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Research Article

Biology and the Statistic Demographic of *Aphis glycines* Matsumura (Hemiptera: Aphididae) on the Soybean with Plant Growth Promoting Rhizobacteria (PGPR) Treatment

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ABSTRACT

Plant Growth Promoting Rhizobacteria (PGPR) applied to different plants may suppress pests population developments.This research was to study the capability of a commercial PGPR product contained *Bacillus polymyxa* and *Pseudomonas fluorescens* in suppressing population developments of *Aphis glycines* Matsumura (Hemiptera: Aphididae). The biology and demographic statistics of *A. glycines* reared on soybean with and without the PGPR applications were compared. The PGPR suspensions of 5 g formulation per liter water were used to soak soybean seed for 15 minutes and to water soybean plant 2 weeks after transplanting. Cohorts of 65 first instar *A. glycines* of each treatment were observed daily and individual mortality, molting, and fecundity were recorded until the last individual dead. Second instar stadium of *A. glycines* reared on treated plant lasted longer than those reared on untreated plant, i.e. 1.4 and 1.1 days, respectively. These resulted on a longer life cycle for *A. glycines* reared on treated plant than on untreated plant, i.e. 4.9 and 4.5 days, respectively. In turn, it caused the *A. glycines* population to experienceslower growth on treated plants than on untreated plants. The values of *A. glycines* GRR, Ro, rm, T and DT on treated plants were 71.834, 57.780, 0.557, 7.287 and 1.245, consecutively; whilst that of untreated plants were 104.861, 63.326, 0.586, 7.084 and 1.184, respectively.

Keywords: *Aphis glycines*; *Bacillus polymyxa*; PGPR; *Pseudomonas fluorescens*

INTRODUCTION

Indonesia heavily relies on soybeans as a source of food, feed for livestrocks, or raw materials for industries. Indonesia's soybean production has fluctuated in five years between 2013–2018. Although Indonesia's soybean production has been reported to reach approximately 982,598 ton in 2018, it was still not sufficient for domestic needs which was predicted to reach 2.8 million tonnes per year (*Pusat Pengkajian Perdagangan dalam Negeri*, 2019). Production fluctuation of soybean is caused by several factors including damage caused by plant pest and disease.

Aphis glycines (Hemiptera: Aphididae) is a major pest on soybeans. *A. glycines* damage plant directly and indirectly by sucking leaf and stem fluids and decreasing quality and quantity of soybean productions. In addition, *A. glycines* cause indirect damage by being vectors of Soybean Mosaic Virus (SMV) (Widariyanto *et al.*, 2017).

Soybean yield loss by *A. glycines* has been reported to reach 58% (Wang *et al.,* 1994); thus, *A. glycines* populations must be managed to stay below economic thresholds. Intergated Pest Management (IPM) is a pest management method based on environmental knowledge and is declared to be the main pest management approach in Indonesia. Good agricultural practice in maintaining plants' health is the main component in IPM, since healthy crops are more resistant to pest damage. Plant growth promoting rhizobacteria (PGPR) is a method to increase plant resistances, using material that consisted of rhizophere microorganisms with activities that benefit crops (Kafrawi *et al.*, 2015).

PGPR can induce plant resistances to suppress pest populations (Soesanto, 2008). Resistances is the plant's ability to harm pest and withstand pest attacks. Induced resistances on vegetative tissues will disturb feeding process and pest's lives. Disturbed feeding will then affect the growth, development, and reproduction of the pest. Therefore, plant resistance is a limiting factor in the development of pest populations (Hutasoit & Sitanggang, 2018). Latifah *et al.* (2018) reported lower *Bemisia tabaci*

and leaf spot incidences on tomatoes treated with PGPR compared to the untreated control. Rhizobacteria, component in a PGPR, can affect the interactions between plants and insects. Rhizobacteria are able to increase nutrition uptakes in plants causing an increase of food quality for insects. This is an example of induced tolerance due to rhizobacteria (Rashid & Chung, 2017).

The effects of PGPR on the survivorship and fecundity of *A. glycines* can be evaluated using life tables. Life tables can provide information on natality, development, reproduction, and mortality of each individual in a population. This study aimed to observe the ability of a commercially available PGPR product, containing *Bacillus polymyxa* and *Pseudomonas fluorescens,* in suppressing *Aphis glycines* Matsumura (Hemiptera: Aphididae) populations.

MATERIALS AND METHODS

This study was done at the Department of Plant Protection, Faculty of Agriculture, IPB University, Dramaga between January–April 2013.

A. glycines *Rearing*

Soybean seeds, of Grobogan variety, were used for *A. glycines* rearing and planted in 15 30× 30 cm polybags filled with soil and compose (2:1) at rates of 4 kg/polybag, compound fertilizer (containing nitrogen, phosphate, and potassium) 16-16-16 at rates of 0.5 g/polybag. Each polybag contained 6 soybean seeds and was watered every day. Initial populations of *A. glycines* were obtained from soybean fields located in Megamendung, Bogor. *A. glycines* were infested onto two-weeks-old soybean plants and allowed to reproduce (Figure 1). Soybeans were closed with plastic cylinders and cloth mesh were placed on top. *A. glycines* were allowed to reproduce until required numbers were reached.

Effect of PGPR on A. glycines *Biology*

All surviving *A. glycines* were observed everyday to check whether individuals were alive or dead, molted (indicated by exuviae), and a count of the number of nymphs was done. *A. glycine*s life cycles were observed since 1st instar nymphs were infested onto plants until they reach imagoes. *A. glycines* went through 4 nymph instars before reaching imago. Development between instars were indicated by the existing of exuviae. Pre-oviposition stages were assumed since individuals reached imagoes until the first offspring were produced. Imago life time was counted since individuals reached imagoes until mortality. Fecundity were obtained from the number of nymphs produced by each *A. glycines* during its lifetime. Observation data were organized into a biological life table of *A. glycines*. Observations included the length of the 1st, 2nd, 3rd, and 4th nymph instars, life cycle, pre-oviposition, life length, and fecundity on PGPR treated and untreated plants.

A. glycines *Cohort Rearing*

Treatments used in this experiment were a PGPR treated and untreated control with 65 replications for each treatment. As much as 240 soybean seeds, variety Grobogan, were wash using clean water and air-dried on sterile paper for 15 minutes. Half of the seed batch were treated using PGPR and the other half was untreated as a control. The PGPR used in this experiment was Rhizomax®. Its formulation was powdery and contained the active ingredients of *B. polymyxa* dan *P. Fluorescens*. PGPR suspension were made form 50 g of PGPR products mixed into 5 L of sterilized water. Soybeans seeds used for the treated treatment were immersed in the PGPR

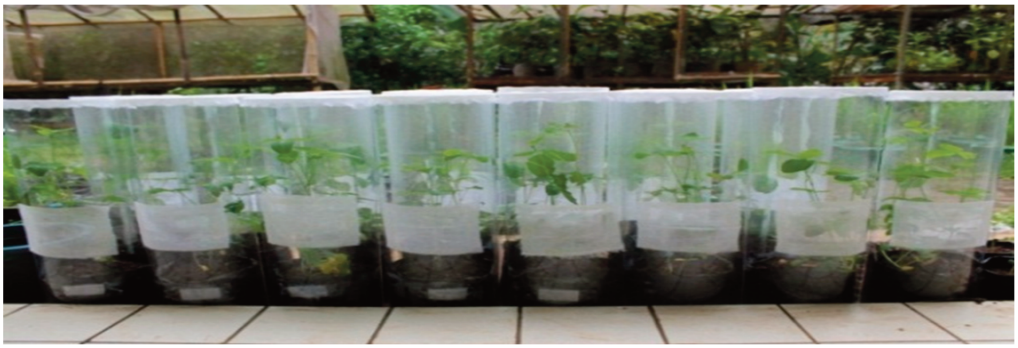

Figure 1. Two-week-old soybean plants *Aphis glycines* rearing

suspension for 15 minutes, while untreated seeds were immersed in sterile water. Seeds were than air-dried for 15 minutes. Soybean seeds were planted in 60 30 × 30 cm polybags. As much as 150 mL of the PGPR suspension remains was watered onto PGPR treated soybean plants, while sterile water was treated on the untreated control. This same watering treatment was done when soybeans reached 2 weeks-old.

Soybean seeds used to feed *A. glycines* imagoes and produce 1st instar nymphs were planted in 25 200 mL plastic cups filled with 200 g of growing media consisting of soil and compose (2:1). Two soybean seeds were planted in each cup. Soybean plant that have reached 7 days old were covered with plastic cylinders with cloth meshes on their top as previously mentioned (Figure 2). The following day after plants were covered, two *A. Glycines* imagoes were placed on plants and newly produced 1st instar nymphs were obtained the next day.

First *A. glycines* nymphs were infested on 3-week-old soybean shoots that have been treated or not treated with PGPR. Plants were covered with plastic cylinders with the top and bottom covered with cloth meshes and polybags were placed on top of black cardboard.

Life Tables and Demographic Statistics of A. glycines

Surviving individual were counted every day to obtain survivorship data (l_x) of *A. glycine*s. Daily fecundity (m_x) were calculated from the average nymphs produce by each imago at every stage (x). Survivorship and daily fecundity were organized into curves and life tables were obtained. Life table of cohorts are the life tables that records the development of each cohort by recording the survivorship of individuals until mortality of all individuals (Begon *et al*., 2006). Life table parameters of 1 generation of *A. glycine* were divided into 2-week periods starting from week 1 until week 37 (Price, 1997; Wilson & Bossert, 1971). Insect's demographic statistics according to Zeng *et al*., (1983) are quantitative analytic parameters of insect populations regarding to its survivalship, fecundity, and population growth patterns. These parameters include:

1. Gross Reproduction Rate (GRR) = $\sum m_x$
2. Net Reproduction Rate (Ro) = $\sum l_x m_x$
3. Intrinsic addition rate (r_m) = $\sum l_x m_x e^{-rmx} = 1$
4. Average Generation Length (T) = $(\ln Ro)/r_m$
5. Doubling Time (DT) = $\ln (2)/r_m$

Net reproduction rates (Ro) is the average offspring produces by each imago (Begon *et al*., 2006).

Data Analysis

Data variances were processed using Microsoft Excel 2007 and analyzed using a two-sample t test at $\alpha = 5\%$ using Minitab 16.

RESULTS AND DISCUSSION

The Effect of PGPR Treatment on A. glycines *Biology*

Results showed that life cycles of *A. glycines* were significantly different between the two treatments. Life cycles of *A. glycines* were longer on plants treated with PGPR compared to ones reared on untreated plants. This may be explained by the results from a study by Hutasoit & Sitanggang (2018)

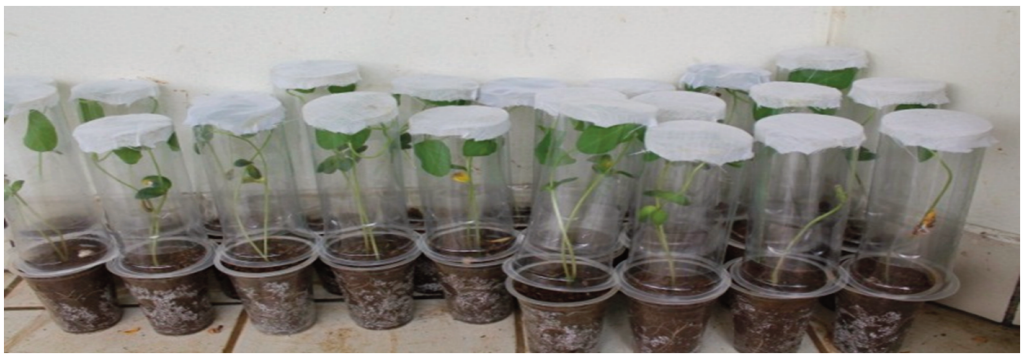

Figure 2. Soybean plants in plastic cages used to supply 1st instar *Aphis glycines* nymphs

which showed that PGPR applications were able to affect the development time of 1st and 2nd instar nymphs, pre-pupa, and *T. parvispinus* imagoes. According to Tétard-Jones *et al.* (2012), PGPR application had indirect effects on aphid that caused development times to be longer. Longer life cycles and time required to reach imago stages will directly delay individual to reproduce, which is an important factor for insects to successfully infest plants. Kozlowski (1992) stated that delays of individuals to reach reproduction stages will increase mortality before reproduction, length of reproduction stages, offspring numbers and longer generation length.

Life time of *A. glycines* were not significantly different between treatments at all life stages, except 2nd nymph instars (Table 1). *A. glycines* molt 4 times during its development into imagoes. Development time of 2nd instar *A. glycines* on untreated plants were significantly lower compared from insects on PGPR treated plants causing individuals taking longer to reach imago stages. PGPR application may inihibit fluid sucking by *A. glycines* causing nutrient deficiency of individuals and hinder development. Research by Fahimi *et al.,* (2013) demonstrated that PGPR application significantly affected 1st and 3rd instar, but not significantly affect 2nd and 4th instar *A. gossypii*.

Short life time will affect fecundity. Fecundity and the length of pre-oviposition of *A. glycines* were not significantly different between the two treatments (Figure 3). Food affect growth, development, fertility, mortality, and fecundity of insects (Begon *et al.,* 2006).

Survivorship and mortality of *A. glycines* were similar between the two treatments used in the experiments (Figure 4). Survivorship of *A. glycines* from untreated plants reached 37 days, while 25 days on PGPR treated plants. Rhizobacteria possess antagonistic properties that suppress growth of phytopathogens by competing for nutrient and habitat,

Table 1. *Aphis glycines* biologi on untreated and PGPR treated soybean plants

Stage	Control (days)	PGPR (days)
1st Instar	1.2	1.3 ns
2nd Instar	1.1	1.4 *
3rd Instar	1.1	1.1 ns
4th Instar	1.1	1.1 ns
Life cycles	4.5	4.9 *
Pre-oviposition	0.4	0.2 ns
Life length	14.6	14.1 ns
Fecundity	66.6	65.3 ns

Information: ns = no significances, *treatments were sigficantly different based on t-test at α = 5%.

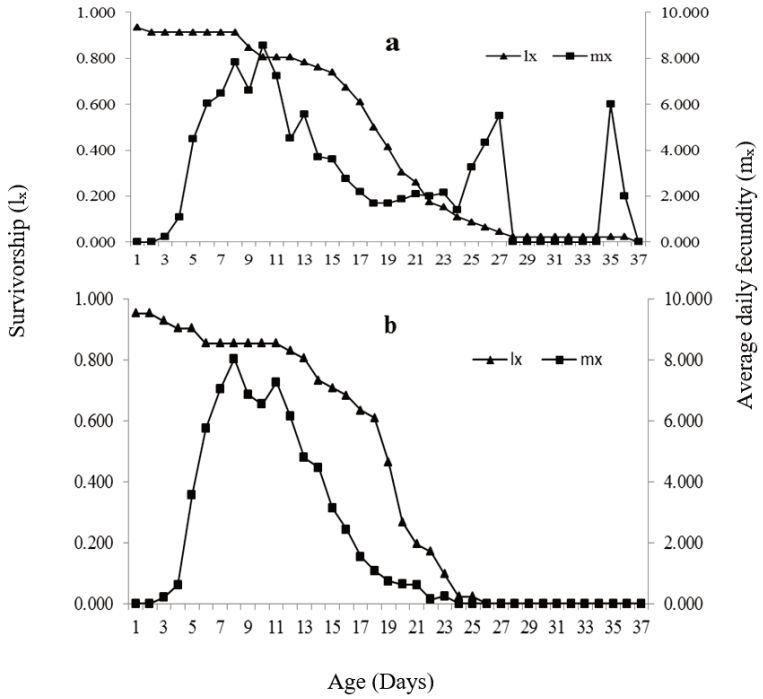

Figure 3. Survivalship and daily fecundity of *Aphis glycines* on untreated plants (control) (a) and PGPR treated soybeans (b)

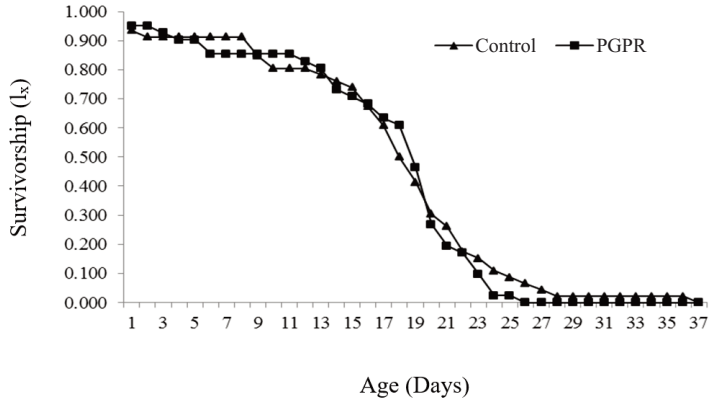

Figure 4. Survivalship of *Aphis glycines* on untreated and PGPR treated soybean plants

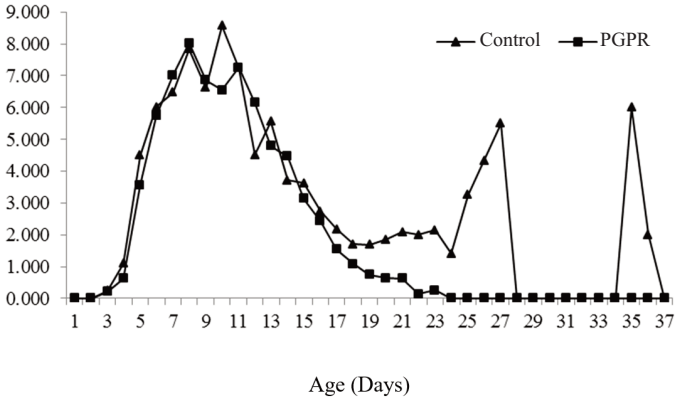

Figure 5. Daily fecundity of *Aphis glycines* on untreated and PGPR treated soybean plants

supply Fe/iron to plants, produce lytic enzymes, and antibiotic properties (Jing *et al*., 2007). This shows that PGPR are able to induce plant resistances and accelerate *A. glycines* mortality.

A. glycines survival type can be categorized as type I. Many nymphs were produced daily based on the fecundity curves (m_x) (Figure 5). In some occasions, there were *A. glycines* nymphs that were reared on both treatmetns that were able to produce offsprings. Food availability is an extrinsic factor that affect developmental time and reproduction of insects. Fecundity curve (m_x) continually increased after *A. glycines* individuals reach imagoes stage.

The highest fecundity reached 8.5 nymphs in one day was from untreated soybean plants and eight nymphs on PGPR treated soybean plants. These daily fecundities occurred peak several times on populations reared on untreated soybean plant, while from populations reared on PGPR treated plants only occurred once. This demonstrated that PGPR treatments were able to reduce the occurrences and level of maximum daily fecundity of *A. glycines*.

The Effect of PGPR on A. glycines *Demographic Statistics*

The value of *A. glycines* GRR on untreated soybeans was larger than ones treated with PGPR, reaching 104.861 and 71.834 individual/generation respectively (Table 2). The number of female individuals produced by female imagoes (Ro) increased on untreated plants. Ro values from populations reared on untreated plants imply that the next *A. glycines* generation will increased by 63.326 folds from the previous generation and by 57.780 fold on PGPR treated plants. High GRR and Ro values indicate suitability of host for insects (Hidayat *et al.*, 2019). The value of rm are related to mortality, natality, and developmental time of an organism. On untreated plants, rm value was 0.586 nymphs/day in optimum environmental conditions and unlimited resources and 0.557 on PGPR treated plants. Longer life cycles of *A. glycines* reared on PGPR treated soybean plants causes lower intrinsic growth rate compared to populations reared on untreated plants. Intrinsic growth rate can be used to predict insect

Table 2. *Aphis glycines* demographic statistics on untreated and PGPR treated soybean plants

No.	Parameter	Treatment	
		Control	PGPR
1.	Gross reproduction rates (GRR)	104.861	71.834
2.	Net reproduction rates (Ro)	63.326	57.780
3.	Intrinsic growth rates (rm)	0.586	0.557
4.	Average generation lenght (T)	7.084	7.287
5.	Doubling time (DT)	1.184	1.245

population growth for a certain period and compare reaction of populations to temperature, humidity, nutrient levels, or secondary metabolites from plants (Hutasoit & Sitanggang, 2018; Havlickova, 1987). High rm values indicate that populations may continuously increase (Gill *et al*., 1989).

Doubling time of *A. glycines* reared on untreated plants was 1.184 days and 1.245 days from populations reared on PGPR treated plants. Low DT values may increase GRR and Ro (Efendi *et al*., 2018). The value of rm and DT can explain population growth in constant environmental condition and unlimited resources (Price, 1997; Southwood & Henderson, 2000).

PGPR is a soil rhizosphere microbes, which may increase plant growth and its resistances against pest and disease. Application of PGPR will directly affect plants by increasing the availability and mobilization or facilitate nutrients absorption, synthesize and alternate concentrations of various phytohormones that induce growth, and indirectly affect plants by suppressing activities of pest and disease by producing compounds and metabolites, such as antibiotics and siderophore, and systemically induce plant resistances (Zainudin *et al.,* 2014; Walida *et al.*, 2018).

The PGPR bacterial group *Bacillus* sp. and *Pseudomonas* sp. are the most studied genus due to their potential as biocontrol agents (Manik *et al*., 2018). Antibiosis is a suppressing mechanism used by the *Bacillus* sp. and *Pseudomonas* sp. Antibiosis is a PGPR mediated resistance mechanism against insect on plants that produces allelochemicals, such as chitinase enzymes, hydrogen cyanide (HCN), and siderophore. These allelochemicals have been reported to suppress reproduction, modify physiology, delay matureness, and induce physical behavior or abnormality on insects that eventually inhibit abundances of insect pest (Tuhuteru *et al*., 2019; Disi *et al*., 2019).

CONCLUSION

PGPR containing *B. polymyxa* and *P. fluorescens* were able to suppress *A. glycines* populations. Applications of PGPR caused longer development of 2nd instar nymphs and eventually causing longer life cycles. This caused populations to grow slower than the untreated control.

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