, although it did not show statistically significant difference.

Cuaterno [4] reported that *M. anisopliae* spraying with a concentration of  $1 \times 10^8$  spores are very effective in controlling rice black bugs. *M. anisopliae* is a systemic fungal and it Infest and grow well on the rice black bug. The effectiveness of endophytic fungus *B. bassiana* and *Trichoderma* sp. increases with increasing number of days of observation, it confirms that this fungus works well on the control system of rice black bug. Aguda [1] reported that *B. bassiana* is a fungus with the potential as a biocontrol agent against brown plant hopper pest. The results obtained in this study are consistent with findings of other researchers [2; 20]. Kiran and Veeranna [6] reported that the effectiveness of *M. anisopliae* was not significantly different from the treatment of Thiomethoxam and Imidacloprid. Increased effectiveness of *B. bassiana* and *Trichoderma* sp. based on the observations fit well with several other researchers [8 and 21]. The success of control using endophytic fungi in the system can not be separated from the system of planting rice plants, which are always in a state of high humidity that endophytic fungi can easily grow and develop in rice.

The occurrence of mortality was greater in the treatment of *Trichoderma* sp. compared to treatment of *B. bassiana* in the early observations of the workings of a well due to secondary metabolites produced toxin or endophytic fungus *Trichoderma* sp. compared with *Beuveria bassiana* in rice plants. Several investigators have reported the production of secondary metabolites or toxins produced by the fungus endophyte that is as anti-insect and anti microbial: are terpenoids [5], alkaloids [3 and 19].

#### CONCLUSION

Both the endophytic fungi *Beuveria bassiana* and *Trichoderma* sp statistically different on mortality of the rice black bugs to compare with the control. Both the endophytic fungi *Beuveria bassiana* and *Trichoderma* sp *Beuveria* sp., was found back in the dead of the insect rice black bugs. The endophytic fungi *Beuveria bassiana* and *Trichoderma* sp are endophytic fungus which provide potential biological control for rice black bugs.

#### ACKNOWLEDGEMENTS

We would like to thank the State Minister of Research and Technology, Republic of Indonesia for the financial support provided for the study, under the Contract of Insentif RisetSinan 38/SEK/INSINAS/PPK/I/2014. We also expand our thanks to the head of Hasanuddin University Research Institute for his valuable advice during the study.

#### REFERENCES

- [1] Aguda, R.M., Litsinger, J.A. and Roberts, D.W. 1984. Pathogenicity of *Beuveria bassiana* on brown planthopper (BPH), whitebacked planthopper (WBHP), and green leafhopper (GLH). *International Rice Research Newsletter*. 9 :20.
- [2] Aguda, R.M., Rombach, M.C., Im, D.J. and Shepard, B.M. 1987. Suppression of populations of the brown planthopper, *Nilaparvata lugens* (Stal.) (Homoptera.: Delphacidae) in field cages by entomogenous fungi (Deuteromycotina) on rice in Korea. *Journal of Applied Entomology*, 104 : 167-172.
- [3] Clay,K.and Cheplick,G.P. 1989 Ergot alkaloids from fungal endophyte-infected grasses on fall army worm (Spodopterafrugiperda). *Journal of Chemical Ecology*,15,169Ð182.
- [4] Cuaterno, W.R. 2007. Current Status of Rice Black Bug and its Management in the Philippines. In: Rice black bugs (Ravindra, C. Joshi, Alberto T. Barrion and Leocadio S. Sebastian. eds.), *Phil Rice, Philippines*. 653-660
- [5] Hu, Z.-Y.; Li, Y.-Y.; Huang, Y.-J.; Su, W.-J.; Shen, Y.-M. 2008. Three new sesquiterpenoids from *Xylaria* sp. NCY2. *Helv. Chim. Acta*, 91, 46-52.
- [6] Kiran, R. and Veernna, R. 2012. Evaluation of bio-pesticide *Metarhizium anisopliae* against brown plant hopper (*Nilaparvatha lugens*) and its efficiency on the improvement of the productivity of paddy. *International Journal of Plant Protection*, 15 : 81-83.

- [7] La Daha, Nur Amin and Abdullah, T. The study on the roles of predators on asian corn stem borer, *Ostrinia furnacalis* guenee (Lepidoptera: Pyralidae). *On Line Journal of Biological Sciences*, Volume 16 (1) : 49 - 55
- [8] Li Mao-Ye, LIN Hua-Feng, LI Shi-Guang. and Jin Li. 2012. Virulence of *Metarhizium flavoviride* 82 to different developmental stages of *Nilaparvata lugens* (Hemiptera: Delphacidae). *Acta Entomologica Sinica*, 55 (3) : 316-323.
- [9] Nur Amin. 2013 a. Diversity of endophytic fungi from root of Maize var. Pulut (waxy corn local variety of South Sulawesi, Indonesia). *International Journal of Current Microbiology and Applied Science*, Vol 2 (8) : 148-154.
- [10] Nur Amin. 2013 b. Investigation of Culture Filtrate of Endophytic Fungi *Nigrospora* sp. Isolate RS10 in Different Concentrations towards Root-Knot Nematode *Meloidogyne* spp. *Indian Journal of Science and Technology*, Vol 6, (9) : 5177 – 5181
- [11] Nur Amin; La Daha; A. Nasruddin, M.Junaed and Andi Iqbal. 2013. The Use of Endophytic Fungi as Biopesticide Against Downy Mildew *Perenosclerospora* spp. On Maize. *Academic Research International*. Vol 4 (4) : 153 - 159
- [12] Nur Amin., Salam, M., Junaid, M., Asman and Baco, MS. 2014a. Isolation and Identification of Endophytic Fungi From Cocoa Plant Resistant VSD M.05 and Cocoa Plant Susceptible VSD M.01 in South Sulawesi, Indonesia. *International Journal of Current Microbiology and Applied Science*, Vol 3 (2) : 459 – 467
- [13] Nur Amin, La Daha and Nurariaty Agus. 2014b. The Study on the Role of Entomopathogenic Fungal Endophytes in Controlling the Cocoa Pod Borer (*Conopomorpha cramerella* (Snellen)) Lepidoptera : Gracillariidae) on Cocoa Plant. *Journal of Entomology* Vol. 11 (3) : 142 - 152
- [14] Nur Amin. 2015. Nematicidal activity of root exudates of sengon plant inoculated with endophytic fungi *Nigrospora* sp. to control of root-knot nematode *Meloidogyne* spp. *Journal of Chemical and Pharmaceutical Research*, 7(3): 307 - 310
- [15] Nur Amin, La Daha, Nurariaty Agus, Ade Rosmana and Muh. Fadlan. 2015a. Diversity of some endophytic fungi associated with rice black bug *Paraeucosmetus pallicornis* on rice plant. *Journal of Chemical and Pharmaceutical Research*, 7(4):1246-1253
- [16] Nur Amin; Muslim Salam; Asman; Ryan and Rahim Danial. 2015 b. Investigation of Endophytic Fungi Towards Vascular Streck Dieback *Oncobasidium theobromae* on Seedling of Cocoa Plant. *Journal of applied biological science*, 9(2) : 86 - 89
- [17] Nur Amin. 2016. Endophytic Fungi to Control of Cocoa Pod Borer (*Conopomorpha cramerella*) on Cocoa Plantation. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 7(6) : 1496 – 1501.
- [18] Nurariaty Agus; Itji Diana Daud; Nur Amin, and Sri Nur Aminah. 2016. Attractiveness And Feeding Ability Of *Coccinella* Sp. On Pellet Added Natural Preservative. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, Volume 7 (5) : 2867 – 2872
- [19] Potter, D.A., Stokes, J.T., Redmond, C.T., Schardl, C.L. & Panaccione, D.G. 2008. Contribution of ergot alkaloids to suppression of a grass-feeding caterpillar assessed with gene-knock out endophytes in perennial rye grass. *Entomologia Experimentalis et Applicata*, 126: 138 - 147.
- [20] Rammohan Rao, P. 1989. Study on culture techniques, safety and control potential of certain entomopathogenic fungi of rice pests. Thesis, Ph.D., Tamilnadu Agric. Univ., Coimbatore, 212p
- [21] Rombach, M.C., Aguda, R.M., Shepard, B.M. and Roberts, D.W. 1986. Infestation of rice brown planthopper, *Nilaparvata legens* (Homoptera: Delphacidae), by field application of entomopathogenic hyphomycetes (Deutero-mycotina). *Entomological Society of America*. 15 : 1070-1073.