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Induction of Resistance to Larvae *Crocidolomia pavonana* F. (Lepidoptera: Crambidae) using Rhizobacteria to the Cabbage

Indri Yanil Vajri[1,2)], Trizelia[2], Haliatur Rahma[2]*

[1] Faculty of Agriculture, Medan Area University, Medan, North Sumatera, Indonesia
[2] Faculty of Agriculture, Unand University, Padang, West Sumatera, Indonesia

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Correspondence

Indri Yanil Vajri
E-mail: indriyanilvajri@staff.uma.ac.id

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Abstract

Crocidolomia pavonana is a significant pest on cabbage that reduces the quality and quantity of cabbage. Utilizing microorganisms such as rhizobacteria is an alternative environmentally friendly control that can potentially suppress the development of this pest. The study aimed to obtain rhizobacteria isolates capable of colonizing cabbage tissue and inducing plant resistance to *C. pavonana* larvae. The research was conducted at the Biological Control Laboratory and Greenhouse, Faculty of Agriculture, Universitas Andalas, Padang. The study used a Completely Randomized Design (CRD) with ten treatments and five replications. The treatment consisted of rhizobacteria isolates, including *Bacillus thuringiensis*, *Bacillus subtilis*, *Serratia marcescens*, *Stenotrophomonas maltophilia*, as well as a negative control (aquadest sterile) and a positive control (Cypermethrin insecticide). The test was carried out by soaking the seeds in a suspension containing rhizobacteria with a population density of 10^8 cells/ml. The variables observed were larval mortality, pupa and imago formation percentage, and increased salicylic acid production. The data were analyzed using variance and continued with the LSD further test at the 5% level. The results showed that all rhizobacteria isolates colonized into cabbage plant tissue could kill *C. pavonana* larvae and inhibit these insects' biological development. *B. thuringiensis* KJKB7.3 showed better results with the highest mortality value (62.67%). Soaking cabbage seeds with rhizobacteria can increase the content of salicylic acid. Based on this research, the rhizobacteria used in the research have the potential to be developed as biological agents to control *C. pavonana*.

Keywords

Biological control, *crocidolomia pavonana*, induction of resistance, patogenecity, rhizobacteria

INTRODUCTION

Crocidolomia pavonana (F) (Lepidoptera: Crambidae) is a major pest in cabbage cultivation. This pest can attack other plants from the Cruciferae family, such as Chinese cabbage, mustard greens, broccoli, radishes, mustard greens, and watercress. The spread of this pest includes South Africa, Southeast Asia, Australia, and the Pacific Islands. The damage is caused by eating the leaves, especially the young leaves, and heading to the growing point so that the growing point runs out and the plant can die. If the population is abundant and the environment is supportive, the attack rate can cause farmers to fail to harvest large areas quickly because the damage can reach 100% (Kalshoven, 1981; Paat et al., 2012). Various control techniques for *C. pavonana* have been carried out, but farmers are more likely to spray with synthetic pesticides due to the high population and rapid life growth. This condition triggers new problems such as environmental pollution, resistant pest species, secondary pest explosions, and resurgence, destroying the balance of the ecosystem and the impact on society (Adriyani, 2006).

Therefore, an environmentally friendly control technique is necessary, namely by using biological agents. Biological agents against insect pests are frequently used as biopesticides to control insect pests in various cropping systems (Lacey et al., 2015; Azizoglu et al., 2020). Recently, it has been discovered that biological agents can not only directly infect insects but also exist as rhizosphere colonizers and endophytes, providing multiple benefits to plants (Regaiolo et al., 2020; Azizoglu et al., 2020).

Rhizobacteria are bacteria that live around plant roots, and their activities are influenced by root exudates (Gnanamanickam, 2006). Rhizobacteria have been widely used and developed as biological agents. The use of rhizobacteria as biological agents includes the Genus Agrobacterium, Alkaligenes, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Beijerinkia, Burkholderia, Caulobacter, Chromobacterium, Clostridium, Enterobacter, Erwinia, Flavobacterium, Klebsiella, Micrococcus, Phyllobacterium, Pseudomonas, Serratia, Pseudomonas, Serratia, and Xanthomonas (Grupta *et al.,* 2015).

Besides being able to kill insect pests directly, rhizobacteria are known to induce plant resistance. Praca *et al*. (2012) showed that the application of *B. thuringiensis* Brasil strain S1450, which was applied through seeds, could colonize cabbage plants and potentially control *Plutella xylostella* pests. Li *et al.* (2015) also reported that the introduction of *Bacillus amyloliquefaciens* to the leaves of the hosta plant (*Hosta* spp.; Asparagaceae) was able to influence the feeding preferences of *Spodoptera frugiperda* larvae, where the larvae were more likely to eat the leaves of plants that were not applied to bacteria in the choice test. Induction of resistance was also reported through a study of *Serratia marcescens* strain 90-166, which effectively reduced the cucumber beetle *Acalyma vittatum* population compared to other strains (Zehnder *et al.,* 1997). Previous studies showed that rhizobacterium *S. marcescens* increased plant growth and induced systemic defense responses in the host plant (Wei *et al.,* 1996). *S. marcescens* is an ecologically and genetically diverse species that includes rhizobacteria, plant endophytes, and insect pathogens with a high potential for biological control (Gyaneshwar *et al.,* 2001; Grimont & Grimont, 2006).

Plants' ability to inhibit pests' development is a complex process regulated by various compounds, such as salicylic acid, jasmonic acid, and ethylene. Salicylic acid is a secondary signal mainly induced by the translocation signal generated at the site of damage (Vallad & Goodman, 2004). Bacteria produce volatile compounds including pheromones, allomon, kairomone, attractants and repellents. Volatile compounds produced by bacteria will stimulate insect behavior in choosing food sources, oviposition preferences, oviposition regulation, male orientation to find female suitable host locations, and the presence of predators (Leroy *et al.,* 2011).

The objectives of the study were to clarify whether cabbage plants that have been inoculated with rhizobacterial isolates through seed soaking applications as insect larvae feed can affect the biology of *S. frugiperda* insects and can affect the increase in plant salicylic acid production related to its role in inducing plant resistance to herbivorous insects.

METHODS

Preparation and rejuvenation of bacterial isolates

The rhizobacteria isolates were from the Haliatur Rahma collection (Table 1). Bacterial isolates were prepared by scratching them on Lactose Broth (LB) media and incubating them for 2 x 24 hours at room temperature. A single-grown colony of bacteria is transferred back to the new LB media with the same method to use as a collection in subsequent tests.

Table 1. Code and Origin of Rhizobacteria used in the Study

Bacterial isolate	Isolate code	Isolate source
B. thuringiensis	KJKB7.2	Corn Plant Rhizosphere
	KJKB7.3	Corn Plant Rhizosphere
	BAKB7.1	Corn Plant Rhizosphere
B. subtilis	KJTSB7.2	Corn Plant Rhizosphere
S. marcescens	AR1	Grassroots Endophytes
	AR2	Grassroots Endophytes
	RK10	Corn Plant Rhizosphere
S. maltophilia	LMTSA5.4	Corn Plant Rhizosphere

Provision of host plants and larvae of C. pavonana

Cabbage seeds of the Grand II variety were sown in seed trays filled with soil and sterilized manure in a ratio of 2:1 (v/v). Cabbage plant seeds that have been 14 days old are transferred into polybags measuring 35 cm x 35 cm. Plants are maintained by watering and weeding. *C. pavonana* larvae were provided from

the center of cabbage cultivation in the Batu Palano Village, Sungai Pua Subdistrict, Agam District. Instar 3 and 4 larvae were collected in plastic boxes measuring 30 cm x 20 cm x 7 cm and fed fresh cabbage. The top box was covered with gauze, and the larvae were reared until the second generation and ready to be used as test insects.

Application of bacterial isolation on cabbage seed

Bacterial rejuvenation was done by culturing bacterial isolates using the scratch method on LB media and then incubating at room temperature for 2 x 24 hours. A single colony of grown bacteria was transferred back to the new LB medium by scratch and incubated for 2 x 24 hours. The pure culture of bacteria in the petri dish was added with 9 ml of distilled water and scraped using a sterile loop. The rhizobacteria suspension was transferred into a test tube using a micropipette, homogenized with a vortex, and compared for turbidity with Mc. Farland. If the turbidity is the same in Mc. Farland's Solution scale of 8, then the population density is estimated at 10^8cells/ml (Klement *et al.,* 1990). The application method by Praca *et al*. (2012) was used in this study; the surface of cabbage seeds was sterilized by seed treatment in 70% ethanol solution for 5 minutes, then in 2% NaOCL solution to which Triton X100 0.01% was added for 30 minutes. The seeds were washed with sterile distilled water thrice and dried on filter paper for 15 minutes to remove the remaining water. Bacterial isolates were introduced to the seeds by soaking 50 cabbage seeds in each bacterial suspension for 5 minutes (population 10^8 cells/ml); as a control, the seeds were soaked in sterile distilled water and positive control in the Insecticide Cypermethrin. Each was then dried for 15 minutes on filter paper, and the work was carried out under sterile conditions in Laminar Air Flow. Afterward, the seeds are sown in a seed tray at a depth of 5 cm.

Mortality and development of C. pavonana

The no-choice method was used in this experiment. One more old cabbage plant that were introduced as a treatment for *C. pavonana*. Two pieces of 4 cm x 4 cm leaves were put in a box containing one group of eggs and then left to hatch. The eggs hatched (instar 1) were fed freshly colonized leaves until they became instar 2. Fiveteen of second instar larvae were separated and then starved for an hour. Two freshly colonized leaf pieces of the same size were fed to the larvae, and the feed was changed daily until the larvae pupae. The larvae were given cabbage feed that did not contain bacteria as a control. The positive control larvae were fed with cabbage, the seeds soaked with the insecticide Cypermethrin. The test was repeated five times by observing anti-feeding Activity, larval mortality, larval lifetime, pupa and imago weight, and the percentage of pupa and imago formation.

Analysis of salicylic acid content

Salicylic acid content was analyzed on 56 days-old cabbage leaves after being inoculated with bacteria with a combination of 5 plants for each treatment. Salicylic acid Extraction and quantification were carried out using the modified method of Rasmussen *et al*. (1991). A total of 5 cabbage leaves were crushed and then extracted with methanol. Salicylic acid content was analyzed by High-Performance Liquid Chromatography (HPLC). Five liters of methanol-extract cabbage leaf was injected into a C18 column (4.6 ID x 250 mm; Lichrospher 100 Rp 18, Altech, Deerfield, IL), equilibrated with 5% (v/v) acetonitrile buffer (50 mm sodium acetate buffer, pH 4.5). Salicylic acid was eluted isocratically 15 min after injection and detected by fluorescence (245 nm excitation; 4 nm emission).

RESULTS AND DISCUSSION

Result Antifeedant Activity

Table 2. Effect of Treatment on Antifeedant Activity of *C.pavonana* Larvae through Seed Immersion

Treatment	Leaf Area Eaten (mm^2)	Antifeedant Activity (%)
AR2	1138.40 a	-5.21
Control	1082.00 a	-
LMTSA5.4	1031.80 a	4.64
RK10	893.90 a	17.38
BAKB7.1	827.00 a	23.57
KJTSB7.2	280.20 b	74.10
KJKB7.3	223.40 b	79.35
AR1	213.50 b	80.27
KJKB7.2	160.30 b	85.19
Sipermetrin	101.30 b	90.64

Figures in the same column followed by the same lowercase letters were not significantly different according to the 5% level LSD follow-up test.

Analysis of variance showed that all treatments had significantly different effects on the antifeedant Activity of *C. pavonana* larvae. The rhizobacteria *B. thuringiensis* KJKB7.2 treatment showed the highest feeding inhibition index among other rhizobacteria treatments, namely 85.19%, with the lowest edible

leaf area of 160.30 mm². This result significantly differed from the control with the edible leaf area value of 1082 mm² (Table 2).

Larval mortality

The analysis of variance showed that all treatments had a significantly different effect on the mortality of *C. pavonana* larvae. The application of *B. thuringiensis* KJKB7.3 bacteria showed better results because it could cause mortality up to 62.67%. At the same time, the application of *S. maltophilia* LMTSA5.4 isolates caused the lowest mortality, namely 30.67%. This result differed significantly from the control with a mortality value of 5.30%. Cypermethrin treatment caused a mortality of 28%. Testing of rhizobacteria isolates on the length of stadia and mortality of larvae can be seen in Table 3.

Length of larval stage

The analysis of variance showed that all treatments had a significantly different effect on the length of the larval stadia of *C. pavonana*. Generally, rhizobacteria isolates were able to prolong the length of the larval stadia of *C. pavonana* from instars 2-3 and 2-4 and gave a significantly different effect from the control, except for the treatment of *S. maltophili*a LMTSA5.4 isolates only able to affect the length of the larval stages from instars 2-4 (5.88 days). The KJKB7.2 isolate showed better results than other isolate treatments because it prolonged the 2-3 instar and 2-4 instar larval stages, which were 4.97 and 7.94 days, respectively, compared to the control, which was 2.47 and 4.90 days (Table 3).

Table 3. Effect of Treatment on *C. pavonana* Larvae through Seed Immersion

Treatment	Length of Larval Stage (Day)		Mortality (% ± SD)
	Instar II-III	Instar II-IV	
KJKB7.3	4.91 a	7.73 a	62.67 ± 9.40 a
AR1	4.13 bc	6.61 bc	54.67 ± 8.20 ab
KJTSB7.2	4.02 bc	6.78 b	40.00 ± 6.00 bc
KJKB7.2	4.97 a	7.94 a	38.67 ± 5.80 bc
BAKB7.1	3.88 cd	6.41 bcd	32.00 ± 4.80 c
AR2	4.10 bc	6.71 bc	30.67 ± 4.60 c
RK10	4.34 b	6.70 bc	30.67 ± 4.60 c
LMTSA5.4	3.33 e	5.88 d	30.67 ± 4.60 c
Sipermetrin	3.50 de	6.18 cd	28.00 ± 4.20 c
Control	2.47 e	4.90 e	5.30 ± 0.80 d

Figures in the same column followed by the same lowercase letters were not significantly different according to the 5% level LSD follow-up test.

Percentage of pupae and imago formed

Tests of rhizobacteria isolates and control of *C. pavonana* pupae formed after being analyzed by variance gave significantly different results. The treatment of *B. thuringiensis* KJKB7.3 isolate showed the best results because it inhibited pupae formation by up to 70%, with the percentage of pupae formed by 29.33%. Isolate treatment of *B. thuringiensis* KJKB7.2 and *S. marcescens* AR1 was able to suppress pupa formation below 50%, and this result was significantly different from the control (89.33%).

The results of normal pupae formed after being analyzed by variance showed that all treatments showed significantly different results. Of the eight treated rhizobacteria isolates, *B. thuringiensis* KJKB7.3 isolate showed better results with the lowest percentage of normal pupae formation (26.67%). Isolates of *S. marcescens* RK10 and *B. thuringiensis* BAKB7.1, the highest percentage of normal pupae formation was 42.67%. This result is different from the control, which is 84%. Abnormal pupa formation, from the analysis of variance, showed that the treatment of rhizobacteria isolates showed significantly different effects. The application of *S. marcescens* RK10 isolate was the better isolate with the highest percentage of abnormal pupae formed, which was 26.67%. This result differed from the control, namely 5.33% and isolate *B. thuringiensis* KJKB7.3; the lowest percentage of abnormal pupa formation was 2.67%.

After being analyzed by variance, the pupa weight of *C. pavonana* treated with several rhizobacteria isolates and controls gave significantly different effects. All treatments were able to increase pupal weight compared to controls. The application of *S. marcescens* AR1 isolates had the highest pupal weight value of 0.033 g, and the results were significantly different from the control, which was 0.023 g.

The effect of applying several rhizobacteria isolates on the length of the pupa stage showed that some treatments could prolong the pupa stage. The longest pupal stage was the application of *B. thuringiensis* KJKB7.3 isolate with a pupal stage of 9.20 days compared to 7.85 days for control. The effect of treating rhizobacteria isolates on the formed *C. pavonana* pupae can be seen in Table 4. Some larvae that succeeded in becoming pupae were usually formed, and some were formed abnormally. The typically formed pupa is reddish-brown, and the tail moves when touched. While the abnormal pupa

body is formed imperfectly, the surface is wrinkled, half of the body is flat, does not move when touched, blackens, and over time, the body becomes soft, oozes fluid when pressed, and smells terrible.

Table 4. Percentage of *C. pavonana* Pupae Formed after Treatment by Seed Soaking

Treatment	Pupae formed (%) ± SD			Weight (g)	Pupa stage duration (days)
	Normal	Abnormal	Total		
Control	84.00 ± 12.6 a	5.33 ± 0.80 e	89.33 ± 13.40 a	0.023 e	7.85 bc
RK10	42.67 ± 6.40 c	26.67 ± 4.00 a	69.33 ± 10.40 b	0.028 cd	8.66 ab
Sipermetrin	60.00 ± 9.00 b	6.67 ± 1.00 de	66.67 ± 10.00 b	0.039 a	8.00 bc
AR2	44.00 ± 6.60 c	14.67 ± 2.80 abcd	62.67 ± 9.40 bc	0.028 cd	8.75 ab
BAKB 7.1	42.67 ± 6.40 c	20.00 ± 3.00 abc	62.67 ± 9.40 bc	0.028 cde	7.81 bc
LMTSA5.4	37.33 ± 5.00 cd	24.00 ± 3.60 ab	58.67 ± 8.80 bcd	0.027 de	7.99 bc
KJTSB 7.2	37.33 ± 5.60 cd	13.33 ± 2.00 bcde	50.67 ± 7.60 bcd	0.038 ab	7.79 bc
KJKB 7.2	37.33 ± 5.60 cd	9.33 ± 1.40 cde	46.67 ± 7.00 cde	0.028 cde	7.61 c
AR1	30.67 ± 4.60 cd	10.67 ± 1.60 cde	41.33 ± 6.20 de	0.033 bc	7.78 bc
KJKB 7.3	26.67 ± 4.00 d	2.67 ± 0.40 e	29.33 ± 4.40 e	0.030 cd	9.20 a

Figures in the same column followed by the same lowercase letters were not significantly different according to the 5% level LSD follow-up test.

The application of several rhizobacteria isolates to the percentage of *C. pavonana* imago formed after being analyzed by variance showed significantly different results. *B. thuringiensis* isolates KJKB7.3 showed better results with the lowest percentage of imago (26.67%). This result was significantly different from the control, with the percentage of imago appearing at 54.64%.

After variance analysis, tests of several rhizobacteria isolates and controls on the percentage of normal imago formation showed significantly different results. *B. thuringiensis* KJKB7.3 isolate was the better isolate with the lowest percentage of normal imago formation, 17.33%. This result differed from the control, with the percentage of normal imago formed at 52%.

The percentage of abnormal imago that appeared after being analyzed by variance showed significantly different results. The application of *S. marcescens* RK10 isolate was the better isolate with the highest percentage formation, which was 21.33%, significantly different from the control, which was 2.67%.

Table 5. Percentage of *C. pavonana* Imago Formed after Treatment by Seeds Soaking

Treatment	Imago formed (%) ± SD			Long stadia (days)
	Normal	Abnormal	Total	
Sipermetrin	50.67 ± 7.60 a	12.00 ± 1.80 bc	62.67 ± 9.40 a	5.130 a
Control	52.00 ± 7.80 a	2.67 ± 0.40 d	54.67 ± 8.20 ab	2.036 e
AR2	30.67 ± 4.60 bc	20.00 ± 3.00 ab	50.67 ± 7.60 abc	2.327 de
RK10	28.00 ± 4.20 bcd	21.33 ± 3.20 a	49.33 ± 7.40 abc	2.744 de
BAKB 7.1	37.33 ± 5.60 b	9.33 ± 1.40 cd	46.67 ± 7.00 abcd	2.188 e
KJTSB7.2	25.33 ± 6.00 cd	14.67 ± 2.20 abc	40.00 ± 6.00 bcde	5.400 a
LMTSA5.4	24.00 ± 3.60 cd	14.67 ± 2.20 abc	38.67 ± 5.80 bcde	2.674 de
KJKB 7.2	21.33 ± 3.20 cd	16.00 ± 2.40 abc	37.33 ± 5.60 cde	3.166 cd
AR1	17.33 ± 2.60 d	13.33 ± 2.00 abc	30.67 ± 4.60 de	3.846 bc
KJKB 7.3	17.33 ± 2.60 d	9.33 ± 1.40 cd	26.67 ± 4.00 e	4.124 b

Figures in the same column followed by the same lowercase letters were not significantly different according to the 5% level LSD follow-up test.

The results of the analysis of the length of the imago stage of C. pavonana showed significantly different results. All treatments were able to prolong the imago stage compared to the control. The isolate of *B.*

subtilis KJTSB7.2 showed better results because it could prolong the imago period by 5.4 days, compared to the control, which was 2,036 days. The effect of isolates on the imago formed can be seen in Table 5.

For pupae that successfully become adults, some are formed typically, and some are formed abnormally. Adults that are formed normally have a perfect body shape, and there are no damaged or deformed body parts. In contrast to the abnormal adults, body parts such as wings are damaged, or death can occur when the adults are almost out of the pupa.

Salicylic acid content in cabbage plant leaves

The results of the analysis of the content of salicylic acid in cabbage leaves can be seen in Table 6. The analysis results on the salicylic acid production in cabbage leaves showed that all cabbage leaves inoculated with rhizobacteria isolates produced higher salicylic acid than controls. The highest salicylic acid content was isolated *B. subtilis* KJTSB7.2, with a salicylic acid content of 185.073 ppm compared to the control of 94,581 ppm. The application of several rhizobacteria isolates to cabbage seeds induced plants to produce salicylic acid; it could be seen from the increase in salicylic acid production after introducing rhizobacteria through seed soaking. The analysis of the salicylic acid content showed that the cabbage plant was able to produce its salicylic acid. However, the addition of rhizobacteria caused the salicylic acid content to increase.

Table 6. Effect of treatment on salicylic acid content in cabbage plants aged 56daa

Treatment	Salicylic acid content (ppm)
KJTSB7.2	185.073
KJKB7.2	157.478
AR2	144.702
AR1	132.594
Sipermetrin	132.575
KJKB7.3	121.527
BAKB7.1	118.352
RK10	114.594
LMTSA5.4	111.450
Control	94.581

Discussion

The application of rhizobacterial isolates through soaking the seeds in this study generally affected the antifeedant Activity of C. pavonana larvae. The application *of B. thuringiensis* KJKB7.2 isolate showed the lowest consumption rate with the most extensive feeding inhibition index. This is in line with the research by Li *et al*. (2015) that the application of *Bacillus* sp. on the leaves of the hosta plant reduced the consumption rate of *S. frugiperda* larvae by 228 ± 38.4 cm^2 compared to the control 309 ± 57.0 cm^2. This is presumably because the introduction of rhizobacteria affects plants to produce secondary metabolites, namely sesquiterpenoid compounds. Scoonhoven *et al*. (2005) stated that plants produce sesquiterpenoid compounds, which are deterrents in nature that inhibit the feeding activity of herbivorous insects.

The application of rhizobacteria through seed soaking prolonged the larval stage in instars 2-3 and 2-4. Application of isolate *B. thuringiensis* KJKB7.2 showed the longest stadia. These results are in line with research by ElSayed and Edrees (2016) that the application of *B. thuringiensis* isolates at 75 ppm was able to extend the duration of development of *Spodoptera littoralis* larvae. Trizelia (1994) stated that the longer larval stage that received *B. thuringiensis* treatment was thought to be due to the inhibition of larval feeding activity so that larval growth became slower and the availability of juvenile hormone lasted longer so that the larval characteristics lasted longer. Abdel-Aal *et al.* (2009) stated that B. thuringiensis is a chitinase enzyme-producing bacterium, and some chitin inhibitors of synthesis will increase the chitinase activity of the larvae. Chitinase is very important for the endocuticle, where changes in this enzyme will interfere with insect molting (Azizoglu *et al.,* 2020).

Application of rhizobacteria isolates to *C. pavonana* larvae showed that rhizobacteria could kill *C. pavonana* larvae. The mortality of *C. pavonana* larvae was affected by rhizobacteria isolates. The occurrence of death in larvae is thought to be due to the presence of toxins produced by bacteria that can damage the digestive tract of insects and cause death of insects. The application of rhizobacteria isolates *B. thuringiensis* KJKB7.3 caused the highest mortality through soaking seeds, which was 62.67%. This indicates that the bacteria can live in plant tissues and cause mortality in *C. pavonana* larvae that eat leaves on food that has been applied to rhizobacteria. Praca *et al.* (2012) reported that Brazilian *B. thuringiensis* strain S1450 was able to colonize cabbage plants

through seed application and potentially control *P. xylostella* pests. This is indicated by bacteria that colonize parts of the cabbage seedling tissue through scanning electronic micrographs. Others have demonstrated that the treatment of potato plants with endophytic bacterial strains *Bacillus* spp. Decreased the survival rate of the plant feeder Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Sorokan *et al.,* 2018).

Applying *S. marcescens* AR1 isolate through seed soaking kills up to 55% of larvae. Niu *et al.* (2020) reported the effectiveness of S. marcescens (S-JS1) in reducing the resistance of Nilaparvata lugens nymphs after being fed rice plants that had been introduced to S. marcescens through soaking seeds at a density of 109 cfu/ml compared to that of uninoculated plants. Wari *et al.* (2019) found that *S. marcescens* subsp. *marcescens* from the oral secretions of *N. lugens* can effectively elicit rice defenses. Asano *et al*. (1999) in Zulfiana *et al. (*2017*)* stated that the ability of *S. marcescens* as a larvacide was better than that of *B. thuringiensis*. It is suspected that these bacteria need time to reproduce (septicemia) before causing death in insects. Tanada and Kaya (1993) stated that when *S. marcescens* enters the body tissues of insects, it will multiply and cause death in one to three days.

The high percentage of larval mortality will affect the rate of pupae formed and the imago that appear. The application of *B. thuringiensis* KJKB7.3 isolate, which had the most increased mortality percentage, also reduced the rate of pupa and imago formation and the portion of typical pupa and imago formation. This is thought to be due to the Cry toxin's secondary effect, which disrupts larvae's metabolism to metamorphose into pupae. Tanada and Kaya (1993) stated that the impact of the toxin produced by *B. thuringiensis* would interfere with the RNA transcription process so that it interferes with cell division, especially during skin turnover and metamorphosis, if the larvae do not die, the pupa or imago will be formed abnormally.

The highest salicylic acid content was found in isolates of *B. subtilis* KJTSB7.2, which was 185,073 ppm. The high salicylic acid content introduced by rhizobacteria is thought to be related to the level of plant resistance to pest attacks. These results are in line with the research of Rashid and Chung (2017), who reported that *B. subtilis* BEB-DN can induce resistance of tomato plants (*Solanum lycopersicum*) to *Bemisia tabaci* and increase the production of jasmonic acid and salicylic acid. In rice, the endophytic strain *Bacillus velezensis* YC7010 can enhance defenses against *N. lugens* by inducing changes in secondary metabolites of rice plants (Harun-Or-Rashid *et al.,* 2018). Treating plants with bacterial endophytes may render the seedlings unsuitable for insect feeding and development (Niu *et al.,* 2020). Colonization of plants by endophytic entomopathogens alters the concentrations of secondary metabolites involved in priming plant defense responses that modulate plant-insect interactions (Disi *et al.,* 2019; Qin *et al.,* 2021). Several secondary metabolites protect the rice plants from insect herbivores (Qi *et al.,* 2013; Harun-Or-Rashid *et al.,* 2018; Wari *et al.,* 2019; Wu *et al.,* 2021). Induced resistance can go through the SAR (Systemic Acquired Resistance) and ISR (Induced Systemic Resistance) processes. In ISR-induced resistance, resistance to non-pathogenic microbial infections is induced. In the ISR process, the activation of defense compounds is not connected with defense genes' role; the defense compounds formed include jasmonic acid and ethylene compounds (Pieters *el al.* 2009).

Heil (2014) stated that plants produce volatile compounds in salicylic acid to defend against herbivorous insects. Lepidoptera insects biting and chewing mouthparts such as *C. pavonana* will stimulate the activation of the salicylic acid pathway after the damage caused to plants (Rodriguez-Saona *et al.,* 2010). Induction of plant resistance does not kill pests but instead increases phytohormones in plants that will increase signals for the defense, such as changes in morphology or physiology of the host plant. The level of resistance is determined by the expression of salicylic acid, which is positively correlated with salicylic acid content (Silverman *et al.,* 1995). The application of endophytic microbes is a promising strategy for controlling pest insects and has driven studies on their ecology in crop ecosystems (Harun-Or-Rashid *et al.,* 2018; Jaber & Ownley, 2018; White *et al.,* 2019).

This study proved that the rhizobacteria derived from the corn plant rhizosphere were *B. thuringiensis* (KJKB7.2, KJKB7.3 and BAKB7.1), *B. subtilis* KJTSB7.2, *S. marcescens* RK10 and *S. maltophilia* LMTSA5.4 and grassroots endophytes (*S. marcescens* AR1 and AR2) have the ability as entomopathogens against *C. pavonana* and can affect the production of salicylic acid in cabbage leaves.

CONCLUSIONS

The results showed that the application of rhizobacteria can kill the larvae of *C. pavonana*. The mortality of *C. pavonana* larvae was strongly influenced by the type of rhizobacteria. *B. thuringiensis* KJKB7.3 is a more virulent isolate, which can cause the highest mortality. Rhizobacteria also inhibited the formation of the pupa and imago of *C. pavonana*. Applying rhizobacteria on cabbage seeds can increase the salicylic acid content of cabbage leaves. Besides being pathogenic to insects, some rhizobacteria isolates also increased the growth of cabbage seedlings.

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