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THE USE OF GOLDEN SNAIL MEAL TO ENRICH *Bacillus thuringiensis* CULTURE MEDIA AND ITS EFFECT ON THE BACTERIAL TOXICITY AGAINST *Spodoptera litura*

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ABSTRACT

***The use of golden snail meal to enrich* Bacillus thuringiensis *culture media and its effect on the bacterial toxicity against* Spodoptera litura.** *Bacillus thuringiensis* is an entomopathogenic bacterium producing spore and protein at sporulation. There has been limited research on using golden snail as protein resource to enrich bacterial culture media. This research was aimed at studying bacterial cell/spore production in culture media made from coconut water media and liquid waste of tofu industry (tofu whey) enriched with golden snail meal (GSM), as well as its toxicity against *Spodoptera litura*. The research was conducted in the Laboratory of Entomology, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University, from June to September 2017. The experiment was arranged in a Factorial Completely Randomized Design (FCRD) with two factors and three replications. The first factor was the addition of GSM (0, 5, 9, 13 and 17 g) and the second was storage longevity (0, 1, and 2 months). Number of test insect (third instar of *S. litura*) was 30 larvae per treatment. Parameter observed were spore density, larval mortality, and symptom of infected larvae. The results showed that *B. thuringiensis* cultured in culture media enriched with 13 g golden snail meal produced the highest spore density, amounted to 14.14 x 10^7spores/ml and caused larval mortality up to 86.67%. After one and two month storage treatments, the spore density in the cultures reduced to 2.51 x 10^7 and 1.49 x 10^7 spores/ml, respectively. There was a tendency of reduction in spore density under longer storage of the culture. Survived larvae developed abnormally, failed to transform to pupa or imago.

Key words: *Bacillus thuringiensis*, golden snail meal, *Spodoptera litura*, storage longevity

ABSTRAK

***Penambahan tepung daging keong emas pada media pertumbuhan* Bacillus thuringiensis *dan toksisitasnya terhadap* Spodoptera litura.** *Bacillus thuringiensis* merupakan bakteri entomopatogen yang menghasilkan spora dan protein pada waktu sporulasi. Media perbanyakan berupa limbah pertanian dengan penambahan sumber protein dari keong emas belum banyak dipelajari. Penelitian bertujuan untuk mempelajari produksi sel/spora bakteri dalam media air kelapa, limbah cair tahu dan penambahan tepung daging keong emas (TDKE) serta toksisitasnya terhadap ulat grayak *Spodoptera litura*. Penelitian dilakukan di Laboratorium Entomologi Program Studi Proteksi Tanaman FP UNSRI, dari bulan Mei-Juli 2017. Penelitian menggunakan Rancangan Acak Lengkap (RAL) dengan 10 perlakuan, yang diulang sebanyak 3 kali. Perlakuan berupa penambahan tepung daging keong emas berturut-turut dari 1, 3, 5, 7, 9, 11, 13, 15, dan 17 g. Jumlah serangga uji berupa *S. litura* instar 3 sebanyak 30 ekor/perlakuan. Parameter yang diamati adalah kerapatan spora, mortalitas larva, gejala kematian, berat daun yang dikonsumsi, dan nilai LT_{50}. Hasil penelitian menunjukkan bahwa *B. thuringiensis* yang ditumbuhkan pada media dengan penambahan tepung daging keong mas memiliki kerapatan spora tertinggi, yaitu 15,75 x 10^7 spora/ml pada penambahan 11 g TDKE), dan setelah disimpan 2 bulan mampu membunuh larva sampai 86,67 %. LT_{50} terendah terjadi pada bioinsektisida yang telah disimpan selama 2 bulan (0 g TDKE) yaitu 99,50 jam. Berat terendah daun yang dimakan pada bioinsektisida yang disimpan selama 2 bulan (penambahan 5 g) yaitu 49,67 g/30 ekor larva.

Kata kunci: *Bacillus thuringiensis*, tepung daging keong emas, *Spodoptera litura*, lama penyimpanan

INTRODUCTION

Bacillus thuringiensis is a gram positive bacteria producing spore and toxic protein at sporulation (Bagari *et al*., 2013). Method of the bacterium to kill host insect is through food digesting system at higher pH, so that insects with low pH digesting system are usually not sensitive to *B. thuringiensis* (Bravo *et al*., 2011). Mass production of *B. thuringensis* to be used as bio-insecticide is usually of high cost due to the expensive bacterial growth media (Prabakaran *et al*., 2008). Therefore, researches opportunity on mass production of *B. thuringiensis* is still widely opened. Researchers had used agricultural products such as soy meal (Valicente *et al*., 2010), agricultural by product such as molasses and liquid by product of maize (*nejayote*) (Salazar-Magallon *et al*., 2015; Jouzani *et al*., 2015), and by product of agricultural industry such as wheat bran and rice bran, to produce spore and protein crystal of *B. thuringiensis* for bio-insecticide (Marzban, 2012).

Bacillus thuringiensis spore and protein crystal production using agricultural by product has been conducted by some researchers, but there have been only few reports in using raw material of animal origin to produce the bacterium. Industrial waste of animal origin (*cheese whey*) (Salazar-Magallon *et al*., 2015) and swine bio-urine (Fernandes *et al*., 2010) ere reported to be suitable to be used as culture media of *B. thuringiensis*. Therefore, material of animal origin, especially agricultural pest such as golden snail (*Pomacea canaliculata* Lamarck) might be potentially used as culture media for *B. thuringiensis*. So far, golden snail has been used to produce liquid fertilizer (Siregar *et al*., 2017) and animal feed (Kurniawati, 1997). Golden snail meal (GSM) which rich of animal protein (Siregar *et al*., 2017) has an opportunity to be used to speed up the growth and development of *B. thuringiensis*. Reports stated that, in order to have optimal result in the production of *B. thuringiensis*, we need carbohydrate as the source of carbon and protein as the source of nitrogen (Purnawati *et al*., 2014; Salazar-Magallon *et al*., 2015; Valicente *et al*., 2010).

The objective of this research was to investigate the spore production of *B. thuringiensis* grown in culture media made from industrial waste enriched with golden snail meal and its toxicity against army worm (*Spodoptera litura*) under laboratory conditions.

MATERIALS AND METHODS

Research Site. The research was conducted in the Laboratory of Entomology, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University. Army worms (*Spodoptera litura*) were collected from water spinach cultivated in Village Inderalaya Baru, Sub-District Inderalaya Utara, District Ogan Ilir, South Sumatra. Golden snail, used as raw material of GSM, in this research was collected from tidal swamp near Jembatan Musi Dua, Village Karang Jaya, Sub-District Gandus, Palembang City. The research was conducted from June to September 2017.

Research Methods. The research was arranged in a Factorial Completely Randomized Design (FCRD) with 2 factors and 3 replication. The factors were addition of golden snail meal to bacterial culture (0, 5, 9, 13 and 17 g) and storage longevity of the culture (0, 1 and 2 months). Media used to culture *B. thuringiensis* was a standard media containing 25 ml coconut water + 25 ml liquid waste of tofu industry + mineral salts (50 mg $CaCl_2$ + 50 mg $MgSO_4$ + 50 mg K_2HPO_4 + 50 mg KH_2PO_4) (Table 1).

Preparation of Golden Snail Meal (GSM). Golden snails were collected from swampy land surrounding Musi Dua Bridge, Village Karang Jaya, Sub-District Gandus, Palembang City. The snails were placed in a plastic bucket of 50 cm height and 30 cm diameter and were soaked in water. The snails were fasted for 48 hours to make them clean. The snails were then washed and cooked in boiling water for 15–20 minutes. After being cooled, the snail flesh was separated from the shell and digestion organs. The flesh was then grilled in an oven at 80 °C for 18 hours. The dried flesh was then

Table 1. Treatments used in the experiment

Factor 1 Addition of GSM into stock solution	Factor 2 storage longevity
B0 : 0 g GSM	T0 : 0 month
B5 : 5 g GSM	T1 : 1 month
B9 : 9 g GSM	T2 : 2 months
B13 : 13 g GSM	
B17 : 17 g GSM	

blended and sieved to obtain finer meal of the snail flesh. The golden snail meal (GSM) was ready to be used to make bio-insecticide.

Insect Rearing. Eggs and larvae of *S. litura* were collected from water spinach cultivation in Village Inderalaya Baru, Sub-District Inderalaya Utara, District Ogan Ilir, South Sumatra. The insect rearing was conducted in the Laboratory of Entomology, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University. The reared insect was placed in a plastic container (20 cm diameter and 25 cm height) covered with cheese cloth. Larvae of *S. litura* were fed daily with fresh water spinach leaves free from chemical insecticide. The container was cleaned every day. When the larvae reached their pre-pupal stage (characterized by having less feeding appetite and tend to be inactive), sterile soil was placed in the container for pupation. Imagoes of *S. litura* emerged from pupae were fed with 10% honey solution. The larvae treated in the experiment were the instar 3 of the third generation.

Bio-insecticide Formulation. Production of *B. thuringiensis* spores in agriculture and industrial waste based media was started with the preparation of coconut water and liquid waste of tofu industry. In order to have the same quality, coconut water was collected from 26 Ilir Market Palembang, and liquid waste of tofu industry was collected from home industry of Sumedang Tofu in Jalan Raya Palembang-Prabumulih Km 20, Sub-District Inderalaya, District Ogan Ilir, South Sumatra. The isolate of *B. thuringiensis* used in the experiment was Isolate SMR-04, a collection of the Laboratory of Entomologi, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University. The formulation of bio-insecticide was begun with the preparation of seed culture (Valicente *et al*., 2010) by placing one lope of *B. thuringiensis* Isolate SMR-04 in 10 ml Nutrient Broth (NB) media and was shaken for 12 hours at 200 rpm. Afterward, 5 ml of this culture was taken and transferred to 10 ml NB media and was shaken for 12 hours at 200 rpm. Seed culture was ready to be used to make bio-insecticide.

Growth media of *B. thuringiensis* was a standard media made of 25 ml coconut water + 25 ml liquid waste of tofu industry + mineral salts (50 mg $CaCl_2$ + 50 mg $MgSO_4$ + 50 mg K_2HPO_4 + 50 mg KH_2PO_4), in 250 ml Erlenmeyer flask (modification of Valicente *et al*., 2010). Forty five Erlenmeyer flasks were prepared in accordance to the need of the treatments and replication, i.e. the addition of GSM (0, 5, 9, 13 and 17 g), three level of storage longevity, and 3 replicates. The Erlenmeyer flasks were covered with aluminium foil and tighten with rubber band. The growth media were then sterilized using autoclave at 121 °C and 1 atmosphere of pressure for 20 minutes. After the sterilized growth media were cool enough, 5 ml of seed culture was poured into each flak of treatment. The growth media were then shaken in the shaker at 200 rpm for 72 hours. The storage of the culture as the second factor consisted of 0 month (applied directly after preparation), 1 month and 2 months. Spores counting was conducted by using haemocytometer at 400 x magnification at the time of bioassay.

Bioassay. For mortality assay of army worms (*S. litura*), the army worms were fed with water spinach leaves (Bonab *et al*., 2016). Five grams of water spinach leaves were soaked in bio-insecticide prepared according to each treatment for 1 minute. After being air dried, the leaves were then placed in 20 x 10 cm square plastic Petri dish and 5 *S. litura* larvae of 3rd instar were released into the dish. This treatment was replicated 3 times. After this application, fresh water spinach leaves were replaced every day for 7 days, with the fresh ones. The observation of larval mortality was done every 24 hours. Larval mortality was determined by using the following formula:

$$\text{Mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae observed}} \times 100\%$$

Data Analysis. Data of spore density and larval mortality were analyzed according to Analysis of Variance (ANOVA) using computer program of SPSS 16.00. Symptoms of infected larvae were observed, documented and descripted.

RESULTS AND DISCUSSION

Spore Density. Media used to grow *B. thuringiensis* usually required certain requirements such as carbon : nitrogen ratio = 1.26 : 1 in LB broth (Valicente *et al*., 2010). Chemical compounds produced by factories usually meet the such requirement of carbon : nitrogen ratio with high quality, but their price are expensive. Therefore, it is important to look for alternative media which can be used as growth media for *B. thuringiensis*.

Coconut water media (Prabakaran *et al*., 2008), liquid waste of tofu industry (Purnawati *et al*., 2014), soybean meal and swine bio-urine (Fernandes *et al*., 2010) were used as alternative of factory produced

media. In this research we used coconut water and liquid waste of tofu industry as growth media of *B. thuringiensis*. Coconut water was abundantly available and easily obtained because coconut water is an unused waste. Furthermore, the material was available every time during the year because coconut is a continuously fruiting crop and grow well in the tropical area. Coconut water contains 30-50% crude protein Tand various minerals useful in crystal protein synthesis (Chuku & Kalagbor, 2014). Liquid waste of tofu industry contains carbon, nitrogen and minerals as required by growth media of *B. thuringiensis*, and its content of calcium can support sporulation of the bacterium. So, the ratio of carbon and nitrogen in liquid waste of tofu industry is 5.4:1 (Purnawati *et al*., 2014).

The calculation of spore density of *B. thuringiensis* grown in coconut water media and liquid waste of tofu industry with various levels of GSM addition showed that the spore production tent to increase with the increase of GSM addition. Treatment B17 i.e. the addition of 17 g GSM resulted in the highest spore density (14.31×10^7 spores mL^{-1}) at 0 month of storage longevity (without storage) (Table 2). The spore density decreased drastically when the media was stored for 1 month (3.46×10^7 spores mL^{-1}) and 2 months (1.39×10^7 spores mL^{-1}).

Generally, spore density of *B. thuringiensis* in all treatment decreased during in the storage. The longer the media was stored, the higher the decrease of spore density. Statistical analyses results showed that there was significant difference amongst storage treatments (HSD 5% = 0.04; P value = 0.00), with average spore density of 0 month storage (without storage) was 11.97×10^7 spores mL^{-1}, followed by 1 month storage (2.67×10^7 spores mL^{-1}) and 2 months storage (1.24×10^7 spores mL^{-1}). This might be due to the decrease of the media components. The difference and reduction of bacterial spore density might be because of the decline of nutrient content of the growth media.

Nutrition content highly influenced the process of cell and spore production of *B. thuringiensis* (Purnawati *et al*., 2014). Furthermore, it was stated that the concentration of nutritional components in the growth media of *B. thuringiensis*, such as glucose and mineral salts, could increase the spore production (Amin *et al*., 2008; Sarrafzadeh, 2012). The development of *B. thuringiensis* cells was better when the nutritional content of the growth media was more complete, including vitamins and minerals (Paul *et al*., 2011).

The treatment of GSM addition resulted in the significant different of average spore density between control and treatments B5 (5 g), B13 (13 g) and B17 (17 g) (BNJ 5% = 0.05; P value = 1.4×10^{-8}), but was not significantly different from treatment B9 (9 g). In the control media (without GSM addition), there were components such as carbon, nitrogen and other elements so that the spore density produced was quite high (4.39×10^7 spores mL^{-1}), even higher than that of treatment B5 (5 g) (4.14×10^7 spores mL^{-1}). Meanwhile, statistical analyses showed that control (0 g) and treatment B9 (9 g) resulted in not significantly different spore density.

It was also noticed that the addition of golden snail meal would only have effect on increasing spore density at levels of 13 g and 17 g. These two treatments were significantly different from control. Therefore, it was suggested that the practical use of golden snail meal should be started from 13 g. By adding golden snail meal containing more than 56% crude protein (Kurniawati, 1997; Dewi, 2014) into the growth media of *B. thuringiensis*, the spore production will also increase. The data is presented in Table 2.

Larval Mortality. Larval mortality of *B. thuringiensis* in the treatment of 0 month storage (without storage) was higher than that of treatment of 1 and 2 months (Table 3). Statistical analyses showed that there was significant different of larval mortality (HSD 5% = 4.46; P value = 0.5×10^{-4}) between was treatment of 0, 1 and 2 months amounted to 52.67%; 44.675% and 31.33% respectively. This might be caused by the spore density of 0 month storage treatment (11.97×10^7 spores mL^{-1}), as presented in Table 2, was higher than that of 1 and 2 months storage treatments (2.67×10^7 spores mL^{-1} and 1.24×10^7 spores mL^{-1}, respectively).

Number of spores affects the toxicity against targeted insect. Based on the mode of action of *B. thuringiensis*, the high number of spores would make more germination than the lower number of spores (Melo *et al*., 2016). Devi *et al*. (2005) also reported that higher number of spores would cause higher insect mortality.

In one and two month storage treatments, even though larval mortality was lower than that of 0 month storage treatment, the treatments still exposed toxicity of *B. thuringiensis*. Abdel-Razek (2002) stated that *B. thuringiensis* subsp. *morrisoni* gave low mortality effect (35%) on *Tribolium confusum* after 28 days. Blanc *et al*. (2002) reported the same result on the spore viability of *B. thuringiensis* subsp. *tenebrionis* isolated from dry tobacco stored for 30 months. Galán-Wong *et al*. (2017) also reported similar finding that *B. thuringiensis* subsp. *israelensis* stored for 30 years

still had toxicity against larvae of mosquito, even though the toxicity was lower than before long storage. van Frankenhuyzen *et al*. (1993) observed the mortality of *B. thuringiensis* stored for 7 months, and found that there was reduction in the mortality of 6 forest pest species i.e. *Choristoneura fumiferana*, *Orgyia leucostigma*, *Lymantria dispar*, *Malacosoma disstria*, *Lambdina fiscellaria fiscellaria*, and *Actebia fennica*, but still caused high mortality of silk worm *Bombyx mori*.

Result of larval mortality observation as presented in Table 3, it can be seen that the addition of GSM increased toxicity of *B. thuringiensis*. The addition of GSM treatment resulted in significant different of larval mortality (HSD 5% = 5.76; P value = 0.0). The highest mortality was found in treatment B13 (13 g GSM addition) amounted to 68.89%. The average spore density of treatment B13 (6.05×10^7 spores mL^{-1}) was not significantly different from that of treatment B17 (Table 2), but larval mortality only 50%.

In Table 3, it can be seen that larval mortality of treatment B17 (17 g) at 1 month storage decreased to 53.33%, while at 2 month storage, the mortality was only 31.33%. It could be caused by the decrease of toxicity. There are several factors causing reduction of toxicity. Elleuch *et al*. (2015) stated that toxicity affected not only by the number of spore but also by the stability of the protein, a toxic component against insect, which could be produced during storage, because protein

Table 2. Spore density of *B. thuringiensis* propagated on coconut water and tofu whey media enriched with golden snail meat meal

GSM treatment	Spore density ($\times 10^7$ spores/ml) at length of storage (months)			Mean (Tukey's test 5% = 0.05)
	0	1	2	
B0 (0 g)	9.22 (7.96)	2.71 (7.43)	1.24 (7.09)	4.39 (7.49) b
B5 (5 g)	9.24 (7.97)	2.46 (7.38)	0.71 (6.85)	4.14 (7.40) a
B9 (9 g)	12.93 (8.11)	2.22 (7.35)	0.88 (6.94)	5.34 (7.47) b
B13 (13 g)	14.14 (8.15)	2.51 (7.40)	1.49 (7.17)	6.05 (7.57) c
B17 (17 g)	14.31 (8.16)	3.46 (7.54)	1.39 (7.14)	6.39 (7.61) c
Mean (Tukey's test 5%=0.04)	11.97 (8.07) c	2.67 (7.42) b	1.24 (7.04) a	

Number in parantheses were log transformed. Numbers in the same column and raws followed by different letters (a-c) are significantly different at the level of P < 0.05 according to Tukey's test. GSM: golden snail meat meal

Table 3. Mortality of *Spodoptera litura* larvae treated with *Bacillus thuringiensis* propagated on coconut water and tofu whey media enriched with golden snail meat meal

GSM treatment	Mortality (%) at length of storage (months)			Mean (Tukey's test 5% = 5.76)
	0	1	2	
B0 (0 g)	3.33 (6.75)	10.00 (15.30)	13.33 (21.14)	8.89 (14.40) a
B5 (5 g)	43.33 (41.07)	36.67 (37.22)	23.33 (28.78)	34.44 (35.69) b
B9 (9 g)	66.67 (54.78)	53.33 (46.92)	36.67 (37.22)	52.22 (46.31) c
B13 (13 g)	86.67 (68.86)	70.00 (57.00)	50.00 (45.00)	68.89 (56.95) d
B17 (17 g)	63.33 (52.78)	53.33 (46.92)	33.33 (35.22)	50.00 (44.97) c
Mean Tukey's test 5% = 4.46)	52.67 (44.85)	44.67 (40.67)	31.33 (33.47)	

Number in parantheses were log transformed. Numbers in the same column and raws followed by different letters (a-c) are significantly different at the level of P < 0.05 according to Tukey's test. GSM: golden snail meat meal

crystal was not analyzed in this research. This appraisal is in accordance to statement of Osman *et al*. (2015) that decrease in protein would affect its toxicity.

Infection Symptoms. Initial symptoms of infection were shown by less motion, less appetite, and no response to touch. All dead larvae in this research had the same conditions, body color changed to pale and gradually turned to darker and shriveled. The texture of dead larvae was getting squashy when being touched. Some dead larvae had their body very soft, fragile and leaky (Figure 1B). Few larvae were survive and successfully passed pre-pupal stadia, but they failed to transfer to pupal stadia (Figure 1C).

Infected larvae successfully transferred to pupal stadia had body color darker than the normal and healthy pupae (Figure 2A). Not all pupae of infected larvae could successfully become imago. Imagoes born from infected pupae had smaller and abnormal body, the wings were trimmer. Pupae which failed to become imago had part of their body still inside the pupal skeleton. The emerging imago died before totally emerged so that the part of the body was still inside the pupal skeleton (Figure 2B). If the larvae had been contaminated by *B. thuringiensis*, and could successfully transform to imago, the body of the imago was abnormal. The abnormal imago usually could not live longer as normal imago and failed to reproduce.

Mortality of *S. litura* larvae treated with various treatments of GSM raged from 34.44 to 68.89% (Table 3). Therefore, the survived larvae ranged from 31.11 to 65.56%. Some larvae fail to complete their life cycle due to toxicity of *B. thuringiensis*. Bernardi *et al*. (2016) reported that 49% of treated *S. frugiferda* larvae could not complete their life cycle.

Figure 1. *Spodoptera litura* larva treated with *B. thuringiensis*. (A) healthy larvae; (B) *B. thuringiensis*-infected larvae: 3 days after treatment/DAT (1), 4 DAT (2), 5 DAT (3), 6 DAT (4), dan 7 DAT (5); (C) larvae failed to become pupae

Figure 2. *Bacillus thuringiensis- treated Spodoptera litura* pupae (A) healthy pupae (1) and infected pupae (2); (B) Pupae failed to become adult; (C) abnormal imago

CONCLUSION

The result of this paper showed that *B. thuringiensis* grown in media enriched with 13 g GSM produced highest spore density amounted to 14.14 × 10^7 spores mL^{-1} and caused larval mortality up to 86.67%. After storage for one and two months, the cultures could produce 2.51 × 10^7 spores mL^{-1} and 1.49 × 10^7 spores mL^{-1}, respectively. There was a tendency of spore density reduction under longer storage of the culture. Survived infected larvae developed abnormally and failed to transform to pupa or imago.

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REFERENCES

Abdel-Razek AS. 2002. Comparative study on the effect of two *Bacillus thuringiensis* strains of the same serotype on three coleopteran pests of stored wheat. *J. Egypt Soc. Parasitol.* 32(2): 415–424.

Amin G, Alotaibi S, Narmen YA, & Saleh WD. 2008. Bioinsecticide production by the bacterium *Bacillus thuringiensis*. 1. pattern of cell growth, toxin production and by-product synthesis. *Arch. Agron. Soil Sci.* 54(4): 387–394.

Bagari L, Syedy M, Sharma GP, Sharma P, & Soni P. 2013. Isolation of crystal protein from *Bacillus thuringiensis*. *Int. J. Pure App. Biosci.* 1(2): 44–47.

Bernardi D, Bernardi O, Horikoshi RJ, Salmeron E, Okuma DM, & Omoto C. 2016. Biological activity of Bt proteins expressed in different structures of transgenic corn against Spodoptera *frugiperda*. *Ciência Rural* 46(6): 1019–1024.

Blanc M, Kaelin P, & Gadani F. 2002. *Bacillus thuringiensis* (Bt) for the control of insect pests in stored tobacco: a review. *Beiträge zur Tabakforschung International-Contributions to Tobacco Research* 20(1): 15–22.

Bonab SH, Moravvej G, & Namaghi HS. 2016. Comparative study on the efficacy of *Bacillus thuringiensis* var. *tenebrionis* and a neem based insecticide on adults and larvae of *Xanthogaleruca luteola* (Mull) (Col/: Chyrsomelidae) in laboratory conditions. *J. Entomol. Zool. Stud.* 4(4): 1122–1125.

Bravo A, Likitvivatanavong S, Gill SS, & Soberón M. 2011. *Bacillus thuringiensis*: A story of a sucessful bioinsecticide. *Insect Biochem. Mol. Biol.* 41(7): 423–431.

Chuku LC & Kalagbor GI. 2014. Protein and mineral element content of coconut (*Cocos nucifera*) water from different species. *Am. J. Advanced Drug Delivery* 11(2): 5–7.

Devi PSV, Ravinder T, & Jaidev C. 2005. Barley-based medium for the cost-effective production of *Bacillus thuringiensis*. *World J. Microbiol. Biotechnol.* 21(2): 173–78.

Dewi FS. 2014. Pemanfaatan tepung keong mas (*Pomacea canaliculata*) sebagai substitusi tepung ikan pada pakan udang vannamei (*Litopenaeus vannamei*) terhadap nilai kecernaan serat kasar dan bahan ekstrak tanpa nitrogen (BETN). Skripsi. Universitas Airlangga. Surabaya.

Elleuch J, Zghal RZ, Lacoix MN, Chandre F, Tounsi S, & Jaoua S. 2015. Evidence of two mechanisms involved in *Bacillus thuringiensis israelensis* decreased toxicity against mosquito larvae: genome dynamic and toxins stability. *Microbiol. Res.* 176: 48–54.

Fernandes SCM, Freire SCR, Silvestre AJD, Desbrières J, Gandini A, & Neto CP. 2010. Production of coated papers with improved properties by using a water-soluble chitosan derivative. *Ind. Eng. Chem. Res.* 49(14): 6432–6438.

Galán-Wong LJ, Gamiño-Hernández R, Fernández-Chapa D, García-Díaz G, Myriam A. De La Garza-Ramos, Claudio Guajardo-Barbosa, & Luna-Olvera1 HA. 2017. Persistence of toxic activity of fermentation extracts from *Bacillus thuringiensis* var. *israelensis* after more than three decades of storage. *Int. J. Microbiol.* 2017: 1–6.

Jouzani GS, Abbasalizadeh S, Moradali MF, & Morsali H. 2015. Development of a cost effective bioprocess for production of an Iranian anti-Coleoptera *Bacillus thuringiensis* strain. *J. Agr. Sci. Tech.* 17: 1183–1196.

Kurniawati N. 1997. Potensi dan pemanfaatan keong mas sebagai bahan pakan ternak. *Balai Besar Penelitian Padi*: 47–58.

Marzban R. 2012. Investigation on the suitable isolate and medium for production of *Bacillus thuringiensis*. *J. Biopest*. 5(2): 144–147.

Melo ALDA, Soccol VT, & Soccol CR. 2016. *Bacillus thuringiensis*: mechanism of action, resistance, and new applications: a review. *Crit. Rev. Biotechnol*. 36(2): 317–326.

Osman GEH, Already R, Assaeedi ASA, Organji SR, El-Ghareeb D, Abulreesh HH, & Althubiani AS. 2015. Bioinsecticide *Bacillus thuringiensis* a comprehensive review. *Egypt J. Biol. Pest Co*. 25(1): 271–288.

Paul B, Paul S, & Khan MdA. 2011. A potential economical substrate for large-scale production of *Bacillus thuringiensis* var. *kurstaki* for caterpillar control. *Biocontrol Sci. Technol*. 21(11): 1363–1368.

Prabakaran G, Hoti SL, Manonmani AM, & Balaraman K. 2008. Coconut water as a cheap source for the production of δendotoxin of *Bacillus thuringiensis* var. israelensis, a mosquito control agent. *Acta Trop*. 105(1): 35–38.

Purnawati R, Sunarti TC, Syamsu K, & Rahayuningsih M. 2014. Characterization of novel *Bacillus thuringiensis* isolated from *Attacus atlas* and its growth kinetics in the cultivation media of tofu whey for bioinsecticide production. *J. Biol. Agri. Healthcare* 4(16): 33–40.

Salazar-Magallon JA, Hernandez-Velazquez VM, Alvear-Garcia A, Arenas-Sosa I, & Pena-Chora G. 2015. Evaluation of industrial by-products for the production of *Bacillus thuringiensis* strain GP139 and the pathogenicity when applied to *Bemisia tabaci* nymphs. *Bull. Insectology* 68(1): 103–1099.

Sarrafzadeh MH. 2012. Nutritional Requirements of *Bacillus thuringiensis* during different phases of growth, sporulation and germination evaluated by plackett-burman method. *Iran J. Chem. Chem. Eng*. 31(4): 131–136.

Siregar AZ, Tulus, & Lubis KS. 2017. Utilization of golden snail as alternative liquid organic fertilizer (LOF) on paddy farmers in Dairi, Indonesia. *IJSTR*. 6(11): 17–21.

Valicente FH, Tuelher EDS, Leite MIS, Freire FL, & Vieira CM. 2010. Production of *Bacillus thuringiensis* biopesticide using commercial lab medium and agricultural by-products as nutrient sources. *Revista Brasileira de Milho e Sorgo* 9(1): 1–11.

van Frankenhuyzen K, Gringorten JL, Gauthier D, Milne RM, Masson L, & Peferoen M. 1993. Toxicity of activated CryI protein from *Bacillus thuringiensis* to six forest Lepidoptera and *Bombyx mori*. *J. Invertebr. Pathol*. 62(3): 295–301.

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