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TOMATO (*LYCOPERSICUM ESCULENTUM* MILL.) RESILIENCE ENHANCEMENT WITH INDIGENOUS ENDOPHYTIC BACTERIA AGAINST *BEMISIA TABACI* (HEMIPTERA: ALEYRODIDAE)

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ABSTRACT

The use of indigenous endophytic bacteria (IEB) isolates as PGPR and resistance induction has been conducted to wilt in tomatoes (*Lycopersicum esculentum* Mill). This study aimed to obtain the best endophytic bacteria isolates in increasing the resistance of tomato plants to *B. tabaci*. The research used a completely randomized design (CRD) with ten treatments and three replications. The treatment consisted of eight isolates of IEB, positive controls (without using insecticides), and negative controls (using insecticides). The study was conducted at the Microbiology and Greenhouse Laboratory, Faculty of Agriculture, Universitas Andalas from May to July 2018. The parameters observed were the number of individuals who survived per day and the length of days needed to develop per stage. The results showed that EPL 1.1.4 and KLE 3.3 isolates inhibited egg laying, whereas EI.AB 2.1, EI.AB 1.2, KLE 3.3, and SNE 2.2 inhibited the development of *B. tabaci*. There was no difference in the length of day between IEB isolates and controls. EI.AB 2.1 showed the most significant adverse effect on the success of *B. tabaci* life with a value of lx = 0.0 achieved on nymph of instar 3. The result indicated that IEB could be developed in increasing the tomato plants resistance to *B. tabaci*.

Keywords: *Bemisia tabaci*, development stage, endophytic bacteria, resistance, tomato.

INTRODUCTION

B. tabaci is an important pest on tomato plants. *B. tabaci*'s attack can cause yield loss to range from 20-100% (Setiawati *et al*. 2007). *B. tabaci* causes damage to plants in two ways. Directly effect as a result of their eating activities, that is the closure of the stomata by the honeydew released by the nymph, formation of chlorotic spots on the leaves as a result of damage to part of the tissue due to stethicle puncture, and falling leaves and can inhibit growth plants (DeBarro 1995). A secondary effect, because they act as essential vectors of virus diseases. There are 100 types of viruses that can be transmitted by *B. tabaci* (Byrne and Bellows 1990).

Control measures taken to overcome *B. tabaci*'s attacks include mechanical control, technical culture, planting resistant varieties, and spraying insecticides. The farmers are more likely to use synthetic pesticides because they are more practical and easy to apply. Improper use of insecticides can have adverse effects on the environment. The negative effects caused by pesticides include water, soil, and air pollution, the emergence of resistant pest species, the emergence of new pest species or the explosion of secondary pests, resurgence, damaging the balance of ecosystems, and the impact on public health (Adriyani, 2006). To avoid the harmful effects of insecticides, environmentally friendly controls can be used, namely by using biocontrol biological agents, one of which is Plant Growth Promoting Rhizobacteria (PGPR).

The presence of bacteria acting as PGPR in plants can be grouped according to their place of colonization, namely rhizosphere, rhizoplane, and endophytes (Soesanto, 2008). Endophytic bacteria are microorganisms that live and colonize host tissues without causing adverse effects. Endophytic bacteria have been used in controlling plant pathogens (Marwan *et al*. 2011; Munif *et al*. 2015). Endophytic bacteria are also able to induce plant resistance to suppress the development and cause the death of plant pests (Rajendran *et al*. 2011; Pineda *et al*. 2012; Praca 2012; Munif *et al*. 2015; Utami 2018). The results of the study by Yanti *et al*. (2017) obtained eight isolates of indigenous endophytic bacteria from tomato plants which were able to suppress the development of *Fusarium oxysforum* and *Ralstonia syzyggii* in planta. These isolates need to be tested in increasing the resistance of tomato plants to *B. tabaci* attacks. The purpose of this study was to obtain the best endophytic bacteria isolates in improving the resilience of tomato plants to *B. tabaci*

MATERIALS AND METHODS

Study area: The study was conducted at the Microbiology and Greenhouse Laboratory, Faculty of Agriculture, Universitas Andalas, West Sumatera from January to July 2019.

Methodology: The research was the experimental method used a completely randomized design (CRD) with10 treatments and three replications. The treatment consisted of 8 isolates of IEB (see Table 1), positive controls (Control) (without using insecticides), and negative controls (Control N) (using insecticides). For negative control, insecticides were applied by hand sprayer from 1 WAP (Week After Planting) with intervals were one week. The parameters observed were the number of individuals who survived per day and the length of days needed to develop per stage.

Table 1. Name and origin of selected indigenous endophytic bacterial (IEB) isolates.

Isolates code	Source of isolate
EPL 1.1.3	Padang Lua, Agam, West Sumatera
EPL 1.1.4	Padang Lua, Agam, West Sumatera
EI.AB 2.1	Aia Batumbuak, Solok, West Sumatera
EI.AB 1.2	Aia Batumbuak, Solok, West Sumatera
SNE 2.2	Sungai Nanam, Solok, West Sumatera
TLE 1.1	Taluak, Agam, West Sumatera
TLE 2.3	Taluak, Agam, West Sumatera
EKL 3.3	Koto Laweh, Solok, West Sumatera

Procedures

Rejuvenation and multiplication of endophytic bacterial isolates: The isolates of indigenous endophytic bacteria were obtained from Yanti's collection (2017). IEB isolates were rejuvenated using one bacterial ose transferred to the NA medium in a Petri dish with a scratch method and incubated at room temperature for 2 x 24 hours. The multiplication of endophytic bacteria was conducted in 2 stages, namely: (1) pre-culture, one indigenous endophytic bacteria colonies from pure culture were transferred into 10 ml NB medium in a culture bottle and incubated on a rotary shaker at 150 rpm for 24 hours at room temperature. (2) Main culture, 1 ml of the suspension from preculture was transferred to 25 ml of sterile coconut water in a bottle of culture and incubated in the same manner for 3 × 24 hours (Habazar *et al*. 2007). The suspension of endophytic bacteria from the main culture was determined by population density based on a comparison with a scale 8 McFarland solution (BaCl 0.8 g + H_2 〖SO 44 1% 9.2 g) (bacterial population density estimated at 108cell /ml) (Klement *et al*. 1990)

Inoculation of Indigenous Endophytic Bacteria (IEB) isolates: The inoculation of endophytic bacteria isolates was conducted for two times, namely in tomato seeds and seedling.

Introduction of *B. tabaci* on tomato plants: *B. tabaci* was founded from tomato and chili plants around the Padang. *B. tabaci* which have been obtained was rearing in a wooden box measuring 60 x 75 x 100 cm^3 and covered with gauze. *B. tabaci* was maintained in tomato plants until imago was formed. Imago formed was then used in endurance testing. Each experimental treatment has inserted a pair of imago *B. tabaci* on plants aged 1 WAP which had been given a plastic-coated cage on each side with the top coated with gauze.

Data analysis: The parameters observed were the number of individuals who survived per day and the length of days needed to develop per stage. Data were analyzed by analysis of variance with 5% level, if there was a difference, it was followed by a Least Significance Different Test (LSD) at the level of 5%.

RESULTS AND DISCUSSION

Result: Based on experiments conducted with a pair of *B.tabaci* placed on tomato plants that have been introduced with eight isolates of indigenous endophytic bacteria (IEB) showed a significantly different effect on the number of individuals produced with controls without treatment and control sprayed with insecticides. The number of *B. tabaci* individuals on tomato plants introduced by IEB can be seen in Table 2. The number of eggs placed by *B. tabaci* on plants introduced by IEB isolates was significantly different than those of controls. Plants that were introduced SNE 2.2 isolates and controls were the plants with the highest number of eggs, which averaged 13-grain crops, while the plants that were introduced were EPL 1.1.4 and KLE 3.3 isolates with the lowest quantity of eggs, which averaged five eggs. In addition to influencing the number of individuals, IEB isolates also affect the amount of *B. tabaci* individuals who can develop into an imago. Plants introduced by IEB isolates showed a markedly different development compared to controls. Introduction of EI.AB.2.1 Isolates, KLE 3.3, SNE 2.2, EI.AB 1.2 and control with insecticides shows the development of *B. tabaci* least developed, whereas control plants show the most developed *B. tabaci*. EI.AB 2.1 isolate is the best isolate in suppressing the development of *B. tabaci*.

The length of the day required by *B. tabaci* to develop every stage is not significantly different. The plants that were introduced were EI.AB 2.1 isolates lacking the production of the eggs to become the imago for the longest, namely 23 days, while the treatment without treatment, control with insecticides and EPL 1.1.3 needed the fastest time, 19 days. The time required

by *B. tabaci* to develop each stage (eggs, nymph instar 1, nymph instar 2, nymph Instar 3, pupae and imago) in plants introduced by endophytic bacteria indigenous and controls can be seen in Table 3

Table 2. The average number of *B. tabaci* individuals per stage in several isolates of indigenous endophytic bacteria on tomato plants.

Treatments	Development Stadia (individual)											
	Egg		Nymph instar 1		Nymph instar 2		Nymph instar 3		Pupae		Imago	
EI.AB 1.2	7,667	abcd	6,333	abcd	4,000	ab	1,667	b	0,667	de	0,333	cd
EI.AB 2.1	7,000	bcd	4,333	cd	0,667	c	0,000	c	0,000	e	0,000	d
EPL 1.1.3	6,667	cd	5,667	bcd	3,333	bc	2,333	b	2,667	abcd	2,333	abc
EPL 1.1.4	5,667	D	3,667	d	3,333	bc	2,667	b	2,000	bcde	2,000	bc
KLE 3.3	5,667	D	4,667	bcd	3,667	ab	1,333	bc	67	de	0,333	cd
Control N	10,333	abc	9,333	abc	4,000	ab	3,000	ab	1,667	cde	0,667	cd
SNE 2.2	6,667	cd	6,333	abcd	3,667	ab	1,333	bc	1,000	de	0,333	cd
TLE 1.1	12,667	ab	10,000	ab	9,000	a	6,667	a	4,667	abc	4,333	ab
TLE 2.3	13,333	A	13,667	a	10,667	a	8,000	a	6,333	a	4,333	b
Control	13,000	A	12,000	a	9,333	a	7,333	a	5,667	ab	5,000	a

Note: The numbers followed by the same letters on the same line are not significantly different according to LSD at the level of 5%

Table 3. The average number of days needed by *B. tabaci* to develop on several isolates of indigenous endophytic bacteria to tomato plants.

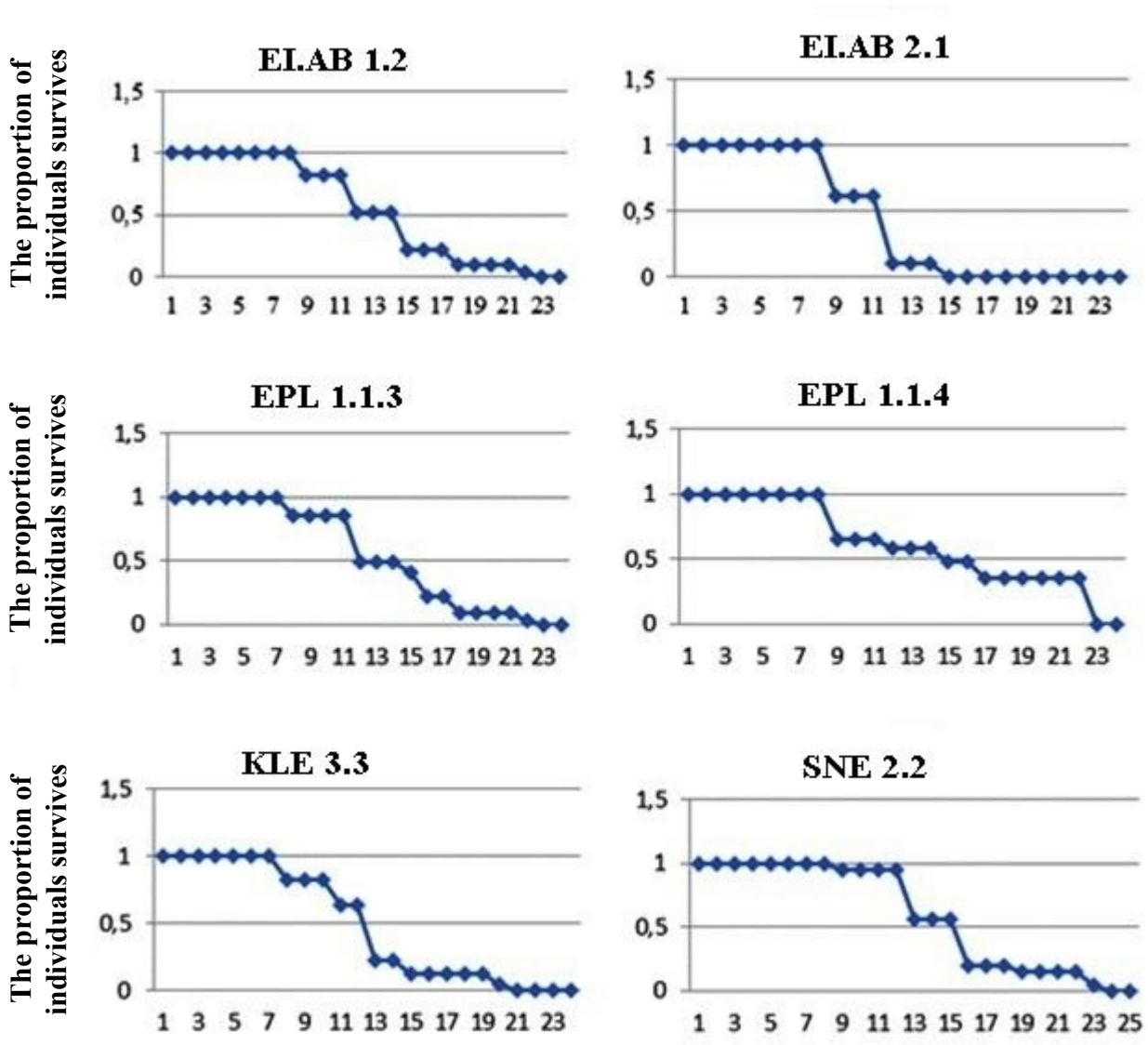
Treatments	Development Stadia (day)				
	Egg	Nymph instar 1	Nymph instar 2	Nymph instar 3	Pupae
EI.AB 1.2	7,3±0,58	3,3±0,58	2,6±0,58	2,3±0,58	1,6±2,89
EI.AB2.1	7,6± 0,58	1,3±2,31	1 ±1,73	0,6± 1,1531	1,3 ±2,31
EPL 1.1.3	7±1,00	2,6±2,31	2±1,73	1,3±1,15	3±2,65
EPL 1.1.4	7,3±1,15	2±1,73	1,6±1,53	1,3±1,15	3±2,65
KLE 3.3	7,3±0,58	3±0	3±0	1,3±1,15	3±2,65
Control N	7±1	3,3±0,58	2,6±0,58	2,6±0,58	4,3±0,58
SNE 2.2	7±1	3,6±0,58	2,6±0,58	2,6±0,58	5±0
TLE 1.1	7,3±0,58	3,3±0,58	3±0	2,3±0,58	4±0
TLE 2.3	7±1	4±0	2,6±0,58	3±0	4,6±0,58
Control	7,3±0,58	3,6±0,58	2,6±0,58	2±0	4,6±0,58

The proportion of *B. tabaci* individuals who survived (Lx) in each stage of development can be seen in Table 4. Based on the calculation of Lx, the proportion of *B. tabaci* individuals in the nymph 1 phase which showed the highest value was the treatment of SNE 2.2 isolates with Lx 0.954 and the lowest is EI.AB 2.1 with Lx 0.614. In the 2nd instar nymph stage, the proportion of individuals who survived the highest was obtained in the treatment of TLE 2.2 with Lx 0.764, and the lowest was treatment with isolates EI.AB 2.1 with Lx 0.100. In the instar three nymph stadia, the proportion of individuals survived the highest was obtained in the control treatment with a value of Lx 0.562 and the lowest Lx value was found in the isolate EI.AB.2.1 with a value of Lx 0,000. The highest Lx value of pupae development was observed in TLE 2.2 treatment with a value of 0.450 and the lowest treatment with isolates EI.AB 2.1 with an amount of 0,000. At the imago stage, the highest Lx value was found in control with Lx value of 0.385, and the lowest was in the treatment with isolates EI.AB 2.1 with a value of Lx 0,000. From all stages of development, based on the value of the proportion of individuals who survived the best isolates in suppressing individuals were EI.AB 2.1. This isolate can lower the individual to survive so that it can only develop until the instar 2 of nymph and nothing survives to become an imago.

Based on the Lx value, it can be seen that the individual pattern of surviving from *B. tabaci* can be described in the form of a life span in Figure 1. The proportion of individuals surviving *B. tabaci* on tomato plants introduced by isolates EI.AB.2.1 immediately decreased dramatically at 0.1 values on 12th day, Isolate KLE 3.3, SNE 2.2 and EI.AB 1.2 on the chart looks down dramatically on the 15th day, while isolates of EPL 1.1.4, EPL 1.1.3, TLE 1.1, TLE 2.2, negative controls and controls have almost similar chart, namely a gradual decrease in the pattern of sustained individuals and there are still individuals surviving live to become an imago.

Table 4. The survivorship of *B. tabaci* which was introduced by several indigenous endophytic bacterial isolates and controls.

Treatments	Development Stadia (proportion)					
	Egg	Nymph instar 1	Nymph instar 2	Nymph instar 3	Pupae	Imago
EI.AB.1.2	1	0,818	0,519	0,221	0,09	0,039
EI.AB.2.1	1	0,614	0,100	0,000	0,000	0,000
EPL 1.1.3	1	0,850	0,493	0,403	0,34	0,299
EPL 1.1.4	1	0,649	0,579	0,474	0,35	0,351
KLE 3.3	1	0,824	0,632	0,228	0,12	0,053
Control N	1	0,902	0,388	0,291	0,17	0,068
SNE 2.2	1	0,954	0,561	0,197	0,15	0,045
TLE 1.1	1	0,793	0,714	0,532	0,37	0,341
TLE 2.3	1	0,809	0,764	0,571	0,45	0,307
Control	1	0,923	0,715	0,562	0,44	0,385



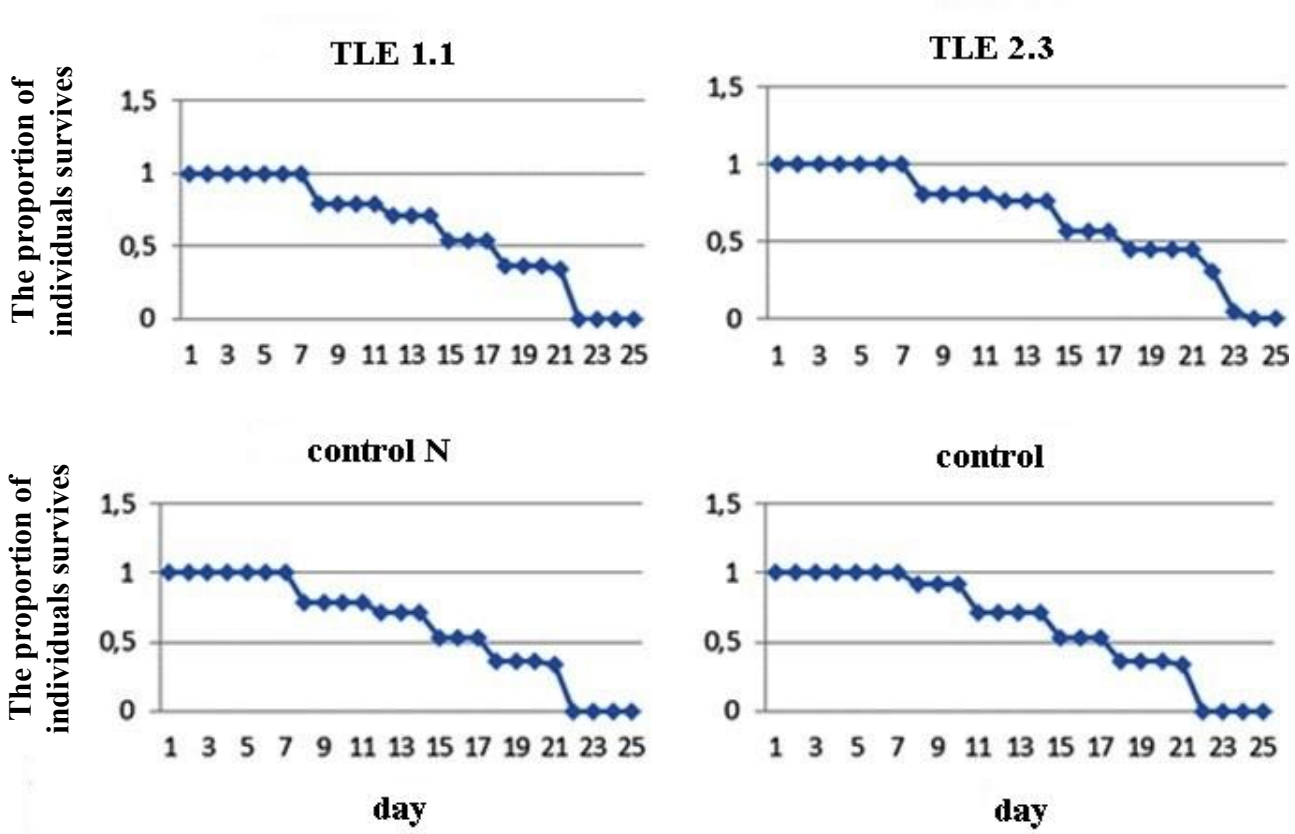


Figure 1. The life span (Lx) of *B. tabaci* per day based on differences in isolates.

DISCUSSION

The introduction of IEB in tomato plants can increase the resistance of tomato plants to *B. tabaci*. Tomato plants introduced with IEB can suppress *B. tabaci* egg laying and reduce the number of surviving individuals. Tomatoes given IEB showed fewer eggs than controls and controls sprayed with insecticides. The administration of IEB isolates can also reduce the amount of *B. tabaci* nymphs that develop in tomato plants, the number of individuals who succeeded in becoming imago in plants introduced with IEB was less than the control. EI.AB.2.1 isolate is the best isolate in suppressing the amount of *B. tabaci* individuals. This isolate showed *B. tabaci,* which survived only until the instar two nymphs did not develop into an imago. Besides EI.AB 2.1 There are isolates of KLE 3.3, SNE 2.2, and EI.AB 1.2, which show significantly different effects from controls but do not affect substantially the control sprayed with insecticides. IEB can influence the number of *B. tabaci* nymphs that develop; this is alleged because the giving of IEB isolates will activate plant resistance signals that will affect the response of plants so that it can suppress the development of *B. tabaci*. According to Kloepper and Ryu (2006) states that non-pathogenic microorganisms will induce plant resistance by activating signals from jasmonic and ethylene acids which are then responded to by plants with the production of chemical compounds or cytological changes. Production of defense-related chemical compounds, such as flavonoids, lignin, and other secondary metabolites, which produce effective defenses against various plant pathogens and insect herbivores arranged in the pathways of JA/ET and SA (Valenzuela-Soto *et al*. 2010). IEB can suppress *B. tabaci* attacks on tomato plants; this is in line with Murphy *et al*. (2000) stating that administration of endophytic bacteria suppresses the number of white flea nymphs that act as TOMV virus vectors.

The introduction of IEB in tomato plants did not affect the length of the day required by *B. tabaci* for development per stage. The average time needed to develop per phase can be seen in Table 3. Days needed by *B. tabaci* to develop at each stage are almost the same, with a range of distance differences of approximately 1-2 days. This is because the resistance of plants induced by IEB does not affect the duration of *B. tabaci* development days. The length of the day required by *B. tabaci* is more influenced by other environmental factors such as temperature and type of plants. In line with this, Kurniawan (2007) reports that *B. tabaci* have a faster generation time in cucumber plants than chili plants. Purbosari (2008), also showed that the imago *B. tabaci*

life cycle at 29 ° C was more rapid than at room and 23 ° C temperature.

B. tabaci on plants introduced by IEB isolates in the life table showed the highest individual mortality per development compared to controls, whereas controls showed that individuals survived the highest in each stage of development. EI.AB 2.1 isolate shows individuals surviving with a value of Lx 0.0 on the stage of development of instar three nymphs, pupae, and imago. In the pattern of sustained individual velocities (Lx), it is seen that the life rate of *B. tabaci*, which was introduced by isolates EI.AB 2.1, KLE 3.3, SNE 2.2, and EI. 1.1 decreased sharply while in controls, insecticide control had a regular pattern and not until zero, there are still individuals who survive to become imago. *B. tabaci* could not survive on plants that had been induced by IEB, presumably because tomato plants introduced by IEB isolates could increase jasmonic acid signals in plants so that plants produced toxic secondary metabolites and inhibitor genes to suppress *B. tabaci* attacks. As revealed by Howe (2004), antagonistic bacteria produce compounds of salicylic acid, jasmonic acid, and ethylene, which influence the eating activity of pest insects by providing chemicals that act as secondary metabolites. This is in line with the study of Valenzuela-Soto *et al*. (2010) that *Bacillus subtilis* on tomato plants will induce resistance to white flea insects by increasing the expression of JA-independent genes (including photosynthetic genes, phenylpropanoids, and terpenoid pathway biosynthetic genes) and genes JA-dependent genes include protease and proteinase inhibitor coding, thereby reducing the attack of white lice in tomatoes. Pineda *et al*. (2012) also reported that Arabidopsis induced by *P. fluorescens* WCS417r had increased resistance to the liquid-sucking aphid of the *Myzus persicae* plant, plants introduced by *P. fluorescens* showed stronger expression after the attack.

The introduction of IEB isolates in plants will work indirectly, IEB will induce resistance by activating the jasmonic and ethylene acid signals and the production of secondary metabolites. Secondary metabolite compounds produced by plants will affect the work of *B.tabaci* cells; this will cause physiological disorders that lead to the death of *B.tabaci*. Also, secondary metabolites can also reduce the birth rate, increase the birth of disabled individuals, and affect the sex of the individual produced. This was revealed by Chowa´nski *et al*. (2016) that secondary metabolite compounds produced by plants have a broad spectrum. Secondary metabolite compounds will disrupt the work of the cells so that it will disturb physiologically from insect and will cause death, disrupting the birth rate, causing the birth of individuals defects, affect the individual sex produced, reduce the number of individuals and affect the stage of development of insects.

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