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Phytochemical and toxicity test of secondary metabolites extracted from *Streptomyces* bacteria against *Anopheles larvae* in Lampung, Indonesia

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Abstract

This research carried out to determine the chemical composition of *Streptomyces* sp extract and the toxicity activity of the secondary metabolite against mosquito larvae of *Anopheles sundaicus*. By using a completely randomized design (CRD), the study was conducted from June to November 2021. The results of the chemical test showed that the isolate of *Streptomyces* bacteria contains alkaloids, flavonoids and terpenoids. The results of the larvicidal test of the extracts against *Anopheles larvae* were significantly different ($p<0.05$) in the number of mortalities between concentrations. The maximum mortality was shown by the extract at a concentration of 500 ppm with an exposing time of 48 hours. In conclusion, the secondary metabolite of *Streptomyces* bacteria is potential as a larvicide candidate for Anopheline mosquitoes.

Keywords: Toxicity test; Phytochemical Test; *Streptomyces*; Larvicides; *Anopheles larvae; Anpheles sundaicus*

1. Introduction

Some areas in Indonesia are still endemic for malaria, generally in remote areas and some of the sufferers are from weak economic groups. The most endemic areas malaria infects all age groups of the 33 provinces, 15 provinces in Indonesia have a prevalence above the national rate and most of them are in East of Indonesia [1,2]. Lampung Province is one of the malaria endemic areas with an API rate of 7.5 percent in 2017 [3]. The high API rate in Lampung Province exceeds the national API rate due to several factors including climate change, community nutritional status, and the occurrence of resistance to larvicides or insecticides

The use of insecticides by most people is suspected because it widely available in the market, have many variations in formulations, and are easy to apply. Most people use insecticides almost every day. The use of insecticides in high frequency and in the long term can harm the community itself. The use of chemical insecticides is not only to kill, in its development there are many insecticides whose ways of working include attracting, repelling, dispelling, or disturbing the growth of insects [3]. Insecticides are the largest group of pesticides and consist of several different types of chemicals, including organochlorines, organophosphates, kabamat, pyrethroids, and diethyl-meta toluamide (DEET). Insecticidal poisons from these various active substances are not only felt by the target insects but can also affect pets and humans. Prolonged use of a chemical insecticide can cause resistance in target insects and health problems in humans. Chemical insecticides can accumulate in body tissues which will later become chronic diseases, abnormalities in newborns, cancer, poisoning in pets, water pollution, environmental damage, food contamination, and residues on the soil surface

Due to the many impacts caused by insecticides with synthetic larvicides, vector control need to be carried out biologically. Biological control is an effort to use biological agents for mosquito control. Several biological agents that

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have been used and proven to be able to control populations are from groups of bacteria and predators such as larvae-eating fish and copepods. There are fishes known to eat mosquito larvae, not only a small type of fishes but also can a big-economic fish such as tilapia, carp, and catfish [4]. Microbial mosquito control recommended as an alternative method, and microbial-based larvicides used to minimize mosquito population provides an effectives, environmentally friendly approach to bringing the mosquito population to the lowest level.

2. Materials and Methods

2.1. Characterization Test

S. hygroscopicus sub sp Jinggangensis bacteria were rejuvenated in the prepared media (ISP 1, ISP 4, ISP6, ISP9, ISP 9+Mannitol, ISP 9+Galactose, ISP 9+Lactose, ISP 9+Sucrose, SCA, and Strepto). All media were sterilized by autoclaving at 121oC at 15 Psi pressure for 15 minutes. 15mL of media was poured into a petri dish and allowed to solidify. Observations were made for 3 consecutive days.

2.2. Biochemical Test

2.2.1. Sugar Fermentation

Arose of bacterial isolate was taken and then inoculated into NB (Nutrient Broth). Media added with 1% sugar sucrose, lactose, fructose, galactose, glucose in a test tube. Previously, the media had been given bromothymol blue indicator as an indicator of fermentation. Then the tube was homogenized and then incubated for 72 hours at a temperature of 25-28^{0}C. A positive result is indicated when there is a color changed and there are also gas bubbles.

2.2.2. Indole test

A colony of bacteria isolates was inserted and then inoculated into SIM media and incubated for 24 hours at 37ºC. Observation of indole test results was carried out by adding 10-12 drops of Kovac's reagent.

2.2.3. Mortality test

A colony of bacteria isolates in SIM media was then incubated for 48 hours at 37oC. Bacterial growth around the puncture showed a negative test result. The growth of bacteria that spread on the media showed a positive test result.

2.2.4. Data analysis

The analysis was carried out, using a completely randomized design (CRD).The treatment was in the form of extract concentration *Streptomyces*sp and time of observation on the mortality of *Anopheles larvae*.

3. Results and Discussion

The results of the first step were the cultures of several *Streptomyces* sp. isolates, namely strain I18 on Yeast Starch Agar (YSA) model, International *Streptomyces* Project (ISP) 1, 2, 4. and Starch Casein Agar (SCA) as shown in Figure 1.

Streptomyces sp. isolates. Strain i18 grows well on ISP 2 media because this media contains rich nutrients yeast extract and malt extract which is a nitrogen source and dextrose which is used as a carbon source. *Streptomyces* sp. isolates. grow on ISP 2 media within 2 days, while on other media it takes more than 3 days and the number of colonies is not as much as compared to ISP2 media.

The increased growth of strain i18 isolates using ISP2 media was good because the media was the standard medium used to culture *Streptomyces* sp. isolates. which contains Malt extract 10 g, Yeast extracts 4 g, dextrose 4 g and Agar 20 g in 1L distilled water.

Production of secondary metabolites was carried out by pre-cultivation of *Streptomyces* sp. strain i18 in the fermentation media used was composed of Soluble starch 20.0 g/L, Soya bean powder 10.0 g/L, KNO_3 1.0 g/L, KH_2PO_4 0.5 g/L, NaCl 0.5 g/L, $MgSO_4$ $7H_2O$ 0.5 g/L, $FeSO_4$ $.7.H_2O$ 0.01 g/L. The starter was made by adding 1 mL of stock isolate (Cell turbidity was adjusted at 0.5 McFarland standard) into 9 mL of fermentation medium at room temperature and incubated for 10 days. The 10-day-old starter was put into 990 mL of fermentation medium and incubated for 10 days. The culture was extracted by centrifugation at 10,000 rpm for 20 minutes. Mycelium debris was filtered with filter paper to obtain the filtrate. The filtrate was added with 70% ethanol and extracted using ethyl acetate and methanol as

solvents. The supernatant which had been added with methanol and ethyl acetate was then allowed to stand for 24 hours, after that it was evaporated to separate the solvent.

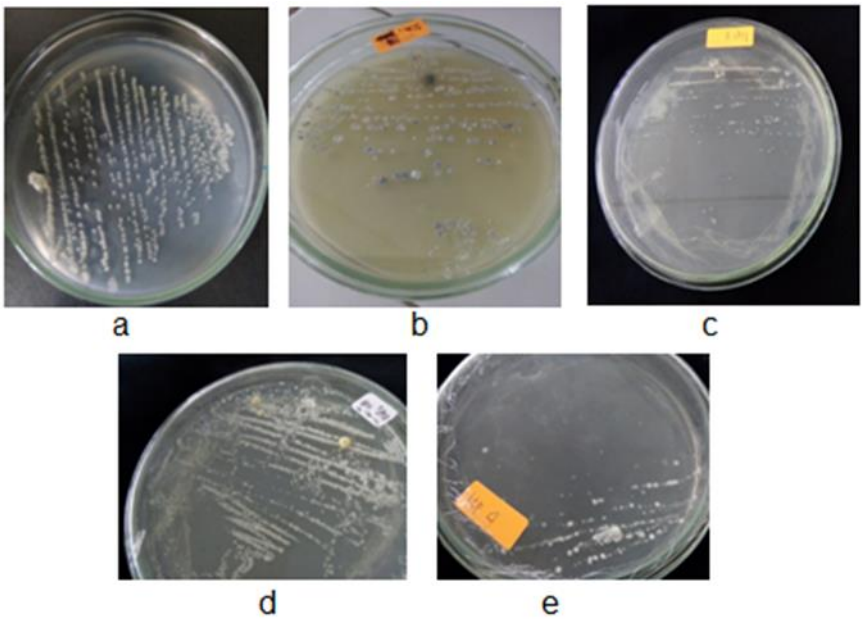


Figure 1 Results of I18 isolates on media (a) YSA (b) SCA (c) ISP1 (d) ISP2 (e) ISP4

Figure 2 Secondary metabolites of *Streptomyces* sp. strain I18 (a) Starter (b) Production yield (Supernatant) (c) Evaporation yield

The media used for sub-culture included Yeast Starch Agar (YSA), International *Streptomyces* Project (ISP 1, ISP 6, ISP 9 lactose), and Mueler Hinton Agar (MHA). After obtaining the production of *Streptomyces* sp. in large quantities then extraction of secondary metabolites of *Streptomyces* sp. The extraction results were then analyzed for phytochemicals.

Phytochemical tests of *Streptomyces* sp. and secondary metabolites were obtained to determine the type of secondary metabolites contained in the isolates of *Streptomyces* sp. such as flavonoids, alkaloids, tannins, saponins, and triterpenoids. The following phytochemical test results configure in Table 1.

The content of chemical compounds in secondary metabolites is contained in plants and microorganisms in the form of flavonoid compounds, terpenoids, alkaloids, steroids, and so on. These compounds give rise to the antioxidant and antibacterial activity of a secondary metabolite so that it has an effect as a drug. Phytochemical test results showed that *Streptomyces* sp. contains alkaloids, flavonoids, and terpenoids. The content of secondary metabolites from *Streptomyces* sp. can be used as a larvicide candidate.

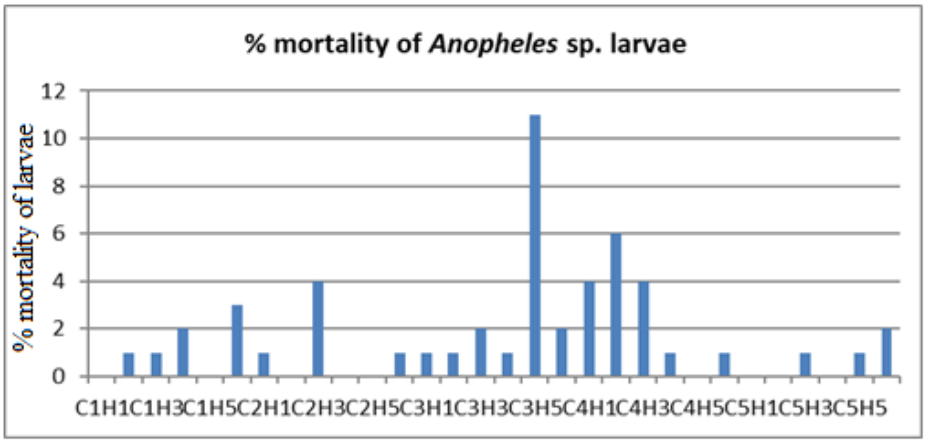
Table 1 Results of Phytochemical Tests for Secondary Metabolites of *Streptomyces* sp. isolates

Test	***Streptomyces*sp.**
Alkaloid	+
Tanin	-
Saponin	-
Flavanoid	+
Trierpenoid	+

Note: + means detected, - means not detected

In this study, alkaloids, saponins, and flavonoids were the most dominating compounds in the isolates of *Streptomyces* sp. The actinobacteria group includes *Streptomyces* sp. reported to be an alternative source of larvicides because it has the potential as a source of bioactive chemicals and is generally free from harmful effects. The function of using these microbes as mosquito control substitutes for insecticide thereby reducing costs and environmental pollution.

The results of the next step of this research were the results of the larvacide test of the secondary metabolite extract of *Streptomyces* sp. concentrations of 125 ppm, 250 ppm, 500 ppm, and 1,000 ppm, showed that the extract was able to kill the larvae of *Anopheles* sp. Instar III, although very low, even the highest mortality was only obtained at an average rate of 2.75, namely the concentration of 500 ppm, with observation time 12 hours after treatment. The complete data can be seen in Figure 3.



C = concentration (125, 250, 500, 1000 and control); H = Observation Time (3, 6, 12, 24, 48 and 72 h)

Figure 3 Percent (%) mortality of *Anopheles* larvae by extract concentration and duration of treatment.

Figure 3 shows the highest percentage value of *Anopheles* sp. larvae mortality on secondary treatment metabolite extract of *Streptomyces* sp. The highest was in the C3H5 treatment, then C4H2, C4H23, and C4H1. According to Kahar [5], *Streptomyces* bacteria contain prodigiosin compounds so that they can kill coconut beetle larvae by 92.5% of the sample population. In addition, the larvae themselves also have varying resistance to insecticides. The application the insecticide will also stimulate selection in the target larval population [6]. Larvae are susceptible to these insecticides will die, while those have experienced resistance will remain alive.

However, after being tested with ANOVA, it appears that the observation time does not affect the mortality of *Anopheles* sp. Based on the result of the analysis of variance, it appears the treatment in the form of concentration and its interaction has a significant effect on the mortality of *Anopheles*sp larvae so there are differences in the number of larvae that die in each treatment. To find out which treatment showed the highest number of dead larvae, then the data continued to the LSD test.

The average number of larvae that died was the most (3,333) at the concentration of C3 then 2,667 at the concentration of C4 and statistically, there was no significant difference between the treatments between these concentrations (C3 and C4), as well as the number of larvae that died at concentrations of C1, C2. and C5. However, the number of larvae

that died at C1 and C3 was significantly different between concentrations of 125 ppm and 500 ppm; concentration of 125 ppm with a concentration of 1000 ppm; concentration of 125 ppm with a concentration of 1000 ppm; concentration of 250 ppm with a concentration of 500 ppm; negative control with a concentration of 1000 ppm; and negative control with a concentration of 500 ppm.

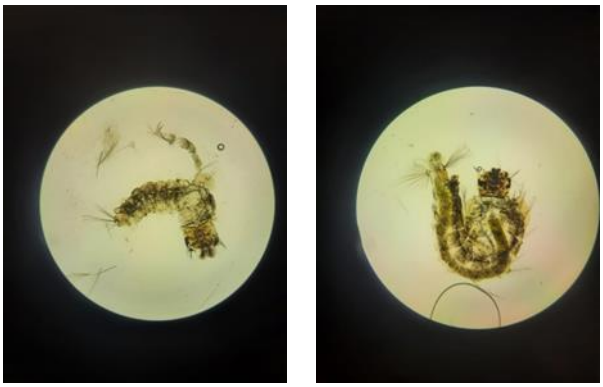
Table 2 Test of LSD Concentration on Mortality of *Anopheles* sp. Larvae

Konsentrasi	**Mean *)**
C1 (125 ppm)	1,167[b]
C2 (250 ppm)	1,000[b]
C3 (500 ppm)	3,333[a]
C4(1000 ppm)	2,667[a]
C5 (K-)	1,333[b]

*) Values followed by the same superscript are not different statistically

According to Samrot [7], the increase in the number of prodigiosin compounds produced by these bacteria plays a role in the detoxification process by inducing the esterase enzyme so that it can block the work of compounds that act as larvicides[7]. According to Suari*et al*.[8], an increase in the esterase enzyme identified an insect detoxification process. Esterase is an enzyme that plays a role in the mechanism of insect resistance to insecticides from the organophosphate group. The mechanism of detoxification in insects, namely the carboxyl esterase enzyme will hydrolyze the carboxylic acid ester group of the organophosphate group such as malathion. If the group in the malathion changes, the insecticidal compound will lose its function as a larvicide. Resistance caused by enzyme activity occurs when the enzyme blocks the insecticide compound from reaching its target [9]. Another opinion from Suryanthi[10], showed prodigiosin can increase the activity of phosphate protease up to 36%. These phosphate proteases play a role in the development of resistance in insects [10].

In a study on extract of *Streptomyces* bacteria strain AB8 against morphological damage of *Anopheles* sp.morphology of the larvae that were treated well using the secondary metabolite extract of *Streptomyces* sp. strain AB8 showed damage in the abdomen while the larval morphology in the control was not damaged (Figure 4).



A **B**

Figure 4 (A) Larval morphology of *Anopheles* sp. instar III given the extract of *Streptomyces* sp. strain AB8, and (B) Larval morphology of *Anopheles* sp. instar III on negative control

The effect of the secondary metabolite extract of *Streptomyces* sp. strain AB8 on larval morphology can be seen by the occurrence of damage to the abdomen, the larvae are pale in color, and the larval shape is shriveled and shortened (Figure 4). Morphological damage to the larvae of *Anopheles* sp. instar III due to the administration of secondary metabolite extract of *Streptomyces* sp. strain AB8 is thought to be due to the presence of compounds that are toxic to larvae. The result of the compound content test conducted by Arifiyanto et al. [11] in the progress of the secondary metabolite extract of *Streptomyces* sp. strain AB8 contains alkaloids and tannins. Alkaloid and tannin compounds are digestive and nervous poison, which allows the inhibition of larval growth to death.

Alkaloid compounds are often found in *Streptomyces*. The mechanism action of this alkaloid is to inhibit the action of the enzyme Acetylcholinesterase (AChE) which causes acetylcholine to accumulate so that it disrupts the impulse delivery system to muscle cells. This compound can also damage cell membranes to enter the larva's body, then it will damage cells and disrupt the larval nervous system. This will cause the larvae to experience spasms and if this situation continues it will cause the death of the larvae. Alkaloids can also cause changes in the color of the larva's body to become pale and transparent and make the larva's body movement slow down with the body position always bent [12]. This is in line with the results of research conducted by Cania and Setyaningrum, that alkaloid compounds enter through the membrane by damaging the larval cell membrane [13]. This is thought to cause the morphological structure of *Anopheles* sp. instar III on the administration of secondary metabolite extract of *Streptomyces* sp. strain AB8 to experience a pale color change and a bent and shortened body shape (Figure 4).

Tannin compounds act as stomach poisons that enter along with food and then damage the digestive system of the larvae. According to Kartini*et al*. the mechanism of action of this compound is to inhibit the work of insect digestive enzymes by forming enzyme protein bonds that cause food to be indigestible by insects[14]. Yuliasih and Widawati also said that tannins are toxic to insects which causes a decrease in growth rate and nutritional disturbances, followed by the death of larvae. It works by binding to proteins in the salivary glands and lowering the activity of digestive enzymes [15]. This is following the research of Waskito and Cahyati, tannin compounds are digestive toxins that can inhibit the growth of larvae [16].

Saponins are bioactive compounds toxic and belong to the class of contact poisons. This compound can reduce the surface tension of the mucous membrane of the digestive tract in insects so that parts of the wall become damaged and metabolic disorders occur [13]. In addition, saponins can also damage the cuticle layer of the larvae, causing the death of the larvae [12]. The mechanism of action saponin compounds is by entering diffusion into the cuticle, then a binding process occurs which can disrupt the stability of cell membranes and the metabolic process of larvae. The color change in larval morphology becomes pale due to alkaloid compounds, alkaloids have a pharmacological effect that can enter the larva's body by degrading the cell membrane and making the larva's body pale and then it will disrupt the larval nervous system so that the larvae's movement becomes slow [17].

The morphology of the larvae showed damage at the abdomen which was thought due to thinning of the cuticle from enzymatic activity. One of the enzymes that play a role in damage to insect cell walls is chitinase [18]. Another opinion from Okay *et al*. showed that bacteria can produce chitinase enzymes and become one of the most effective bacteria in degrading chitin [19]. This is in line with the results of research from Aggarwal *et al*. showed that the chitinolytic activity produced by *S. marcescens* was able to inhibit the growth of larvae and pupae [20]. The mechanism of action of the chitinase enzyme is that the chitinase enzyme will induce peritrophic membrane damage in the insect digestive tract and result in a high reduction in nutrient absorption [21].

4. Conclusion

The conclusions of this study are:

- The extract of isolates of *Streptomyces* bacteria contains secondary metabolites i.e. Alkaloids, flavonoids, and terpenoids.
- Larvicidal test of the metabolites on the mosquito larvae of *Anopheles* had a significant effects on mortality in a concentration manner ($p<0.05$). The highest mortality effect was shown by the extract of 500 ppm in 48 hours of expose.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

References

[1] Septiani L, Setyaningrum . and Ernawati K. 2012. Ecological study of malaria vector breeding sites in Sukamaju Village, Punduh Pidada District, Pesawaran Regency, Lampung Province.

[2] Anasis AM, Setyaningrum E and Umar S. 2014. Ecological study of malaria vector breeding sites in the swamp area of Lempasing Village, Padang Cermin District, Pesawaran Regency, Lampung Province.

[3] Suwandi JF and Setyaningrum E. 2015. Buffering and clustering patterns of malaria sufferers in the South Coast of Bandar Lampung City.

[4] Suwandi JF, Supargiyono, Asmara W, and Kusnanto H. 2014. Mapping and prevalence of malaria Falciparum patients with ACT failed therapy in Hanura public . center. Open J. Epidemiol. 4:169-77

[5] Kahar SRS, Hasan A and Lamangantjo C. 2019. Entomopathogenic activity of *Serratia marcescens* Bizio against coconut beetle larvae mortality (*Brontispa longissima*) Gestro. Jambura Edu Biosfer Journal, 1(2), 64–71.

[6] Sinaga LS, Martini, and Saraswati LD. 2016. Resistance status of Aedes aegypti (Linnaeus) larvae to temephos (Study in Jatiasih Village, Jatiasih District, Bekasi City, West Java Province).Jurnal Kesehatan Masyarakat, 4(1), 142–152.

[7] Samrot A. V. 2011. Optimized production of prodigiosin from *Serratia marcescens* SU-10 grown as batch culture and evaluation of bioactivity of produced prodigiosin. International Journal of Medicobiological Research. 1(3) : 145-150.

[8] Suari LGSA, Haq AD, and Rahayu LAD. 2021. Potential of kamboja flower extract (Plumeria Sp.) and kluwih flower (*Artocarpus camansi*) as biolarvicidal on mosquitoes Anopheles in efforts to prevent malaria. JIMKI. 8(3) : 137–145.

[9] Widiastuti D, and Ikawati B. 2018. Malathion resistance and esterase enzyme activity in *Aedes aegypti* mosquito populations in Pekalongan Regency. Balaba: Jurnal Litbang Pengendalian Penyakit Bersumber Binatang Banjarnegara. 61–70.

[10] Suryawanshi RK, Patil CD, Borase HP, Narkhede CP, Salunke BK and Patil SV. 2015. Mosquito larvicidal and pupaecidal potential of prodigiosin from *Serratia marcescens* and understanding its mechanism of action. Pesticide Biochemistry and Physiology.123 : 49–55.

[11] Arifiyanto A, Surtiningsih T, Ni'matuzaroh, Fatimah, Agustina D, and Alami N.H. 2010. Antimicrobial activity of biosurfactans produced by Actinomycetes isolated from rhizosphere of Sidoarjo mug region. Biocatalys and Agricultural Biotechnology. 24(2020)101513;1-7

[12] Bisyaro, N. 2020. Toxicity test of Kelor seed (Moringa oleifera) against mosquito larvae of Aedes aegypti. Jurnal Farmasi Tinctura. 1(2) : 34–44.

[13] Cania E and Setyaningrum E. 2013. Larvicidal test of legundi leaf extract (*Vitex trifolia*) against *Aedes aegypti* larvae. Journal Medical of Lampung University. 2(4) : 52–60.

[14] Kartini S, Pratiwi D and Atina Z. 2020. Mortality test of Anopheles mosquito larvae with salam plant leaf ethanol extract (*Syzygium polyantum*). Klinikal Sains : Jurnal Analis Kesehatan. 8(1) : 41–48.

[15] Yuliasih Y and Widawati M. 2017. Larvicidal activity of various solvents of seed extract of kayu besi (*Pongamia pinnata*) on mortality of Aedes spp larvae. Balaba: Jurnal Litbang Pengendalian Penyakit Bersumber Binatang Banjarnegara.13(2) : 125–132.

[16] Waskito P and Cahyati W. 2018. Effectiveness of plant leaves granule of salam plant (*Eugenia polyantha* Wight) as *Aedes aegypti* mosquito larvicidal. SPIRAKEL.10(1) : 12–20.

[17] Ridjal ATM, Kasma AY, and Risda M. 2019. Effectiveness of pandan wangi leaf extract (*Pandanus amaryllifolius*) on mortality of Aedes sp and Anopheles larvae. Jurnal Vektor Penyakit, 13(2), 107–114.

[18] Krishanti NPRA, Wikantyoso B, Zulfitri A and Zulfiana D 2017. Entomopathogenic bacteria as biocontrol agents against *Spodoptera litura* larvae (F.). Jurnal Ilmu-Ilmu Hayati. 16(1).

[19] Okay S, Özdal M, and Kurbanoğlu EB. 2013. Characterization, antifungal activity, and cell immobilization of a chitinase from Serratiamarcescens MO-1. Turkish Journal of Biology. 37(6) : 639-644.

[20] Aggarwal C, Paul S, Tripathi V, Paul B, and Khan MA. 2015. Chitinolytic activity in Serratiamarcescens (strain SEN) and potency against different larval instars of Spodoptera litura with effect of sublethal doses on insect development. BioControl. 60(5) : 631–640.

[21] Gilbert GI, Latrou K., and Gill SS. 2005. Biochemistry of Digestion, in Comprehensive Molecular Insect science Biochemical and Molecular Biology. 171-224. Elsevier Press. Oxford. UK.