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Potential of bacterial consortium in controlling oil palm bagworm, *Metisa plana*

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Elaeis guinensis is known as African oil palm, is the major industrial crop in Malaysia. However, one of the important pests that could seriously decrease the yield of oil palm production in Malaysia is the bagworm which is *Metisa plana*. Up to now, the bagworm infestation is controlled by using synthetic insecticides such as trichlorfon in various ways. However, the excessive uses of chemical pesticides have led to the larvae resistance and give negative effects to the environment. Thus, bacteria strains such as *Bacillus thuringiensis*, *Bacillus toyonensis*, *Serratia marcescens*, and *Paenibacillus* sp. were evaluated as potential eco friendly biopesticides to control *M. plana* effectively. Bacterial insecticidal activity against *M. plana* were assessed at different concentrations and different stages. Then, the synergistic pathogenicity of bacterial consortium against bagworm, *M. plana* was evaluated. The bacterial strains that grown in nutrient broth were sprayed on the larvae of *M. plana* with different concentrations and the result indicates that the highest mortality was caused by the concentration of 10^9 cfu mL^{-1} with the effectiveness, 68% in seven days. The larvae instars of *M. plana* were divided into two stages, Stage 1 (consisting of larvae on levels 2, 3, and 4) and Stage 2 (consist of larvae in levels 5, 6, and 7). The result showed that the highest mortality rate against *M. plana* in Stage 1, with an effectiveness of 54.8% in seven days. Bacterial Consortium 4 (*B. thuringiensis* and *Paenibacillus* sp.) have the highest antagonistic potentiality with a 92.3% mortality rate in four days. Overall, the consortium of bacteria treatment caused higher mortality of *M. plana* compared to single bacterial strains. This study have the potential biological approach for sustainable pest management in the oil palm industry in Malaysia.

Keywords: ***Metisa plana*****, oil palm, bacterial consortium, synergistic pathogenicity, insecticidal activity.**

INTRODUCTION

Eleis guinensis is known as African oil palm which is an essential crop in the agriculture sector in Malaysia, and the oil palm industry remains an important economic asset in Malaysia since the 1960s (Parveez et al. 2019). Malaysia is one of the largest producers and exporters of palm oil in the world. There are about 4.49 million hectares of land in Malaysia under oil palm plantation, where Malaysia produces 17.73 million tonnes of oil palm and 2.13 kernel oil (Malaysian Palm Oil Council, 2019). However, one of the important pests that could seriously decrease 43% of the yield of oil palm production in Malaysia over two years after a severe infestation is the bagworm, *M. plana* (Lepidoptera: Psychidae). The bagworm, *M. plana* is an insect that feeds the oil palm leaves for their growth and development (Hamim and Hariri, 2011; Halim et al. 2017). In Malaysia, bagworm infestations and outbreaks have occurred for over five decades (Cheong and Tey, 2012). Bagworm has been declared as a dangerous pest on 15 November 2013 under Malaysia Act 167, Plant Quarantine Act 1976 where under this act, the planter will be fined not more than RM 10,000 or to be jailed for two years if failed to control bagworm infestation after receiving the notice (Kamarudin et al. 2017). The major control mechanism in managing bagworm outbreaks in most plantations as compared to smallholdings is chemical pesticides (Hasber, 2010). However, over-reliance on chemical pesticides to control *M. plana* will lead more persistent problems such as resistance of *M. plana* towards oil palm, an abundance of chemical residues in the environment, and the disruption of beneficial insects such as parasitoid (Kamarudin et al. 2017; Hamim & Hariri, 2011). In addition, continuously using chemical pesticides may toxicate the environment and humans (Noorshilawati et al. 2015). Thus, biopesticides is used as an alternative for controlling *M. plana* as niopestic as it is beneficial for environmental sustainability and human health (Hasber, 2012; Noorshilawati et al. 2015).

Biopesticides are natural biological agents used to control pests that could be derived from microbes and some plants. The usage of biopesticide is recommended for the usage in agriculture field to make sure the targeted pest species is reduced (Sethi and Gupta, 2013; Kamarudin et al. 2017). One of the effective biocontrol agents against bagworms is *B. thuringiensis* (Ramlah and Basri, 1997; Ramlah, 2000; Ramlah and Mahadi, 2001). According to Yanti et al. (2018), *B. thuringiensis* also acts as biocontrol of *Ralstonia syigii* and promotes the growth of tomato. In addition, *S. marcescens* also have been used as biopesticides in the control of insect because it produces chitinase enzyme where the enzyme hydrolyzes the chitin into its monomers and produce antibiotic substances (Korany et al. 2019). The larvae of *Spodoptera litura* decrease in their larval and pupal weight, percent normal pupation, and adult emergence has shown by insecticidal activity of *S. marcescens* (Aggarwal et al. 2015). Additionally, *Bacillus toyonensis* also has broad-spectrum antagonisms promising to control various diseases in crops (Fan et al. 2015). According to Yanti et al. (2018), *B. toyonensis* can promote growth and control *Ralstonia syzgii* subs. *indonesiensis*. Moreover, *B. toyonensis* have antagonistic activity toward *Fusarium oxysporum f.* sp**.** *lycopersici* and can control fusarium wilt caused by fungus (Rocha et al. 2017). Furthermore, *B. toyonensis* also can resist root rot disease of *Panax notoginseng* caused by a fungus (Fan et al. 2015). Besides, another endophytic bacteria is *Paenibacillus* sp. As an example, *Paenibacillus polymyxa* is an endospore-forming bacterium that can act as a biocontrol agent against a wide array of plant pathogens such as *Aspergillus niger* (the pathogenic fungus that causes crown rot disease of the peanut) both in vitro and in vivo (Padda and Puri, 2017). Apart from being a potent biocontrol agent, *P. polymyxa* strains also have their ability to fix atmospheric nitrogen, increase seedling length and biomass which will be an effective biofertilizer in commercial agriculture (Puri et al. 2015; Padda, 2015; Padda et al. 2016). The study by Sajili et al. (2018) proved that some bacteria have ability in controlling plant pathogen such as *Fusarium* wilt disease in melon industry.

Recently, most biocontrol agents were reported by researchers for their inconsistent performance because they used a single biocontrol agent such as one species of bacteria to control a pathogen and pest. Therefore, the emerging strategies for controlling plant pathogens and pests are by applying antagonistic microorganisms in combination or consortium. The consortium gives a better effect when compared to a single introduction because the organisms work together in a complex and synergistic way (Yanti et al. 2020; Sudhani et al. 2014). Also, it is supported by Rajasekar and Thaiyalnayagi (2018), and Bashan (2015), a consortium of bacterial gives better results than a single bacterial application. There are two types of mechanisms explained by Wang et al. (2010) which are direct mechanism and indirect mechanism. The direct mechanism is the endophytic bacteria control plant pathogen by producing antimicrobial, siderophore, and chitinase enzymes. Meanwhile, the indirect mechanism is through the induction of systemic resistance in plants (Wang et al. 2010). The mechanism of action involves solubilization of the parasporal crystals and activation of the released proteins in the midgut of the target insect. The active toxins will then bind with specific receptor proteins and form pores through the membrane of the gut lining, which led to osmotic lysis. Besides, the spores present in the product formulation can germinate, forming more *B. thuringiensis* cells resulting in septicemia and killing the target insect

(Aronson et al. 2002). Thus, this study aimed to determine the insecticidal activity in different concentrations against bagworm, determine the insecticidal activity of bacteria against different stages of bagworm, and to evaluate the synergistic pathogenicity of bacterial consortium against bagworm.

MATERIALS AND METHODS

Sample Collection and Rearing of *Metisa plana*

Cultures of *M. plana* were established by collecting the larvae of *M. plana* from oil palm plantation at Ladang Besout Jaya, Sungkai Perak. The larvae of the *M. plana* were collected from middle and lower fronds of oil palm and placed in plastic containers covered with nets for ventilation. The larvae were fed with fresh oil palm leaflets to ensure enough food supply for the translocation from the site sample to the laboratory. The *M. plana* were reared under standard laboratory conditions 27.8℃ ± 0.5 and 77.3% relative humidity on a 12:12 photo regime. Then, the larvae were fed on fresh oil palm leaflets which will be replenished every three days until pupation. Besides, to avoid cannibalism, larvae were reared according to their stages. The oil palm leaves were cut into 20-30 cm length sections in order to feed 20 larvae of the *M. plana*.

Activation of bacterial strains

Four types of potential bacteria were tested as biocontrol to *M. plana* which are *Bacillus thuringiensis*, *Bacillus toyonensis*, *Paenibacillus* sp., and *Serratia marcescens*. All the strains were provided from the laboratory where the molecular identification and biochemical characterization have been conducted (Badaluddin et al. 2020). The strains were activated on Nutrient Agar (NA) that was mixed with 23 g of BD Difco™ Nutrient Agar in 1 L of purified water. The strains were activated by streaking of bacterial strain on NA and incubated for three days at 30℃. The contaminations were checked and identified by naked eyes, and all the contaminations were discarded.

Preparation of the bacterial suspension

The bacterial isolates were prepared by inoculating one pure colony from NA with bacteria growth into 50 mL Falcon tube containing 10 mL of Nutrient Broth (NB) composed of Nutrient Broth, 8g in 1 L of purified water (BD DifcoTM Nutrient Broth). The Falcon tubes were incubated at 30 °C overnight. After incubation, the concentrations of bacterial suspensions were measured using a spectrophotometer (Eppendorf BioPhotometer plus) at OD_{600} and serial dilution of pure culture (10^{-7} until 10^{-9} dilutions) were conducted.

Sorting of *M. plana*

The larvae were sorted into two stages based on their size of larvae. Stage 1 consists of larvae in level 2, 3 and 4 and stage 2 consists of larvae in level 5, 6 and 7 **(Figure 1**). According to Kok et al. (2011), instar level of *M. plana* from first larvae instar to fourth larvae have highest feeding activity rate and actively consumed oil palm leaflets for their case construction. Meanwhile, after fifth larvae levels are similar in size where they start to become pupae and remains inactive in their case.

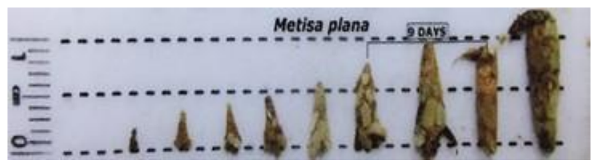

Figure 1: The larvae of *M. plana* with different size according to their level. The instar level 2, 3, and 4 are sorted into stage 1; meanwhile, instar level 5, 6, and 7 are stage 2.

Bioassay of insecticidal activity

The bioassay of the insecticidal activity of different concentrations was carried out by spraying 3 mL of a bacterial suspension of $10^{7,}$ 10^{8}, and 10^{9} cfu mL^{-1} on *M. plana* and both sides of the oil palm leaflet. The healthy larvae of *M. plana* were randomly selected. Then, the mortalities of *M. plana* were recorded daily until all the bagworms died. Then, the insecticisidal activity of different stages were carried out by spraying 3 mL of the best bacterial suspension which was 10^{9} cfu mL^{-1}, on *M. plana* and both sides of the oil palm leaflet. The healthy larvae of *M. plana* were randomly selected and the mortalities of *M. plana* were recorded daily until all the bagworms died. The bioassay of insecticidal activity of bacterial consortium was carried out by spraying 3 mL of 10^{9} cfu mL^{-1} bacterial consortia on *M. plana* in Stage 1 which is the best susceptible stage and both sides of oil palm leaflet. Then, mortalities of *M. plana* were recorded daily until all the bagworms died and dead larvae were removed from the containers.All bioassays were conducted in five replicates where each replicate contained ten larvae. The insecticidal activity experiment was tested under laboratory conditions. The sterile water was used as a negative control.

Compatibility test of bacterial strains

The compatibility between bacterial strains was tested for their antagonistic and synergistic bacterial consortium development. The strains were inoculated into NB and incubated at 30℃ for 24 hours. After incubation, the bacterial suspension of two different strains was resuspended with sterile water in order to obtain 10^9 cfu mL^{-1}. After that, 0.5 mL of each strain with 10^9 cfu mL^{-1} will be inoculated on NA and homogenize strains using a hockey stick and incubated at 30℃ for 24 hours. The presence of an inhibition zone will be observed for each strain. Each treatment has three replicates. Then, the bacterial consortia were developed based on the compatibility of the strains (Kumar et al. 2011).

Preparation of bacterial consortia

After determined the best optimum concentration and the best susceptible stage, the experiment was conducted to evaluate the effective bacterial consortium. The bacterial consortiums were made by mixing with two different bacteria suspensions with a concentration of 10^9 cfu mL^{-1} with the same ratio (1:1) (Sayed & Behle, 2017). Six bacterial consortium which had potential to control bagworms had been listed in Table 1.

Table 1. List of bacterial consortia

Code	Strain
C1	*B. toyonensis* & *Paenibacillus* sp.
C2	*B. thuringiensis* & *B. toyonensis*
C3	*S. marcescens* & *Paenibacillus* sp.
C4	*B. thuringiensis* & *Paenibacillus* sp.
C5	*B. thuringiensis* & *S. marcescens*
C6	*S. marcescens* & *B. toyonensis*

Non Host Test

The effect of bacterial suspension and the bacterial consortium on oil palm leaflets tested. The oil palm leaflets without injury were cut into 20 - 30 cm and placed in plastic containers. Then, the oil palm leaflets were sprayed with 10^9 cfu mL^{-1} of bacterial suspension to observe the changes of oil palm leaflets compared with control (sterile water). Each treatment has three replicates. The percentage of disease incidence (DI) was calculated using this formula:

Disease Incidence = (Number of plants infected)/ (Number of plants inoculated) x 100

Statistical Analysis

The parameter that observed in this research was the mortalities of larvae. The data were analyzed using one-way ANOVA. All data were analyzed using IBM SPSS Statistics 22 Software. The effectiveness of all the treatment towards control, sterile water also calculated.

RESULTS AND DISCUSSIONS

Effectiveness of bacterial in different concentration against bagworms in 7 days.

The larvae mortality was found all the bacterial strains in different concentrations used in this study have insecticidal activity against bagworms where an average mortality rate of 68.0% in 10^9 cfu mL^{-1}, 60.8% in 10^8 cfu mL^{-1} and 48% in 10^7 cfu mL^{-1} of bacteria suspensions. The mortality of the larvae in control (sterile water) was 0% **(Figure 2).** A one-way ANOVA was used to test the hypothesis of whether there is a significant difference in the mortality rate of *M. plana* that exposed with three types of concentration than with the control. The mean mortality rate differed significantly compared to the control; hence the test was significant.

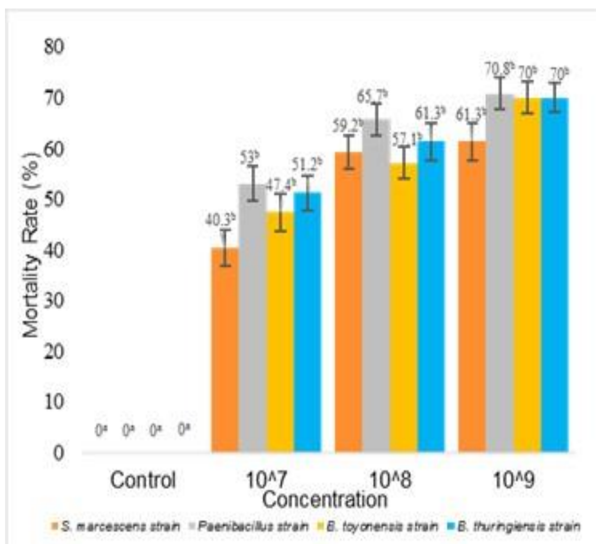

Figure 2: The mortality rate of *M. plana* in different concentration on controlling bagworm.

Paenibacillus sp. has the highest mortality rate on controlling bagworm in all three concentrations compared with control and others concentration (Figure 2). *Paenibacillus* sp. produces resistant spore and hydrolytic enzymes such as protease which are toxic to insects and pests (Harrison et al. 2010). The result shown in this study is supported by Eski et al. (2018) revealed that the effectiveness of *B. thuringiensis* against armyworm was 100% mortality when exposed to 10^9 cfu mL^{-1} bacterial concentration compared with other concentration such as 96% mortality at 10^8 cfu mL^{-1}. It contradicts with Sayed & Behle (2017), where he mixed of 10^6 concentration of spores from *B. thuringiensis* and *B. bassiana* strain treatment caused 80% mortality of *Trichoplusia* neonates after three days exposure. Each bacteria has its growth rate and the diversity of growth rate in bacteria involving fluctuation in gene expression and metabolic cycles (Peterson et al. 2015). The biocontrol pesticides are on the exponential (log) phase because, during this phase, bacteria rapidly grow and replicates which involves multiple rounds of DNA synthesis coupled with transcription and translation to synthesize macromolecule and enzyme (Rolfe et al. 2012). The effectiveness of each bacterial strain has specifically different antagonistic activities towards a specific type of pests, whether insect or pathogenic microorganisms. Hence, the concentration of 10^9 cfu mL^{-1} was chosen for determining the best stage against bagworm and for evaluating the synergistic pathogenicity of bacterial consortium against bagworms.

Rate of *M. plana* mortality in different stages.

Three replicate of ten larvae of *M. plana* according to the size introduced with the bacterial strains showed the effectiveness to control the pests. (Table 2). The mortality rate of *M. plana* introduced with *S. marcescens*, *B. toyonensis*, and *B. thuringiensis* were faster in Stage 1 (11 – 14 days) compared to Stage 2 (11 – 14 days). Meanwhile, the mortality rate of *M. plana* introduced with *Paenibacillus* sp. strain was faster in Stage 2 (13 days after inoculation) compared to Stage 1. The larval control by *B. thuringiensis* strain was slow, and it usually takes two to three days, or sometimes it even extended beyond one week for larger larvae (Knowles, 1994; Glare & Callaghan, 2000).

Table 2: Rate of *M. plana* mortality in different stages.

Bacterial strain	Total Days of Mortality	
	Stage 1	**Stage 2**
Control	0	0
Serratia marcescens	12	14
Paenibacillus sp.	16	13
Bacillus toyonensis	14	15
Bacillus thuringiensis	11	14

Effectiveness of bacterial strains on controlling different stages of *M. plana* in 7 days

The mortality rate of the larvae in Stage 1 after seven days exposure was the highest with average mortality rate (54.8%) compared to the Stage 2 after seven days exposure with an average mortality rate (48.9%) and control (0%) **(Figure 3).**

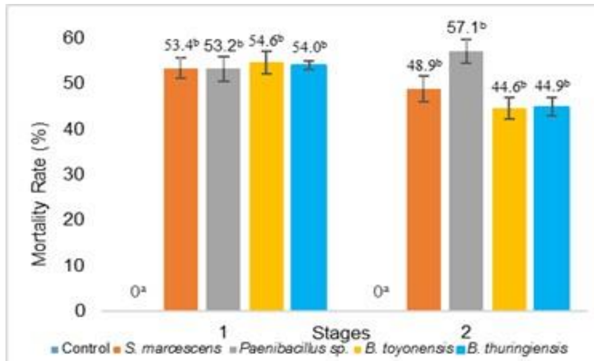

Figure 3: The mortality rate of bacterial suspension (10^9 cfu mL^{-1}) in controlling different stages of bagworm.

A one-way ANOVA was used to test the hypothesis that whether there is a significant difference in the mortality rate of different stages of *M. plana* that exposed with different bacteria with concentrations of 10^9 cfu mL^{-1} with the control. The mean mortality rate differed significantly compared to the control; hence the test was significant. From the result, Stage 1 was chosen for evaluating the synergistic pathogenicity of bacterial consortium against bagworm. The result shown in this study was supported by Yee et al. (2008) where the third instar larvae were more susceptible to *B. thuringiensis* than fifth instar larvae. Hellberg et al. (2015) also reported that early larvae are more sensitive than late larvae. In his experiment, the worm of *Caenorhabditis elegans* from larval stage 1 until larval stage 4 were more susceptible to the *Serratia plymuthica* A153. The *C. elegans* susceptibility to the *S. plymuthica* is inversely associated with the developmental stage and age of worms (Hellberg et al. 2015). According to Yee et al. (2008), the younger and small larvae were easier and faster to kill compared to matured larvae or late instar larvae. Kok et al. (2011) also revealed that the most effective instar level to control *M. plana* by the chemical is from first larvae instar to fourth larvae instar because of the increment of their feeding activities for their growth of development and cases construction. This result shows the effectiveness of bacterial strains to control *M. plana* correlated to the instar level of larvae.

Compatibility among bacterial strains.

All the potential bacteria were compatible with each other with no inhibition zone formed (Table 3). No inhibition zone indicates that the strains were not an antagonist to one another and can grow together. (Kumar et al. 2011). *B. thuringiensis* was compatible with two other bacillus strains which are *B. toyonensis* strain and *Paenibacillus sp*. and one *Serratia* sp. which is *S. marcescens*.

Based on compatibility between bacterial strains grow on NA, six consortia obtained consisted of C1 (*B. thuringiensis* and *S. marcescens*), C2 (*B. thuringiensis* and *B. toyonensis*), C3 (*B. thuringiensis* and *Paenibacillus* sp.), C4 (*S. marcescens* and *B. toyonensis*), C5 (*S. marcescens* and *Paenibacillus* and C6 (*B. toyonensis* and *Paenibacillus* sp.). According to Kumar and Jagadesh (2016), the evaluation of the compatibility of microbial components is required in the ability of the microorganism consortium used as biocontrol agents and biofertilizers. The bacterial consortium that interacts synergistically have better control of plant pathogens compared to a single bacterial application.

The synergistic pathogenicity of bacterial consortium against bagworm

All bagworms that introduced with the bacterial consortium showed the synergistic effectiveness pathogenicity of bacterial consortium against bagworms (Table 4). The fastest incubation period of bacterial consortium introduced against bagworm was four days, which were C3 (*Paenibacillus* and *S. marcescens*) and C4 (*Paenibacillus* and *B. thuringiensis*) compared to controls (seven days). The most effective bacterial consortium against bagworms was C4 with 10^9 concentration and the mortality rate of 92.3% compared with others bacterial consortiums which were C1 (*Paenibacillus* sp. and *B. toyonensis*) with mortality rate 80.3%, C2 (*B. thuringiensis* and *B. toyonensis*) and C6 (*S. marcescens* and *B. toyonensis*) with mortality rate 91.7%, C3 with mortality rate 91.4% and C5 (*B. thuringiensis* and *S. marcescens*) with mortality rate 91.10%. A one-way ANOVA was used to test the hypothesis whether there is a significant difference in the mortality rate of *M. plana* that exposed with six types of bacterial consortia than with the control. The mean mortality rate differed significantly compared to the control hence the test was significant.

Table 3: Compatibility between bacterial strains

Bacterial strain	*B. thuringiensis*	*S.marcescens*	*B. toyonensis*	*Paenibacillus sp.*
B. thuringiensis		+	+	+
S. marcescens	+		+	+
B. toyonensis	+	+		+
Paenibacillus sp.	+	+	+	

Note: +: compatible, -: incompatible

Table 4: **The total days of mortality and the effectiveness (%) on controlling *M. plana* introduced with the bacteria consortia.**

Bacteria consortium	Rate of *M. plana* mortality	Effectiveness (%)
Control	0	0^a
C1	7	80.28^b
C2	5	91.70^b
C3	4	91.40^b
C4	4	**92.30^b**
C5	5	91.10^b
C6	5	91.70^b

B. thuringiensis and *Paenibacillus* sp. are aerobic endospore-forming bacteria and can suppress insect's pests and plant pathogens by producing enzymes (Govindasamy et al. 2010; Govindasamy et al. 2008). According to Govindasamy et al. (2010), multiple species of *Bacillus* spp. and *Paenibacillus* spp. can control pests and affect crop growth by three different ecological mechanisms which are the promotion of plant growth and nutrition, antagonism for pathogens, insects, and nematodes, and stimulation of host defence mechanisms. According to Sajili et al. (2017), *Bacillus* spp. Produced antibiotic substances in vitro that can inhibiting mycelial growth and spore germination of *Colletotrichum gleosporioides*, causal agent of anthracnose disease of *Carica papaya* in Malaysia.

The result shown by Yanti et al. (2020) contradicted with this study, which most effective consortium was a combination of *Bacillus pseudomycoides* strain SLBE 3.1 AP, *Bacillus thuringiensis* strain SLBE 2.3 BB and *Bacillus toyonensis* strain AGBE 2.1 TL with the effectiveness of 57.80%. Yanti et al. (2018) revealed that five consortia were consisting of two to three compatible bacillus strains useful in controlling bacterial wilt and fusarium wilt with the effectiveness of 100%. Hence, the effectiveness of each consortium of *B*. *thuringiensis* has specifically different antagonistic activities towards a specific type of pest, whether insect or pathogenic microorganism. The consortium of *Bacillus* spp. such as *B. thuringiensis* sp. is an excellent biocontrol reagent. Additionally, bacterial consortiums have their interaction mechanism when exposed to insect pests and pathogenic pests.

In recent studies by Eski et al. (2018) and Ni et al. (2015), B. *thuringiensis* produces endotoxin, insecticidal crystal proteins (ICP) during sporulation such as cry1 genes and cry2 genes. It makes *B*. *thuringiensis* has highest insecticidal activity because the genes are toxins to insects. When the larvae eat the leaves exposed to pathogenic bacteria, the ICPs will degrade the gut and lead to insect starvation (Eski et al. 2018). Moreover, *B. thuringiensis* also produces chitinase, where the chitinase has the ability as pest control (Gohel et al. 2006). In the insect gut such as larvae of lepidopteran is composed of a chitinous matrix and the presence of chitinase enzyme may increase the uptake of ICP and enhanced insecticidal activity of *B. thuringiensis*. (Ni et al. 2015). Meanwhile, the mechanism of bacterial strain for controlling plant pathogen is by producing antimicrobial, siderophores, chitinase enzyme and toxins that act against the growth of plant pathogens by degrading the pathogenic bacterial cell walls and the change the cell morphology (Wang et al. 2010; Chen et al. 2013; Elshakh et al. 2016).

In this study, all the bacteria consortia have high effectiveness against the bagworm compared with applying antagonistic microorganisms alone. The consortium of bacteria that work together in a complex and synergistic way gives better results compared with a single bacterial application (Sudharani et al. 2014; Bashan, 2005). The mortality rate of bagworm introduced with the bacterial consortium was higher (80.28% - 92.30%) than applying bacterial strain alone with concentration 10^9 cfu mL^{-1} (44.5% - 53.5%) (Figure 2). Each bacterial species such as *B. thuringiensis*, *S. marcescens*, *B. toyonensis*, and *Paenibacillus* sp. have their mechanisms to control pests. Hence, by mixing the bacteria will provide various control mechanisms simultaneously; therefore, it is more effective in controlling pathogens. According to Yanti et al. (2018), five out of ten types of consortium were used in controlling *Ralstonia syzygii* subsp. *indonesiensis* with the effectiveness of 100%. Some researchers compile a consortium of biocontrol agents based on their ability to control the specific pathogen and pests. Ouhaibi et al. (2016), used three types of the consortium of selected rhizobacteria to control tomato's wilt by *Sclerotinia sclerotiorum*. Then, Zhang et al. (2019) used consortium of PGPR strain (*Bacillus cereus* AR156, *Bacillus subtilis* SM21, and *Serratia* sp. XY21) to suppress *Phytophthora* blight on sweet potato. The consortium also promotes the growth of tomato by promoting the vegetative phase (Yanti et al. 2020; Yanti et al. 2018; Zhang et al. 2019; Ouhaibi et al. 2016).

In this study, the behaviours of the *M. plana* before applying bacterial consortium were very active in feeding and actively constructed their case using tissue of oil palm. Then, the behaviour of *M. plana* after applying sterile water (control) has remained active in their feeding for growth development and case construction. However, after one-day application of bacterial consortium towards *M. plana* and the oil palm leaflets, the behaviour of *M. plana* tended to be inactive or started to die and decrease their feeding activities compared to control (**Figure 5**).

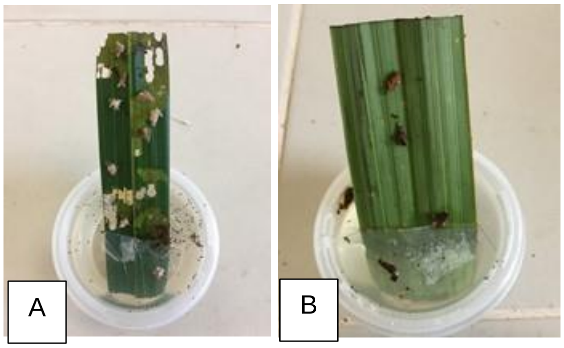

Figure 5: Synergistic bacteria consortium treatments on *M. plana* (A) The condition of *M. plana* after application of sterile water (control). The *M. plana* were very active in their feeding for growth development and case construction (B) The condition of *M. plana* after application of bacterial consortia. The *M. plana* were tended to be inactive and started to die.

Non-Host Pest Testing

All the single bacterial suspensions, which were *B. thuringiensis*, *S. marcescens, Paenibacillus* sp., and *B. toyonensis* showed 0% disease incidence towards oil palm leaflets. In addition, all the bacterial consortium which were C1 (*Paenibacillus* sp. and *B. toyonensis*), C2 (*B. thuringiensis* and *B. toyonensis*), C3 (*Paenibacillus* sp*.* and *S. marcescens*), C4 (*Paenibacillus* sp. and *B. thuringiensis*), C5 (*B. thuringiensis* and S*. marcescens*) and C6 (*S. marcescens* and *B. toyonensis*) showed 0% disease incidence toward oil palm leaflets. The data was recorded for 14 days. It indicates the bacterial strain and bacterial consortium do not affect the oil palm leaflets as non- host pests.

All the strains used in this study are potential plant growth promoter rhizobacteria (PGPR) where the bacterial strains have the ability to promote the growth of crops, meanwhile controlling the pests. PGPR can prevent the deleterious effect of the phytopathogenic organisms from the environment (Chakraborty et al. 2009). According to Govindasamy et al. (2010), *Bacillus* spp*.* and *Paenibacillus* spp. involved in nitrogen fixation, soil phosphorus solubilization, and promote plant growth meanwhile suppress the soil-borne plant pathogens. A variety of studies have been revealed that PGPR bacterial strains as plant growth regulators and as biocontrol agents. Yanti et al. (2020), the consortium of *Bacillus* spp. (*Bacillus pseudomycoides* strain SLBE 3.1 AP, *Bacillus thuringiensis* strain SLBE 2.3 BB and *Bacillus toyonesis* strain AGBE 2.1 TL) increased number of leaves, the length of the root of chilli, and the seedling height of chilli. Likewise, Yanti et al. (2018), the PGPR consortia such as *B. thuringiensis* and *B. toyonensis* increased plant height and number of tomatoes, where the strains used in her study can also produce indole acetic acid and others. A consortium of PGPR bacteria affected the growth of periwinkle better than a single strain (Rajasekar and Thaiyalnayagi, 2018). The study of Shanmugam et al. (2013) revealed that the production of ginger rhizomes increased by 45.8% when introduced with PGPR strain consortium treatment. According to Ouhaibi et al. (2016), the consortium of rhizobacteria can control the wilting of the tomato plants and promote the growth of tomato.

Hence, the strains used in this study do not give negative effects on the oil palm leaflets because the strains used in this study could promote the growth of crops when tested in the field. This study is supported by Kumar and Jangades (2016), the microorganism consortium act as biocontrol agents and also biofertilizers.

CONCLUSION

Based on the above finding, all the bacteria strains were useful as biocontrol agents and had antagonistic characteristics towards bagworm. The bacteria consortium was found to be effective in controlling the *Metisa plana*. The combinations of bacterial strains showed the highest mortality rate (%) on controlling *M. plana* compared to individual agents of bacteria. All consortia in this study can suppressing *M. plana*. In addition, the most effective time on controlling *M. plana* is during the early stage of instar, which in this study was in stage 1 (consists of instar 2, 3, and 4). The highest mortality rate of *M. plana* was 10^9 cfu mL^{-1} compared to 10^8 cfu mL^{-1} and 10^7 cfu mL^{-1} of bacterial strains. All the bacterial strains showed no disease incidence when introduced with bacterial strains and bacterial consortium. These biocontrol agents can be alternatives to chemical pesticides on controlling *M. plana* because the bacterial strains are not harmful to the environment.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

MM performed experiments and data analysis. NAB, SM, MHS and MNL designed experiments and reviewed the manuscript. All authors read and approved the final version.

REFERENCES

Aggarwal C, Paul S, Tripathi V, Paul B, & Khan MA, 2015. "Chitinolytic Activity in *Serratia marcescens* (Strain SEN) and Potency against Different Larval Instars of *Spodoptera litura* with Effect of Sublethal Doses on Insect Development." BioControl 60(5):631–40.

Aronson A, 2002. Sporulation and D-Endotoxin Synthesis by *Bacillus thuringiensis*, *Cell. Mol. Life Sci. 59*: 417-425.

Badaluddin, NA, Yin SY, Umar R, Sabri NH, Hasshim NS, Ali MK, & Rashad Mohamed A. (2020). Isolation and Characterization of High Ambient Electromagnetic Radiation (EMR) Bacteria. Malaysian Applied Biology, 49(4), 165–172.

Bashan LE, Bashan Y, 2005. Bacteria: Plant growth-promoting soil. In: Hillel D (ed). Encyclopedia of Soil in Environment vol 1. Elsevier, Oxford.

Cheong YL & Tey CC, 2012. Understanding Pest Biology And Behaviour For Effective Control of Oil Palm Bagworms. The Planter, 88(1039): 699-715.

Chen Y, Yan F, Chai Y, Liu H, Kolte R, Losick R, and Guo JH, 2013. Biocontrol of Tomato Wilt Disease by *Bacillus subtilis* Isolates From Natural Environments Depends On Conserved Genes Mediating Biofilm Formation. Environ Microbiol 15 (3): 848-864.

Eski A, Demir İ, Güllü M, and Demirbağ Z, 2018. Biodiversity and Pathogenicity of Bacteria Associated with The Gut Microbiota of Beet Armyworm, *Spodoptera exigua Hübner* (Lepidoptera: Noctuidae). *Microbial Pathogenesis*, *121*(October 2017), 350–358. https://doi.org/10.1016/j.micpath.2018.05.012

Elshakh AS, Anjum SI, Qiu W, Almoneafy AA, Li W, Yang Z, et al, 2016. Controlling and Defence-Related Mechanisms of *Bacillus* strains Against Bacterial Leaf Blight Of Rice. J Phytopathol 164 (7-8): 534-546

Fan Z, Cui-ping M, Xin-guo Q, You-kun Z, Hua-hong C, You-wei C, Li-hua X, Li-xing Z and Hui-lin G, 2015. "Diversity , Distribution , and Antagonistic Activities of Rhizobacteria of Panax Notoginseng." Journal of Ginseng Research (June):1–8.

Gohel V, Singh A, Vimal M, Ashwini P, and Chhatpar HS, 2006. Bioprospecting And Antifungal Potential of Chitinolytic Microorganisms. African Journal of Biotechnology **5**: 54–72.

Govindasamy V, Senthilkumar M, Magheshwaran V, Kumar U, Bose P, Sharma VA & Annapurna K, 2010. *Bacillus and Paenibacillus spp.:* Potential PGPR for Sustainable Agriculture (Vol. 18). https://doi.org/10.1007/978-3-642-13612-2

Govindasamy V, Senthilkumar M, and UpendraKumar AK, 2008. PGPR-Biotechnology For Management of Abiotic and Biotic Stresses In Crop Plants. In: Maheshwari DK, Dubey RC (eds) Potential microorganisms for sustainable agriculture. IK International Publishing, New Delhi, pp 26–48

Glare TR, and Callaghan M. O, 2000. *Bacillus thuringiensis*: Biology, ecology and safety. Chichester: John Wiley & Sons, Ltd.

Halim M, Muhaimin AMD, Syarifah Zulaikha SA, Nor Atikah AR, Masri MMM and Yaakop, S, 2017. Evaluation of infestation in parasitoids on *Metisa plana* Walker (Lepidoptera: Psychidae) In Three Oil Palm Plantations In Peninsular Malaysia. Serangga, 22(2), 135–149.

Hamim S and Hariri MA, 2011. "Population Assessment and Appropriate Spraying Technique to Control The Bagworm (*Metisa plana* Walker) in North Sumatra and

Lampung." 33(2):188–98.

Harrison RL, & Bonning BC, 2010. Proteases as Insecticidal Agents. Toxins, 2(5), 935–953.

Hasber S, 2010. Evaluation of Several Chemical Control Approaches against Bagworm, *Metisa plana* Walker (Lepidoptera: Psychidae) in Felda Oil Palm Plantations. M.Sc. thesis, Universiti Sains Malaysia, Pulau Pinang, Malaysia.

Hellberg JEEU, Matilla MA and Salmond GPC, 2015. The Broad-Spectrum Antibiotic, Zeamine, Kills The Nematode Worm *Caenorhabditis elegans*. Frontiers in Microbiology, 6(FEB), 1–13. https://doi.org/10.3389/fmicb.2015.00137

Knowles BH, 1994. Mechanism of action of *Bacillus thuringiensis* Insecticidal δ-endotoxin. Advance in Insect Physiology 24:275–308.

Kok CC, Eng OK, Razak AR and Arshad AM, 2011. "Microstructure and Life Cycle of *Metisa plana* Walker (Lepidoptera : Psychidae)." 6(1):51–59.

Korany SM, Mansour AN, El-Hendawy HH, Kobisi ANA and Aly HH, 2019. "Entomopathogenic Efficacy of the Chitinolytic Bacteria: Aeromonas Hydrophila Isolated from Siwa Oasis, Egypt." Egyptian Journal of Biological Pest Control 29(1).

Kumar H, Dubey RC and Maheshwari DK, 2011. Effect of Plant Growth Promoting Rhizobia on Seed Germination, Growth Promotion and Suppression of Fusarium Wilt of Fenugreek (*Trigonella foenum-graecum* L.). Crop Protect 30 (11): 1396-1403

Kumar KH and Jagadeesh KS, 2016. Microbial Consortia-Mediated Plant Defense Against Phytopathogens and Growth Benefits. South Indian J Biol Sci 2 (4): 395-403.

Ni H, Zeng S, Qin X, Sun X, Zhang S, Zhao X and Li L, 2015. Molecular Docking and Site-Directed Mutagenesis of a *Bacillus thuringiensis* Chitinase to Improve Chitinolytic, Synergistic Lepidopteran-Larvicidal and Nematicidal Activities. International Journal of Biological Sciences, 11(3), 304–315. https://doi.org/10.7150/ijbs.10632

Noorshilawati AA, Umi NH and Suraya A, 2015. "Phytochemical Screening And In Vitro Antibacterial Activity of *Elaeis Guineensis* Leaves Extracts Against Human Pathogenic Bacteria (Penyaringan Fitokimia Dan Aktiviti Antibakteria Daun Sawit (*Elaeis Guineensis*) In Vitro." 19(4):775–80.

Ouhaibi-Ben AN, Renault D, Gerbore J, Vallance J and Rey P, 2016. Evaluation of the Effectiveness of Tomato-Associated Rhizobacteria Applied Singly or As Three-Strain Consortium for Biosuppression of Sclerotinia Stem Rot In Tomato. J Microb Biochem Technol 8: 312-320.

Padda KP, 2015. Impact of GFP-modification of *Paenibacillus polymyxa* on Its Ability to Enhance Growth of Corn, Canola and Tomato Seedlings. Master's thesis, University of British Columbia, Vancouver, Canada. doi: 10.14288/1.0166773

Padda KP, Puri A and Chanway CP, 2016a. Effect of GFP Tagging of *Paenibacillus polymyxa* P2B-2R on Its Ability to Promote Growth of Canola and Tomato Seedlings. Biol Fertil Soils 52:377–387. doi:10.1007/s00374-015-1083-3

Padda KP, Puri A and Chanway CP, 2016b. Plant Growth Promotion and Nitrogen Fixation In Canola By An Endophytic Strain of *Paenibacillus polymyxa* and its GFP-tagged Derivative In A Long-Term Study. Botany 94:1209–1217. doi: 10.1139/cjb-2016-0075

Padda KP, Puri A and Chanway CP, 2017. *Paenibacillus polymyxa*: A Prominent Biofertilizer and Biocontrol Agent for Sustainable Agriculture. In V.S. Meena et al. (eds.), Agriculturally Important Microbes for Sustainable 116- 191. Singapore: Springer Nature Pte Ltd. doi:10.1007/978-981-10-5343-6_6

Peterson JR, Cole JA, Fei J, Ha T and Luthey-Schulten ZA, 2015. Effects of DNA Replication on mRNA Noise. Proc. Natl. Acad. Sci. U.S.A. 112, 15886–15891. 10.1073/pnas.1516246112.

Puri A, Padda KP and Chanway CP, 2015. Can A Diazotrophic Endophyte Originally Isolated From Lodgepole Pine Colonize An Agricultural Crop (Corn) and Promote Its Growth? Soil Biol Biochem 89:210–216. doi:10.1016/j.soilbio.2015.07.012

Ramlah AS, 2000. Mechanism of action of *Bacillus thuringiensis* □-endotoxins: binding studies of □-endotoxins to brush border membrane vesicle of *Metisa plana* 17 (Walker). PhD Thesis. Faculty of Science and Technology. Universiti Kebangsaan Malaysia.

Ramlah AS and Basri MW, 1997. A local *Bacillus thuringiensis* SRBT1 with potential for controlling *Metisa plana* (Wlk). Elaeis **9**(1): 34-45.

Ramlah AA, Mazmira MM, Najib M and Basri MW, 2005. "Potential of Terakil-1 and Teracon-1 : *Bacillus Thuringiensis* for Reducing Chemicals in Bagworms Control Potential of Terakil-1 and Teracon-1 : *Bacillus Thuringiensis* for Reducing Chemicals in Bagworms Control." (January).

Ramlah, AS and Mahadi NM, 2001. Binding of D-Endotoxin of *Bacillus thuringiensis* to BBMV from Susceptible and Resistant *Metisa Plana*. Paper presented at 4th Rim Pacific Conference on the Biotechnology of *Bacillus thuringiensis* and its Environmental Impact, 11-15 November 2001, Canberra, Australia.

Rajasekar S and Thaiyalnayagi S. (2018). "Studies on the Effect of Biofertilizers as Single and Consortium Inoculant on the Protein Content of Catharanthus Roesus." International Journal of Current Research in Life Sciences 07(04):1467–68.

Rolfe MD, Rice CJ, Lucchini S, Pin C, Thompson A, Cameron ADS and Hinton, JCD. (2012). Lag Phase is a Distinct Growth Phase That Prepares Bacteria For Exponential Growth And Involves Transient Metal Accumulation. Journal of Bacteriology, 194(3), 686–701.

Sajili MH, Azman AA, Badaluddin NA, Ghazali ASM, Mohamed S, and Ngah N. (2018). Potential of Pseudomonas sp. (UniSZA-MKB10) and Bacillus spp. (UniSZA-BK3, UniSZA-BK4 and UniSZA-DA) as Biological Control Agent for Controlling Anthracnose Disease of Carica papaya L. Journal Of Agrobiotechnology, 9(1S), 269-282.

Sajili MH, Wahida WN, Badaluddin NA, Abdullah TA, Abdul Aziz ZF, and Jugah K. (2017). Potential of *Pseudomonas sp.* (UniSZA-MKB10) and *Bacillus spp.* (UniSZA-BK3, UniSZA-BK4 and UniSZA-DA) as Biological Control Agent for Controlling Anthracnose Disease of *Carica papaya L.* Journal Of Agrobiotechnology 8(2), 64–76.

Sayed AMM and Behle RW, 2017. Evaluating a Dual Microbial Agent Biopesticide with *Bacillus Thuringiensis* Var. *Kurstaki* And *Beauveria Bassiana Blastospores*. Biocontrol Science and Technology, 27(4), 461–474. https://doi.org/10.1080/09583157.2017.1303662

Sethi S and Gupta S, 2013. Impact of Pesticides And Biopesticides on Soil Microbial Biomass Carbon. J. Environmental Research and Technology, 3(2): 326- 330

Shanmugam V, Thakur H, Kaur J, Gupta S, Rajkumar S and Dohroo NP, 2013. Genetic Diversity of *Fusarium* spp. Inciting Rhizome Rot of Ginger and Its Management by PGPR Consortium in the Western Himalayas. Biol Control 66 (1): 1-7.

Sudharani M, Shivaprakash MK and Prabhavathi MK, 2014. Role of consortia of biocontrol agents and PGPR in the production of cauliflower under field condition. Trends Biosci 7 (22): 3542-3546.

Wang Y, Zeng Q and Zhang Z, 2010. Antagonistic Bioactivity of an Endophytic Bacterium H-6. Afr J Biotechnol 9 (37): 6140-6145.

Yanti Y, Hamid H, Reflin, Warnita R and Habazar T, 2020. The ability of indigenous *bacillus* spp. Consortia to control the anthracnose disease (*Colletotrichum capsici*) and increase the growth of chili plants. Biodiversitas, 21(1), 179–186. https://doi.org/10.13057/biodiv/d210123

Yanti Y, Warnita R and Hamid H. (2018). "Short Communication: Development of Selected PGPR Consortium to Control *Ralstonia Syzygii* Subsp. *Indonesiensis* and Promote the Growth of Tomato." Biodiversitas 19(6):2073–78.

Yanti Y, Astuti FF, Habazar T and Nasution CR, 2017. Screening of Rhizobacteria from Rhizosphere of Healthy Chili to Control Bacterial Wilt Disease and to Promote Growth and Yield of Chili. Biodiversitas 18 (1): 1-9.

Yee TS, Yusof I and Dzolkhifli O, 2008. "Efficacy of *Bacillus thuringiensis* Berliner subspecies *Kurstaki* and *Aizawai* against the Bagworm, *Metisa Plana* Walker on Oil Palm." Journal of Bioscience 19(1):103–14.

Zhang LN, Wang DC, Hu Q, Dai XQ, Xie YS, Li Q, Liu HM and Guo JH, 2019. The consortium of plant growth-promoting *Rhizobacteria* strains suppresses sweet pepper disease by altering the rhizosphere microbiota. Front Microbiol 10 (1668): 1-10.