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# Potential of *Streptomyces spp*. From Peanut Plant Soil as an Entomopathogen of Pests *Spodoptera Litura*

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**Abstract.** Peanuts are agricultural commodities that have high economic value and play an important role in meeting human needs, namely as a source of vegetable protein, oil and other nutrients, but domestic peanut production has not been able to meet the needs of the Indonesian people. The national demand for peanuts reaches 856.1 thousand tons per year, and the average consumption of peeled peanuts is 0.32 kg per capita every year. [1] In 2013 peanut production was 701,680 tons, then there was a decrease in production in 2014 to 638,896 tons, and continued until 2015 to 605,449 tons, thus showing a 13.7% decrease that occurred from 2013 to 2015. Low productivity is caused by various factors, one of which is the attack of pests and plant diseases. This study aims to determine the type of Streptomyces spp. Microbes with high chitinolytic content that can control armyworm pests (S.litura) on peanut plants. and influence of biological control agency Streptomyces spp as an entomopathogen of insect pests S. litura. This study was able to improve understanding of the influence of the biological control agency Streptomyces spp as an entomopathogen of S. litura insect pests to protect plants in the future. The results of the exploration of Streptomyces sp. acquired Colonies are irregular and branched coccus-shaped. The colony is tightly attached to the GNA medium, wavy white with a notched surface and a starchy texture. In addition, it has the largest chitinase enzyme content with a chitin zone diameter of 3 cm. PCR test Based on BLAST® result tabulation data that isolates belong to the genus Streptomyces sp. with an identity percentage of 99.86% with Streptomyces sp. VEL gene 17 to 16 rRNA, partial sequence with access code AB914463.2 The highest mortality was in the treatment (S0 RP K3) and (S1 RP K3) there was 60% this showed that with the administration of Streptomyces sp. With a concentration of 75% can cause very high mortality with abdominal contact poison in S. Litura pests

**Keywords:** Entomopathogen, Peanuts, *Streptomyces spp, S. Litura*

## 1 Introduction

Peanuts are agricultural commodities that have high economic value and play an important role in meeting human needs, namely as a source of vegetable protein, oil and other nutrients, but domestic peanut production has not been able to meet the needs of the Indonesian people. The national demand for peanuts reaches 856.1 thousand tons per year, and the average consumption of peeled peanuts is 0.32 kg per capita every year. [1] In 2013 peanut production was 701,680 tons, then there was a decrease in production in 2014 to 638,896 tons, and continued until 2015 to 605,449 tons, thus showing a 13.7% decrease that occurred from 2013 to 2015. Low productivity is caused by various factors, one of which is the attack of pests and plant diseases.

One of the pests on peanut plants is armyworm (S.litura). Leaf damage by armyworms can interfere with the photosynthesis process and eventually result in yield loss. The magnitude of yield loss depends on the degree of leaf damage and the stage of plant growth when the infestation occurs. Leaf damage of 12.5% can lead to economic losses equivalent to the cost of twice the application of insecticides. Armyworm (S.litura) is a polyphagous pest caterpillar because the caterpillar can eat all types of plants for its survival and is difficult to control [2].

Biocontrol of peanut pests is still limited, although some aspects of pest biology and parasitism on plants have been carried out. To increase the potential of biological control, it is necessary to find alternative ways that are safer for natural enemies and at the same time able to control pests to the limit of natural equilibrium (Natural equilibrium level). One potential way to be developed is by utilizing synthetic non-chemical insecticides such as biological and vegetable control agents. The biological control agent Bacillus thuringiensis has long been known and used commercially, while the vegetable control agent from srikaya and neem has

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traditionally been used by farmers abroad and in Indonesia to control various important agricultural crop pests. Compared to synthetic chemical insecticides, biological and vegetable ingredients, it has several advantages, namely its abundant availability in nature, does not pollute the environment, and is not harmful to humans and other animals as well as natural enemies of insects [3] The above pest control is environmentally friendly by utilizing biological control agents because these agents have the potential to inhibit the development of pests and plant diseases, one of them is Streptomyces spp. The use of Streptomyces spp. as a biological control agent is very potential to control pests and diseases in plants. Streptomyces spp. is a Gram positive bacterium found in soil, compost, water and plants. about 70% of microbes present in soil are Streptomyces spp. The presence of Streptomyces spp. in soil has been widely studied. Streptomyces spp. Classified as entomopathogenic bacteria that have been effective in controlling important pests on agricultural crops. Steptomyces spp. species show high chitin propagation gene efficacy. Chitinase enzyme is an important enzyme needed for insect control. [4,5] revealed that Streptomyces spp. bacteria are able to produce secondary metabolites such as antibiotics so that they can be antagonistic to certain pathogens and can increase the availability of nutrients so as to increase resistance in peanuts.

This study aims to determine the type of Streptomyces spp. Microbes with high chitinolytic content that can control armyworm pests (S.litura) on peanut plants. and influence of biological control agency Streptomyces spp as an entomopathogen of insect pests S. litura. This study was able to improve understanding of the influence of the biological control agency Streptomyces spp as an entomopathogen of S. litura insect pests to protect plants in the future.

# 2 Research method

## 2.1 Material

The materials used in this study are as follows: Soil and Peanut Root samples, S.litura Third Instar Larvae in peanut plants, GNA Media (Glucose (1 gr), KH2PO4 (1.75 gr), NaNO3 (0.85 gr), KCl (0.75 gr), MgSO47H2O (2.5 gr), agar (20 gr), sterile aquadest (1000 ml)), PDA media (Potato Dextrose Agar), CCA Media (colloidal chitin 1%, peptone 0.1%, KH2PO4 0.1%, MgSO4.7H2O 0.05%) , ECG media, plastic, tissue, label paper, alcohol 70%, NaOCl 1%, spirit. Further exploration results were carried out further analysis using PCR techniques using primers 16S rRNA, 27F and 1492R,.

## 2.2 Tool

The tools used in this study include Anumbra 8 cm petri dish, autoclave (All American Model No.50X), shaker (IKA Yelloe line RS 10), stereo microscope, homogenizer/fortex, Laminar Air Flow (LAF) Envirco SV 900 SS and 1300 Series A2, compound microscope (Olympus CX33 RTFS2), eppendorf tube, micropipette (Fisherbrand), IWAKI beaker glass 500 ml and 1000 ml, Erlenmeyer Schot duran 100 ml, 250 ml and 500 ml, glass stirrer, Ose needle, tode, drip pipette, bunsen lamp, preparation glass, cover glass, knife, analytical balance (ACIS Model AD 6001), cotton, aluminum foil, stove, pot, plastic wrap, tweezers, test tube, hoe, shovel, and trowel, Neubauer hemocytometer 0.100 m,/0.0025mm2, hand counter, camera,

In this study, the identification of Streptomyces sp. using PCR techniques using 16S rRNA, 27F and 1492R primers. Technical stages of Streptomyces sp. The identification is as follows:

a. DNA isolation technique Streptomyces sp. performed using Genomic DNA extraction using Quick-DNATM Fungi/Bacteria Miniprep Kit (Zymo Research)
b. PCR amplification using MyTaq HS Red Mix (Bioline, BIO-25047)
c. PCR product purification using Zymoclean™ Gel DNA Recovery Kit (Zymo Research) Bi-drectional Sequencing

## 2.3 Research Design

This study used Factorial Group Randomized Design (RAK) with 3 factors. The first factor is the addition of the biological agency Streptomyces sp. On the soil before planting and after planting. The second factor of application time will be carried out before the infestation and after the infestation, pest infestation is carried out when peanut plants have begun to enter the generative period (40 HST). The third factor is the concentration of biological agency Streptomyces sp. which will be given as much as 25%, 50%, and 75%, with codes K1, K2, K3, and K0 (0 ml) as controls. Aquadest will be used as a solvent The number of experimental units used is 48 plants where per polybag will be planted 3 peanuts with 3 repeats.

## 2.4 Research Time and Location

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This research will be in May 2023 to December 2023 Sampling Preparation Activities in Gunung Dulang Village, Klakah District, Lumajang Regency, Biological Agent Isolation, Bioagent Propagation at the Plant Health Laboratory, Faculty of Agriculture, National Development University "Veteran" East Java and Quality Control Laboratory.

### 2.5 Preparation, Exploration, and Isolation of microorganisms Streptomyces spp. Soil

Microbial isolation of Streptomyces spp. using soil plating method. The soil from the peanut plant field is weighed with an analytical balance of 1 gram, then a suspension is made with a dilution of 10-4. Next, 1 mL is taken aseptically inserted and flattened into the GNA medium in a Petri dish. The biological agents obtained, then purified, and propagated on PDA media in petri dishes and test tubes. isolation of biological agents of Streptomyces sp., rejuvenation of isolates of biological agents, and propagation of biological agents in ECG media[5].

### 2.6 Chitinolytic test

Pure culture of Streptomyces spp. age 5 days on PDA media cut with a drill cutter diameter of 0.5 cm, then inoculated on Colloidal Chitin Agar (CCA) media. Then the culture is incubated for 14 days, until clear zones are visible around and under the culture. The diameter of the clear zone is measured (mm) and is used as an indicator of chitinase activity. Based on these results, three isolates of Streptomyces spp. that produced high chitinase activity were selected as candidates for biological agents. [6]

### 2.7 Breeding Test Insects *(S. litura)*

S. litura larvae from the field are collected and maintained in the laboratory by feeding in the form of natural feed. Natural feed preparation (peanut leaves) is obtained by taking leaves from peanut plants, with criteria: located at the first node (from above), three-stemmed, round / oval, and green. The obtained natural feed is then cleaned under running water and dried. After the leaves dry, the petioles are given a damp cotton swab to keep the leaves fresh. After becoming a pupa, it is then stored on a glass jar covered with gauze. The pupa that has become an imago is then fed honey, in the glass is given opaque paper as a place for the imago to lay eggs. The larval eggs are then separated in a clear plastic jar and covered with gauze. The larvae hatched from the eggs are then fed artificial feed. After entering instar 3, the larvae were ready to be tested using Streptomyces spp. culture. [7]

### 2.8 Propagation of Isolates

Propagation of Streptomyces spp. in media such as ECG (Sugar Potato Extract), Propagation of the three isolates of Streptomyces spp. obtained in ECG culture media (Sugar Potato Extract is done by taking 4 plongs using cork drills (each ± 5 mm in diameter). Then, inoculated into 150 ml ECG culture media (Sugar Potato Extract), at erlenmeyer capacity of 250 ml and gojlog using a shaker at a speed of 120 rpm at room temperature for 14 days [8].

### 2.9 Test the effectiveness of Streptomyces spp. against *S.litura* In Vitro

The biological agent obtained was used by taking as much as 5 ose cultures of Streptomyces spp. from isolates that had been aged 14 days then each was put in 250 ml of ECG media and homogenized using a corner tool at medium speed for 7 days. Then each dissolved in 7 liters of water, applied to 30 peanut leaves, sprayed with three isolates, 20 ml each experiment with the application of instar 3 larvae in each experiment as many as 10 heads. Peanut leaves are changed every three days with the same spraying, then observations are made on mortality rates, symptoms of attack and death, insect behavior. [9].

### 2.10 Data Analysis

The research data were analyzed with Analysis of Varience (ANOVA) to determine whether or not there was an influence from each experiment. If there is a real effect of the treatment, a further test is carried out using the Honest Real Difference Test (BNJ) or Tukey Test, while if there is a real effect of the treatment, the Duncan Multiple Range Test (DMRT) is carried out at the level of 5%.

## 3 Result and Discussion

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### 3.1 Exploration and Isolation of Streptomyces sp.

Exploration of Streptomyces sp. carried out in the summer of March 2023 in the Lumajang area, the area at that time was being planted peanut plants. Sampling is carried out on the basis of healthy plants, not infected with pests and diseases and is carried out randomly. Soil samples taken as much as 1 kg which are then dried by air, dry soil will be carried out a serial dilution process.

Exploration acyl Streptomyces sp. The soil of peanut plants obtained is located in Gunung Dulang Village, Klakah District, Lumajang Regency. Colonies are irregular and branched in the form of coccus. The colony is tightly attached to the GNA medium, wavy white with a notched surface and a starchy texture. Microscopic observation of conidia in the form of coccus, there are conidia arranged in chains at the ends of hyphae and these isolates include Gram positive. [10,11]

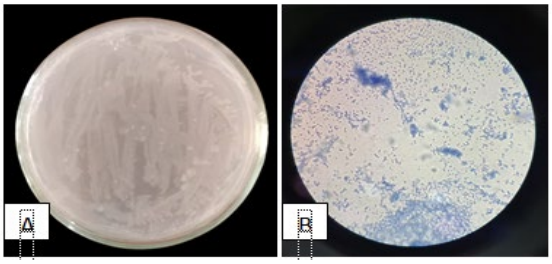


**Figure 1.** (A) Colony of Streptomyces sp.
(B) Microscopic approximation of 100 x spore chains of Streptomyces sp.

### 3.2 Chitinase Test

Test chitinase enzyme activity is carried out to determine the content of chitinase enzyme from Streptomyces sp. isolate.Isolate Streptomyces sp.. (Figure 2) has the largest chitinase enzyme content with a chitin zone diameter of 3 cm.Based on research, the production time of chitinase enzyme from Streptomyces sp. isolate. is 6 days. The time it takes to produce the enzyme chitinase is in the exponential phase, which varies depending on each microbe. The pH and temperature conditions of chitinase enzyme production vary. Chitinase activity is also influenced by other factors such as added nutritional supplements, colloidal chitin concentration, addition of carbon sources, addition of nitrogen sources, and use of detergents [12] Chitinase enzymes that play a role in pest control are able to decompose chitin in fungal cell walls, nematodes and insect exoskeletons into N-acetyl glucoglucominides. Chitinolytic microorganisms degrade chitin by involving the enzyme chitinase. Most of these microorganisms are from the group of bacteria, for example Streptomyces sp. Bacillus, Aeromonas, Serratia, and Enterobacter. Streptomyces sp.. has the ability to produce the enzyme chitinase. Streptomyces sp.. Induces chitinase synthesis by recognizing the physical structure of chitin such as chain arrangements. These bacteria produce lectin-like proteins that bind specifically to α-chitin crystals and during chitin degradation cells can also recognize the degree of deacylation of the relative amounts of glucosamine and G1cNac liberated [13]

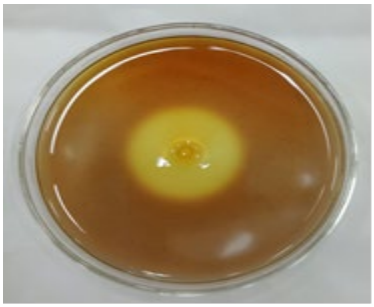

**Figure 2.** Chitinase Test Results of Streptomyces sp..

### 3.3 Identification of Streptomyces sp.

Identification Molecular determination of Streptomyces sp. these were tested using PCR techniques using common 16S rRNA Primers namely: 27F and 1492R. The nucleic acid (DNA genome) quantification (Nanodrop) is as follows: Conc 4.3 ng/ µL, A260/280 2.3, A260/230 0.35, volume 50 µL. Amplification of the 16S rRNA gene was successfully performed using 27F and 1492R primers. 1 µL of PCR product (±1400 bp) was analyzed using agarose gel. 1 µL of PCR product was run on 1% TBE agarose gel at 100 Volt for 30 min

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(Figure 3). [14,15] Phylogenetic relationship of Streptomyces sp and related taxa, based on 16S rRNA analysis. The history of evolution is deduced using the Neighbor-Joining method (Figure 4)

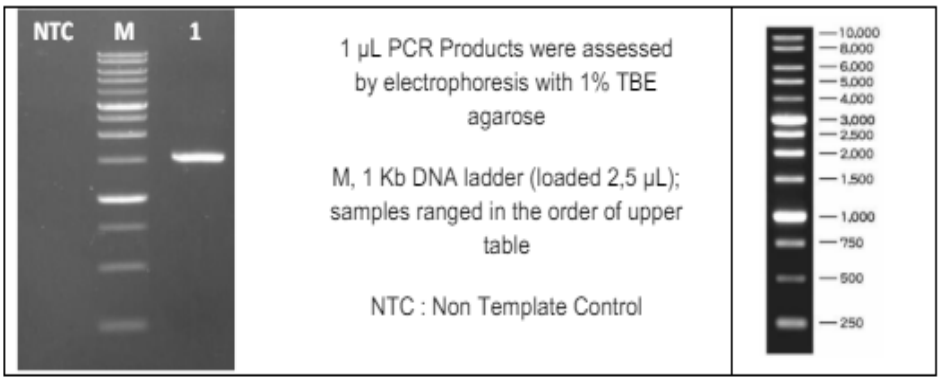


**Figure 3.** Gel Photo – PCRProducts

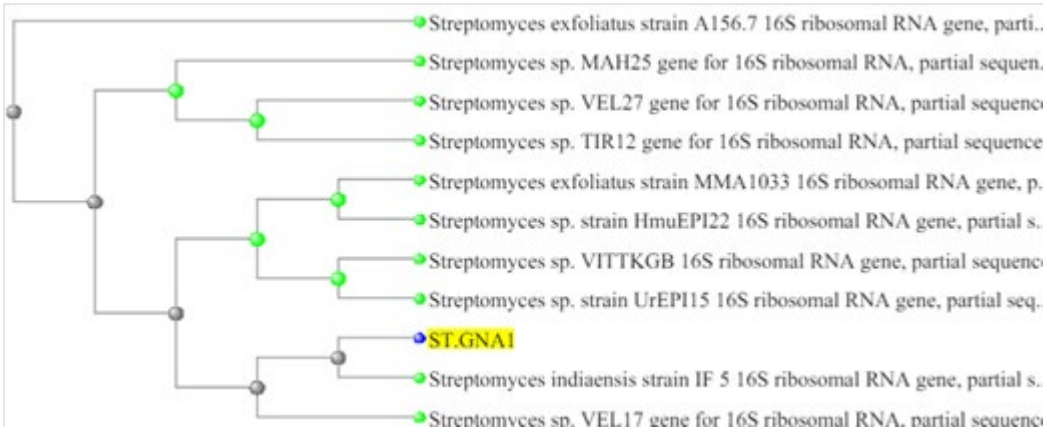


**Figure 4.** Phylogenetic Tree ST. GNA 1

The PCR method has amplified 16S rRNA from isolates. PCR results are purified and cloned to get the best sequence results. The gene sequence length of the 16S rRNA species (1605 bp) is quite informative in identifying species. Based on the tabulation data of BLAST® results that isolates belong to the genus Streptomyces sp. with an identity percentage of 99.86% with Streptomyces sp. VEL gene 17 for 16 rRNA, partial sequence with access code AB914463.2[15]

## 3.4 Test the Effectiveness of Streptomyces spp. against S.litura In Vitro

### 3.4.1 Mortality Rate ( Death Rate ) S.litura.

The mortality rate of armyworm pests (S.litura) is carried out by direct observation of the number of S. litura pests that die after being given Streptomyces sp. Data on dead S. litura were observed and counted daily for 10 days to determine the time of death.

**Table 1.** Mortality table *S.Litura* sec. In. Vitro

| Treatment | Mortality % | Notation |
|---|---|---|
| S0 RK K0 | 13 | a |
| S0 RP K0 | 13 | a |
| S1 RK K0 | 13 | a |
| S1 RP K0 | 13 | a |
| S0 RK K1 | 30 | b |
| S1 RK K1 | 30 | b |
| S0 RK K2 | 33 | bc |
| S1 RP K1 | 33 | bc |
| S0 RK K3 | 36 | bc |
| S0 RP K1 | 36 | bc |
| S1 RK K2 | 40 | bc |

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| | | |
|---|---|---|
| S1 RK K3 | 40 | bc |
| S0 RP K2 | 43 | bc |
| S1 RP K2 | 46 | cd |
| S0 RP K3 | 60 | d |
| S1 RP K3 | 60 | d |

In the table. 1 indicates that the time of death takes place on different days because the RP treatment acts as a stomach poison so that it takes time for the biocide to kill the pest in the abdomen of the pest, while in the RK treatment the time of death occurs at the beginning when given the biocide because the biocide acts as a contact poison, this is in accordance with the statement [16] that contact poison acts directly through the skin of insects, While stomach / stomach toxins work when the part affected by biopesticides is eaten.

The highest mortality was in the treatment (S0 RP K3) and (S1 RP K3) there was 60%, this shows that with the administration of Streptomyces sp. With a concentration of 75% can cause very high mortality with abdominal contact poison in S. Litura pests . treatment (S1 RP K2) there are 46%. And the treatment of S0 RK K2, S1 RP K1, S0 RK K3, S0 RP K1, S1 RK K2, S1 RK K3, S0 RP K2 has the same effect.

Research [18] used the active ingredient Streptomyces sp. In dealing with pests, L. stigma is a contact poison while research by applying directly on the soil surface [19] which also uses the active ingredient Streptomyces sp. In controlling fruit flies (Bactrocera sp.) in the form of contact poison by spraying directly on fruit fly larvae. Based on the mortality results above, it shows that Streptomyces sp. Has the ability as a biocide that can be used as pest control.

### 3.4.2 Observation of insect behavior, Symptoms of Attack and Death.

From observations of the behavior of test insects, the symptoms caused by S. Litura eating leaves that have been applied after investigation and before investment have decreased mobility of *S. litura*. Decreased appetite in *S. litura* is characterized by leaves that are not depleted and *S. litura* is also impaired digestion. While in Control there is no indigestion and larvae develop normally. This is because Streptomyces sp. Produce secondary metabolites that have strong biological potential, including as antimicrobial agents and insecticides. Antimicrobials and insecticides produced will affect digestive disorders so that there is a refusal to eat in S. litura and can cause poisoning in S. litura. In addition to decreased appetite, S.Litura also experienced a slow growth cessation and color changes arising in uninfected *S.Litura* was clearly darker in color, while infected *S.Litura* was clearly visible the color changed paler than usual, white spots appeared, causing partial paralysis, difficulty moving and death.

## 4 Conclusion

The results of the exploration of *Streptomyces sp*. acquired Colonies are irregular and branched coccus-shaped. The colony is tightly attached to the GNA medium, wavy white with a notched surface and a starchy texture. In addition, it has the largest chitinase enzyme content with a chitin zone diameter of 3 cm. PCR test Based on BLAST® result tabulation data that isolates belong to the genus *Streptomyces sp*. with an identity percentage of 99.86% with *Streptomyces sp*. VEL gene 17 to 16 rRNA, partial sequence with access code AB914463.2 The highest mortality was in the treatment (S0 RP K3) and (S1 RP K3) there was 60% this showed that with the administration of Streptomyces sp. With a concentration of 75% can cause very high mortality with abdominal contact poison in *S. Litura* pests.

## References

[1] Kasno, A. and Harnowo, D. 2014. "The characteristics of high-yielding varieties of peanuts and their dough by farmers. Tananaman Research Institute for Various Nuts and Sweet Potatoes". Food Crop Science and Technology 9(1): 13 – 23.

[2] Sari, N. M., R. Kawuri and K. Khalimi. 2012. "Streptomyces sp. As a Biofungicide Pathogenic Fusarium oxysporum (Schlecht.) f.sp. lycopersici (Sacc.) Snyd. et Hans. Causes of wilt disease in tomato plants (Solanum lycopersicum L.)". Agrotopes, 2(2): 161-169.

[3] Eri, D. Salbiah & H. Laoh.2013. "Test several concentrations of areca nut seeds (Areca catechu) to control armyworm pests (Spodoptera litura F.) on mustard plants (Brassica juncea L.)". Faperta Journal. 1(2): 1-9

[4] Hasani, A., A. ”Kariminik dan K. Issazadeh. 2014. Streptomycetes: Characteristics and Their Antimicrobial Activities. Journal of Advanced Biological and Biomedical Research”. 2(1): 63-75.

[5] Gowdar, S. B., H. Deepa and Y. S. Amaresh. 2018. “A Brief Review on Biocontrol Potential and PGPR traits of Streptomyces sp. for the Management of Plant Diseases. Journal Pharmacognosy and Phytochemistry”, 7(5): 3-7.
[6] Suryadi et al in Nafisah, et al. 2017, "Isolation and Activity Test of Chitinase Isolate of Bacteria from Dieng Geothermal Region," Biome: Periodical Scientific Biology, vol. 19, no. 1, pp. 22-29
[7] Mayasanti R, 2012. Nutritional Needs of Natural and Artificial Feed. Web publicationhttps://www.scribd.com/doc/82092 108/Needs-Nutrition-Feed-Natural-andArtificial.
[8] Widiantini, F., Yulia, E. and Nasahi, C., 2018. Potential antagonism of secondary metabolite compounds of endophytic bacterial origin with methanol solvents against the fungus G. boninense Pat. Agriculture. Vol. 29 (1): 55-60
[9] Tyas, Y. P., H. Zayadi, and A. Hayati. 2018. Test of the Combination of Mahogany Seed Juice (Swietenia sp) and Garlic Skin (Allium sativum) against Crop Caterpillar Pests (Crocidolomia pavonana Fab.) on Cabbage Plants (Brassica oleraceae L). e-Scientific Journal BIOSAINTROPIC Volume 4, No. 1 Pages 60 – 65
[10] Suryaminarsih and Mujoko. 2012. "Multiantagonistic population development of Streptomyces sp. Gliocladium sp and Trichoderma harzianum as biological agents of fusarium wilt disease on semi-natural media and pellet formula aaket". Plumula, Pages 202-210.
[11] Suryaminarsih, P., Harjani, W. S. and W. Mindari. 2018. "Multi Antagonists Streptomyces sp. and Trichoderma sp. against Horticultural Plant Disease Pests in Marginal Soils". Stranas Report
[12] Bhattacharya S., S. Chakrabortty, and A. Das. 2012. Optimization of Process Parameters for Chitinase Production by a Marine Isolate of Serratia marcescens. J. Pharm. Biol. Sci, 2(2):8-20.
[13] Goddess, I.M. 2008. Bacterial Isolation and Crude Thermophilic Chitinase Activity Test from Raja High Hot Spring, Simalungun, North Sumatra (Thesis), Medan: Postgraduate University of North Sumatra.
[14] Palazzotto E, Renzone G, Fontana P, et al. Tryptophan promotes morphological and physiological differentiation in Streptomyces coelicolor. Appl Microbiol Biotechnol. 2015;99(23):10177-10189.
[15] Dhanjal DS, Chopra C, Anand P, Chopra RS. Accessing the microbial diversity of sugarcane fields from Gujjarwal village, Ludhiana and their molecular identification. Res J Pharm Technol. 2017;10(10):3439-3442.
[16] Djojosumarto, P. 2008. Complete Guide to Pesticides & Their Applications. Agromedia. 1, 13-31.
[17] Ancient, S. 2007. Test the Effectiveness of Noni Leaf Extract (Morinda citrifolia) Against Plutella xylostella L. (Lepidoptera: Plutellidae) in the Laboratory. Thesis. University of North Sumatra. Terrain. pp 29-35
[18] Hidayah, A., Harijani, W., Widajati, W., and Ernawati, D. 2019. Potential entomopathogenic fungi Metarhizium anisopliae, Beauveria bassiana and Streptomyces sp. against Lepidiota stigma mortality on sugarcane plants. Plumula 7(2): 64– 72.
[19] Safri, M., Harijani, W. S., & Suryaminarsih, P. (2017)." Test of Life Expectancy of Fruit Fly Pupa (Bactrocera Sp.) into Imago by Administering Bioagents Streptomyces sp". Agrotechnology Scientific Periodical-Plumula, 5(1).