

## The Combination of Entomopathogenic Fungus of *Beauveria bassiana* (Balls) Vuill. with the Insect Growth Regulator (IGR) of Lufenuron Against Reproductive of *Bactrocera carambolae* Fruit Flies (Diptera: Tephritidae)

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### Abstract

The study aimed to determine the reproductive ability of fruit flies *B. carambolae* treated with *B. bassiana* and Lufenuron. This study is conducted at the Laboratory of Pest. Department of Plant Pests and Diseases, Faculty of Agriculture, University of Brawijaya, Malang. This study used a completely randomized design with nine treatment and three replications. The study was trying to evaluate the effect of *B. bassiana* and Lufenuron on the reproduction capacity of *B. carambolae*. Results showed that adults of *B. carambolae* to applied combination of *B. bassiana* and Lufenuron immediately after coming out of the pupae until day eighth have the average number of eggs laid is 7.69%, a decrease of fecundity 92.40%, egg fertility by 61.38% and 95.24% decrease of reproduction. Adults of *B. carambolae* applied of *B. bassiana* and Lufenuron on day eighth until day sixteenth (for 8 days), show a decrease in the number of eggs laid by 13.63%, the decrease of fecundity 88.50%, egg fertility by 50.16% and decrease of reproduction by 93.12%.

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

### INTRODUCTION

The fruit fly is a pest so much affecting the horticultural crops. Under condition where the fruit fly populations are high, the intensity of the attack can reach 100% [1]. One type of fruit flies that need attention is *Bactrocera carambolae* (Diptera: Tephritidae). Fruit attacked by *B. carambolae* looks intact from the outside, but the inside of the fruit is actually destroyed as it has been eaten by the larvae of *B. carambolae* [2].

*Bactrocera carambolae* larvae control using pathogenic microorganisms is more effective because it is environmentally friendly and does not cause resistance on the species. One of pathogenic microorganisms that can be used for larval control of *B. carambolae* is the fungus *Beauveria bassiana* (Bals) Vuill. The pathogenicity of this fungus is not consistent when applied in the field, due to the influence of environmental conditions that do not support especially temperature, humidity, and the intensity of sunlight. The pathogenicity of the fungus *B. bassiana* can be improved by formulation of

isolates with the addition of Insect Growth Regulator (IGR).

IGR is a product or material that interferes with or inhibits the life cycle of pests, such that pests cannot reach imago, and unable to reproduce [3]. One of the insecticide active ingredients included in the IGR is Lufenuron. Lufenuron works by inhibiting the synthesis of chitin in the process of ecdysis. In addition to inhibiting the synthesis of chitin, Lufenuron also interferes with the reproductive system of the insects pest target [4]. The combination of *B. bassiana* fungus with the addition of IGR is expected to be a new approach for controlling *B. carambolae* effectively, environmentally friendly. Which in turn does not cause resistance and does not cause the death of natural enemies of both predators and parasitoids as well as to improve the quality of fruits and vegetables [5]. Increased pathogenicity of entomopathogenic fungi by the addition of insecticides can fix isolate and improve the performance of these isolates [6].

According to above previous research, there is a need for research on the combination of entomopathogenic fungus *Beuaveria bassiana* (Balls) Vuill with the Insect Growth Regulator (IGR) of Lufenuron against reproductive of *Bactrocera carambolae* fruit flies (Diptera: Tephritidae). This study aims to determine the

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reproductive ability of fruit flies *B. carambolae* treated with *B. bassiana* and Lufenuron.

## MATERIALS AND METHODS

### Study Object

Male and female imago were treated in separate cages. Each cage consisted of 10 imago. Imago separation was done because there was a difference in treatment between male and female imago. Male and female imago of *B. carambolae* respectively were treated with *B. bassiana* + Lufenuron with 1.5 mL.L<sup>-1</sup> concentration. *Beauveria bassiana* was added with Lufenuron for imago of *B. carambolae* for applications. Applications to *B. carambolae* was done by using a saturated sponge and placed on top of the treatment cage. Differences in the treatment of adult males and females were conducted to determine the effect of *B. bassiana* + Lufenuron to the death of *B. carambolae*. Each treatment in the imago is presented in Table 2.

**Table 1.** Treatment of *B. bassiana* and Lufenuron on the Imago of *B. Carambolae*

| Treatment                           | Application (Day) |
|-------------------------------------|-------------------|
| ♂ BL1 >< ♀ Normal (P <sub>1</sub> ) | 1 - 8             |
| ♀ BL1 >< ♂ Normal (P <sub>2</sub> ) | 1 - 8             |
| ♂ BL1 >< ♀ BL1 (P <sub>3</sub> )    | 1 - 8             |
| ♂ BL8 >< ♀ Normal (P <sub>4</sub> ) | 8 - 16            |
| ♀ BL8 >< ♂ Normal (P <sub>5</sub> ) | 8 - 16            |
| ♂ BL8 >< ♀ BL8 (P <sub>6</sub> )    | 8 - 16            |
| Control (P <sub>7</sub> )           | 0                 |
| Control (P <sub>8</sub> )           | 1 - 8             |
| Control (P <sub>9</sub> )           | 8 - 16            |

### Description :

BL : *Beauveria bassiana* and Lufenuron

BL1 : Application the first day until the eighth day

BL8 : Application eight day until the sixteenth day

♂ : Male Imago

♀ : Female imago

### Data Collection and Analysis

Date of the eggs number and formed imago were obtained by direct observation. The calculation was conducted on the eggs number placed by the *B. carambolae* imago and then counts the number of eggs capable of being larva, pupa, up until the imago. Data were analyzed using analysis of variance (ANOVA), if there is a significant difference then continued with Duncan's Multiple Range Test at 5% level.

## RESULT AND DISCUSSION

### Fecundity of Fruit Flies *B. carambolae*

Fecundity of fruit flies that were applied the combination *B. bassiana* and Lufenuron can be seen in the Table 2. The lowest mean number of

eggs was for P<sub>3</sub> (male and female were alike given *B. bassiana* combined with Lufenuron 1.5 mL.L<sup>-1</sup> in the medium for pupation for effective pupation in suppressing the formation of pupae. The lowest number of eggs was for treatment P<sub>6</sub> (male and female were alike given *B. bassiana* combined with Lufenuron), which was 131.67. All treatments provided real difference to the average number of eggs laid by imago *B. carambolae* on controls. The decline in fecundity of *B. carambolae* imago can be known by subtracting the average number of eggs in the control group with the one in the experiment group divided by the number of eggs in control group and then multiplied by one hundred.

In Table 2, the highest decrease in fecundity of *B. carambolae* imago for those mated on day eighth after the treatment was in P<sub>3</sub> (male and female were alike given *B. bassiana* combined with Lufenuron) which was 92.40%. While the highest fecundity for those mated on day sixteenth after the treatment was in P<sub>6</sub> (male and female were alike given *B. bassiana* combined with Lufenuron) which was 88.50%. This shows that the male and female imago of *B. carambolae* treated with *B. bassiana* and lufenuron was effective in reducing fecundity of fruit fly *B. carambolae*. The decline in fecundity was also due to premature death of imago of *B. carambolae* given *B. bassiana* and Lufenuron.

**Table 2.** The Average Fecundity and the Decrease in Fecundity of Fruit Fly *B. carambolae* Treated with *B. bassiana* and Lufenuron on Different Ages

| Treatment                           | Average Number of Eggs (Pcs) | Decrease in Fecundity (%) |
|-------------------------------------|------------------------------|---------------------------|
| ♂ BL1 >< ♀ BL1 (P <sub>3</sub> )    | 290.00 abcd                  | 92.40                     |
| ♂ BL8 >< ♀ Normal (P <sub>4</sub> ) | 213.00 abc                   | 81.40                     |
| ♀ BL8 >< ♂ Normal (P <sub>5</sub> ) | 154.67 ab                    | 86.50                     |
| ♂ BL8 >< ♀ BL8 (P <sub>6</sub> )    | 131.67 a                     | 88.50                     |
| Control (P <sub>7</sub> )           | 6651.33 i                    | 0.00                      |
| Control (P <sub>8</sub> )           | 3813.33 h                    | 0.00                      |
| Control (P <sub>9</sub> )           | 1145.33 fg                   | 0.00                      |

### Description :

BL : *Beauveria bassiana* and Lufenuron

BL1 : Application the first day until the eighth day

BL8 : Application eight day until the sixteenth day

♂ : Male Imago

♀ : Female imago

Different notation indicates a significant difference (P<0.05).

*Beauveria bassiana* applied to the imago of *Tetranychus urticae* reduces the number of eggs placed by the imago of *T. urticae* up to 98% [10]. Combination of fungus of *B. bassiana* and

Lufenuron  $1.5 \text{ mL.L}^{-1}$  results in the average number of eggs by 12.42% compared with the untreated imago. This means that the decline in the number of eggs is 87.58% [5]. Application of *B. bassiana* with concentration of spores at  $2.0 \times 10^7$  is able to reduce female fecundity of green leaf hoppers up to 58% [7].

Observation on present studies shows that the infected imago of *B. carambolae* mostly die prematurely. This is because *B. bassiana* enters the insect host's body through the skin, gastrointestinal tract, spiracles, and other openings (Fig. 1). In addition, inoculum of fungi that attach to the body of the insect host can germinate and grow to form a tubular sprouts, then penetrate through the cuticle of the insect body. The penetration is done mechanically or chemically by enzymes or toxins [8].



Figure 1. Imago of *B. carambolae* that Infected with *B. bassiana* and Lufenuron Combination

#### Fertility of Fruit Flies *B. carambolae*

The results show that there were differences between the mean on fertility of treated and untreated eggs of *B. carambolae* fruit fly. This indicates that *B. bassiana* combined with Lufenuron affected fertility of eggs. Table 3 shows that the lowest percentage of egg hatching on imago of *B. carambolae* mated on day eighth after being treated was in  $P_3$  (female treatment vs male treatment) reaching 55.39% when compared to the imago of *B. carambolae* mated at same age, which was 99.30%. While in the imago of *B. carambolae* mated on day sixteenth after the treatment, the lowest average of egg hatching was in  $P_6$  (male and female treated) which reached 50.16% when compared to the control group of imago of *B. carambolae* which reached 99.27%. Table 3 also shows that the highest decrease in reproduction was in  $P_3$  (male and female equally treated) which reached 95.24%. However, almost all treatments could

affect the reproductive decline when compared to the imago of *B. carambolae* in control.

*Beauveria bassiana* fungus will further produce beauverin toxins making damage to the insect tissue. Within days, the insects will die (Fig. 2). The mycelium of the fungus will come out of the host's body, grow over the host's body, and produce conidium. Insects attacked by *Beauveria bassiana* will die with a hardened body like a mummy and covered by threads of white hyphae. Lufenuron combined with *B. bassiana* will release toxins that cause blood clotting and cessation of blood circulation to the insect that the insect will die [5].

Table 3. The Average Fertility and the Decrease in Reproductive Function of Fruit Fly *B. carambolae* Treated with *B. bassiana* and Lufenuron on Different Ages

| Treatment                  | Average Number of Egg Hatching (Pcs) | Decrease in Reproductive Function (%) |
|----------------------------|--------------------------------------|---------------------------------------|
| ♂ BL1 > ♀ Normal ( $P_1$ ) | 451.33 ef                            | 85.26                                 |
| ♀ BL1 > ♂ Normal ( $P_2$ ) | 217.67 cde                           | 92.89                                 |
| ♂ BL1 > ♀ BL1 ( $P_3$ )    | 145.67 bc                            | 95.24                                 |
| ♂ BL8 > ♀ Normal ( $P_4$ ) | 98.33 ab                             | 87.07                                 |
| ♀ BL8 > ♂ Normal ( $P_5$ ) | 67 a                                 | 91.19                                 |
| ♂ BL8 > ♀ BL8 ( $P_6$ )    | 52.33 a                              | 93.12                                 |
| Control ( $P_7$ )          | 5341.67 i                            | 0                                     |
| Control ( $P_8$ )          | 3062.33 h                            | 0                                     |
| Control ( $P_9$ )          | 760.33 g                             | 0                                     |

#### Description :

BL : *Beauveria bassiana* and Lufenuron

BL1 : Application the first day until the eighth day

BL8 : Application eight day until the sixteenth day

♂ : Male Imago

♀ : Female imago

Different notation indicates a significant difference ( $P < 0.05$ ).



Figure 2. Imago *B. bassiana* dead Stricken by the Combination of *B. bassiana* and Lufenuron

*Beauveria bassiana* can produce mycotoxins in the form of beauvericin toxins that cause

damage to the parts of the egg and the embryo causing disruption in the function of the haemolymph and nucleus of the insects. In addition, *Beauveria bassiana* can also generate secondary bassianolid metabolites like beauverolite, isorolite, and oxalic acid that its mechanism leads to the increase in pH of haemolymph, clumping of haemolymph, and cessation in the circulation of haemocytes as well as tissue or mechanical organ damage such as the gastrointestinal tract, muscles, nervous system, respiratory system and these disorders cause death [9].

### CONCLUSION

Combination treatment of *B. bassiana* and Lufenuron influence the fecundity and fertility of *B. carambolae* imago, i.e. 92.40% decline in fecundity and fertility decline 93.12%. The combination of fungus *B. bassiana* and IGR Lufenuron that applied on fruit fly imago *B. carambolae* can inhibit the reproduction of fruit flies experiments in the laboratory. However, it needs further research on the field application thus it can be used for pest control in the agriculture practices.

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