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Research Article
Diamondback Moth, *Plutella xylostella* (Linnaeus) Responses on Chinese Kale (*Brassica oleracea* Var. *alboglabra*) Treated by Plant Growth Promoting Rhizobacteria

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Abstract

Background and Objective: Plant Growth Promoting Rhizobacteria (PGPR) is a new and potential tool of the biocontrol for pests on crops. *Pseudomonas fluorescens* (*P. fluorescens*) and *Bacillus subtilis* (*B. subtilis*) are two species of PGPR that proved in biocontrol of crop pests and diseases. This research aimed to investigate the diamondback moth (DBM) responses on the Chinese kale that treated by *P. fluorescens*, *B. subtilis* and combination between both species. **Materials and Methods:** Research was conducted in the Pest Laboratory, Department of Plant Pests and Diseases, University of Brawijaya from January-April, 2015. Completely randomized design was adopted with four treatments (immersed seeds into *P. fluorescens*, *B. subtilis*, *P. fluorescens* and *B. subtilis* combination and control) and four replications to investigate the feeding activities, period of larval instars and oviposition of diamondback moth. In addition, phenolic and wax content tests were performed to get additional data related to the diamondback moth responses. **Results:** Result showed that feeding activities of *Plutella xylostella* (*P. xylostella*) was inhibited by application of PGPR. Concentration of phenolic compound contained in the plant tissue which treated by PGPR was higher than control. Period of larva become shorter after application of *B. subtilis*, *P. fluorescens* and combination of *P. fluorescens* and *B. subtilis*. Oviposition of *P. xylostella* was affected by PGPR and application of single species of PGPR reduced oviposition better than combination application. *Pseudomonas fluorescens* produced the lowest oviposition. Phenolic and wax compounds were produced higher than control for all PGPR treatments and it may be related to plant defense mechanisms against the diamondback moth. **Conclusion:** Application of PGPR cause the changes of DBM responses such as feeding and oviposition activities and it reduce period of larva. The high concentration of phenolic and wax compounds on PGPR application is related to the changes of DBM responses.

Key words: Feeding, oviposition, larval period, phenolic content, wax content

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plutella xylostella Linnaeus is commonly known as the Diamondback Moth, an oligophagous species of Lepidoptera that feeds on Brassicaceae crops, such as cabbage, cauliflower, broccoli and Chinese kale. Based on worldwide condition, to control the diamondback needs over US$ 1 billion annually. In the past, the use of synthetic chemical insecticides intensively for long time produce the resistance of pests to all synthetic insecticides. There were several reports related to resistances of the diamondback moth to synthetic insecticides in the world such as in India, Taiwan, Brazil, Indonesia and China[1–5]. In India, fenvalerate, quinalphos, monocrotophos, cartap hydrochloride and carbosulfan are known as ordered resistance synthetic insecticides from very high to low levels[1]. In Central China, spinosad, abamectin, chlorfluazuron, indoxacarb and beta-cypermethrin are resistance insecticides[5].

Plant growth promoting rhizobacteria are bioresources as a potential tool that provide the substantial benefits in the agriculture such as to support emergence, colonize roots, promote growth and increase yield. In addition, resistance against various plant pathogens and pests in different crops i.e., cereals, pulses, ornamentals, vegetables, plantation crops, spices and some trees are provided by PGPR[6-8].

To reduce chemical input though increasing plant fitness, productivity and resistance to pests and diseases by biological control in sustainable agriculture[9]. Biological control as new biotechnological methods for crop protection are based on the use of beneficial microorganisms such as biofertilizers and biocontrol agents, this approach could reduce chemical fertilizer use, control crop pests and diseases and reduce environmental pollution[10]. Plant Growth Promoting Rhizobacteria (PGPR) is a new and potential tool of the biocontrol for pests on crops and it can provide substantial benefits to the agriculture such as enhancing emergence, colonizing roots, stimulating growth and increasing yield[6]. *Pseudomonas putida, P. fluorescens*, *Serratia marcescens*, *Bacillus amyloliquefaciens*, *B. subtilis* and *B. cereus* were the six species of PGPR that were studied to control the root knot nematodes in Saudi Arabia[11].

Related to the effectiveness of PGRP on previous research on root knot nematodes, it can be applied to other pests such as *P. xylostella*. Single use and applications rely on the use of interest and compatibility among isolates from one another. Related to two models of application, it is necessary to confirm the single and combined treatments which can produce the better control for *P. xylostella*. Based on the potential of PGPR, research aimed to distinguish the factors that cause the diamondback moth give responses on the Chinese kale that treated by PGPR, because in the previous research there was no reason related to this matter.

MATERIALS AND METHODS

Research was conducted in the Pest Laboratory, Department of Plant Pests and Diseases, University of Brawijaya from January-April, 2015. Shovel, poly bags (3 kg), brushes, dissecting microscope, measuring glass, plastic, wooden stick as a hook feed honey for imago of diamondback moth, petri dish (9 cm in the diameter), ruler, gauze cage, digital scales and hand counter were used as tools in this research. The soil, compost, seeds of Chinese kale and 5% formalin were prepared for soil sterilization. Alcohol 70%, larvae of *P. xylostella*, graph paper, tissue, cotton and honey were prepared for materials. *Pseudomonas fluorescense* (Pf) and *Bacillus subtilis* (Bs) were provided by Department of Plant Pest and Disease, Faculty of Agriculture, University of Brawijaya.

Completely randomized design was adopted with four treatments and replications, respectively to investigate the feeding activities and period of larval instars. Plants maintained by means of watered after every two days and given fertilizer once for planting. NPK fertilizer was given as much (3.5 g L^{-1}) and drained on the surface of the soil near the roots. In case of oviposition, preferences of imago was conducted in the insect cages based on each treatment and four replications. Treatments were: Immersed seed into *P. fluorescens*, *B. subtilis*, combination of *P. fluorescens* and *B. subtilis* and tap water as control.

Planting medium consisted of a mixture of soil and compost in the ratio 1: 1. Land for previous studies had been sterilized using 5% formalin for one week. Furthermore, a mixture of soil and compost put in a polybag 3 kg. Before the Chinese kale seeds put in a polybag, seeds soaked with warm water to determine good quality of seeds. The floated seeds were removed and the remained seeds that sink to the bottom were selected and used for research. The selected seeds were then immersed in to the PGPR solution based on the treatments. The PGPR isolates were 10^7 Colonies Form Unit (cfu) in densities. Concentration of PGPR solution as a soaking treatment was 30 mL L^{-1}. Ten minutes was time of immersion of seeds into PGPR solution. After steps of immersion, seeds were planted on the nursery tray and then transplanted after 10 days on polybags.

Rearing of *P. xylostella*: Rearing was done by collecting larvae, eggs and imago *P. xylostella* affected plants. Larvae kept in a plastic jar and covered with gauze. Larvae feed

preserved kale seeds. Instar larvae were separated according to stadia. In cages, cotton dipped into honey was used as feed for the imago stage.

Test of feeding inhibition: Feeding inhibition test was done by taking leaves of Chinese kale for each treatment, further weighed 2 g of leaves and put on a petri dish. Previous leaf of Chinese kale was weighed on millimeter paper. Then the millimeter paper was also weighed as the initial weight before the larvae infested. Furthermore, one third instar of larvae was put on a petri dish. Each treatment was repeated three times. For three days observation, affected leaves (feeding activity) were measured by using Pandey and Singh method[12]. Estimates of leaf area were measured by the equation:

$$\text{Leaf area } (cm^2) = \frac{x}{y}$$

Where:

x = Weight (g) of the area covered by the leaf outline on a millimeter graph paper

y = Weight of one cm^2 of the same graph paper

Replacing old instar larvae of *P. xylostella*: Observations of instar were done by taking the first leaves of Chinese kale, for further treatment, leaves put on a petri dish, then one larvae of newly hatched *P. xylostella* entered into each petri dish. Weakened leaves of kale were replaced with fresh ones. Parameters measured were old instar turnover from first to fourth instars larvae.

Oviposition preferences of *P. xylostella*: A pair of adults were infested into the cage in which there were four plastic bags for each treatment. Cotton that has been dipped in honey was hung in cage as feed for adults. The number of eggs laid on each plant was counted.

Phenolic content test: Phenolic content test was done by taking a leaf Chinese kale on each 0.5 g sample and then pulverized using liquid nitrogen. Additionally, Chinese kale leaves were homogenized by 5 mL ethanol and centrifuged at 10,000 rpm for 20 min. This stage was repeated twice, taken sediment under tube and then add 3 mL of distilled water and 0.5 mL reagent Folin-Ciocalteu. The solution was boiled in water bath for 1 min at 70°C, then the solution was cooled and absorbance was measured using spectrophotometer at 650 nm wavelength.

Wax content test: Materials used in this test were knife, three units of 1 L beaker glass, electrical balance, six units of 500 mL beaker glass, tweezers, three units of 500 mL of Erlenmeyer, spoon, funnel, filter papers, three units of small bottles, three plastics and rubber bands. Firstly, leaves were taken and soaked into a tube filled with water, before that each leaf was weighed with analytic scales. The leaves were immersed in n-hexane for 10 sec (3 times into different containers). After soaking, the leaves were weighed again, to know the difference in leaf weight. The extraction result of the wax dissolution with n-hexane solvent was made one for each leaf and then sodium sulfite was added and filtered. Then the solution was evaporated to get how much wax for each leaf. Finally, the leaf weight differences or the weight of the wax extract was calculated[13].

Statistical analysis: Data were analyzed by one way-analysis of variance at $\alpha = 5\%$. If there were differences between each treatment, 5% of Least Significant Different (LSD) was used. R-Studio[14] and Sigma plot 12 were used for analysis and create graphics.

RESULTS

Feeding activities of *P. xylostella* was inhibited by application of PGPR and it was described in Fig. 1. Application of PGPR especially *P. fluorescens* inhibited feeding activities of the diamondback moth almost 15% compared to control. However, application of *B. subtilis* and *P. fluorescens* and *B. subtilis* combination were not different to control.

Based on Fig. 2, concentration of phenolic compound contained in the plant tissue which was treated by *B. subtilis* and *P. fluorescens* were higher than treatment of *B. subtilis* and *P. fluorescens* combination. All treatments were higher than control. It seems that single treatment for both *B. subtilis* and *P. fluorescens*, stimulated plant to increase the production of phenolic compound. Phenolic content of each treatment was 1.4, 1.4, 1.1 and 1.0 mg/plant tissue for *B. subtilis*, *P. fluorescens*, *P. fluorescens-B. subtilis* combination and control, respectively.

Period of larva become shorter after application of *B. subtilis*, *P. fluorescens* and combination of *P. fluorescens* and *B. subtilis*, based on Fig. 3. It can be seen when all applications of PGPR compared to control. Shorter period of larva is clearly caused by period of first instar (Fig. 4), where

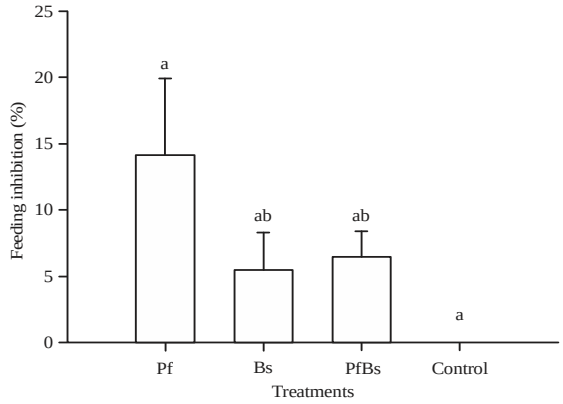

Fig. 1: Feeding inhibition of *P. xylostella* on each treatment such as *Bacillus subtilis* (Bs), *Pseudomonas fluorescens* (Pf), *P. fluorescens-B. subtilis* combination (PfBs) and control

The different letters show the differences between each treatment by 5% LSD

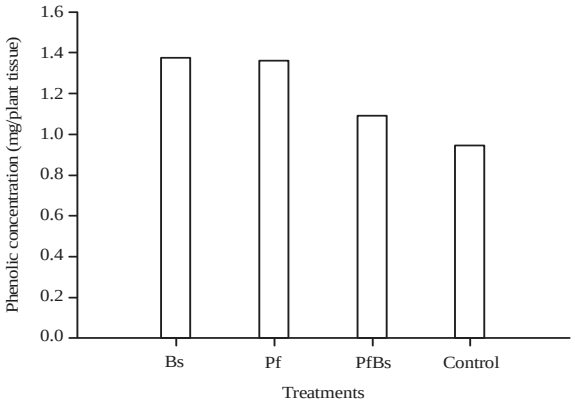

Fig. 2: Concentration of phenolic compound contained in the plant tissue which treated by each treatment such as *Bacillus subtilis* (Bs), *Pseudomonas fluorescens* (Pf), *P. fluorescens-B. subtilis* combination (PfBs) and control

all applications of PGPR such as *P. fluorescens*, *B. subtilis* and combination of both produced shorter period of first instar than control (Fig. 5).

Figure 4a shows that oviposition of *P. xylostella* affected by all treatments of PGPR. Application of single species of PGPR reduced oviposition better than combination application. *Pseudomonas fluorescens* produced the lowest oviposition. There will be several reasons such as phenolic compounds as deterrent for oviposition activities of herbivores.

Related to deterrent activities of oviposition, wax content is also important variable to describe oviposition deterrent of

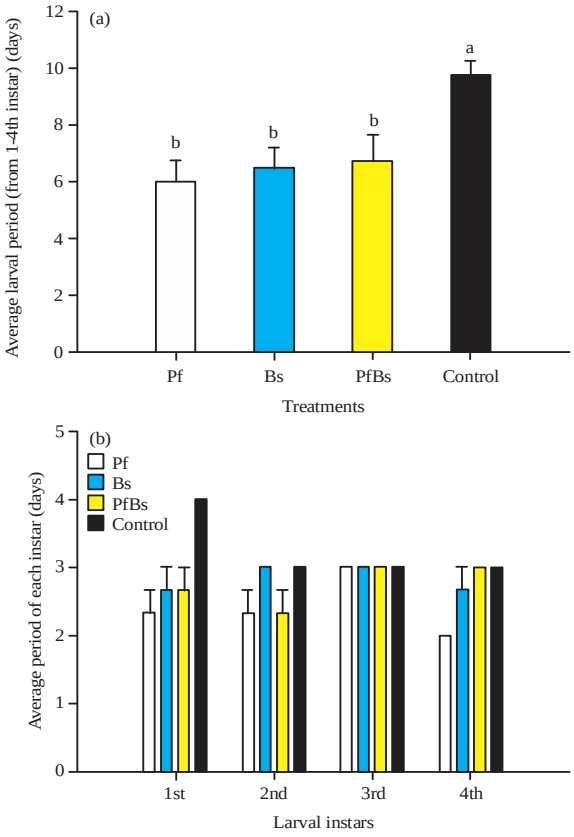

Fig. 3(a-b): (a)Average larval period and (b) Each larval instar on each treatment such as *Bacillus subtilis* (Bs), *Pseudomonas fluorescens* (Pf), *Bacillus subtilis-Pseudomonas fluorescens* combination (PfBs) and control

The different letters show the differences between each treatment by 5% LSD

P. xylostella. Based on Fig. 5, *P. fluorescens* produced the highest wax content than the control for all PGPR treatments followed by *B. subtilis*.

DISCUSSION

Generally, application of PGPR cause the changes on DBM responses such as feeding and oviposition and reduce the period of larva. Phenolic and wax compounds were produced higher than control for all PGPR treatments and it's related to the changes of DBM responses.

Related to the feeding activities, according to Rashid and Chung[15], soil microorganisms such as rhizobacteria can increase plant health in a variety of different ways and these microorganisms may build broad-spectrum resistance to insect herbivores. Biosynthesis of secondary metabolites and plant defensive

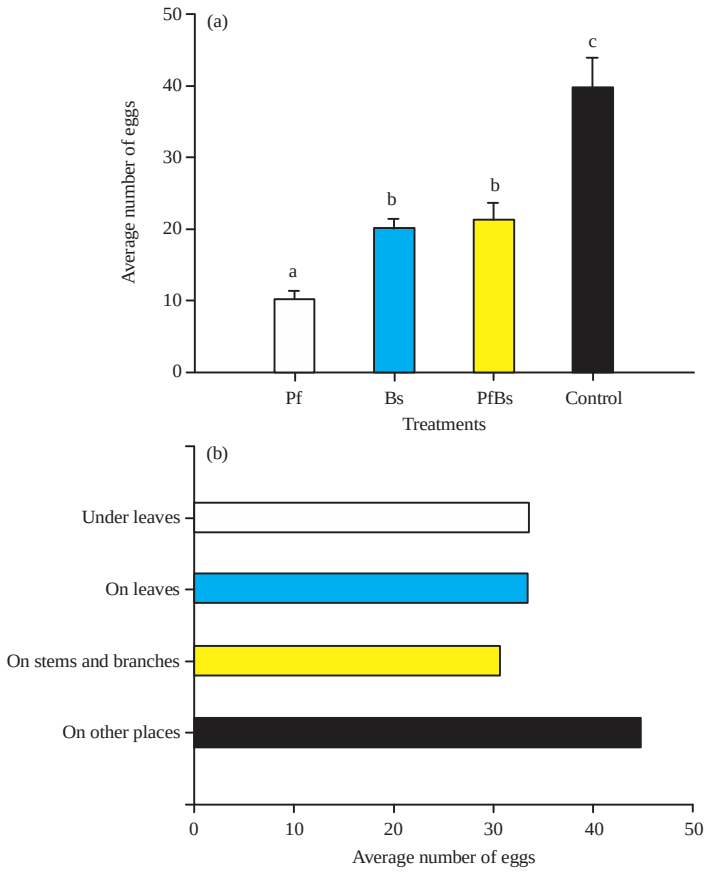

Fig. 4(a-b): Average number of *Plutella xylostella* eggs based on oviposition between each treatment such as B*acillus subtilis* (Bs), *Pseudomonas fluorescens* (Pf), *P. fluorescens-B. subtilis* combination (PfBs) and control, (a) Number of eggs based and (b) On oviposition sites

The different letters show the differences between each treatment by 5% LSD

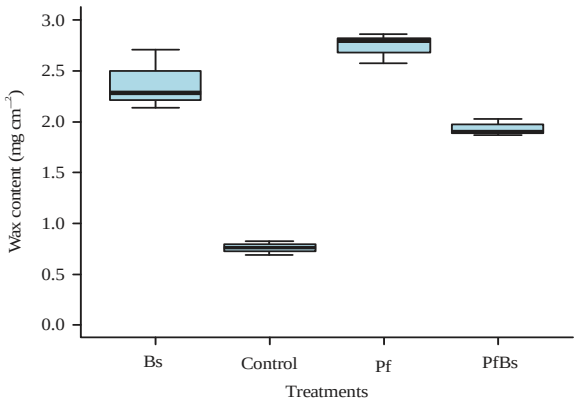

Fig. 5: Wax contents of leaves on each treatment such as *Bacillus subtilis* (Bs), *Pseudomonas fluorescens* (Pf), *P. fluorescens-B. subtilis* combination (PfBs) and control

proteins can be regulated by rhizobacteria that may induce defenses against leaf-chewing herbivores[15]. Kordan and Gabrys[16] reported that chemical substances that effected behavior and physiology of herbivores can be divided into highly active deterrents (α-phellandrene and β-ionone), strong deterrents [α-terpinene and α-ionone], relatively strong deterrents (citronellol, (-)-linalool, p-cymene), moderate deterrents [(+)-fenchone, (+)-R-limonene, γ-terpinene and (S)-(+)-carvone] and inactive substances (α-pinene, eucalyptol, bornyl acetate, geraniol, thymol and L-menthol). As the plant secondary metabolites, phenolic compounds such as coumarins, tannins, flavonoids and phenolic acids are commonly included in interaction between plant and insect[17]. Amount of defense metabolites such as phenolic compound is commonly produced by slow-growing species of plant, however, fast-growing species also produce with low concentration[18]. Phenolic compounds are needed by plant for growth, resistance to pathogens and many other functions[19]. According to Kumar *et al.*[20], phenolic compounds play a major role in plant defense system to plant pathogens and pests. *Pseudomonas fluorescens* and *P. aeruginosa* applied on leaves can reduce the powdery mildew pathogen

Erysiphe pisi, on pea (*Pisum sativum*) by increased accumulation of phenolics[21]. It also happen for insect herbivores, decrease in phenolics and total tannins can increase leaf area lost to herbivores [22].

Related to period of larva, flavonoids and other phenolic compounds in *Alibertia intermedia* and *Alternanthera sessilis* gave harmful effects on the *P. xylostella* life cycle[23]. On *Aedes aegypti* (Diptera: Culicidae), secondary metabolite fractions i.e., alkaloid, phenolics and terpenoid caused mortality at larval and pupal stages[24]. In addition, the effectiveness of phenolics as a resistance factors to insect feeding is increased by oxidation to polymers, which reduce nutritional value, palatability and digestibility[20].

Plant phenolics act as deterrent, repellent and enzyme inactivator for insect herbivores[20]. The oviposition deterrent effects of four phenolic compounds i.e., quercetin, rutin, gallic acid and tannic acid were investigated against the melon fruit fly, *Bactrocera cucurbitae*. All the phenolic compounds effectively reduced egg laying in choice and no-choice conditions except rutin[25]. In addition, deterrent effect was shown by evident that *P. xylostella* put eggs on the other places compared to leaf more than 40 eggs (Fig. 4b). Phenolic compounds produced by plant was distributed systematically in all parts of plant include leaves and stem. Secondary metabolites produced by most plants are toxic to plant pathogens and pests, either as part of their normal program of growth and development or in response to biotic stress[20].

According to Bennett and Wallsgrove[26], deterrent oviposition can cause physical defense mechanisms, such as cuticular waxes, leaf hairs, thorns and barbs, secondary thickening and other structural factors recognized to protect plants. In this research, cuticular wax was described by wax contents. It is closed to be evident that wax content related to the physical defense mechanism for *P. xylostella*. The higher content of wax of leaves resulted in the lower oviposition activities. Larval survival on normal wax genotypes of *Brassica* is about 50% of survival on susceptible[27]. In neonate larvae, glossy leaf waxes apparently elicit nonacceptance behaviors which effect in their failure to successfully establish on plants. The development of *Brassica oleracea* cultivars resistant to diamondback moth will be facilitated by knowledge of resistance mechanisms[27].

CONCLUSION

It is concluded that the application of PGPR changed DBM responses such as feeding and oviposition and decrease the period of larva. High concentrations of phenolic and wax compounds were produced for all PGPR treatments and it's related to the changes of DBM responses.

SIGNIFICANCE STATEMENT

This study provides the information related to the beneficial responses of DBM on Chinese kale crops. Application of PGPR can stimulate plant defense mechanisms by reducing of feeding and oviposition of DBM and it is related to phenolic and wax contents.

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