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Combination Potential of some Bacteria Insulated From LMO Banana Stem Bud As a Bio-control Agents to *Spodoptera litura* Fabricius

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ABSTRACT

Spodoptera litura Fabricius is widespread in Indonesia, covering 22 provinces with an average attack area of 11,163 ha/year. This is a polyphagous insect pest causing defoliation with a crop loss of 85% to 100%. The application of biotechnology derived from local resources is a very appropriate alternative to controlling *Spodoptera litura* Fabricius, namely *Bacillus cereus* strain ATCC 14579, *Bacillus subtillis* subsp. subtilis strain 168 *Bacillus siamensis* strain KCTC13613, *Azotobacter* sp. and *Pseudomonas fl*uorescens isolated from the LMO banana stem bud. The study aims to determine the potential of 5 types of bacteria that were combined as *S. litura* pest bio-control agents. The research consisted of stages; 1) bacterial compatibility test, which would be combined; 2) in-vitro bacterial combination potential test on *S. litura* F. larvae. The results showed that the bacteria *Bacillus cereus* strain ATCC 14579, *Bacillus subtillis* subsp. Subtilis strain 168 *Bacillus siamensis* strain KCTC13613, *Azotobacter* sp. and *Pseudomonas fluorescens* are compatible with each other so that they can be combined. These five types of bacteria are single less potential as *Spodoptera litura* pest biocontrol agents due to cause low larval mortality, but a combination of 5 bacteria cause larval mortality by 70% its potential for shallow swampland. Although this line was not the best, it showed better overall agronomic performances than the check variety.

INTRODUCTION

Loss of food crops production due to pests and plant diseases is estimated by the FAO around 40% annually or equivalent to US$ 220 billion. One strategy to increase global food demand is to reduce yield losses due to Pest attacks (FAO, 2013). *Spodoptera litura* Fabricius. is a pest in various types of important plants. This leaf-eating pest is an important pest because it causes crop failure if it is not controlled. Spodoptera litura attacks of 20-80% in soybean plants while in cabbage was approximately 70% (Rosmiati et al. 1818).

To control Spodoptera litura, farmers generally use synthetic insecticides because more effective and fast results are quickly identified, and the application is relatively easy. However, synthetic insecticides that are not wise can cause adverse effects such as immunity on pests, resurgence, and pollution in the environment, both abiotic and biotic. Therefore, ways to control pests based on

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ecological considerations and economic efficiency in managing environmentally friendly and sustainable ecosystems need to be developed, such as pest bio-control agents such as bacteria, fungi, and viruses. However, microorganisms as biological agents are susceptible to environmental factors. Therefore, it would be more effective to use local microorganisms or microorganisms indigenous, such as a bacterial combination consisting of *Bacillus cereus* strain ATCC 14579 and *Bacillus subtillis* subsp. subtilis strain 168 *Bacillus siamensis* strain KCTC13613, *Azotobacter sp.* and *Pseudomonas fluorescens* isolated from LOM banana stem bud. These bacteria, besides *Azotobacter*, can secrete extracellular enzymes such as *chitinase, protease*, and *cellulose* that can integrate host cells (Yulensri et al., 2018). The three types of *Bacillus* and *Pseudomonas fluorescent* are widely reported as controlling disease, while *Azotobacter sp*. is an Airborne N fixation bacteria. To increase these bacteria's killing power or virulence, the five bacteria were consecrated in one community. The use of microbial combinations tends to give better results than using a single isolate because it is expected that the work of enzymes of each type of microbe can complement each other to survive using nutrient sources (Komaaawijaya, 2009).

This study aims to determine the potential of 5 types of bacteria and their combination to control *Spodoptera litura*.

MATERIALS AND METHOD

The study was conducted at the Biology Laboratory Politeknik Pertanian Negeri Payakumbuh from February to June 2020. Isolates of *Bacillus cereus* strain ATCC 14579, *Bacillus subtillis* subsp. Subtilis strain 168 and *Bacillus siamensis* strain KCTC13613, *Azotobacter sp*. and *Pseudomonas fluorescens* media *Tryptone Soy Agar* (TSA). Petri dish, one needle, cork borer with a diameter of 8 mm, *Spodoptera litura cabbage leaf*, paddy leaves, honey bee, and Whatman paper no one was used in this study. Compatibility test of *Bacillus cereus* strain ATCC 14579, *Bacillus subtillis* subsp. Subtilis strain 168 and *Bacillus siamensis* strain KCTC13613, *Azotobacter sp*. And *Pseudomonas fluorescens*in vitro were performed using the dual culture method with Tryptone Soy Agar (TSA) media. The TSA media was heated until it thawed; after that, it was poured into a 15 ml/Petri dish, then allowed to stand for 10 minutes until it was frozen. The isolates of each bacterium used for treatment were two days old. Each bacteria was tested for compatibility by etching the first isolate on TSA media. While the second bacterial isolate was inoculated by making a suspension well using a cork borer with a diameter of 8 mm. Isolates are stated to be compatible if there are no inhibition zones (clear zones) around the suspension well and are stated to be incompatible if there are inhibition zones in the area where the two isolates meet the suspension wells (Djainuddin and Faisal, 2015).

The effectiveness test of *Spodoptera lititur* F in vitro uses a completely randomized design with seven treatments, and three replications of treatment are A). *Bacillus cereus* strain ATCC 14579, B). *Bacillus subtillis* subsp. subtilis strain 168, C) *Bacillus siamensis* strain KCTC13613, D) *Azotobacter sp*. E) *Pseudomonas fluorescens*, F) Combination of 5 bacteria G) Control. Observation of larval mortality (P) began 24 hours after inoculation. (Priyono, in IPM, 1999). To test the treatment effect on the observed responses, analysis of variance was performed by using the Statistical analysis system (SAS) program. The Duncan New Multiple Range Test is then tested for multiple regions to see differences in treatment at the 5% level.

Egg groups are taken from the field and maintained in the laboratory. The eggs are put into a Petri dish that has been covered with wet filter paper, and the eggs are left until they hatch. After the eggs hatch, the larvae are kept in plastic boxes of 10 larvae per box and covered with gauze. Larvae are fed with cabbage leaves and soybean leaves which are free of pesticides. When larvae enter the pupa stage, they are moved to another box with sawdust as a place for pupae. The emerging imago is maintained in a plastic box that has

been coated with filter paper for the female imago to lay eggs. The number of imagos placed in each box is three animals with a ratio of 2 females and one male. Imago is fed with 10% honey liquid fed through cotton wool moistened with honey, and placed on plastic in the box. The groups of eggs produced are maintained until they hatch into larvae. The resulting larvae are kept until they reach instar -2 as they are treated; the larvae are fed with pesticide-free cabbage leaves and soybeans (Basana and Prijono, 1994 in Lina et al., 2012).

This observation is carried out on pests that have died, and new larvae are said to be dead when touched did not react anymore. Finally, the data obtained is calculated as a percentage using the formula:

P = (A/B) x 100%
P = Percentage of dead larvae
A = Number of dead larvae
B = The number of larvae observed

If there are deaths in the control treatment, then the percentage of deaths is corrected by the Aboot formula (1995) in the Integrated Pest Control Center, i.e.:

$$Pt = \frac{Po - Pc}{100 - Pc} x100\%$$

Pt = Percentage of deaths corrected
Po= Percentage of deaths at treatment
Pc= Percentage of deaths in controls

RESULTS AND DISCUSSION

The results of observations of the bacterial compatibility test are presented in Figure 1. It showed that *Bacillus cereus* strain ATCC 14579, *Bacillus Subtillis* subsp. Subtilis strain 168, *Bacillus siamensis* strain KCTC13613, Azotobacter sp. and *Pseudomonas fluorescens* are compatible because there is no clear zone or clear halo circle around the suspension nicely.

Spodoptera litura pest mortality caused by a single colony of *Pseudomonas fluorescens*, *Bacillus cereus* strain ATCC 14579, *Bacillus substilis* subsp. Subtilis strain 168, *Bacillus siamensis Bacillus Siamensis* strain KCTC13613, and Azotobacter sp in vitro were relatively low at 20 -46.7%; according to Duncan, the larval mortality test was not significantly different from control. Mortality of *Spodoptera litura* larvae caused by a combination of 5 bacteria is relatively high at 70% and significantly different from the control according to Duncan's test (Table 1)

The highest percentage of *Spodoptera litura* larvae mortality was in the bacterial combination treatment followed by *Pseudomonas fluorescens, Bacillus cereus* strain ATCC 14579, *Bacillus substilis* subsp. Subtilis strain 168, *Bacillus siamensis*, *Bacillus siamensis* strain KCTC13613, and *Azotobacter sp*.

Bacillus cereus strain ATCC 14579*, Bacillus subtillis* subsp. *Subtilis strain 168* and *Bacillus siamensis strain KCTC13613, Azotobacter sp.* and *Ppodoptera fluorescens* are compatible to be combined in one community. For controlling *Spodoptera litura,* all bacterial isolate combinations tested did not show any inhibition zone or clear zone (Figure 1). The clearing zone is formed due to competition in obtaining nutrition in the culture media by these isolates. The population growth of one bacterium becomes faster than other bacteria, which results in the availability of bacterial food being limited and hindered its development. Due to the antagonistic nature of these bacterial isolates (Djainuddin and Faisal, 2015).

Bacillus cereus strain ATCC 14579*, Bacillus subtillis subsp. Subtilis strain 168 and Bacillus siamensis strain KCTC13613 and Pseudomonas fluorescens* single isolate were ineffective for controlling *Spodoptera litura* pests because larval mortality was not significantly different from control. Single isolates of these four bacteria are widely reported as bio-control agents of plant diseases. *Bacillus spp, Bacillus cereus, Bacillus substilis,* and *Pseudomonas fluorescens* can control the bacterial wilt disease of *Ralstonia solanacearum* (Istiqomah et al., 2018). Some bacteria of the genus Bacillus, such as *Bacillus subtilis, Bacillus cereus, Bacillus licheniformis, Bacillus megaterium,* and *Bacillus pumilus*, can act as biocontrol agents to control the growth of Fusarium sp (Deng and Huang, 2016). *Bacillus substilis* causes antagonistic activity

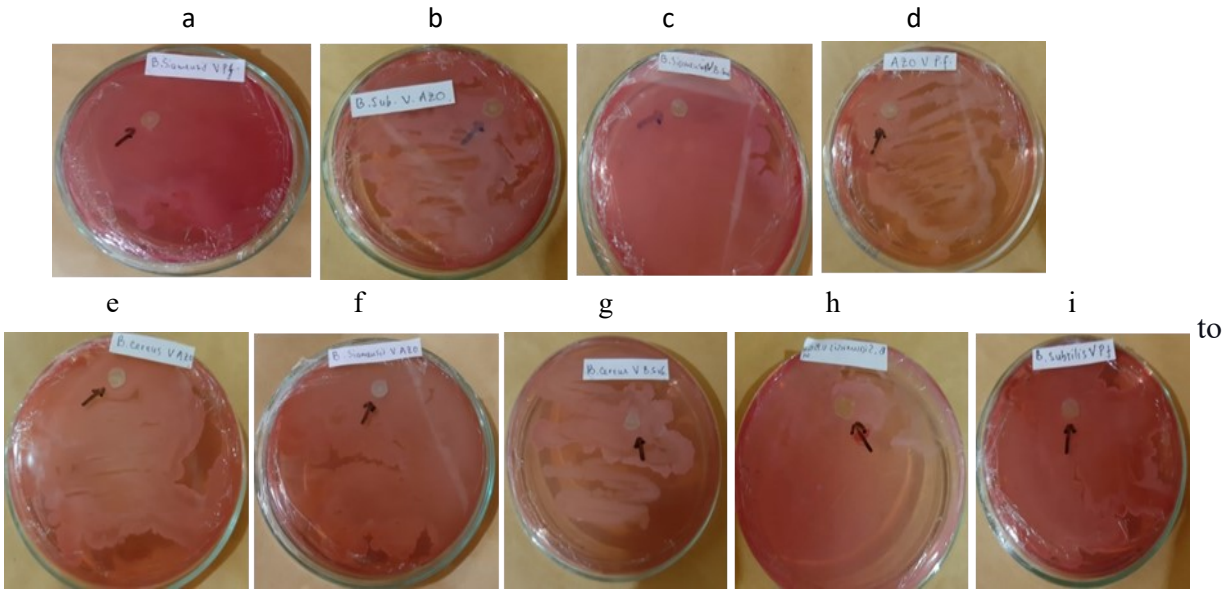

Figure 1. Compatibility test of *Bacillus cereus* strain ATCC 14579, *Bacillus Subtillis* subsp. Subtilis strain 168 and *Bacillus siamensis* strain KCTC13613, *Azotobacter sp*. and *Pseudomonas fluorescens*
a. *Bacillus siamensis* strain KCTC13613 compatibility with *Pseudomonas fluorescens*
b. *Bacillus substilis* subsp. Subtilis strain 168 compatibility with *Azotobacter sp*
c. *Bacillus siamensis* strain KCTC13613 compatibility with *Bacillus substilis* subsp. Subtilis strain 168.
d. *Azotobacter sp* compatibility with *Pseudomonas fluorescens.*
e. *Bacillus cereuscereus* strain ATCC 14579 compatibility with *Azotobacter sp.*
f. *Bacillus siamensis* strain KCTC13613compatibility with *Azotobacter sp.*
g. *Bacillus cereus* strain ATCC 14579compatibility with *Bacillus substilis*subsp. Subtilis strain168.
h. *Bacillus siamensis* strain KCTC13613 compatibility with *Bacillus cereus cereus* strain ATCC14579.
i. *Bacillus substilis* subsp. Subtilis strain 168compatibility with *Azotobacter sp.*

against phytopathogenic fungi and bacteria. *Bacillus cereus* can reduce the growth of the fungus mycelium *Sclerotium rolfsii, Fusarium oxysporuium, Phytium apanidermatum, Helminthosprium maydis* (Muhammad and Amusa, 2003). *Bacillus subti*tlis can produce dissolved compounds that do not evaporate and have high antifungal activity. This bacterium can also play a role in suppressing some pathogenic fungi such as Rhizoctonia and Fusarium (Suryadi et al., 2015). *Pseudomonas fluorescence*, five isolates of *Bacillus spp*. Able to inhibit the growth of *Fusarium solani* but has not found any bacteria that can suppress the development of *Meloidogyne incognita* larvae in pepper (Whardika et al. 2014). The antagonistic formula of *Bacillus subtilis* TM4 effectively suppresses leaf blight and leaf blight disease through seed treatment. However, it is not effective in suppressing the development of leaf blight disease by spraying the formula (Djainuddin et al., 2017). Single isolates of *Bacillus cereus, Pseudomonas aeruginosa,* and *Pseudomonas fluorescens* were also reported have suppressive bacterial leaf blight in vivo respectively of 20.57%, 30.67%, and 36.48% (Zuraidah, 2012).

The effectiveness of these bacteria combinations is increased the mortality of *Spodoptera litura* 2nd instar larvae is 70% and significantly different according to the Duncan tests compared to larval mortality caused by a single isolate to these four bacteria (Table 1 and Figure 2).

Table 1. Effectiveness of biocontrol agents on *S. litura* pest mortality

Treatments	Larval mortality *S.litura* (%)
Bacterial Combination	70.0 a
Pseudomonas fluorescens	46.7 b
Bacillus cereus strain ATCC 14579	45.0 b
Bacillus substilis subsp. Subtilis strain 168	36.7 bc
Bacillus siamensis strain KCTC13613	30.0 bc
Azotobacter sp	20.0 b
Control	20.0 b
CV	19,7

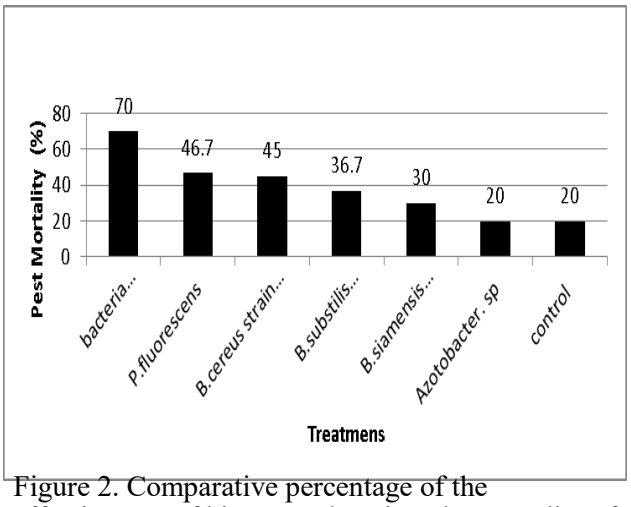

Figure 2. Comparative percentage of the effectiveness of bio-control against the mortality of *Spodoptera litura*

A bacterial combination is a collection of bacteria that work together to form a community, to produce significant products (Asri and Zulaika, 2016). The compatibility or synergism of two or more inoculated bacteria is a significant factor so that the bacteria can work well together (Elfiati, 2015).

They were increasing the effectiveness of the *Bacillus cereus* strain ATCC 14579, *Bacillus subtillis* subsp. subtilis strain 168 and *Bacillus siamensis* strain KCTC13613 and *Pseudomonas fluorescens* against *Spodoptera litura* larvae compared to singularly proving the role of synergy, cooperation, and complementarity between isolates in improving the ability virulence against *Spodoptera litura* larvae. The mechanism of the virulence ability of the bacterial combination is to be further studied; so far, there have been no reports of synergism between isolates in the combination. Allegations of several studies due to several factors, namely: (Rokhzadi et al., 2008) one member of the genus can provide one or more nutritional factors that cannot be synthesized by other members of the genus (Zulaika and Laili, 2015) one member of the genus not being able to degrade certain organic materials will depend on members of the genus that can provide the results of the degradation of organic material, (Okoh, 2006)) one member of the genus protects other genus members who are sensitive to certain organic materials by reducing the concentration of toxic organic material by producing specific and non-specific protective factors (Deng and Wang, 2016). The combination test shows that complementary isolates are working to degrade propoxur as a growth substrate. (Syahlan et al. 2014).

Incorporation of microbes into the formulation of the bacterial combination *Pseudomonas aeruginosa* C32b, *Serratia marcescens* E31, *Bacillus firmus* E65, proves the existence of a synergistic and complementary role among bacterial isolates in enhancing the antagonistic ability of a higher level of protection against leaf blast disease, blight leaf blight caused by *Rhizoctonia solani*, and bacterial leaf blight by *Xanthomonas oryzae* due to the integrated mechanism of each bacterial isolate. In vivo tests on the three pathogens in rice plants showed average suppression effectiveness above 50% for two diseases, namely leaf blast and leaf blight. This bacterial combination formulation has a higher emphasis on controlling blast disease and leaf blight than previous studies reported using a single biocontrol agent (Trianggana, 2013). In vitro, the A6 combination consisting of *Bacillus. cereus, Bacillus firmus*, and *Pseudomonas aeruginosa* had inhibitory activity against *Pseudomonas oryzae*, *Rhizoctonia solani*, and *Xanthomonas oryzae*, respectively by 37.78%, 41.67%, and 21.05%. (Djainuddin et al., 2017). In vitro testing of the combination of *Bacillus firmus*, *Bacillus cereus*, and *Pseudomonas aeruginosa* can suppress the growth of *Xanthomonas oryzae* fungi that cause blast disease 69.92% (Riana, 2011).

CONCLUSION

The results showed that the bacteria *Bacillus cereus* strain ATCC 14579, *Bacillus subtillis* subsp. Subtilis strain 168 *Bacillus siamensis* strain KCTC13613, *Azotobacter* sp. and *Pseudomonas fluorescens* are compatible with each other to be mixed. These five types of bacteria have less potential than *Spodoptera litura* pest bio-control agents because they cause low larval mortality, but a combination of 5 bacteria causes larval mortality by 70%.

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