EFFECTIVITY OF ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA* TO CONTROL WHITE GRUB *LEPIDIOTA* SP.

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ABSTRACT

Entomopathogenic fungus *Beauveria bassiana* is soil inhabited fungus that known to have a wide range of hosts. Previous work showed that this fungus can be cultured in solid media using rice bran. The objective of this research was to study the effectivity of *B. bassiana* cultured in rice bran to control *Lepidiota* sp. in semi field experiment. Two grubs were inoculated into seven-weeks old soybean planted in a polybag. The fungus was applied one week after grub inoculation, in three levels of dose, i.e. 1 g cultured fungus/100 g soil; 1.5 g cultured fungus/100 g soil; 2 g cultured fungus/100 g soil, and control with no fungal application. The applications of the fungus were conducted with two methods, first, the solid cultured fungus was applied directly into soil around root area, and second, the solid cultured fungus was diluted into 400 mL water and the fungal solution was poured on the soil. The results showed that the mortality of treated grubs was not significantly different from those untreated grubs. However, the weight of fresh root, fresh plant and dry plant of plant treated directly with 1.5 g cultured fungus/100 g soil was significantly higher than those of other treated plants and untreated plant. The direct application of 1.5 g cultured fungus/100 g soil resulted in higher number of seed per plant than other applications. In conclusion, this fungus can be used to control *Lepidiota* sp., although higher dose may be needed.

Key words: Effectivity, Entomopathogenic Fungus, *Beauveria bassiana*, White Grub, *Lepidiota* sp

INTRODUCTION

Soybean is an important crop in Indonesia; however, its productivity is low. Average national yield was 1.3 juta ton/ha (BPS, 2007), while the potential yield could reach 2.5-3.0 ton/ha. Indonesia imports soybean 2.1 million tons/year that equal to Rp. 4.6 billion (Mashar, 2008). The white grub *Lepidiota* sp. is one of the pests limiting the production of soybean. The beetles appear during the first month of west monsoon. The female beetles prefer to lay their eggs in the fields with vegetation to bare soil or those cover with mulches. The grubs grow rapidly and within 2.5 month they develop about 4 cm long (Kalshoven 1981). This makes them very destructive when feeding the root of the plant. The root damage can be very severe that cause the plant die (Pers. Observation).

White grub is difficult to control, because they live in soil. Control of this pest is usually conducted by using systemic insecticides applied into soil. Synthetic insecticides could

harm the environment due to insecticides residue, therefore alternative control method should be sought. The grubs can be controlled biologically using their natural enemies, including entomopathogen.

Beauveria bassiana is entomopathogen fungi that attack more than 700 insect spesies (Li 1988; Glare and Milner 1991; Humber 1991; Goettel *et al*., 2000). Although attacking insect, this fungi can be isolated from soil around plant root (rhizosphere) and living associated with plant without causing diseases (endophyte) (Devi *et al*., 2008). Previous study in Indonesia showed that *B. bassiana* can be used to control paddy bug *Lepotocorisa acuta* Thumberg, sweet potato borer *Cylas formicarius* (F.), termites, army worm *Spodoptera litura* F., small pepper weevil *Lophobaris piperis* Marsh., corn ear worm *Helicoverpa armigera*, semi-looper *Chrysodeixis chalcites*, brown plant hopper *Nilaparvata lugens*, coffee berry borer *Hypothenemus hampei*, cacao pod borer *Conopomorpha cramerella*, diamondback moth *Plutella xylostella* (Tohidin, & Machdar, 1993; Priyanto *et al*., 1996; Saranga, 1996; Jauharlina & Chamzurni, 1999; Suprapto & Suroso, 1999; Soetopo *et al*., 2005; Thalib *et al*., 2005).

Beauveria bassiana produces toxin beauvericin (Kučera dan Samšiňáková, 1968). This toxin interfere the function of insect haemolymph and nucleus. Infected insect become swollen and harden. The fungi could infect the insect through contact and food contamination. Inside host body, the fungi multiply quickly so the whole insect cells are infected. Infected insect will stop eating, become weaken and then die (Broome *et al*., 1976). The body of died insect is usually covered with the white conidia of *B. bassiana* (Cheung & Grula, 1982). Although in some cases, this symptom is not always appears (Plate, 1976).

The use of *B. bassiana* to control white grubs in Indonesia has not been reported. In Nepal, indigenous *B. bassiana* isolates are reported to be effective to control several grub species, such as *Anomala dimidiata, Adoretus lasiopygus* and *Phyllognathus dionysius* (Dhoj *et al*., 2008). Therefore, it is important to study the patogenicity of *B. bassiana* against white grub *Lepidiota* sp. The aim of this study is to find the effective dose of *B. bassiana* to control *Lepidiota* sp.

MATERIALS AND METHODS

Experimental materials

The white grubs *Lepidiota* sp. were collected from the field in Wedomartani, Yogyakarta, Indonesia. Grubs were maintained individually in a plastic container (diam. 10 cm; height 10 cm) filled with soil (300 g) and fed with carrot. The soil was kept moist by spraying water and carrot was changed weekly.

Beauveria bassiana culture was obtained from Plant Protection laboratory at Universitas Pembangunan Nasional "Veteran" Yogyakarta, Indonesia. The fungi were isolated from grubs infested by *B. bassiana*. Rice bran was used as a solid media for mass production of *B. bassiana*. Rice bran was steamed for 45 min. One glass of the steamed rice bran was filled into 1 kg plastic bag then sterilized with autoclave for 1 hour (t: 121° C). Conidia of *B. bassiana* were harvested from Potato Dextrose Agar medium with a sterile aquadest blended with 1% Tween® 80 and 5 mL fungal suspension was taken to inoculate solid media using syringe. The inoculated rice bran was incubated for two

weeks (t: 28±1° C) until the fungi covering the media. To dry up the fungi, kaolin was mixed with *B. bassiana* culture (w/w=2:1), then spread onto plastic tray and kept in air conditioned room (t: 18° C) until dry. The number of conidia was counted using a haemocytometer, in 1 g dried mixed culture containing 2.2×10^6 conidia.

Mortality Test in Laboratory

Dried *B. bassiana* culture was mixed thoroughly with soil (1 g cultured fungus /100 g soil; 1,5 g cultured fungus /100 g soil and 2,0 g cultured fungus /100 g soil, depending on treatment). The grub was placed individually in a plastic container (diam. 10 cm; height 10 cm) filled with treated soil (300 g) and fed with carrot. The carrot was changed weekly. The soil humidity was kept about 75% by spraying water twice a week. Control treatment was conducted with same manner but without *B. bassiana* culture. Each treatment was replicated four times, each consisting 20 grubs. Mortality was observed weekly for 8 weeks.

Semi-field Experiment

Soybean var. Anjasmoro was used as a host plant. It was planted in a polybag (diam. 30 cm) containing a mixture of soil, compost and manure (1:1:1). When the plant was 9 weeks old, two grubs were infested into soil (20 cm depth). One week after grubs infestation, dried *B. bassiana* culture (1 g cultured fungus /100 g soil; 1,5 g cultured fungus /100 g soil and 2,0 g cultured fungus /100 g soil, depending on treatment) was applied with two methods. The first method was direct application of dried *B. bassiana* culture into soil around plant root. The second method was conducting by diluting the fungi (depending on treatment) with 400 mL water/pot mixed with 1% Tween® 80 using a blender. The fungal solution was poured on the soil. Control treatment was conducted with same manner but without *B. bassiana* culture. Each treatment was replicated seven times. The experiment were arrange according to completely randomized design. The observations were conducted two times, first, at two week after application (12 weeks after planting) and second, at four weeks after application (12 weeks after planting). Four plants were sampled destructively for each treatment during the observation. The parameters observed were larval mortality, root weight (fresh and dry), plant weight (fresh and dry), pods/plant, seeds/plant, seed weight/plant, weight of 100 seeds, and empty pods.

Data Analysis

The data obtained in each experiment was submitted to analyses of variance and means compared by the Least Significance Different (LSD) using SPSS 15. Statistically significant differences refer to P-values ≤ 0.05.

RESULTS AND DISCUSSION

Mortality Test in Laboratory

One week after of *B. bassiana* application, there was no mortality observed on treatment with 1,0 g cultured fungus/100 g soil and control. Low mortality level occurred on treatment with 1,5 g cultured fungus/100 g soil and 2,0 g cultured fungus/100 g soil. Two weeks after application all dose application caused mortality less than 10%. Grubs mortality increased gradually until eight weeks after application. The highest mortality

(30%) was caused by the application of 1,5 g cultured fungus/100 g soil or equal to $3,3\times10^6$ conidia (Fig. 1).

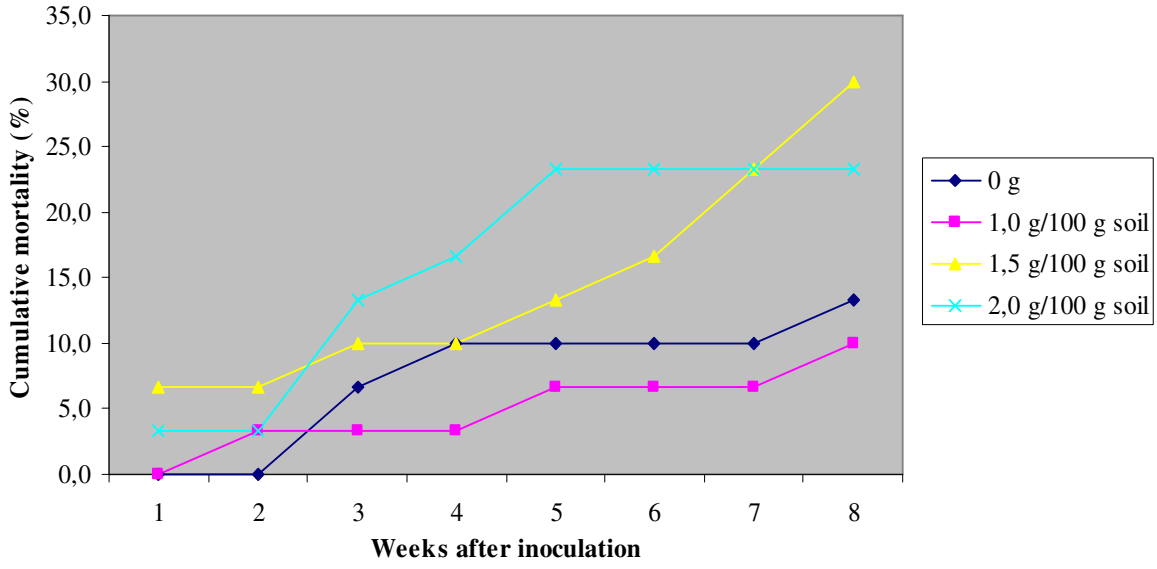

Fig. 1. Grubs mortality in laboratorium

The low level of mortality occurred could be caused by low number of conidia applied, so there is no sufficient contact between grubs and *B. bassiana* conidia to cause infection. Research on other white grub species, *Cyclocephala signaticollis* Burmeister (Coleoptera: Scarabidae), showed that *B. bassiana* caused 70% mortality on the third instar at 40 days after application with $1x10^8$ conidia/ml (Beron dan Diaz, 2005). Furthermore, environmental condition especially soil humidity and temperature also affect infection process and the sporulation of entomopathogenic fungi (Roberts & Campbell, 1977; McCoy *et al*., 1988). Spores germination and sporulation on insect cuticle need high humidity (>90% RH) (Millstein *et al*., 1983; Nordin *et al*., 1983), although to release *B. bassiana* spores from conidifore only need 50% RH (Gottwald & Tedders, 1982). In addition, the movement of grubs in soil could detach the spores that germinate on insect cuticle before penetration occurred, so infection did not happen. In the infection process, the conidia of entomopathogenic fungi need to be remained on the cuticles of host insect to germinate, and then penetrate the cuticle and proliferate in the body of host insects (St. Leger *et al*., 1989). Imidachloprid treatment on grubs *Anomala cuprea* inoculated with entomopathogenic fungi *Beauveria amorpha* could suppress grubs movement by paralyzing. Number of conidia attached on cuticle of those grubs was remained stable after 24 hours after treatment, whereas the conidia on non-paralyzed grubs were detached from the cuticle 12 hours after application (Yaginuma *et al.*, 2006).

Grubs mortality also observed in control (Fig. 1), although the soil was not inoculated with *B. bassiana.* The mortality might be caused by other entomopathogen exist in the soil. Dead grubs in control were rotten showing symptom of bacterial infection; those were different from fungal infection. Grubs infected by *B. bassiana* were swollen, harden and sometimes showing hyphal growth with white spores (Kučera dan Samšiňáková, 1968; Cheung dan Grula, 1982). However, in some cases, mycosis with

white spores growing in dead insect is not apparent. Aphids infected by *B. bassiana* were swollen without colour changes. Also, fly maggots infected by *B. bassiana* that were found on grasses have no spores appear (Plate, 1976).

Semi-field Experiment

Two weeks after application (12 weeks after planting), the grub mortality was variable. No grub mortality observed in polybag treated with 2.0 g cultured fungus/100 g soil, with the fungi diluted with water. However, all grubs were died in untreated soil (control; 100% mortality). Four weeks after treatment, there was no significant different on the mortality rate between grubs infested in soil treated with *B. bassiana* and grubs infested in untreated soil (control). In general, higher mortality rate observed four weeks after fungi treatment (Table 1). As, in laboratory experiment, dead grubs that found in untreated soil were not showing mycosis symptom. The mortality might be caused by other factor, such as bacterial infection. In soil, there are many entomopathogen bacteria capable to attack grubs (Hochberg, 1989; Jackson, 1999). In soil treated with *B. bassiana*, dead grubs were showing mycosis with the grub's body became swollen and harden.

Table 1. Grub mortality occured in semi-field experiment

Treatment	2 weeks after treatment	4 weeks after treatment
Direct application		
2.0 g/100 g soil	66.7±16.7 ab	62.5±12.5 a
1.5 g/100 g soil	16.7±16.7 a	87.5±12.5 a
1.0 g/100 g soil	66.7±16.7 ab	87.5±12.5 a
Diluted with water		
2.0 g/100 g soil	0.0±0.0 a	75.0±25.0 a
1.5 g/100 g soil	33.3±16.7 ab	100.0±0.0 a
1.0 g/100 g soil	33.3±16.7 ab	62.5±12.5 a
Control	100.0±0.0 b	62.5±23.9 a

Means followed by same letter within a column are not significantly different from one another at $P \leq 0.05$ (LSD).

Two weeks after application (12 weeks after planting), the fresh and dry weight of root of soybean treated with fungi diluted with water dose 2.0 g cultured fungus/100 g soil or direct application of 1.5 g cultured fungus/100 g soil was significantly higher than that treated with direct application of 1.0 g cultured fungus/100 g soil. The fresh weight of plant of soybean treated with fungi diluted with water dose 2.0 g cultured fungus/100 g soil was significantly higher than those treated with direct application of 1.0 g cultured fungus/100 g soil or fungi diluted with water dose 1.5 g cultured fungus/100 g soil (Table 2). In general plants with higher root weight were also had higher plant weight. Root function is to absorb nutrients from soil. The plants that have more roots will grow better than those that have fewer roots. Interestingly, low level of mortality was not necessarily correlated with low root weight and plant weight. This could be because the plants were already fully developed, so they can compensate the damage. Study on

artificial root damage by mechanical pruning of corn root, showed that when three quarter of root were pruned, the remaining root could compensate damage by growing more lateral root, resulting in a 30% reduction on dry weight of root (Gavloski *et al*., 1992).

Table 2. Fresh and dry weight of roots and plants of soybean treated with *Beauveria bassiana* two weeks after treatment (12 weeks after planting)

Treatment	Fresh Root (g)/plant	Dry Root (g)/ plant	Fresh plant (g)/ plant	Dry Plant (g)/ plant
Direct application				
2.0 g/100 g soil	3.5±0.4 ab	1.4±0.4 c	8.4±0.9 ab	2.7±0.4 a
1.5 g/100 g soil	5.3±1.8 b	0.9±0.2 bc	7.7±1.0 ab	2.7±0.3 a
1.0 g/100 g soil	1.0±0.5 a	0.2±0.1 a	4.7±1.8 a	2.0±0.5 a
Diluted with water				
2.0 g/100 g soil	5.6±0.3 b	0.8±0.0 bc	10.9±2.0 b	3.9±0.5 a
1.5 g/100 g soil	1.7±0.8 a	0.6±0.1 b	4.2±2.3 a	1.8±1.3 a
1.0 g/100 g soil	2.7±1.3 ab	0.9±0.2 bc	6.6±1.8 ab	2.3±0.4 a
Control	3.7±1.4 ab	0.6±0.2 b	7.2±2.0 ab	4.3±1.8 a

Means followed by same letter within a column are not significantly different from one another at P≤ 0.05 (LSD).

Table 3. Yield of soybean treated with with *Beauveria bassiana* two weeks after treatment (12 weeks after planting)

Treatment	Number Pods/plant	Number Seeds/plant	Seed weight /plant (g)	Weight of 100 seeds (g)	Empty Pods/plant
Direct application					
2.0 g/100 g soil	37.7±7.2 a	74.3±14.8 b	7.4±1.7 b	9.7±1.0 ab	1.0±0.7 a
1.5 g/100 g soil	20.7±4.9 a	32.3±7.9 a	3.8±1.0 ab	11.7±0.6 ab	1.0±0.4 a
1.0 g/100 g soil	34.0±11.7 a	50.7±13.3 ab	4.2±0.9 ab	8.5±0.9 a	6.3±3.8 b
Diluted with water					
2.0 g/100 g soil	29.3±0.7 a	55.0±3.5 ab	6.0±0.1 ab	11.0±0.6 ab	1.7±0.9 ab
1.5 g/100 g soil	20.7±10.7 a	38.0±15.6 ab	3.3±1.6 a	8.0±2.0 a	1.3±0.9 a
1.0 g/100 g soil	30.0±5.3 a	42.3±11.6 ab	3.2±0.6 a	7.8±0.8 a	3.3±0.9 ab
Control	19.3±4.4 a	28.0±11.9 a	3.2±0.9 a	12.8±1.9 b	2.7±0.3 ab

Means followed by same letter within a column are not significantly different from one another at P≤ 0.05 (LSD).

Two weeks after application (12 weeks after planting), the application of *B. bassiana* did not affect significantly the number of pods/plant The highest number seeds/plant and seed weight/plant were resulted from soybean treated with direct application of 2.0 g

cultured fungus/100 g soil (Table 3). These plants also had the highest dry weight of root (Table 2). Plant with high root volume has the high ability to absorb nutrient for plant growth and production. Direct application of 1.0 g cultured fungus/100 g soil resulted in the lowest number of empty pods/plant (Table 3). These plants also had the lowest weight dry of root (Table 2). Poor root may cause plant absorb the nutrient inadequately that the plant do not have enough sink (photosynthate) for pod filling.

Tabel 4. Fresh and dry weight of roots and plants of soybean treated with *Beauveria bassiana* four weeks after treatment (14 weeks after planting)

Treatment	Fresh Root (g)/plant	Dry Root (g)/ plant	Fresh plant (g/ plant)	Dry Plant (g/ plant)
Direct application				
2.0 g/100 g soil	3.5±0.3 a	0.6±0.1 a	3.4±1.0 a	4.1±0.9 bc
1.5 g/100 g soil	7.2±2.1 b	2.3±0.6 b	16.4±2.6 c	5.9±1.0 c
1.0 g/100 g soil	1.0±0.2 a	1.5±0.2 ab	11.9±2.7 bc	1.7±0.4 a
Diluted with water				
2.0 g/100 g soil	3.3±1.0 a	1.2±0.1 a	7.6±1.3 ab	3.0±0.4 ab
1.5 g/100 g soil	3.0±0.6 a	1.4±0.6 ab	6.2±2.3 ab	2.6±0.6 ab
1.0 g/100 g soil	4.0±1.7 ab	1.4±0.2 ab	5.4±0.8 a	2.4±0.6 ab
Control	2.4±0.6 a	1.4±0.3 ab	8.2±2.5 ab	3.6±0.6 ab

Means followed by same letter within a column are not significantly different from one another at P≤ 0.05 (LSD).

Tabel 5. Yield of soybean treated with with *Beauveria bassiana* four weeks after treatment (14 weeks after planting)

Treatment	Number Pods/plant	Number Seeds/plant	Seed weight /plant (g)	Weight of 100 seeds (g)	Empty Pods/plant
Direct application					
2.0 g/100 g soil	32.5±2.0 a	90.5±12.9 c	9.1±0.7 bc	10.6±1.4 a	3.3±0.9bc
1.5 g/100 g soil	75.3±30.3b	91.3±20.6 c	11.0±3.8 c	11.6±1.9 a	3.5±0.3bc
1.0 g/100 g soil	21.5±3.7 a	34.3±4.1 a	3.7±0.5 a	10.7±0.8 a	2.3±1.0ab
Diluted with water					
2.0 g/100 g soil	42.0±8.1 ab	66.0±11.6abc	7.2±0.9abc	11.3±1.1 a	2.5±0.5abc
1.5 g/100 g soil	39.8±9.9 ab	74.8±17.8 bc	8.8±1.8abc	12.2±1.3 a	1.0±0.6 a
1.0 g/100 g soil	22.3±3.6 a	38.0±3.4 ab	4.1±0.5 ab	10.8±0.6 a	1.8±0.5 ab
Control	32.3±8.5 a	46.8±15.0 ab	5.4±1.7 ab	11.3±0.8 a	4.5±0.9 c

Means followed by same letter within a column are not significantly different from one another at P≤ 0.05 (LSD).

Four weeks after treatment, plants with direct application of 1.5 g cultured fungus/100 g soil had highest fresh and dry weight of root and plant (Table 4). These plants also produced highest number of pods/plant, seeds/plant and seed weight/plant (Table 5). As

the results at two weeks after treatment, soybean with better plant growth was resulted in better yield.

CONCLUSION

This study showed that *B. bassiana* could infect *Lepidiota* sp. However, the fungi application at 2.0 g /100 g soil (4.4×10^6 conidia) did not sufficiently control the white grub. Yet, soybean treated with *B. bassiana* at 1.5-2.0 g cultured fungus/100 g soil at 10 weeks after planting yielded higher number of pods/plant, seeds/plant and seed weight/plant than soybean treated with 1.0 g cultured fungus/100 g soil and untreated soybean. Research to investigate the most vulnerable instar of *Lepidiota* sp. toward *B. bassiana* and the effective dose of *B. bassiana* against this grubs is needed.

ACKNOWLEDGMENT

This research was funded by Institute for Research and Community Service, Universitas Pembangunan Nasional "Veteran" Yogyakarta, Indonesia, Research Grant No. B/65A/V/2010/LPPM.

REFERENCES

Beron, C.M. & B. M. Diaz. 2005. Pathogenicity of hypothenemus fungi against Cyclocephala signaticollis. Bio Control 50: 143-150.

Broome, J.R., P.P. Sikorowski, & B.R. Norment. 1976. A mechanism of pathogenicity of *Beauveria bassiana* on larvae of the imported fire ant, *Solenopsis richteri*. Journal of Invertebrate Pathology 28: 87-91.

Cheung, P.Y.K. & E.A. Grula. 1982. In vivo events associated with entomopathology of *Beauveria bassiana* for the corn earworm (*Heliothis zea*). Journal of Invertebrate Pathology 39: 303-313.

Devi, K.U., J. Padmavathi, C.U.M. Rao, A.A.P. Khan, M.C. Mohan. 2008. A study of host specificity in the entomopathogenic fungus *Beauveria bassiana* (Hypocreales, Clavicipitaceae), Biocontrol Science and Technology 18: 975-989

Dhoj, G.C.Y., S. Siegfried Keller, P. Nagel, & L.Kafle. 2008. Virulence of *Metarhizium anisopliae* and *Beauveria bassiana* against common white grubs in Nepal. Formosan Entomology 28: 11-20.

Gavlosky, J.E., G.H. Whitfield, & C.R. Ellis. 1992. Effect of larvae of Western corn rootworm (Coleoptera: Chrysomelidae) and of mechanical root pruning on sap flow and growth of corn. Journal of Economic Entomology 85: 1434-1441.

Glare, T.R., & R.J. Milner. 1991. Ecology of Entomopathogenic Fungi. *In*: D.K. Arora, K.G. Mukherji and E. Drouhet (Eds.). *Handbook of Applied Mycology Humans Animals and Insects*. Marcel Dekker, New York. pp. 547-612.

Goettel, M.S., G.D. Inglis, & S.P. Wraight, 2000. Fungi. *In*: L.A. Lacey and H.K. Kaya (Eds.), *Field Manual in Invertebrate Pathology.* Kluwer Academic Press, Dordrecht, The Netherlands. pp. 255_282.

Gottwald, T.R. & W.L. Tedders. 1982. Studies on the conidia release by the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) from adult pecan weevil (Coleoptera: Curculionidae) cadavers. Environmental Entomology 11: 1274- 1279.

Hochberg, ME. 1989. The potential role of pathogens in biological control. Nature. 337:262–265.

Humber, R.A. 1991. Fungal Pathogens of Aphids. Proceedings of an OSU Centennial Event, pp.45-56.

Jackson, TA. 1999. Factors in the success and failure of microbial control agents for soil dwelling pests. Integrated Pest Manage Review 4:281–285.

Jauharlina & Chamzurni, T. 1999. Potensi *Beauveria bassiana* sebagai cendawan entomopatogen pada hama ulat grayak (*Spodoptera litura* F.). Seminar Peranan Entomologi dalam Pengendalian Hama yang ramah Lingkungan dan Ekonomis. Bogor, 16 Februari 1999.

Kučera, M. & A. Samšiňáková. 1968. Toxins of the entomophagous fungus *Beauveria bassiana*. Journal of Invertebrate Pathology 12: 316-320.

Mashar, A.Z. 2008. Teknologi untuk percepatan swasembada pangan dalam pembangunan ketahan pangan masional. Pangan 51:66-80.

McCoy, C.W., R.A. Samson, & D.G. Boucias. 1988. Entomogenous Fungi. In: C.M. Ignoffo. *CRC Handbook of Natural Pesticides. Microbial Insecticides, Part A. Entomogenous Protozoa and Fungi* (Ed.). CRC Press, Boca Raton, Florida. Vol. 5, pp. 151-236.

Millstein, J.A., G.C. Brown, & G.L. Nordin. 1983. Microclimatic moisture and conidial production in *Erynia* sp. (Entomophthorales: Entomophthoraceae): In vivo moisture balance and conidiation phenology. Environmental Entomology. 12: 1339-1343.

Nordin, G.L., G.C. Brown, & J.A. Millstein. 1983. Epizootic phenology of Erynia disease of the alfalfa weevil, Hypera postica (Gyllenhal) (Coleoptera: Curculionidae), in central Kentucky. Environmental Entomology 12: 1350-1355.

Plate, J. 1976. Fungi. Biological Control: A guide to natural enemies in North America. Cornel University. 4pp.

Priyanto, T.P., E. Priatna, M.K. Kardin, & A.R. Braun. 1996. Efektifitas *Metarhizium anisopliae* dan *Beauveria bassiana* untuk mengendalikan hama lanas ubijalar (*Cylas formicarius*). Seminar Nasional Pengendalian Hayati. Yogyakarta, 25-26 November 1996.

Roberts, D.W. & A.S. Campbell. 1977. Stability of entomopathogenic fungi. Miscellaneous Publication of Entomological Society of America 10: 19-76.

Saranga, A.P. 1996. Uji patogenitas jamur *Beauveria bassiana* Vulli (Hypomycetes: Moniliales) pada dua species rayap (Isoptera). Seminar Nasional Pengendalian Hayati. Yogyakarta, 25-26 November 1996.

Soetopo, D., S.G. Reyes, & D.R. Santiago. 2005. Laboratory assay of *Beauveria bassiana* isolates against *Helicoverpa armigera*. Proceedings of The 1st International Conference of Crop Security. Brawijaya, Malang, 20-22 September 2005. pp.46-55.

St. Leger, R. J., T. Q. Butt, M. S. Goettel, R. C. Staples & D. W. Roberts. 1989 Production *in vitro* of appressoria by the entomopathogenic fungus *Metarhizium anisopliae. Experimental Mycology* 13: 274–288.

Suprapto & Suroso. 1999. Pengaruh konsentrasi cendawan *Beauveria bassiana* Vulli terhadap aspek biologi penggerek batang lada (*Lophobaris piperis* Marsh.) (Curculionidae; Coleoptera). Seminar Peranan Entomologi dalam Pengendalian Hama yang ramah Lingkungan dan Ekonomis. Bogor, 16 Februari 1999. 8hal.

Thalib, R., A. Adam, Suwandi, Y. Pujiastuti, & S.Herlinda. 2005. Fitness of *Beauveria basiana* isolates from Sumatera and Java on *Plutella xylostella* larvae. Proceedings of The 1st International Conference of Crop Security. Brawijaya, Malang, 20-22 September 2005. pp. 105-107.

Tohidin, A.T. & B.P. Machdar. 1993. Daya bunuh jamur entomopatogen *Beauveria bassiana* (Balsamo) Vuillemin (Moniliales, Moniliaceae) terhadap *Leptocorisa acuta* Thumberg (Hemiptera: Alydiadae) di rumah kaca. Prosiding Makalah Simposium Patologi Serangga I, Yogyakarta, 12-13 Oktober 1993. p.135-143.

Yaginuma, D., H. Hiromori., & M. Hatsukade. 2006. Friction-associated conidial detachment of the entomopathogenic fungus *Beauveria amorpha* from the cuticle of a scarab grub, *Anomala cuprea*, in the soil. Applied Entomology and Zoology 41: 613–620