Identification and pathogenicity of entomopathogenic fungi for controlling the beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae)

[1]Ahsol Hasyim, [1]Wiwin Setiawati, [2]Hadis Jayanti, [3]Nusyirwan Hasan, [4]Muhammad Syakir

[1] Indonesia Vegetable Research Institute (IVEGRI), West Java, Indonesia; [2] Bali Assessment Institute for Agricultural Technology (Bali AIAT), Denpasar Selatan, Bali, Indonesia; [3] West Sumatra Assessment Institute for Agricultural Technology, West Sumatra, Indonesia; [4] Indonesian Agency for Agricultural Research and Development, Jakarta, Indonesia. Corresponding author: A. Hasyim, ahsolhasyim@yahoo.co.id

Abstract. The identification and pathogenicity of fungal entomopathogens aim for controlling the beet armyworm *Spodoptera exigua* L. Recently, the control of shallot pest *S. exigua* keeps using chemical insecticides. Chemical insecticides can cause pest insects to become resistant, demise of useful insects and environmental problem by abundant residue. Therefore, a biological control agent which is safe and effective needs to be sought and developed as an alternative for pest control. In this case, an entomopathogenic fungi is one of the alternatives for controlling shallot pest *S. exigua*. Many entomopathogenic fungi are associated with soil ecosystems. Fungal entomopathogens are obtained from the land planted with crops and fallow land. Entomopathogen fungi appear from the ground to catching the insect pest, usually *Tenebrio molitor* larvae was used. This research aims to isolate, characterize, and identify entomopathogen fungi, as well as figuring out the pathogenicity for controlling shallot pest *S. exigua.* The research results showed that entomopathogen which infects T. ,olitor is the entomopathogen fungi, it caused death in shallot pest S. exigua. Three kinds of entomopathogen fungi were successfully sub-cultured on SDAY media (sabouraud dextrose agar + yeast). These three types of fungi were characterized as *Metarhizium anisopliae*, *Beauveria bassiana* and *Verticillium lecanii*. From the three types of entomopathogen fungi, of which spore germination being tested, *M. anisopliae* has viability over 95% in average. Meanwhile, *B. bassiana* and *V. lecanii* have viability below 95% averagely. Average diameter of each fungal entomopathogen colony of *M. anisopliae*, *B. bassiana* and *V. lecanii* is relatively the same, no siginificant differences were observed. Nevertheless, in general the largest diameter of the colony from early observation until the end of the observation (20 day after inoculation) was found on *M. Anisopliae*, while *V. lecanii* generated more conidia when cultured on rice substrate and *B. bassiana* produced more conidia when cultured on wheat substrate. The observation results on the laboratory indicated 2 g/L water concentration of the entomopathogen fungi of *B. bassiana* and *V. lecanii* is capable of annihilating *S. exigua* larvae until 90%, whereas *M. anisopliae* with the same concentration caused mortality of *S. exigua* up to 98%. The results of this research showed that the three types of entomopathogen fungi obtained (*B. bassiana, M. anisopliae* and *V. lecanii)* cpotentialy to n be considered for development of biological control agents on shallot pest *S. exigua.*
Key Words: entomopathogen, *Metarhizium anisopliae*, *Beauveria bassiana*, *Verticillium lecanii* substrate**,** shallot.

Introduction. In Indonesia, *Spodoptera exigua* (Hübner) is one of the most damaging pests on shallots and leeks (Kalshoven 1981). *S. exigua* can attack shallots since the beginning of the growth and lead to some yield losses. The larvae damage the plants by eating the leaves. According to Sastrosiswojo (1996), the yield loss resulted from *S. exigua* larvae might reach 57%. Even, severe attacks may cause 100% yield loss because the leaves are all consumed by the larvae, making crop failures inevitable. These heavy attacks usually occur during the dry season that potentially decrease crop production (Shepard et al 1999; Setiawati et al 2014).

Entomopathogen fungus that has been used to control pest insects are *Beauveria bassiana* (Balsamo) Vuillemin (Wraight et al 2000; Hasyim & Azwana 2003; Hasyim 2006; Hasyim et al 2005; Hasyim & Gold 1999), *Metarhizium anisopliae* (Metch)

(Widayat & Rayati 1993a; Pendland & Boucias 1996; Gabriel & Riyanto 1989), *Nomuraea rileyi* (Farlow) (Lezama-Guterrez et al 2001), *Paecilomyces fumosoroseus* (Wize) Brown & Smith (Wraight et al 2000), *Fungi* sp., and *Spicaria* sp. The fungus is pathogenic against various types of insects with a broad host range (Lezama-Guterrez et al 2001; Prayogo et al 2005). The highly potential fungus and widely used to controlling the insects are some species of *B. bassiana*. The fungus is reported as a highly effective biological control agent against pest insect species including termites, white flies, and some beetles (Gillespie 1988). As the insect pathogen, *B. bassiana* can naturally be isolated from the field or from the ground. The epizootic in nature is influenced by climatic conditions, as they require warm and moist environment to grow up. In some countries, this fungus has been used as a biological control over a number of pest insects, ranging from food crops, ornamentals, fruits, vegetables, legumes, horticulture, forestry, and desert plantation (Vandenberg **1996; Cagáň** & Švercel 2001; Kouassi et al 2003; Tafoya et al 2004; Bextine & Thorvilson 2004; Sabbahi 2006; Barnet & Hunter 1972).

The main problem in the shallot cultivation is pest attacks, particularly *S. exigua*. This pest can cause damage which is quite detrimental, even causing 100% loss when is out of control. In dealing with the pest, farmers usually use insecticide intensively with high doses, which are very inefficient and potentially polluting the environment.

Controlling pest insects with chemical insecticides may also be problematic and raises many issues: the increasing pest resistance against chemical insecticides, population explosion on secondary pest insects, increasing risk of toxicity in humans and farm animals, contamination on ground water, decrease in biodiversity, and other hazards related to the environment. The emergence of these problems becomes a stimulant that raises the interest in integrated pest management (IPM) efforts. Sustainable agriculture in the 21st century will further put forward an alternative, eco-friendly effort to manage pest insect and minimize contact between humans and chemical insecticides. Insect pathogens (entomopathogens) have the chance to become the alternative pest control but they still need some improvements, including potentialy, production and formulation. Proper understanding on its ability to integrate with ecosystems, compliance with environmental and other components of the IPM, as well as acceptability to farmers or users. One of the alternative pest control by using entomopathogenic fungi, more than 700 species of entomopathogenic fungi are reported to have been isolated from different species of pest insects, but only 10 new species are successfully developed for pest controls (Hajek & St. Leger 1994). The types range from true parasitic pathogens to saprofit or without a host, causing some species of entomopathogenic fungi are very pathogenic against pest insects. Among the highly potential fungi in the attempt to control pest insects, there are *B. bassiana*. The fungi is reported as a highly effective biological control of pest insects species including termites, white flies, and some types of beetles (Gillespie 1988). As the insect pathogen, *B. bassiana* can naturally be isolated from the field or from the ground.

In the utilization of fungal entomopathogen as a biological pest control agent of *S. exigua*, the type and the isolated fungus must be determined first. The virulent entomopathogenic fungi can be obtained from the target pest or from rhizosphere on the plantation ecosystems where the pest lives, because soil is a natural reservoir for fungal entomopathogen. The use of entomopathogen fungi is the main program in the IPM. In addition, in order to the fungi can be used as a biological pest control agent against S. exigua, it is necessary to search for information about the existence, diversity, pathogenicity, as well as stadium of insects which is sensitive to the fungal entomopathogens on ecosystems. Therefore it needs to do pathogenicity tests on entomopathogenic fungus against shallot pest of *S. exigua* either in the laboratory or in the field.

The aim of the study is to identify and characterize entomopathogenic fungi on rhizosphere from the shallot plantation, and to investigate the pathogenicity of entomopathogenic fungi to control *S. exigua* pests in shallots

Material and Method

The collection of entomopathogen fungi. Entomopathogenic fungi used were taken from the rhizosphere of shallot field and the former field for sugar cane used as crop rotation with shallot plants in the region of Cirebon and Brebes, Indonesia with 6 soil samples, respectively. The soil samples were taken at random in two areas above, since each region has its own characteristics from the farming systems. The soil samples were obtained by digging at a depth of 5–10 cm around the shallot plant which was formerly used to plant sugar cane; each sample was as much as 5 x 400 g, then stored in plastic bags and labeled according to the name of location, type of commodity, and date taken. In each area, the soil samples were taken with a diagonal system, and then merged.

Isolation and culturing. Fungi that come from rhizosphere of shallot and sugar cane field were isolated using insects bait method (Goettel & Inglis 1997; Zimmermann 1986; Hasyim & Harlion 2002; Hasyim & Azwana 2003; Trizelia 2005) (Figure 1). Insects used as bait was *Tenebrio molitor*.

The soil samples were cleaned from roots, sifted with 600 mesh sieve and as many as 400 g of the sample was placed into a plastic box measuring 13 x 13 x 10 cm (each sample used 4 pieces box), then given a label in accordance with its area (Papierok & Hajek 1997; Hasyim & Azwana 2003). The soil was moistened with sterile water until moist, then 10 larvae of *T. molitor* instar 3 which just molted (the skin was still white) were brought into the boxes containing the soil samples. The larvae were then covered with an upper ground and moistened by spraying sterile water. The next box was covered with strips of muslin material which was also moistened. The larvae of *T. molitor* allegedly stricken with entomopathogenic fungi would be observed on the third day after treatment, but every day observation was also necessary. The larvae stricken with entomopathogenic fungi were isolated as the object to be tested (Papierok & Hajek 1997; Zimmermann 1986; Nankinga 1999).

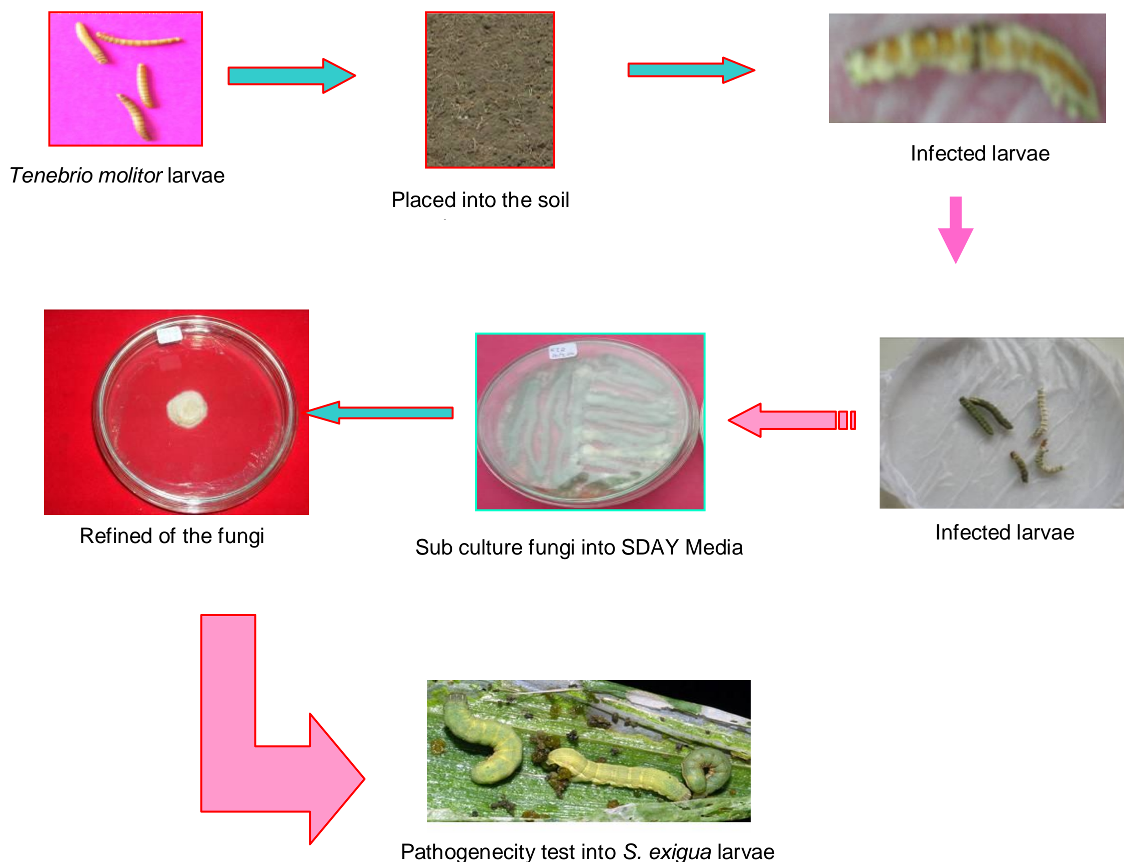


Figure 1. Isolation of entomopathogenic fungi from the soil with insect bait method.

The larvae infected by the entomopathogen fungi was first sterilized with 1% natrium hyphochlorit for 3 minutes, then rinsed with sterile water 3 times and dried on sterile paper filter. The larvae were placed into the petri dish containing a sterile, moist sterile paper filter, and they were incubated to stimulate the growth of the entomopathogenic fungi. Conidia of the entomopathogenic fungi that came out from the body of the infected larvae were taken with a needle loop, and moved onto the medium of sabouraud dextrose agar + yeast (SDAY) with the composition of 40 g dextrose, 10 g peptone, 2.0 g yeast extract 15 g agar and 1 L sterile water (Samson 1981) and incubated for 7 days at a temperature of 23–25°C (Poinar & Thomas 1984; Papierok & Hajek 1997).

The growing fungi were identified and characterized in macroscopic and microscopic ways (Madelin 1963; Barnett & Hunter 1972; Poinar & Thomas 1984; Humber 1997). The identification on each fungus was done by observing the properties, morphology, physiology (viability, rate of growth of the colony and sporulation) of the fungus. Each type of entomopathogen fungi was purified in the SDAY media as the source of the entomopathogen fungi to be used in the testing of the next target pests.

Mass production of Beat army worm (BAW), S. exigua. BAW population was originated from eggs cluster collected from shallot crops in Lembang, Bandung, Indonesia. Eggs were allowed to hatch in a 20 x 15 x 10 cm wooden cage and larvae were reared using shallot leaves as diet until pupae and then imagos. Imagos were reared using honey as diet in the same cage, and then paired to allow copulation. Eggs clusters generated from the copulation were allowed to hatch and larvae were reared as previously until instar 3

The provision of shallot plants. Shallot plants used to feed the larvae of *Spodoptera* were planted in a poly bag. The shallots belong to Sembrani variety.

Implementation of the research

Identification of the entomopathogen fungi. The aim of this studies is to find out variety of entomopathogen fungi found in the soil of the and shallots and sugar cane field that can be used to control shallot pests. Each entomopathogenic fungi was already purified and identified macroscopically and microscopically.

Physiological characterization of entomopathogen fungi. Each of purified fungi was characterized in terms of physiology. Observations were made against the viability, growth rate of the colony, and sporulation.

a. Conidia viability

Evaluation of viability was done according to the method proposed by Junianto & Sukamto (1995). Slab-shaped SDAY medium with the size of 1 cm^2 and 1-2 mm thickness was placed on a sterile glass object. The medium was shed with 10 μL conidia suspension containing 10^6 conidia/mL from each of fungi, and next the medium was placed into a sterile petri dish filled with moist filter paper and incubated at 25°C for 18 hours. Each treatment was repeated 4 times. Observations were through light microscopes with 400 times magnification. The sprout percentage was calculated from 100 conidia. Conidia was considered sproutting when the tube of germinated sprouts has been exceeding the diameter of conidia. The experiment was arranged in a complete random design. Data obtained were processed with analysis of variance (ANOVA) and continued with Tukey's test (HSD) on the 5% significance level.

b. The growth rate of of the colony

Each fungus aged 7 days with a diameter of 10 mm was inoculated on SDAY medium in petri dish and incubated at a temperature of 25°C. Colony diameter from each fungus was measured every 5 days until Day 20. Data obtained were processed with ANOVA and continued with Tukey's test (HSD) on a significance level of 5%.

c. Sporulation on several types of substrate

The experiment used Complete Random Design with factorial pattern consisting of two factors, namely types of fungi and variety of substrates with 5 repetitions. The data

obtained were analyzed with ANOVA, when there were real and significant differences, they were again tested with DNMRT at the 5% significance level.

The substrates used were rice, corn, soybeans and wheat. All the substrates were cleaned. Then, they were boiled until somewhat soft (approximately for 45 minutes). Furthermore the 250 g substrates of corn, rice, soybeans and wheat were placed into a plastic bag, and sterilized in the autoclave with the temperature of 121°C for 60 minutes. Furthermore, they were left to cool for approximately 12 hours. Culture of each entomopathogen fungus aged 15 days or as much as 5 mL suspension conidia (conidia density 10^7) was inoculated into plastic bags containing the substrates. Then, the bag was closed and incubated for 14 days at room temperature (25°C).

The bag was checked every 2 days while stirred it. After 14 days, the fungi culture on substrate were able to harvest. Each entomopathogen fungi culture on each substrate was taken as much as 1 gram to be sent to test tubes, added with 5 mL sterile aquades. The culture was vortexed, filtered and diluted up to 4 times. The number of conidia per gram and substrate per mililiter were calculated using haemocytometer.

Concentration of entomopathogen fungi. The aim is to know the dosage of each entomopathogenic fungi against the larvae of instar 3 S. *exigua*. The fungi were grown on rice and then blended. Every 1 g of fungi was contained $1x10^9$ conidia. The experiment applied Complete Random Design with factorial pattern consisting of two factors, namely the type of entomopathogen fungi and the concentration. The data obtained were analyzed with different ANOVA; if significant differences were found, they were again tested with DNMRT at 5% significance level.

As many as 10 larvae of the same instar were placed into a plastic box with a diameter of 10 cm. Inoculation of entomopathogen fungi was done by spraying the conidia suspension at the dorsal and ventral of larvae body from each dose used. Then, the larvae were fed fresh shallot leaves that were already washed with sterile water. Mortality of the larvae was observed every day up to 7 days after sprayed by entomopathogen fungi.

Larval mortality percentage was calculated by using the formula: $M = A / D \times 100\ \%$

Where: M = mortality percentage
A = number of dead insects due to infected by the fungi
D = number of insects being tested

The mortality percentage obtained was then corrected by using Abbott's formula:

$$P = \frac{P_0 - P_c}{100 - P_c} \times 100\ \%$$

Where: P = percentage of the test insects which died after corrected
P_0 = percentage of the test insects which died on the treatment
P_c = percentage of dead insects on the control

The mortality percentage was then transformed into the probit analysis using statistical analysis system (SAS) version 6.12 software to figure out the pathogenicity of each entomopathogen fungus.

Results and Discussion

Identification of the entomopathogen fungi. Based on the identification results of the fungi on SDAY medium, six genus of fungi with different characteristics and colors of colonies were found (Table 1).

In details, the morphological characteristics of the three fungus are as follows:
B. bassiana, has a short hypha, straight and thick hyalin. The hypha group emerges from the middle with the length of 3-4 µm and a width of 1-2 µm, with white colony, round

conidia with size of 2-3 x 2-2.4 μm hyalin, one-celled, formed in solitary at ends of conidiophor and attached to the short sterigma with intermittent growth patterns, simpodial growth of conidiophor (Vandenberg 1996; Domsch et al 1980; Samson 1981) (Figure 2).

Table 1

Morphological characterization of entomopathogenic fungi of 14 days old on SDAY

Isolate	*Locality*	*Colony color*	*Hypae*	*Conidia*	*Species*
BVL1	Lembang	White	Hyalin	Round, hyalin	*Beauveria bassiana*
BVC1	Cirebon	White	Hyalin	Round, hyalin	*Beauveria bassiana*
MAL 1	Lembang	Light to dark green	Hyalin	Cylindris, hyalin	*Metarhizium anisopliae*
MAC1	Cirebon	Light to dark green	Hyalin	Cylindris, hyalin	*Beauveria bassiana*
VLL	Lembang	White, Orange	Hyalin	Cylindris to elips, contain of single sel, no colour	*Verticillium lecanii*

BVL1 = *Beauveria bassiana* (collected from Lembang), BVC1 = *Beauveria bassiana* (collected from Cirebon), MAL1 = *Metarhizium anisopliae* (Collected from Lembang), MAC1 = *Metarhizium anisopliae* (collected from Cirebon), VLL = *Verticillium lecanii* (collected from Lembang).

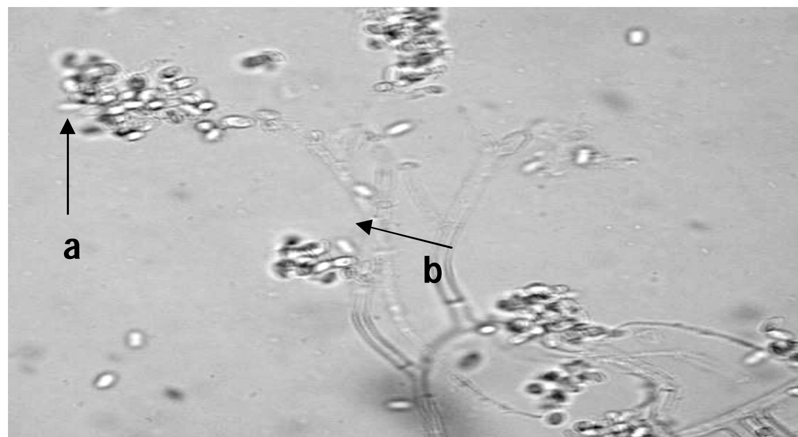


Figure 2. *Beauveria bassiana* (Magnification 100 X); a - Conidia; b - Hyphae (Nuraida 2006).

Metarhizium has insulated mycelium, upright conidiophor with varying sizes between 4.0-13.4 x 1.4-1.6 μm, coated and branched, filled with conidia, single-celled hyaline colored conidia, spherical-shaped cylinders. The conidia is 4-7 μm length and 1.43 x 2.0 μm length. It has a fialide with varying size between 6.2-8.0 x 1.7-3.5 μm. White fungi colony then later changes to dark green when aging (Vandenberg 1996; Domsch et al 1980; Samson 1981.) (Figure 3).

Verticillium lecanii (Monilialis: Moniliaceae) has upright conidiophore, differentiated from the negative hypha, has a lot of branches along the bar and is needle-shaped. Some of them have a flat shape at the base. Fungal colonies grow rapidly in sabouraui dextrose agar (SDA) medium in 23°C for 7 days. The entire surface is covered with smooth colored Hypha (Boucias & Pendland 1998) (Figure 4).

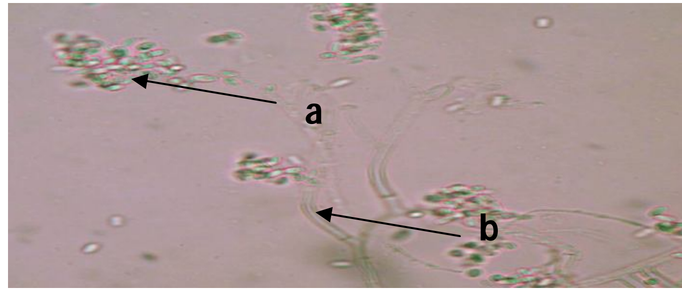


Figure 3. *Metarhizium anisopliae* (Magnification 400 X); a - Conidia; b - Hyphae (Nuraida 2006).

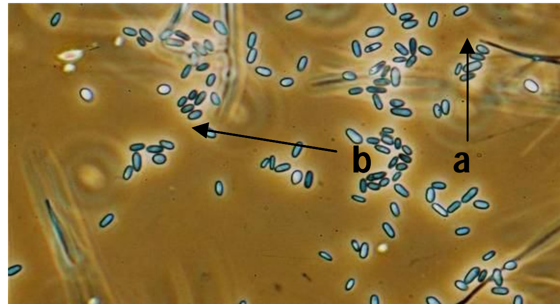
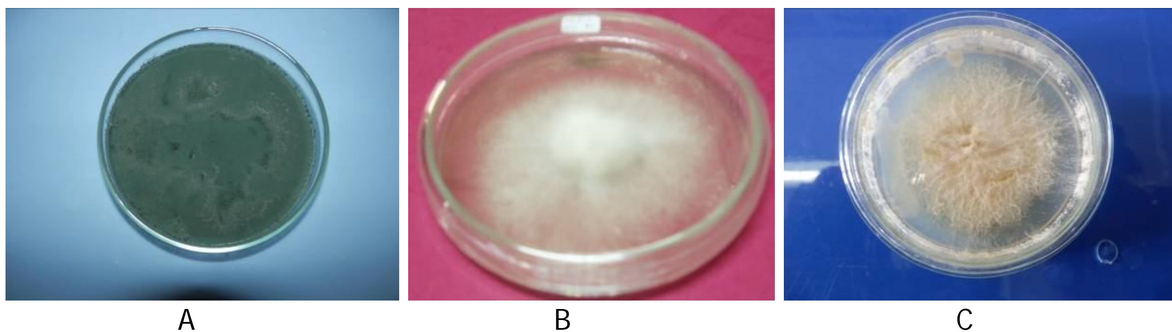


Figure 4. *Verticillium lecanii* (Magnification 400 X); a - Conidia; b - Hyphae (Nuraida 2006).

The growth of the entomopathogenic fungus in sabaroud of dextose agar was done at room temperatures (22-23°C) and took 2-3 weeks to complete the production process (Figure 5).



A B C

Figure 5. Entomopathogenic fungi growth on Sabouraud dextrose agar + Yeast (SDAY); a - *M. anisopliae; b - B. bassiana; c - V. lecanii* (Nuraida 2006).

Physiological characterization of entomopathogen fungi

Viability. Viability is a very important parameter because it is very decisive in the success of fungi growth. Evaluation of viability in conidia needs to do especially when the fungi would be developed as a biological pest control. Conidia is stated viable when the length of the sprout has been exceeding conidia diameter (Junianto & Sukamto 1995) or the length is more than 3 μm. The results of the ANOVA show that the viability of entomopathogen fungi in 18 hours after inoculation (hai) is not significant different among fungi (Table 2). Of the three types of entomopathogen fungi which viability is being tested, *M. anisopliae* has viability above 95% in average. For *B. bassiana* and *V. lecanii*, the viability is mostly under 95%. The results of the other studies reveal that the percentage of viability of entomopathogen fungi after 16 hours varies between 27-100%, depending on the type of the fungi, the composition of the medium especially the content of carbon and nitrogen that are used (Mustafa & Kaur 2009). Kassa (2003) says that fungi which have viability above 80% are qualified to develop as a biological pest control.

Generally, all the isolated fungi from rhizosphere have good viability so they potentially develop as a biological control agent for shallot pest *S. exigua*.

The growth rate of the colony. The observation results on the colony growth rate on each entomopathogenic fungi was conducted every 5 days until the 20th day after inoculation (dai) are not in significant different among entomopathogenic fungi (Table 3).

Table 2

Viability average (percent) of entomopathogenic fungi

Entomopathogenic fungi	*Average viability (%) ± SD*
Metarhizium anisopliae	97.25±1.16[a]
Beauveria bassiana	94.50±2.65[a]
Verticilium lecanii	93.25±1.85[a]

Table 3

The average diameter of entomopathogenic fungi colonies on media SDAY from 5 to 20 dai

Species	*Diameter colonies (cm) day after inoculation (dai)*			
	5 ± SD	*10 ± SD*	*15 ± SD*	*20 ± SD*
Metarhizium anisopliae	5.75±0.97[a]	6.75±1.27[a]	7.12± 1.11[a]	8.25±1.15[a]
Beauveria bassiana	5.25±0.57[a]	6.23±1.25[a]	6.87± 1.12[a]	7.95±1.23[a]
Verticillium lecanii	5.15±1.10[a]	6.50±1.17[a]	6.75 ± 1.20[a]	7.72±1.57[a]

In general, the growth of *M. anisopliae* colony is faster than of the other fungi. On observation of 5 to 20 days after inoculation, average diameter of each colony of *M. Anisopliae*, *B. bassiana* and *V. lecanii* is relatively the same and in fact not significantly different. Nevertheless, overall it can be seen that the largest diameter of the colony from the initial observation until the end of the observation (20 days after inoculation) was found in *M. anisopliae*. *M. anisopliae* can grow well in the temperature range of 20-30°C with 90% humidity (Mc Coy et al 1988). The optimum temperature for the growth of *M. anisopliae* ranges from 22°C to 27°C (Roberts & Humber 1981; Kassa 2003) although some reports state that fungi can still grow in a cooler temperature (Bidocha et al 2000). Conidia will form sprouts on a humidity above 90%. According to Walstad et al (1970), the optimum temperatures for growth and sporulation of *B. bassiana* is between 25-30°C with 100% humidity. Furthermore, Ferron (1981) reported that the optimum temperature for the growth of *B. bassiana* conidia is between 23-25°C. In this study, the growth rate of all fungi colonies was tested in incubation at room temperature (22-25°C) with 70-80% humidity. Some reports suggest that every genus or species needs adequate nutrients, pH, moisture in the medium, optimum temperature, light, aeration, and incubation period for growth and formation of conidia (Pham et al 2009; Barlett & Jaronski 1988; Latgé & Moletta 1988; Kleespies & Zimmermann 1992).

Sporulation on several types of substrate. Each entomopathogen fungus has a different ability to thrive on some types of substrate as shown in Table 4.

Table 4

Average number of conidia at 4 culture substrate types (total conidia/gram substrate)

Entomopathogenic fungi	*Average number of conidia/gram substrate) (X 10^{9})*			
	Rice	*Soybean*	*Corn*	*Wheat*
Vertilicillium lecanii	7.25[b]	6.25[b]	4.50[a]	6.25[b]
Beauveria bassiana	5.75[c]	8.75[a]	5.25[a]	7.25[a]
Metarhizium anisopliae	10.50[a]	5.25[c]	7.00[a]	4.75[c]
CV	23.30%	22.63%	26.40%	16.44%

CV - coeffecient variation.

The result of the ANOVA indicates that there is a difference in the number of conidia between types of fungi and substrate that is used. The substrate that can generate more for *M. anisopliae* and *V. lecanii* conidia is rice substrate, while *B. bassiana* produces more conidia on wheat substrate. Hence, this nutritional difference greatly affects the production of conidia. Therefore, the selection of substrate materials as the medium for augmentation of the entomopathogen fungi has to be done properly, particularly in selecting materials that have the capability of producing conidia consistently. The results of other studies have also proved that rice is the right medium for cultured *B. bassiana* due to high production of conidia (Alves & Pereira 1989; Mendonca 1992; Ibrahim & Low 1993; Milner et al 1993). Moreover, the results of the research conducted by Rachappa et al (2005) denote that the number of conidia produced by *M. anisopliae* per gram of rice substrate is 4.03×10^9, of corn substrate is 2.42×10^9, and of wheat substrate is 2.04×10^9.

Concentrations of entomopathogenic fungi against S. exigua larvae. The observations indicate the concentration of *B. bassiana and V. lecanii* of 2 g/L of water is capable of killing *S. exigua* larvae until above 90%, while *M. anisopliae* with the same concentration caused mortality to *S. exigua* up to 98% (Figure 6). Some research abroad show that mortality of *Varroa destructor* caused by *M. anisopliae, V. lecanii, and B. bassiana* with a concentration of 1×10^8 mL/L can reach 100% after 7 days of treatment (Shaw et al 2002). Further, Smith et al (2006) proves that *B. bassiana* conidia from 0.2 g/g corn substrate can trigger to mortality of *Prostephanus truncatus* up to 90% after one week of treatment.

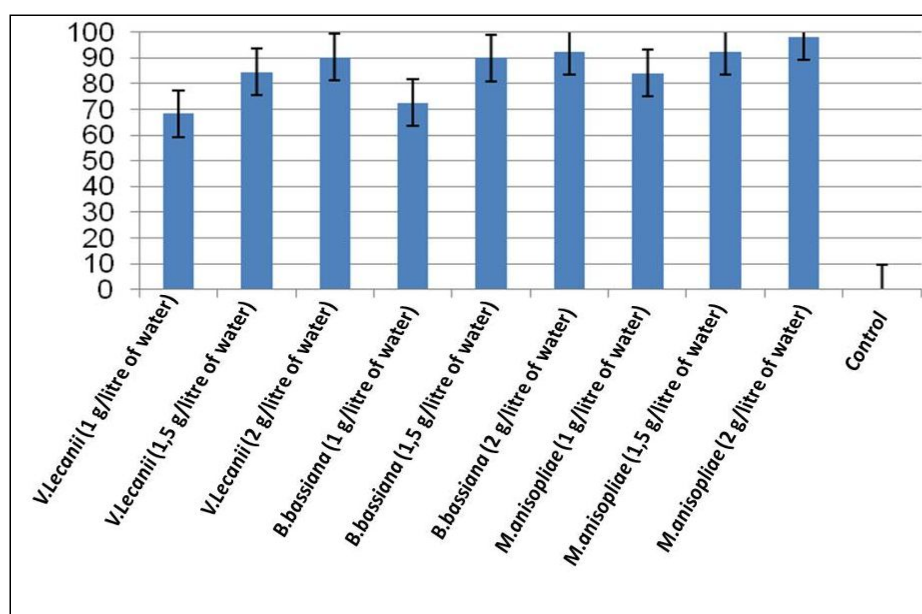


Figure 6. Average mortality of *S.exigua* larvae (percent) on various concentrations of entomopathogenic fungi (n = 10) after 7 days of treatment.

Conclusions. Based on the results of the exploration of entomopathogenic fungi from shallot field, three types of entomopathogen fungi i.e. *Vertilicillium lecanii*, *Beauveria bassiana* and *Metharizium anisopliae* were found. Generally, all the isolated fungi from rhizosphere have a good viability (over 80%) so they are potential to develop as biological pest control agents against *S. exigua* larvae on the shallots. The growth rate of the three types of the ungal colony is relatively the same and does not differ significantly. On the concentration of 2 g/L of water, *B. bassiana* and *V. lecanii* can kill *S. exigua* larvae up to above 90%, and *M. anisopliae* up to 98%.

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Authors:
Ahsol Hasyim, Indonesian Vegetable Research Institute, Indonesia, Lembang – Bandung Barat 40391, Jl. Tangkuban Parahu No. 517, e-mail: ahsolhasyim@yahoo.co.id
Wiwin Setiawati, Indonesian Vegetable Research Institute, Indonesia, Lembang – Bandung Barat 40391, Jl. Tangkuban Parahu No. 517, e-mail: wsetiawati@yahoo.com
Hadis Jayanti, Bali Assessment Institute for Agricultural Technology (Bali AIAT), Indonesia, Jl. By Pass Ngurah Rai, Pesanggaran, Denpasar Selatan 80222, Bali-Indonesia, e-mail: hadisjayanti@yahoo.com
Nusyirwan Hasan, West Sumatera Assessment Institute for Agricultural Technology (West Sumatera AIAT) Jalan Raya Padang-Solok Km. 40, Sukarami Kecamatan Gunung Talang Kabupaten Solok, West Sumatera, Indonesia. e-mail: nusyirwanh@yahoo.com
Muhammad Syakir, Indonesian Agency for Agricultural Research and Development, Indonesia, Jakarta, Jl. Ragunan 29 Pasar Minggu Jakarta 12540, e-mail: kbadan@litbang.pertanian.go.id
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