ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Pathogenicity of Several Fungal Entomopathogenic Species against *Aphis glycines* (Homoptera: Aphididae) in South Sulawesi, Indonesia.

Ahwiyah Ekawaty Said, and Andi Nasruddin*.

Department of Plant Pests and Diseases, Faculty of Agriculture, Hasanuddin University, Makassar 90245, Indonesia

ABSTRACT

The goal of the study was to determine pathogenicity of three locally isolated fungal entomopathogens: *Paecilomyces fumosoroseus*, *Fusarium* sp., and *Beauveria bassiana* against the soybean aphid (*Aphis glycines* Mats.). The virulence of the entomopathogens was determine by applying different conidial concentrations (10^4, 10^5, 10^6, 10^7, and 10^8 conidia/ml aquadest) to the aphid colonies on soybean plants in the laboratary. The most virulent isolates were *P. fumosoroseus* (Pspg and Pwajoisolates) with LC_{50} of 2.3 x 10^5 and 2.6 x 10^5 conidia/ml, and with LT_{50} of 3.7 and 3.9 days, respectively. The next most virulent isolates was *Fusarium* sp. (Fspg and Fwajo isolates) with LC_{50} of 1.8 x $10^7$and 1.9 x 10^7 conidia/ml, and with LT_{50} of 4.9and 4.8days, respectively. The least virulent isolates were *B. bassiana* (Bspg and Bwajo isolates) with LC_{50} of 3.4 x 10^8 and 4.2 x 10^8 conidia/ml, with LT_{50} of 5.3 and 5.4 days, respectively. The numbers of aphids in all treatments were significantly lower than in the control. There was an overall trend showing that the lowest number of aphids were found on plants treated with *P.fumosoroseus* followed by *Fusarium* sp., and *B. bassiana*. Thus, *P. fumosoroseus* was the most potential amongst the entompatohegens tested against *A. glycines*.

Keywords: *LC50, LT50, entomopathogen, Aphis glycines*, virulence

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Soybean aphid, *Aphis glycines* Matsumura (Homoptera: Aphididae), is an important insect pest of soybean that can cause yield loss up to 50% in the absence of control measures ([1]. The insect can directly and indirectly damage plants by sucking plant saps and transmitting plant viral diseases, respectively. High infestation of soybean aphid can cause physiologic stress to plants [2] and substantially reduce plant height, pod number, seed size and quality, and yield [3]. In addition, this aphid also vectors several plant viral diseases, such as soybean mosaic virus (SMV) and alfalfa mosaic virus (AMV) [4] and [5].

To control the aphid, soybean growers rely heavily on insecticide use. Excessive use of insecticides can induce the development of insect populations resistant to the insecticides. In addition, insecticides can also kill non-target organisms and leave hazardous residues on agricultural products [6]. Alternative control measures, which are not only effective against the insect but also safe to non-target organisms and the environment, must be sought. The use of entomopathogenic fungi to control insect pests is promising to fulfill the criteria. Secondary metabolites of several fungal species have been proven to be effective against insect pests [7]. For examples, *Fusarium* spp. produce secondary insecticidal metabolites, such as trichothecenes [8], fumonisins, and beauvericin (BEA) [9]. Some other entomopathogenic fungi also produce BEA, such as *Beauveria bassiana* (Balsamo) Vuill. and *Paecilomyces fumosoroseus* (Wize) Brown & Smith [9].The purpose of the current study was to determine the pathogenic activities of *Fusarium* sp., *P. fumosoroseus*, and *B. bassiana*, against *A. glycines*. The entomopathogenic fungi were isolated from different soybean—producing areas in South Sulawesi, Indonesia.

MATERIALS AND METHODS

Insect Colonies

Colony of *A. glycines* was originally collected from soybean plants in Sub-district of Simbang, Maros District, South Sulawesi Province of Indonesia. The aphid was mass-reared on soybean cv. Mahameru grown in several plastic pots (20 x 20 x 20 cm), placed in a cage (1 x 1 x 1 m) in a greenhouse (27 ±1.2^{o}C, 12L : 12D). The caged plants were replaced with new plants as necessary to sustain enough test insects throughout the experiment.

Fungal Isolates

Isolates of *Paecilomyces fumosoroseus*(Wize) Brown & Smithand *Beauveria bassiana(Bals.-Criv.) Vuill.* were obtained from *Bemisia tabaci*Genn. And *Fusarium* sp. was obtained from *A. glycines* I nfesting soybean in two major soybean-producing areas in South Sulawesi, Districts of Soppeng and Wajo. Two isolates of each entomopathogen were used in this experiment (Table 1).

Table 1: Isolates of entomopathogenic fungi isolated from infected *B. tabaci* dan *A. glycines* collected from the Districts of Wajo and Soppeng

Species	Isolate	Insect Host	Host Plant	Location
B. bassiana	Bspg	*A. glycines*	Soybean	Panincong, Soppeng
	Bwajo	*A. glycines*	Soybean	Sabbangparu, Wajo
Fusarium sp.	Fspg	*A. glycines*	Soybean	Panincong, Soppeng
	Fwajo	*A. glycines*	Soybean	Sabbangparu, Wajo
P. fumosoroseus fumosoroseus	Pspg	*B. tabaci*	Soybean	Panincong, Soppeng
	Pwajo	*B. tabaci*	Soybean	Sabbangparu, Wajo

Preparation for Spore Suspension

Infected soybean aphid and sweet potato whitefly individuals collected from soybean fields in the Districts of Wajo and Soppeng were surface sterilized using 0.5% sodium hypochlorite (NaOCl) . The insects

were individually immersed into the 0.5% sodium hypochlorite (NaOCl) contained in separate beaker (10 ml) for 5 seconds and then placed on a piece of filter paper for 15 minutes to dry under the room temperature. The insects were then individually placed in a Petri dish (9 mm diam) with a potato dextrose agar (PDA) growth medium. The entomopathogen cultures were purified by transferring a single spore into new growth medium. Thirteen to 15 days after the initiation of the culture, the conidia were harvested by gently scraping the surface of the culture using a spatula. The spores were then added into a 20-ml beaker containing 10 ml of sterile distilled water. The conidia suspension was manually agitated using a glass rod for 5 minutes. Spore concentration (conidia per milliliter of suspension) of the stock was determined by using a hemocytometer. The stock suspension was diluted to prepare a series of suspension ranging from 10^4 to 10^8 conidia/ml.

Virulence of the Entomopathogens

Two weeks old soybean seedlings were individually transplanted in 10 x 10 x 6 cm pots. All leaves but two fully expanded were removed. Twenty adult apterae were transferred onto each plant using a fine camel brush. Multiple dosages of entomopathogen were tested to determine their virulence against the soybean aphid. Conidia of each entomopathogen were prepared in five different concentrations: 10^4, 10^5, 10^6, 10^7, dan 10^8 conidia per ml of sterile distilled water. Each concentration treatment had five replications of a plant each. The leaves were sprayed with respective treatment of conidia concentration using a perfume sprayer until run off (spray volume

3 ml/leaflet). Aphids sprayed with the same amount of water without conidia served as control. After the spraying, the plants were then covered with 1.5-l coke bottles. To prevent the formation of water vapor inside of the bottle, four holes (3 x 2 cm) were made on the sides of the bottle. The holes were covered with fine screen cloth to prevent the aphids from escape. The bottles were then placed in room temperature with L:D = 12:12. The numbers of dead insects were determined every 24 h for seven days. The virulence of the entomopathogens (LC_{50} and LT_{50}) was calculated using a statistical package of Biostat [10].

Population Suppression

Three species of fungal entomopathogen (*P. fumosoroseous*, *Fusarium* sp., and *B. bassiana*) were tested to determine their effectiveness in suppressing the aphid population in greenhouse. All entomopathogens were applied using a single conidial concentration (10^6 conidia/ml) and a control applied with sterile distilled water only. Twenty newly formed adult apterae of *A. glycines* were transferred onto a 3-week old soybeanplant cv. Mahameru planted in a 10-cm diameter pot covered with a 1.5 plastic bottle cage as previously described. One week later, each entomopathogen was sprayed to five plants containing the test aphids and each of the plants was considered as a replicate in this experiment. Living aphids (adults and nymphs) on each plant were counted every day for seven days. Aphid counts were transformed using log (x + 1) before they were subjected to an analysis of variance (ANOVA). When a significant difference was detected, the treatment means were separated using a Duncan's multiple range test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Virulence of the Entomopthogens

All three entomopathogenic fungal species tested were pathogenic and causing mortality to the soybean aphid. Virulence of the isolates belonging to the same species was not significantly different (Table 2).

Pwajo and Pspg isolates of *P. fumosoroseus* had LC_{50} of 2.3 x 10^5 and 2.6 x 10^5, respectively; and LT_{50} of 3.9and 3.7 days, respectively; and they were not significantly different each other ($P > 0.5$). Pwajo and Pspg isolates of *Fusarium* sp. had LC_{50} of 1.8 x $10^7$and 1.9 x 10^7, respectively; and LT_{50} of 4.8and 4.9 days, respectively; and they were not significantly different each other ($P > 0.5$). *B. bassiana* isolates of Pwajo and Pspg had LC_{50} of 3.4 x $10^8$and 4.2 x 10^8, respectively; and LT_{50} of 5.3and 5.4 days, respectively; and they were not significantly different each other ($P > 0.5$).

ISSN: 0975-8585

Table 2: Virulence (LC_{50} and LT_{50} values) of entomopathogenic fungi, *P. fumosoroseus*,*Fusarium sp.*, and *B. bassiana*against *A. glycines* adults

Fungal Species	Isolate	No. insects[a]	LC50 (95% C1)[b]	LT50 (days)
P. fumosoroseus	Pspg	500	$2,3 \times 10^5$ ($1,6 \times 10^4$ - $1,2 \times 10^6$)	3,7 (3,6 - 5,1)
	Pwajo	500	$2,6 \times 10^5$ ($1,4 \times 10^4$ - $1,3 \times 10^6$)	3,9 (3,5 - 4,9)
Fusarium sp.	Fspg	500	$1,8x\ 10^7$ ($2,1 \times 10^6$ - $3,0 \times 10^8$)	4,9 (3,8 - 6,3)
	Fwajo	500	$1,9 \times 10^7$ ($2,4 \times 10^6$ - $3,2 \times 10^8$)	4,8 (4,0 - 5,2)
B. bassiana	Bspg	500	$3,4 \times 10^8$ ($2,7 \times 10^8$ - $3,0 \times 10^9$)	5,3 (4.2 - 5.6)
	Bwajo	500	$4,2 \times 10^8$ ($2,1 \times 10^8$ - $3,0 \times 10^9$)	5,4 (4,0 - 6,1)

'Number of adult aphids tested.

[b]LC_{50} expressed in number of conidia per ml of water.

Overlapped 95% CI values indicating no significance difference in LC_{50} or LT_{50} at 5% level

Smaller values of LC_{50} and LT_{50} of an entomopathogen indicate higher virulence against its insect host. A smaller value of LC_{50} mean less active ingredient is needed to inflict 50% mortality to the insect target. Similarly, a lower value of LT_{50} mean less time is requiredto kill 50% of a target population [11]. Thus, the most virulent entomopathogen to *A. glycines* was *P. fumosoroseus* followed by *Fusarium* sp.; and *B. bassiana* was the least virulent amongst entomopathogens tested in this study.

Population Suppression

The number of life soybean aphids per plant were not significantly different between isolates of the same species of fungus ($P > 0.05$). Therefore, the data for the isolates of the same fungal species were pooled (Fig. 1). Aphid population on untreated plants steadily increased and by day-7 the population has increased by about 150% compared to the initial population. On the other hand, there was a general trend reflecting that the aphid populations on the treated plants consistently diminished. Starting from 48 h after the application, in every observation, all treatments showed significantly lower populations than the control did. In all observations starting from 72 h after the application, the lowest population was found on plants treated with *P. fumosoroseus*, followed by *Fusarium* sp. and then *B. bassiana*. On the 7th day after application, population suppressions by *P. fumosoroseus*, *Fusarium* sp., and *B. bassiana* were 50%, 35%, and 22% of the initial populations. However, if compared to average aphid population in the control, population suppressions were 81, 76.4, and 71.6%, for *P. fumosoroseus*,*Fusarium* sp., and *B. bassiana*, respectively.

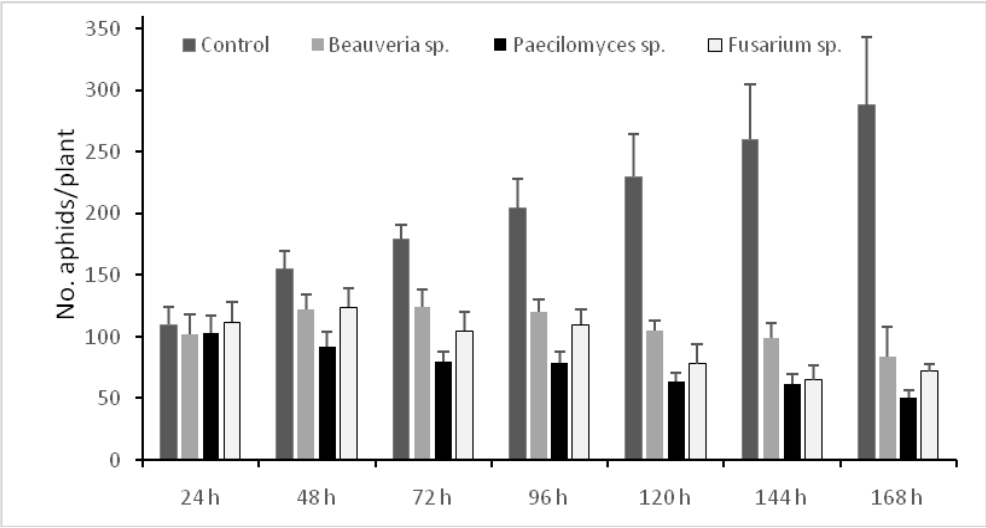

Fig 1: Average number of *A. glycines* per plant on the soybean plants applied with conidial suspension of *B. bassiana*, *P. fumosoroseus*, and *Fusarium* sp. at concentration of 10^6/ml. Data were recorded every 24 h for seven days. Bars are standard errors of means. Treatments showed significantly lower populations than the control did ($P < 0.05$).

Thus, the results showed that all species tested in this experiment were pathogenic against the soybean aphid with different levels of virulence. This agrees with previous reports showing that *P. fumosoroseous* and *B. bassiana* are pathogenic against the Russian wheat aphid, *Diuraphis noxia*, and the former was more virulent than the latter (Kurdjumov) [12]. In addition, *Fusarium semitectum* Berk and Ravenel was virulent against the tobacco aphid, *Myzus persicae* (Sulzer) [13]. Based on the LC_{50} and LT_{50} values, the most virulent entomopathogen in this trial was *P. fumosoroseous,* followed by *Fusarium sp*., and then *B. bassiana.* Similarly, the highest soybean aphid population suppression was provided by *P. fumosoroseous* followed by *Fusarium sp*., and then *B. bassiana.*

ACKNOWLEDGEMENT

We thank Muhammad Said Baco and Jumardi who provided technical assistance during the study. The study was financially supported by the Ministry of Research, Technology and Higher Education, Republic of Indonesia (KEMENRISTEKDIKTI, R.I), administered by the Institute of Research and Extension, Hasanuddin University, Makassar, under the International Collaborative Research and Publication Scheme (KLN) 2016.

REFERENCES

[1] Wang, X. B., V. H. Fang, S. Z. Lin, L. R. Zhang, and H. D. Wang. 1994. A study on the damage and economic threshold of the soybean aphid at the seedling stage. Plant Prot. 20: 12- 13

[2] Macedo, T. B., C. S. Bastos, L. G. Higley, K. R. Ostlie & S. Madhavan. 2003. Photosynthetic responses of soybean to soybean aphid (Homoptera: Aphididae) injury. J. Econ. Entomol. 96: 188–193.

[3] Ostlie,K. (2001). Soybean aphid reduces yields: Harvest results frominsecticide strip trials. University of Minnesota, St.Paul,MN.http://www.soybeans.umn.edu/crop/insects/aphid/studyresults.htm.

[4] Hill, J.H., R. Alleman, D.B. Hogg, and CR Gran. 2001. First report of transmission of soybean mosaic virus and alfalfa mosaic virus by Aphis glycines in New World. Paint Dis. 85:561.

[5] Wang RY, Kritzman A, Hershman DE, Ghabrial SA. 2006. Aphis glycines as a vector of persistently and nonpersistently transmitted viruses and potential risks for soybean and other crops. Plant Dis 90:920-926.

[6] Pedigo, L.P. 1989. Entomology and pest management. Macmillan Publishing Company, New York.

[7] Dowd P. F. 1992. Insect interactions with mycotoxin-producing fungi and their hosts. Handbook of Applied Mycology Vol. 5: Mycotoxins in Ecological Systems, D. Bhatnagar, E. B. Lillehoj, D. K. Arora. Marcel Dekker, New York; 137–155.

[8] Marasas, W.F.O., Nelson, P.E. and Tousson, T.A. 1984. "Toxigenic Fusarium Species: Identity and Mycotoxicology". University Park, Pennsylvania: Pennsylvania State University Press.

[9] Nelson, P.E., Plattner, RD., Shackelford, D.D. & Desjardins, AE. (1992) Fumonisin Bi production by Fusarium species other than F monilifonne ¡n section Liseola and by sorne related species. Appl. environ. Mierobiol., 58, 984-989.

[10] AnalystSoft, Inc. 2009. Statistical Analysis Program. Biostat.

[11] Tanada, Y. and Kaya, H. K.. 1993. Insect Pathology. Gulf Professional Publishing, 1993, 666 pp.

[12] Vandenberg, J.D. 2996. Standardized Bioassay and Screening of *Beauveria bassiana* and *Paecilomyces fumosoroseus* Against the Russian Wheat Aphid (Homoptera: Aphididae). J Econ Entomol (1996) 89 (6): 1418-1423.

[13] Manjunatha, A.M., Naik, M.I., Shivanna,B.K. Deviand, S.G.,and Pradeep, S. 2009. Evaluation of fungal pathogen, Fusarium semitectum Berk and Ravenel against tobacco aphid under laboratory and greenhouse conditions. Karnataka J. Agric. Sci., 22(3-Spl. Issue): (495-498).