
Original Article

The Application of *Beauveria bassiana* and Lufenuron Could Reduce the Reproduction of Fruit Fly (*Bactrocera carambolae*) (Drew dan Hancock) (DIPTERA: TEPHRITIDAE)

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Abstract

This study was conducted to examine the effect of *Beauveria bassiana* and insect growth regulator lufenuron on the fecundity and reproductivity decline of fruit fly *B. carambolae* adults. The study was conducted at the Laboratory of Entomology, Department of Plant Pests and Diseases, Faculty of Agriculture, University of Brawijaya from March 2012 until December 2012. The density of *B. bassiana* spores used in this study was 10^8 spores/ml and the lufenuron concentrations used are 0.5, 1, and 1.5 ml/l. The results showed that the application of *B. bassiana* spores at density of 10^8 spores/ml combined with lufenuron at the concentration of 1.5 ml/l significantly reduced the fecundity, egg fertility and reproduction of *B. carambolae* up to 95.69%.

Keywords: *Beauveria bassiana*, *Bactrocera carambolae*, Lufenuron.

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INTRODUCTION

Fruit fly is one of the harmful pests which affects both quantity and quality of fruits and vegetables. This pest is a key pest on fruit in all over the world, including in Indonesia (Kuswadi *et al.*, 1997). The genus of *Bactrocera* has various types of destructive fruit flies in Indonesia, one of them is *Bactrocera carambolae* Drew and Hancock (Diptera: Tephritidae).

B. carambolae is one of 52 species on the complex group of *B. dorsalis* (Hendel) and could attack a wide range of fruits including papaya and star fruit. This fly has been reported attacked 75% of fruit trees and become second major pest of fruit crops after *B. papayae* (Malavasi *et al.*, 2000 in Rachmawati, 2006).

Damage caused by *B. carambolae* was usually seen from the puncture marks found on fruit skin. Ovipositor punctures of *B. carambolae* females are easily recognized by discoloration of skin fruit around the puncture marks and the fruit will decay quickly.

The damage caused by fruit flies *B. carambolae* can be prevented and reduced by preventive efforts. The control measure for fruit

fly can be done either by physical, chemical and biological. The control of fruit flies was commonly done by using chemical insecticides. However, insecticides application causing a lot of problems such as increasing the insect resistance, environmental pollution, poisoning, and death of non-target animals.

An alternative control of fruit fly *B. carambolae* could be done by using natural enemies either of parasitoids, predators, or microbial pathogens. The most vulnerable life stage of fruit flies which frequently attacked by natural enemies are at the stage of final instar of larva, pupa, and adult. Eggs and early instar of larva are tend to be protected from the attack of natural enemies because they were exist inside the fruits. However, the eggs and early instar of larva could also be attacked by parasitoids, mites and pathogenic microorganisms (Artayasa, 1999). The control of eggs and early instar of larva of *B. carambolae* will be more effective because it can prevent the formation of pupa and adult led to reduce the spread rate of *B. carambolae*.

The control of early instar of *B. carambolae* larva using pathogenic microorganisms is considered more effective because it is

environmental friendly and does not cause resistance. One of beneficial pathogenic microorganisms that can be used to control the larva *B. carambolae* is the fungus *Beauveria bassiana* (Bals) Vuill. This fungus has been widely used in various types of pest control on a wide range of plant species. *B. bassiana* has been proved effective in controlling a variety of pests. Unfortunately, when it is applied in the field, the pathogenicity is not consistent due to the influence of unfavorable environmental conditions, especially temperature, humidity, and solar radiation (English, *et al.*, 1995). To increase the pathogenicity of *B. bassiana*, the formulation of isolates as well as adding a carrier that can improve the performance of the fungus must be done. One of compatible carrier which could increase the pathogenicity of *B. bassiana* is the addition of Insect Growth Regulator (IGR) (Krysan, 2010).

IGR is an insecticide belongs to the new group which has high selectivity towards the target insect that is in accordance with the principles of integrated pest management (IPM). IGR can interfere or inhibit the life cycle of pests, hence the pests can not reach the adulthood and reproduce. One of the insecticides included in the IGR is lufenuron. Lufenuron works by inhibiting the synthesis of chitin for skin replacement process. In addition to inhibiting the synthesis of chitin, lufenuron also interfere the reproductive system of target insect pests (Hoffman, 1998). The combination of *B. bassiana* with IGR is expected to be a new approach for enhancing the effective control of *B. carambolae*.

MATERIALS AND METHODS

Propagation of Fruit Flies *carambolae*

The fruit flies *B. carambolae* used in this study were mass propagated in rearing laboratory of Department of Plant Pests and Diseases, Faculty of Agriculture, University of Brawijaya. Pupa of *B. carambolae* which almost ready to be adult were placed in a Petri dish and put in a maintenance cage (Figure 3a). Maintenance cage with 45 cm long, 35 cm wide and 50 cm high walls are made of polywood and the other side is made of gauze for aeration and the entry of light into the cage. The back wall is made shaped door to enter the pupa and food for adult *B. carambolae*. The front wall consist of four holes with diameter of each 5 cm as the installation of nesting cups (Figure 3b). On the inside of the glass made nesting small holes with a diameter of 0.5 mm. Before being installed, the nesting cups were filled with water-saturated sponge pieces. In the culture cage, 20-watt fluorescent lamp at 20 cm above the surface of the cage was mounted to add lighting maintenance (Kuswadi *et al.*, 1997).

Feed used for maintenance adult *B. carambolae* was a mixture of sugar and protein hydrolysate with a ratio of 4:1. After weighed, the feed is placed on a Petri dish and put in the culture cage (Kuswadi *et al.*, 2000). Feeding on the insects affect the growth, fecundity and survival of insects. Sugar or sucrose is a form of carbohydrate that is needed by the female fruit flies to produce eggs, while the protein was required for sexual maturity and egg production (Crown, 1997).

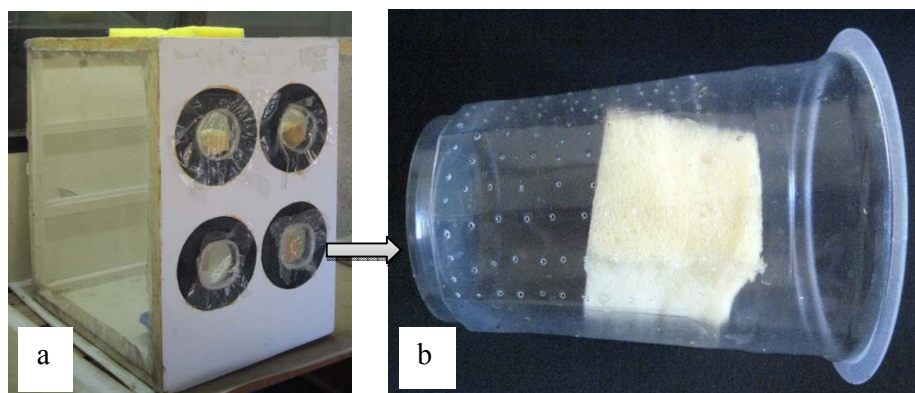


Figure 3. (a) Culture cage of *B. carambolae* adult; (b) The adult glass egg for laying female fruit flies

Female *B. carambolae* will lay eggs on nesting glasses after the age of 10 days. Egg harvesting was done by placing a glass nesting in holes that already exist in the cage wall. Before being installed, nesting cups filled with pieces of water-saturated sponge to retain moisture in the glass so that the laid eggs were not dry. Laying glass installed for 24 hours starting at 08.00 am. Eggs laid on the inside of nesting glass wall were collected by washed and filtered using a black fabric so that the egg could be seen and then the egg were ready to be infested or used for the treatment (Kuswadi *et al.*, 1997).

Treatment cages of *B. carambolae* adult were transparent plastic cups and covered with gauze (Figure 2). In the cage wall was made a hole for installed tube with a diameter of 3 cm as a place for adult to lay the eggs. Inside of the tube was perforated using a needle with a diameter of 0.5 mm as a place for fruit fly *B. carambolae* oviposition.

Propagation *B. bassiana*

The fungus *B. bassiana* used in this study is a collection of Plant Pest and Disease Laboratory Faculty of Agriculture, University of Brawijaya Malang. Isolates were propagated using Potato Dextrose liquid medium, in a fermenter. Fermenter is tank or container which contain media for the entire cell (microbial) to transform raw material into biochemical products with or without byproducts. The first step for fermentation was incorporation of pure *B. bassiana* into the fermenter containing Potato Dextrose liquid media (200 g potato, 20 g dextrose, in 1 liter of distilled water) and aerated

using aerators for 3-4 days then the conidia were collected and calculated using haemocytometer.

Application of *B. bassiana* and Lufenuron on Fruit Flies *B. carambolae* adult.

Adult *B. carambolae* used in this study were male and female adult emerging from pupa and old alike. In order to obtain the adult of the same age, pupa of *B. carambolae* were harvested on the same day, hence the adults which came out on the same day is calculated as the adult of the same age. Male and female adult of *B. carambolae* which came out of the pupa were immediately taken out using the respirator and put in treatment cage. *B. carambolae* adult fed a sugar and protein hydrolysis with 4: 1 ratio.

Males and females adult are treated in a separate cage. Each cage was inserted with 10 adult. Adult separation have to be done because there was a difference treatment between male and female adult. *B. carambolae* male and female adults are treated with *B. bassiana* and Lufenuron with different concentrations. Lufenuron was dissolved on water and then used for application onto *B. carambolae* adult. Administration of *B. carambolae* was done by using a saturated sponge placed in the treatment cage. The difference treatments for male and female adults were conducted to determine the effect of *B. bassiana* and Lufenuron with various concentrations on the death and sterility of *B. carambolae*. The concentration of *B. bassiana* and Lufenuron used in each treatment are presented in Table 3.

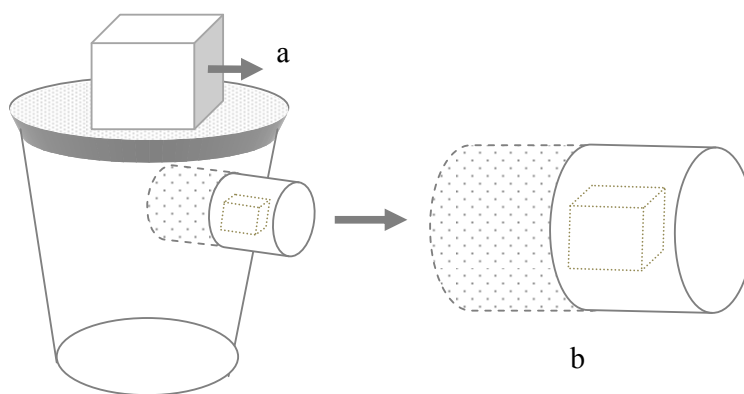


Figure 2. Treatment cage of *B. carambolae* adult. (a) Saturated sponge for drinking of *B. carambolae* adult; (b) Nesting place tube of female adult.

Table 3. Administration of *B. bassiana* with various concentration level of Lufenuron on *B. carambolae* adults

<i>B. carambolae</i> Adult	<i>B. bassiana</i> (spore/ml)	Lufenuron (ml/l)
Male	10 ⁸	0,5 1 1,5
Female	10 ⁸	0,5 1 1,5
Control	0	0

The treatment used in this experiment was completely randomized design (CRD) consisting of three treatments on either female or male adults and one treatment using aquadest as a control, the whole treatment was repeated three times.

B. carambolae adults used as controls were 10 pairs of adult without administration of *B. bassiana* and Lufenuron. On a separate cage, 30 male adults and 30 normal female adults (without administration of *B. bassiana* and lufenuron) were also maintained. The adults will be administered with combined *B. bassiana* and lufenuron. At 2 days old, male and female adults paired were also copulated by placed 10 pairs of adults in each cage. Male adults administered with *B. bassiana* and lufenuron were paired with normal female adults, in contrast the female adults administered with *B. bassiana* and lufenuron were paired with normal male adults. *B. carambolae* adults used as controls were male and female adults both without administration of *B. bassiana* and lufenuron.

The variable observed in this study were the fecundity of *B. carambolae* and egg fertility of fruit fly *B. carambolae*. Fecundity *B. carambolae* was determined by counting the number of eggs laid by the female adults during her lifetime and the number of eggs in the ovaries. Number of eggs of *B. carambolae* female adults laid was calculated and recorded daily. The eggs which were still in the ovary were counted by dissecting the abdominal of

dead adult females. To obtain eggs produced by female adults (female fecundity) the eggs laid by adult females were summed with the eggs in the ovaries

B. carambolae egg fertility was determined by calculating the percentage of the number of eggs that can hatch into larvae. Eggs obtained were placed on a Petri dish which was lined with black fabric to facilitate observation and added with water saturated cotton to retain the moisture. The number of eggs that hatched at 48 hours after treatment was then observed with a magnifying glass and then calculated. The eggs that have hatched will be showed more transparent than eggs that have not hatched. Eggs that did not hatch were showed white in colour.

The formula used to calculate the percentage of hatching eggs is as followed.

$$P = \frac{a}{b} \times 100\%$$

Description:

P: Percentage of egg hatching

a: The number of eggs that hatch

b: The number of eggs laid

The fecundity of *B. carambolae* were calculated to determine the percentage of reduction in fecundity of *B. carambolae* resulted from the administering of *B. bassiana* and lufenuron on *B. carambolae* adults.

The formula used to calculate the reduction percentage in fecundity of *B. carambolae* is as followed.

$$\text{Fecundity reduction (\%)} = \frac{\text{MFC} - \text{MFT}}{\text{MFC}} \times 100\%$$

Description:

MFC : Mean fecundity of *B. carambolae* in Control

MFT : Mean fecundity of *B. carambolae* in Treatment

RESULTS AND DISCUSSION

The Effect of *B. bassiana* and Lufenuron on the Fecundity of *B. carambolae*.

The effectiveness of administration of *B. bassiana* combined with lufenuron on *B. carambolae* fecundity could be known from the number of eggs laid by adult *B. carambolae*. The results showed that the number of eggs laid by adult *B. carambolae* administered with combined *B. bassiana* and lufenuron is less than the number of eggs laid by adult *B. carambolae* without administered with combined *B. bassiana* and lufenuron (Table 5). This result

suggests that *B. bassiana* combined with lufenuron could reduce the number of eggs laid by adult *B. carambolae*.

Data on Table 5 showed that the administering of *B. bassiana* combined with lufenuron at the concentration of 1.5 ml/l revealed the lowest number of eggs produced by both adult females without being administered with *B. bassiana* and lufenuron paired with adult males administered with *B. bassiana* and lufenuron, as well as adult females administered with *B. bassiana* and lufenuron paired with adult males without being administered with *B. bassiana* and lufenuron compared with those at lower concentration of *B. bassiana* and lufenuron. This result suggests that the increase of the lufenuron concentration could reduce the number of eggs laid by the adult female *B. carambolae*.

The administering of *B. bassiana* combined with lufenuron on the adult *B. carambolae* also affected *B. carambolae* fecundity (Table 6). Fecundity of *B. carambolae* could be known by counting the eggs laid and the number of eggs in the ovaries of adult female *B. carambolae*.

Table 5. The Average Number of Eggs Laid by *B. carambolae* administered with combined *B. bassiana* 10⁸ spores / ml and Lufenuron at different concentrations

Treatment	Lufenuron (ml/l)	Number of Laid Eggs (egg)
♀ Normal >< ♂ Given BB + Lufenuron	0,5	6247,00 f
	1	3108,33 d
	1,5	848,33 b
♂ Normal >< ♀ Given BB + Lufenuron	0,5	5080,33 e
	1	1310,33 c
	1,5	447,33 a
Control	0	6829,33 g

Description: The numbers followed by same letter in the same column indicate no significantly different at 5% error level by Duncan Multiple Range Test (DMRT).

Table 6. The mean and decrease in fecundity Fruit Flies *B. carambolae* given drink *B. bassiana* 10⁸ spores / ml and Lufenuron at Different Concentrations

Treatment	Lufenuron (ml/l)	Fecundity (egg)	Decrease In Fecundity (%)
♀ Normal >< ♂ Given BB + Lufenuron	0,5	6383,00 f	11,37
	1	3371,00 d	53,19
	1,5	1054,00 b	85,36
♂ Normal >< ♀ Given BB + Lufenuron	0,5	5212,67 e	27,62
	1	1485,00 c	79,38
	1,5	577,00 a	91,98
Control	0	7202,33 g	0

Description: The numbers followed by same letter in the same column indicate no significantly different at 5% error level by DMRT.

Table 6 showed that the fruit fly *B. carambolae* administered with combination of *B. bassiana* and lufenuron revealed lower fecundity compared with that of *B. carambolae* without administered with *B. bassiana* and lufenuron. The combination of *B. bassiana* with lufenuron administered at a concentration of 1.5 ml/l showed the highest in reducing the fecundity of *B. carambolae*. The percentage of the reduction of the fecundity of *B. carambolae* was also seen the highest reaching 91.98% at the concentration of 1.5 ml/l (Table 6). This suggests that administering the combined *B. bassiana* and lufenuron at concentration of 1.5 ml/l was effective in reducing the *B. carambolae* fecundity.

The reduction of the fecundity of *B. carambolae* administered with combined *B. bassiana* and lufenuron was probably because *B. bassiana* and lufenuron was able to affect the reproductive system and reduce fertility of *B. carambolae*. Budiwati (1997) stated that Lufenuron could influence the reproduction of insect may be by inhibition of oocyte development or egg maturation in the ovaries. Inhibition of primary oocyte can affect the formation of adult females which led to have no follicle cells. Follicle cell function in the process of ovulation and oocyte transportation, serve as endocrine glands as well as the corpus luteum at post-ovulation. Post ovulation corpus luteum produces estrogen and progesterone which are essential for controlling the reproductive cycle, sexual appearance and sexual behavior (Budiwati, 1997).

The Effect of *B. Bassiana* and Lufenuron on the Egg Fertility of *B. carambolae*.

The results showed that *B. carambolae* administered with combined *B. bassiana* and lufenuron had lower egg fertility than those without administered with *B. bassiana* and lufenuron (Table 7). Increased concentrations of lufenuron combined with *B. bassiana* showed more reduction of eggs fertility of *B. carambolae*.

Male or female *B. carambolae* administered with of lufenuron at the concentration of 1.5 ml/l showed consistently able to reduce the eggs fertility of *B. carambolae*. This suggests that *B. bassiana* combined with lufenuron at a concentration of 1.5 ml/l is effective in lowering the egg fertility or increasing the sterility of *B. carambolae*.

The reduction of *B. carambolae* egg fertility indicates the decline on the reproduction *B. carambolae* resulted on the reduction of the number of *B. carambolae* offspring. Lufenuron has been known not only inhibits the formation of chitin but also capable of interfering the formation of egg cells led to the reduction of the egg fertility of *B. carambolae*. Lufenuron inhibits the presence of chitin in the ovary which is an important structural component of the egg and is involved in oogenesis. Lufenuron could inhibits the production of chitin in the insects since at the egg stage when the larvae are still developing. There is a possibility that the decrease in egg hatchability is also caused by a defect in the differentiation of oocytes and sperm (Nehad *et al.*, 2009).

Table 7. The mean of Eggs Fertility and The Reduction of Reproduction of Fruit Flies *B. carambolae* Administered with *B. bassiana* at 10^8 Spores/ml Combined with Lufenuron at Different Concentrations

Treatment	Lufenuron (ml/l)	Fertility (egg)	Reduction of Reproduction (%)
♀ Normal >< ♂ Given BB + Lufenuron	0,5	3215,67 c	32,22
	1	1363,33 b	71,26
	1,5	413,67 a	91,28
♂ Normal >< ♀ Given BB + Lufenuron	0,5	3362,67 c	29,12
	1	627,67 a	86,77
	1,5	204,33 a	95,69
Control	0	4744,67 d	0

Description: The numbers followed by same letter in the same column indicate no significantly different at 5% error level by DMRT.

Other report showed that *B. bassiana* was able to damage the haemocoel tissues, such as the gastrointestinal tract, muscles, nervous system and respiratory system lead to sterility of insects (Robert and Yendol, 1982).

In the previous study (Puspitasari, 2012), showed that the application of lufenuron solely on adult females mated with normal adult males of fruit flies could reduce the reproduction by 42.82%. Mahmoud (2009) showed that the application of *B. bassiana* on adult *Bactrocera spp* could reduce the reproduction by 60.8%. In this study, the combination of *B. bassiana* and lufenuron applied to adult *B. carambolae* could reduce the *B. carambolae* reproduction up to 95.65%, indicating that the combination of *B. bassiana* and lufenuron is more effective than a single application *B. bassiana* or lufenuron in controlling pest fruit fly *B. carambolae*.

CONCLUSION

The application of *B. bassiana* at concentration of 10^8 spores/ml combined with lufenuron at the concentration of 1.5 ml/l on adult *B. carambolae* was significantly effective in reducing the fecundity and the reproduction of fruit fly *B. carambolae* up to 95.69%. It can be suggested that those combination can be used as an alternative and environmental friendly pest control for fruit fly *B. carambolae*.

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