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Isolation and Bioassay of Insect Pathogenic Bacteria in the Rhizosphere of Soybean Crops in Dry Land Central Lombok

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Abstract : *Spodoptera litura are able to destroy 1,5 hectares of soybean crops in 12 hours. The control of this S. litura by using pathogenic bacteria of insects are more efficient compare with using synthetic chemical insecticide. The exploration of pathogenic bacteria of insects is conducted on rhizosphere of soybean crops in dry land. The bacteria are isolated with heating technique and selective medium. Of pathogen bacteria are obtained 9 isolations bacteria, 4 isolations are obtained by the method of heating at 80 ° C for 10 minutes and 5 isolations are gain used selective medium King's B tested for the ability to control S.litura. The screening process is held by measuring remaining larva feed and percentage of dead larva in concentration of .32x10^9 CFU/ml. Remaining larva feed caused by bacteria achieve the number of 9.08 gram and larva mortality percentage is 94.66% in 162 hours.*

Keywords: *Pathogen bacteria of insects, Spodoptera litura, Pathogenesis*

I. Introduction

S. litura attacts are able to destroyed 1,5 hectares of soybean crops area in 12 hours [BP2TP NTB, 2014]. *S. litura* is threatening an area of 66.285 hectares in Central Lombok District. [BP2TP NTB, 2014]. The control og this *S. litura* by using pathogen bacteria of insects are more efficient compare with using synthetic chemical insecticide [Mandal,2003.]. The use of insect pathogen is not only proven effective to target pest but also caused pest resistance and safe for other non-target organism.

Insects pathogen isolation has been done through rhizosphere crops before[Prayogo, 2006]. Insect fungal phatogen of rhizosphere of soybean crops has their habitat in dry land Lampung, and insect fungal pathogen in Lombok has their habitat in orange crops rhizosphere [Rai, 2004]. Pathogen bacteria of insects has its habitat in plant crops rhizosphere. Rhizosphere of soybean crop becomes habitat of *S. litura* (fifth instar pre-pupae and pupae) insects. Bacteria isolat from *S.litura* habitat has higer virulence to control *S. litura* pests [Fargues, 1977].

The aim of this study is to isolate local pathogen bacteria of insects isolation which is found in dry land rhizosphere of soybean crops, Central Lombok, and to define the pathogenicity of pathogen bacteria of insects isolation through *S. litura*.

II. Material And Metods

The sample which is taken from the land is isolated with heating technique [Ohba &Aizawa,1986] by taking 1 gram of soil sample, then put it into test tube filled with 9 ml sterile ringer. The suspension is shaken until homogenous followed by heating in water bath at temperature of 80^{o}C for 10 minutes. Next step is making series of dilution suspension start from 10^{-2}–10^{-4}. From the dilution of 10^{-3}–10^{-4}, it is taken 0,1 ml each and flattened on NA media, after that Petri dish is wrapped with sterile paper coves and laid in an inverted position. This is incubated for 48 hours at room temperature (28^0-30^0C).

Method that use selective media with King-s B as growing media is utilized to select bacteria which had the characteristics of *Pseudomonas* sp. Where dilution suspension is made up to 10^{-7}, taken 0,1 ml and flattened on King's B media. This is incubate for 48 hours at room temperature (28^0-30^0C). This is observed under UV ray to detect whether the bacteria colony produce Florensens ray.

For initial selection, Of numerous colony growing chosen characteristic colony with morphological traits: stem shaped cells, shows motility, gram positive, circular colon, white and yellowish white color of colony. Bacteria colonies which show positive characteristics as bacillus bacteria are made pure culture to put in tube contains liquid with 15% glycerol and keep in refrigerator as stock used along this research (Cristina, 2011) [Christina, 2011].

Initial stage against pathogenic bacteria of insect isolation for *S. litura* larvae is conducted to observe the potential from pathogenic bacteria of insect found. Pathogenic insect isolation which will be tested its level of poison bred in NA media for 24 hours to calculate the number of Colony Forming Unit using Photospectrometer tool. The result of the calculation is drawn on the standard number of the colony that is used.

S. litura larvae that is used as test material is third instar larvae. The test design is completely randomized design (CRD) with dilution of suspension up to 10^{-7}per ml. This is sprayed to the larvae feed *S. litura* done by suspension as much as 2 ml of any experiment unit, whereby on every experiment unit there were 25 larva. The weigh is equal to 10 grams.

The observation is made at the time of treatment and every six hour after treatment up to one week by monitoring *S. litura* larva's appetite, symptoms and dying process.

III. Results And Discussion

Pathogen bacteria of insects are able to grow properly in maximum temperature of 35°c to 45°c and in minimum temperature of 20°c to 10°c [Gordon, 1973]. The average temperature of dry land in Central Lombok is between antara 27,6°C-28,1°C. Rhizosphere is a good habitate for growthh of bacteria because the crops' root provide various organic substances. Root exudates can influence the growth of microorganisms inside the zone. On the other hand the metabolism of crops will be influenced by microorganisms' activity that is in it zone [Soemarno. 2010].

Tabel 1. Isolate colony morphology isolation and characterisation of pathogen bacteria of insect from rhizosphere of soybean crops in dry land Central Lombok.

Isolation	Colony	Colony'sMargin Shape	Colony's Size	Colony's Colour
SP1	Round	Smooth	3.0 mm	Yellow
SP2	Round	Wavy	2.4 mm	Yellow
SP3	Round	Smooth	1.8 mm	Cream
SP4	Round	Smooth	3.0 mm	Yellow
Sp5	Round	Wavy	2.7 mm	Cream
SP6	Round	Smooth	1.3 mm	White
SP7	Round	Wavy	2.5 mm	White
SP8	Round	Smooth	2.0 mm	White
SP9	Round	Smooth	2.5 mm	White

Annotation. SP1-SP5 use King's B medium . SP6-SP9 use NA medium

The spread of pathogen bacteria of insects is trough soilborne which might interact with other micro organism in rhizosfer. The crops are symbiotic with pathogen bacteria of insects because they are able to hinder a plant disease caused by other pathogenic microorganisms and increase the growth of plants at the same time [Kotiranta, 2000]. Virulent pathogen bacteria of insects have ability to decrease appetite and increase larvae dead number percentage.

The nine pathogen bacteria of insects isolation are able to decrese larvae appetite and shows significant differences agains the control which is shown by Diagram 1. Remaining larva feed on larvae treatment which inoculate with pathogen SP1 isolation is 9.08 gram per 6 hours of treatment, while on control treatment, the remaining larva feeds is 6.94 gram per 6 hours of treatment.

The decreasing of insect appetite is caused by increasing the level of acidity digestion of *S. litura*. The infected insects felt paralysis which causing pH increasing in their digestion system and the insects are not able to eat anything due to the influence of a toxin that worked in digestion system of the insects [Steinhaus, 1963].

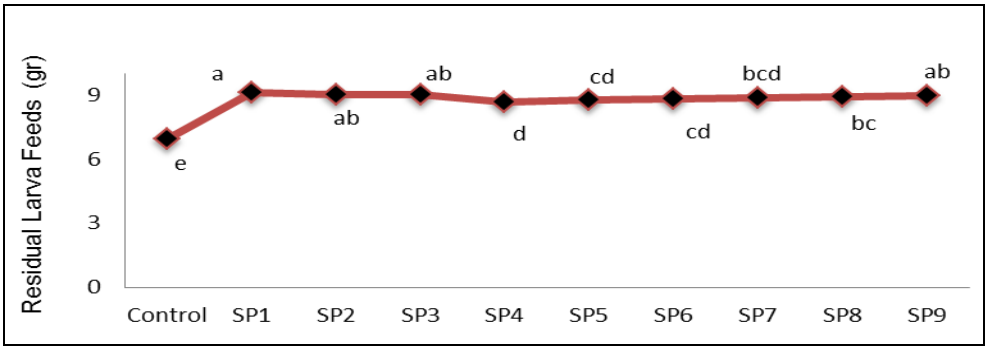

Diagram 1. Residual feeds of *S. litura* due to infection of 9 bacteria isolation collected from dry land
Chart following by letter has no significant differences.

Dead larvae infected with the bacteria has a characteristic that is watery soft, blackened and smelled foul. In accordance with the opinion of Kashwar and Yulianti (2001), the body color of larvae that have died on the first day showed no change but on the second day will show symptoms of reddish-brown discoloration. On the third day the body of the larvae will turn black and secrete a milky white fluid and cause a foul odor. According Poinar and Thomas (1973) dead larvae infected with the bacteria *Pseudomonas entomophila* watery body, her skin color to black, soft, shriveled, stinking, and after a few days to dry out and shrink.

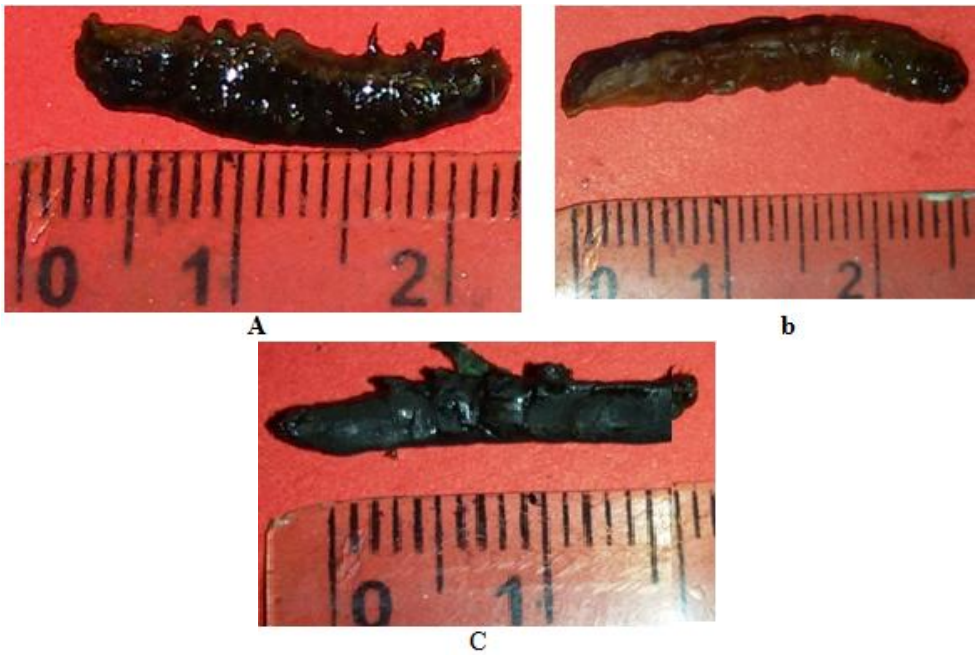

Figure 7.Larva *S. litura* dead insects infected with pathogenic bacterial isolates obtained from dry land. a, Larva soft watery, b, Larva rot, c, Larva blackened dries.

Treatment of 162 hours pathogen bacteria of insects application with $3.32x10^9$ CFU/ml population density causes larvae mortality ranged from 76% - 94.66% showed by Diagram 2. SP6 isolation has the highest dead percentage of *S. litura* larvae as much as 94.66% with significant differences over SP3 and SP4 isolations. It is assumed that the differences are caused due to diversity of types of toxin in different pathogen bacteria of insects. *Pseudomonas entomophila* bacteria produces haemolysin activity which becoming toxin against insects[Vodovar, 2006].

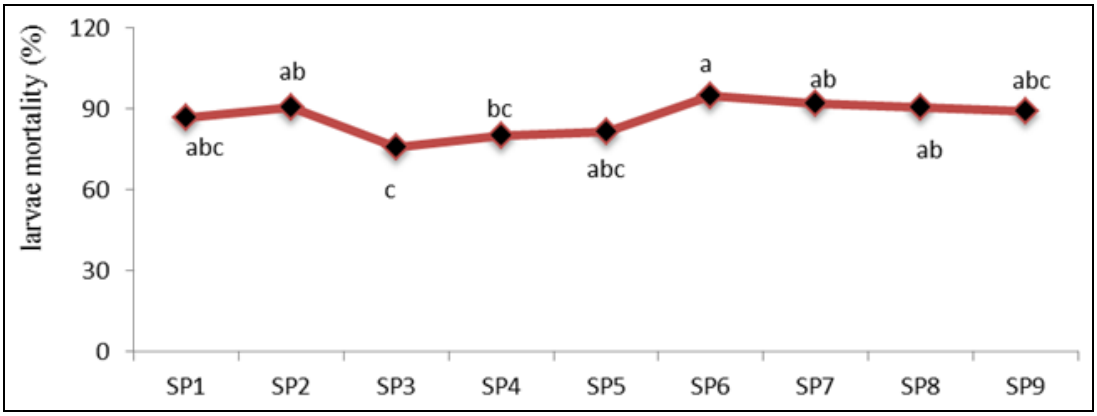

Diagram 2.*S. litura* larvae dead number due to infection of 9 bacteria isolation collected from dry land Chart following by letter has no significant differences.

Bacteria haemolysin act as exotoxin which causes breakage of cells that causes paralysis on insects. In B. Thuringiensis the working power of δ-endotoxin is located in its crystal protein. Various decisive factors on the efficacy of δ-endotoxsin are strain B. Thuringiensis strain, solubility of crystal protein in food channel, and insects' sensibility against toxin [13].

IV. Conclusion

In the effort to search a species of pathogenic bacteria of insects on soybean crop in dry land of Central Lombok, it is found 9 isolations which are potentially control insect pest that is most capable to control the larva is *S. litura* up to 96,66% in period of 162 hours at concentration $3.32x10^9$ CFU/ml.

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