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Potential Endophytic Indigenous Bacteria as Resistant Inducing Shallot Plant in Controlling Spodoptera Exigua Pests (Lepidoptera: Noctuidae)

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Abstract

Shallots (*Allium ascalonicum* L.) are vegetable commodity that have high economic value and are included in important commodity in Indonesia. The research conducted at Agricultural Biological Control Laboratory and in Solok District. The authors had obtained five endophytic bacterial isolates, which have the ability to impact on systemic shallot plant resistance. One of the mechanisms in plant resistance resilience is the change in salicylic acid content. The purpose of this study were to calculate the salicylic acid content of shallots plants introduced by endophytic bacteria isolate and able to affect its resistance to *S.exigua*. This research was conducted by introducing six isolates of endophytic bacteria on shallots tubers and planted in Lipek Pageh village, Solok district. Then the shallots crop were allowed to infected *Spodoptera exigua* naturally on endemic onion crops *Spodoptera exigua*. The study was arranged in a randomized block design (RAK) with 5 treatments and 4 replications. The treatments were B$10^3$4, B$10^5$1, U$10^4$3, U$10^3$1, A$10^3$4. Analysis of salicylic acid content was done on the roots of shallot plants. Extraction and quantification of salicylic acid was done by Rasmussen and his colleagues. (1991) modified. The results showed that endophytic bacteria was able to stimulate the occurrence of resistance to onion plant cultivation of *S. exigua*. This is indicated by the decrease of *S. exigua* attack rate.

Keywords: bacteria; *Spodoptera;*   *exigua*; endophytic.

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1. Introduction

Shallots (*Allium ascalonicum* L.) and leek (*A. fistulosum*) are vegetable commodities that have high economic value and are included in important commodities in Indonesia [1,2].

The main constraint limiting the production of shallots is the disturbance of plant pest organism (OPT). According to [3], one of the most important pests on shallot plants is *Spodotera exigua* (Lepidotera: Noctuidae).

Some of these pest control techniques have been carrying out such as the use of pesticides, technical culture and the use of resistant varieties, but have not yet achieved effective results.

The use of pesticides is known in addition to providing a positive impact also poses a threat to environmental quality, ecosystem balance and human health.

In addition, the treatment of pesticides can stimulate the emergence of pests, new strains that are more resistant to pesticides and the death of natural enemies, useful microorganisms in the soil and the presence of pesticide residues. In addition, the use of pesticides to control *S. exigua* is not optimal because *S. exigua* damages the shallot plants by eating the leaves from the inside until only the outer epidermis is left. So the pesticide does not contact directly with the pest.

Early inoculation of endophytic bacteria will increase the resistance of plant seed to *S. exigua* pest. In addition, the ability of endophytic bacteria to live and thrive in plant tissues can protect host plants from insect pests that can damage crops. Plant tissue provides relatively safe and uniform environments compared to rhizosphere and filoplan regions.

Endophytic attachment to its host, provides more advantages for endophytes than other biological agents because they do not have to compete in new and complex ecosystems [4]. As well as toxic metabolites produced by endophytes in plants can reduce the population of insects residing in plants.

The use of entomopathogenic bacteria has been widely reported to be able to suppress the population of various insect pests in various host plants. The results of research by [5] showed that screening of 141 isolates against *S. litura* larvae contained 80 isolates that could cause disease and kill test larvae> 50%. Red bacteria isolated from brown planthopper (WBC) proved to be pathogenic to WBC and other insects [6].

Utilization of endophytes as biological agents for insect pests *Spodoptera exigua* has not been widely reported. As a country that has high biodiversity, exploration and it is time to be improved to support integrated pest/disease control program.

2. Material and Method

This research conducted from December 2016 to July 2017 at the Biological Control Laboratory of Faculty of Agriculture Andalas University of Padang and in Lipek Pageh village and Alahan Panjang village, Solok

District.

2.1. Material Plant

Seeds of shallot (Cirebon cultivar) were obtained from farmers breeding seeds in the area of Alahan Panjang Solok regency, West Sumatra. Shallot crops were kept in Lipek Pageh village, Solok District.

2.2. Isolate Bacteria Endofit

Five endophytic bacterial isolates used in this study were the best isolates in the shallot resistance resistance to *S.exigua* pests.The isolates were isolated from roots, stems and tubers healthy shallot plants originating from Regency in West Sumatera that is Solok Regency. Endophytic bacterial isolates used were $B10^34$, $B10^51$, $U10^43$, $U10^31$, $A10^34$

Bacterial isolates were rejuvenated with scratch method on TSA media, then incubated 2x24 hours.Next 1 colony was put into 25 ml of NB medium in a 250 ml erlenmeyer flask and incubated on a rotary shaker (preculture).1 ml culture of preculture was transferred into 200 ml of sterile coconut water and incubated in rotary shaker at 110 rpm (mainculture), then incubated 2x24 hours.The endophytic suspension was compared to its incidence with a Mc Farland solution of scale 8, if the turbidity is the same, the endophytic population density was estimated to be 10^8 cells / ml [7] (Klement and his colleagues 1990).

2.3. Introduction of Endophytic Bacteria and Shallot Cultivation

Selected seeds that have almost the same size, clean, no defect, and bright red color. Before planting the seed had been cut 1/3 of the top then soaked in endophytic bacterial suspension for 15 minutes, dried wind.

The seeds were grown on polybag (30x40 cm) with a mixed medium of soil and chicken fertilizer (2: 1 v / v) which had been sterilized. Then the shallot crops were allowed to infected *Spodoptera exigua* naturally on endemic shallot crops *Spodoptera exigua.*

The study was arranged in a randomized block design (RAK) with 5 treatments and 4 replications. The treatments were $B10^34$, $B10^51$, $U10^43$, $U10^31$, $A10^34$. The data obtained from the observations were analyzed by variance, if significantly different, followed by a 5% tukey test.

The observed variables were plant damage caused by S.exigua (Intensity of *Spodoptera exigua* plant pest damage).

The effectiveness of endophytic bacteria isolate in reducing the intensity of *spodoptera exigua* plant pest damage emerged, the first shoot was calculated using the formula..

$$E= \frac{K-P}{K} \text{ x } 100\%$$

2.4. Salicylic acid

Analysis of salicylic acid content was done on the roots of shallot plants. Extraction and quantification of salicylic acid was done by [8] modified. The roots of shallot plant were crushed, then were extracted with methanol. Salicylic acid content was analyzed by High-Performance Liquid Chromatography (HPLC).A total of 5 µl of shallot roots methanol extract was injected in C18 column (4,6 ID x 250 mm; Lichrospher 100 Rp 18, Alltech, Deerfield, IL), was equilibrated with 5% (v / v) acetonitrile buffer (50 mm sodium acetate buffer, pH 4.5).

Salicylic acid were elicited decisively 15 minutes after injection, and were detected with fluorescens (excitation 290 nm, 402 nm emission). Salicylic acid concentrations were measured using linear spacing from calibration standards containing 0-1.3 mg / 50 ml salicylic acid (Sigma-Aldrich, St. Louis). The concentration of salicylic acid was expressed in micrograms per gram of fresh weight.

3. Results

3.1. Intensity of Plant Damage Due to Pest Attack Spodoptera exigua

Introduction of endophytic bacteria on shallot plants shows the intensity of damage due to *S. exigua* is not significantly different between treatments. However, almost all endophytic bacterial isolates on the shallot shows a decrease in the percentage of attacks lower than the control. Shallot plant introduced by isolate B10^{3}4 shows the highest percentage of damage damage that is 21.00% with effectiveness 51,72%.

Table 1: Percentage of shallots leaf damage by *S.exegua* after introduction of some endophytic isolates.

Number	Endophytic bacteria	Percentage of damage (%)		Effectiveness (%)
1	B10^{3}4	21,00 ±	19,77	51,72
2	B10^{5}1	23,25 ±	7,41	46,55
3	U10^{4}3	23,50 ±	11,24	45,98
4	U10^{3}1	25,25 ±	7,80	41,95
5	A10^{3}4	26,75 ±	5,69	38,51
6	Kontrol	43,50 ±	5,32	0,00

Introduction of 5 endophytic isolates obtains higher salicylic acid yields from the control. The highest salicylic acid content is found in Isolate B10^{3}4 (6,799), followed by isolate A10^{3}4 (6.146), then isolates U10^{4}3 (6,273), and B10^{5}1 (6,783), and isolate U10^{3}1 (6,868) while control (3.657).

The results of the introduction of endophytic bacterial isolates on shallot plants increases salicylic acid content in shallot plants (Figure 1).

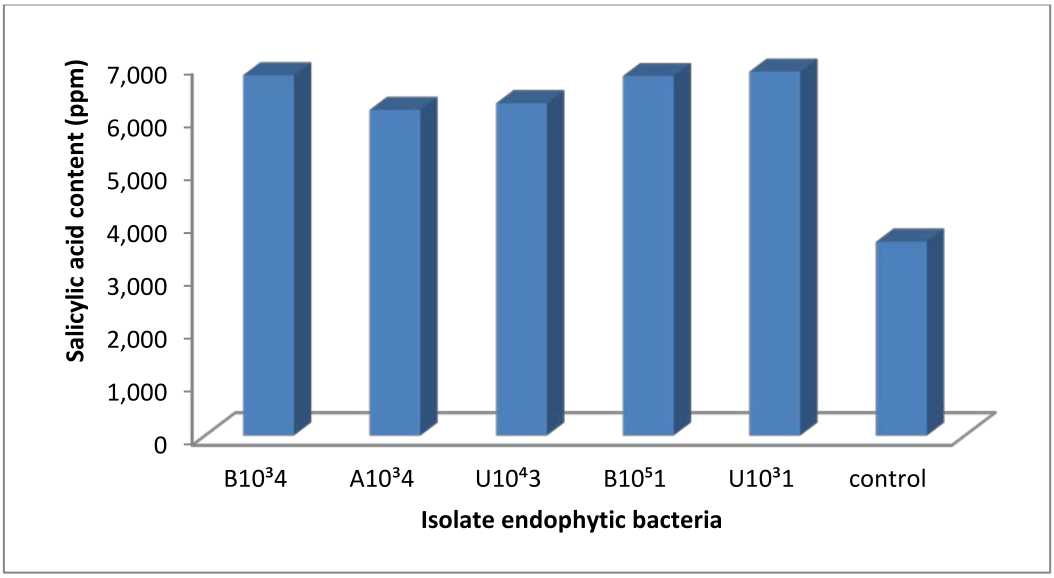

Figure 1: Effect of endophytes on salicylic acid content on shallot plants.

The introduction of endophytes on shallot plants decreases the percentage of shallot plant damage. This is seen from the effectiveness of plant damage percentage ranged from 38.51% to 51.72% compared to control.

The inhibition of *S.exigua* development is probably caused by the induction mechanism of plant endurance stimulated by endophytic bacteria with increased salicylic acid in plant tissues.

This is seen from 5 isolates of endophytic bacteria originating from stems, tubers, and roots ie $B10^34$, $A10^34$, $U10^43$, $B10^51$, and $U10^51$ isolates showed higher salicylic acid content than control (Figure 1).

The ability to induce plant endurance was also one of the advantaged of endophytic bacteria present that can fight the growth of other organisms.

This research is in line with the results of research [9,10], which states that the mechanism of suppression of plant damage by endophytes is thought to occur through the mechanism of inducing plant resistance. [11] also reported that the pepper resistance inducer by endophytic bacteria is characterized by an increase in salicylic acid content in the roots.

The increase in the peroxidase and salicylic acid content occurs as a response to the elicitor produced by endophytic bacteria, allowing plants to activate resilience genes or to react hypersensitivity to pest attacks [12]. Endophytic bacteria that play a role in inducing plant resilience systems, also have special characteristics such as, producing siderophores and producing lipopolysaccharides enzymes. Induction of resilience systems is associated with increased sensitivity resulting in growth hormone to promote plant growth by encouraging partial activation of parental gene [13].

Endophytic bacteria such as *Pseudomonas fluorescens* that produce secondary metabolites 2,4-diacetylpholoroglucinol, are able to reduce egg hatching and cause mortality for juvenile *M. Javanica* (Treub)

Chitwoodi. In greenhouse, the application of this isolate can reduce root-knot formation in tomato plants.

Several other studies shown that the use of endophytic bacteria are isolated from cucumbers and cotton can reduce the population of *Meloidogyne incognita* in cucumbers by 50% [14].

In addition, the ability of endophytic bacterial isolates to inhibit *S. exigua* growth is thought to be caused by certain compounds such as fitoalection produced by endophytic bacterial isolates associated with shallots [15], states that in potato plants found phytoalexin norsesquiterpenoid and risitin. Reference [15] state that large-scale phytoalexin production is only found in a combination of bacterial races.

5. Conclusion

Endophytic bacteria tested was able to stimulate the occurrence of resistance to shallot plant cultivation of *S. exigua*. This was indicated by the decrease in the incidence of *S. exigua* and followed by the high concentration of salicylic acid in onion plants compared to the control.

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