

## Virulence of Five Isolates of Indigenous *Beauveria bassiana* Against Eggs and Nymphs of *Bemisiatabaci* Gennadius (Hemiptera: Aleyrodidae)

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### Abstract

This research aims to study the virulence of five isolates of *Beauveria bassiana* to eggs and nymphs of *Bemisiatabacion* tomato. In the experiment eggs and second instar nymphs of *B. Tabaci* were used. Five isolates of the fungus, i.e., WS, TD312, PD114, PA221, PB211, were tested. Conidial concentration of *B. bassiana* used were  $10^8$  conidia/ml. Experimental parameters included mortality of eggs and nymph and percentage of adult emergence. The results showed that all *B. bassiana* isolates tested were able to kill *B. Tabaci* eggs but with very low mortality (2-19%). Mortality of second instar *B. Tabaci* nymphs was dependent on the fungal isolates. Isolate WS had the highest virulence, which caused 70% mortality of 2<sup>nd</sup> instar nymphs, with aLT50 of 4.87 days. Nymphs of *B. Tabaci* were highly susceptible to *B. bassiana* infection compared with eggs. *B. bassiana* applicated to nymphs of *B. Tabaci* can decrease the percentage of adult emergence.



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### Introduction


*Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) is one of the main pests in tomato plants.<sup>1</sup> *B. tabaci* is a polyphagous insect that has many host plants such as ornamental plants, vegetables, fruits, and wild plants (weeds).<sup>2</sup> This insect make damage directly on plants by sucking the liquid, causing a physiological disturbance on plants, chlorosis on leaves, and disturb ripening of tomatoes.<sup>3,4</sup> In addition to direct damages, the pest cause indirect

damage by the accumulation of honey dew produced by *B. tabaci* which leads to mold growth on foliage, and as a vector of Tomato yellow leaf curl virus (TYLCV) and more than 100 other begomo viruses.<sup>5,6</sup> The loss of yields due to *B. tabaci* attacks and yellow virus ranges from 20 to 100%.<sup>7</sup> This pest was first found in Indonesia in 1938 on a tobacco plant.<sup>8</sup> The reproduction and spread of these pests are very fast, in fact, in a year, is able to produc till 15 generations.<sup>9</sup>

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Traditionally, synthetic insecticides have been used for controlling *B. tabaci*, but excessive and irrational use of insecticides has led to adverse effects on the environment. To reduce the use of pesticides, it is important to developing alternative safety control methods such as the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. *B. bassiana* is considered the most effective alternative control method of *B. tabaci*. It is an entomopathogenic fungus with a wide host range, able to infect various kinds of insects, from pre-adult to adult.<sup>10,11</sup> *B. bassiana* infects insect by digestion, respiration, and particularly through the integuments of insect.<sup>12</sup>

*Beauveria bassiana* have been used as a biological agent to control several insect pests. In laboratory condition, *B. bassiana* was able to kill *Crociodolomiapavonanalarvae* till 80%. The mortality of larvae depends on isolates.<sup>13</sup> *B. bassiana* can kill several kinds of vegetable pests such as *Spodoptera exigua*,<sup>14</sup> *S. litura*,<sup>15</sup> *Nezaraviridula*,<sup>16</sup> and *Eurydemapulchrum*.<sup>17</sup> The dose of *B. bassiana* had a significant effect on adult of *Aphis crassivora* and *B. tabaci*. An increase

of *B. bassiana* concentration markedly decreased adult longevity, period of reproduction, and fecundity of the two insects.<sup>18</sup> One of the essential criteria in selecting entomopathogenic fungi for commercial development is to have high virulenceto target insects. In order to evaluate the importance of virulent strains for an efficient biological control of *B. tabaci* on tomato, we analyzed under laboratory conditions the effect of five fungal isolates of indigenous *B. bassiana* on eggs and nymphs of *B. tabaci*.

## Materials and Methods

### *Beauveria Bassiana* Isolates

Five fungal isolates of *B. bassiana* were obtained from the collection of entomopathogenic fungal culture maintained by the Biological Control Laboratory, Department of Pests and Plant Diseases, Faculty of Agriculture, Andalas University. (Table 1). *B. bassiana* isolates were obtained from plants and pest insects. Isolates were cultivated on the Sabouraud dextrose agar + yeast extract (SDAY) medium.

**Table 1: List of *B. bassiana* isolates used in research**

Isolates code	Source	Location
WS	<i>Leptocorisaoratorius</i>	Duku (Padang Pariaman), West Sumatra, Indonesia
TD312	Wheat stems endophyte	Koto Laweh (Tanah Datar), West Sumatra, Indonesia
PB211	Chili stems endophyte	Parabek (Agam), West Sumatra, Indonesia
PD114	Chili leaves endophyte	Parabek (Agam), West Sumatra, Indonesia
PA221	Chili roots endophyte	Parabek (Agam), West Sumatra, Indonesia

The conidial suspension of *B. bassiana* was obtained by adding 10 ml of distilled water and 0.1% Tween 80 to Petri dishes containing the fungus culture, and conidia were harvested by scraping the surface of the plate with a steril spatula. The conidial concentration was determined using an improved Neubauer haemocytometer and adjusted to 10<sup>8</sup> conidia / mL.

### Insects

*Bemisia tabaci* adults were collected from chili plants in Padang areas, West Sumatra, Indonesia and insects mass-reared on tomato plants. The tomato plants were planted in the polybag and put into large screen cages (60 x 75 x 100 cm). A large colony

of *B. tabaci* adults was introduced into the screen cages for three days. After that, whitefly adults were removed and tomato plants infested by eggs were transferred to other cages. The eggs laid by female adults of *B. tabaci* were used for *B. bassiana* bioassay on eggs. Other eggs were also kept until nymphs emergence to evaluate the efficacy of the fungus against nymphal stages of *B. tabaci*.

### Bioassay of *B. Bassiana* against *B. Tabaci* Eggs

The tomato leaf contained 20 eggs selected and treated with the fungus. Eggs were sprayed by conidial suspension of *B. bassiana*, then eggs were put into Petri dish on a moist filter paper. The assays was repeated five times. As control, the same

number of eggs were treated with distilled water. The eggs were reared till their hatching. Eggs mortality and infection of first instar nymphs were assessed and recorded daily for eight days.

### Bioassay of *B. Bassiana* against *B. Tabaci* Nymphs

The second instar nymphs of *B. tabaci* were moved on 30 days old tomato plants using a soft brush. Furthermore, 2 ml of the *B. bassiana* fungal suspensions, for each unit test, were sprayed on the insects using hand sprayer. For the control, the insects were sprayed with distillate water. The tomato plants were put into a cylindrical tubular-shaped plastic mica cage (high 60 cm, diameter 45 cm) and covered with gauze. The treatments were repeated five times, and every unit of treatment consisted of 10 nymphs. The mortality of nymphs was observed every day by counting the numbers of test insects that died until seven days after application (DAA).

### Data Analysis

The data obtained were analyzed by ANOVA and followed by a test of Duncan's New Multiple Range Test (DNMRT) a significant level of 5%.

**Table 1: Mortality of *B. tabaci* eggs after the application of *B. bassiana* isolates**

Isolates	Eggs mortality $\pm$ SE
WS	19.00 $\pm$ 1.00 <sup>a</sup>
TD312	17.00 $\pm$ 2.55 <sup>a</sup>
PB211	5.00 $\pm$ 1.58 <sup>b</sup>
PD114	4.00 $\pm$ 1.87 <sup>bc</sup>
PA221	2.00 $\pm$ 1.22 <sup>bc</sup>
Control	0.00 $\pm$ 0.00

Means followed by the same letter are not significantly different ( $P < 0.05$ ) by Duncan's Multiple Range Test.

### Results and Discussion

#### Eggs Mortality

Results of the virulence test of five *B. bassiana* isolates against *B. tabaci* eggs showed that all tested isolates kill *B. tabaci* eggs, but with very low mortality. Statistical analysis showed a significant effect of *B. bassiana* isolate on the mortality of *B. tabaci* eggs ( $F = 26.15$ ;  $db = 5, 24$ ;  $P < 0.0001$ ).

The mortality of *B. tabaci* eggs after *B. bassiana* application reported in Table 1.

Treatment of WS isolates on *B. tabaci* eggs resulted in the highest egg mortality (19%) compared to other isolates. Treatment with PA221 isolate resulted in the lowest egg mortality, namely 2%. In control, there was no death of the eggs. All the eggs hatched into nymphs. Low mortality of *B. tabaci* eggs after the fungal application is thought to be caused by the eggshell of *B. tabaci* which has a specific layer that can prevent the conidial tube from penetrating to the inside of the egg. The wax coating on insects could inhibit the germination of *B. bassiana* conidia. In addition, the failure of the fungus to infect eggs can be related to the presence of anti-fungal compounds found on the surface of the eggshell, which inhibits the germination process of fungal conidia. Eggs of *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae) were resistant to *B. bassiana* infection. Electron microscopy observations showed that only 13.0% of *B. bassiana* conidia germinated on eggs.<sup>11</sup>

The results of this study are similar to the results of Al-Deghairi<sup>19</sup> who observed a mortality of *B. tabaci* eggs after *B. bassiana* application of only 4.49%. Eggs of *B. tabaci* was more tolerant to *B. bassiana* infection and were not easily killed even by the highest conidial concentration. Furthermore, Islam *et al.*<sup>20</sup> also reported that applying *B. bassiana* to *B. tabaci* eggs with a concentration of 108 conidia/ml resulted in a mortality of 25.2%. and Trizelia *et al.*<sup>21</sup> reported that *B. bassiana* could not infect *Crocidolomia pavonana* eggs. In eggs of *Blissus antillus* (Leonard) (Hemiptera: Lygaeidae), egg mortality due to *B. bassiana* infection varied between isolates. Isolate CG24 can cause egg infections by 43.3%, while isolates CG04 and ARSEF792 were only 7.8%. The results of observations with fluorescent microscopy showed that the difference in virulence was due to differences in the ability of the fungal conidia to adhere to the surface of the egg and then penetrate the chorion.<sup>22</sup>

Based on macroscopic observations, the fungal mycelial covered the eggs. Fungal mycelia on the surface of the eggs were seen four days after the inoculation of the fungus. Al-Deghairi<sup>19</sup> also reported that infection symptoms on the eggs of *B. tabaci* were observed on the 3<sup>rd</sup> day of inoculation. Within

four days from the treatment, the eggs that became subsequently infected by the fungal had little color change but appeared slightly shrunk when observed under the microscope. One week after treatment, most of the unhatched eggs became conspicuously shrunk and had fewer fungal out growths on the surface.

**Table 2: Mortality of the first-instar nymph of *B. tabaci***

Isolates	Mortality of first nymphs ±SE
WS	7.43±1.27 <sup>a</sup>
TD312	5.97±1.87 <sup>ab</sup>
PB211	4.16±1.96 <sup>abc</sup>
PD114	3.10±1.27 <sup>abc</sup>
PA221	2.05±1.26 <sup>bc</sup>
Control	0.00±0.00 <sup>c</sup>

Means followed by the same letter are not significantly different (P<0.05) by Duncan's Multiple Range Test.

**Table 3: Mortality and LT<sub>50</sub> values of 2<sup>nd</sup> instar nymphs of *B. tabaci* treated with 10<sup>8</sup> conidia/ml of *B. bassiana* isolates at seven days post-inoculation**

Isolates	2 <sup>nd</sup> instar nymph mortality (%) ± SE	LT <sub>50</sub> (Day)
WS	70.00 ± 4.47 <sup>a</sup>	4.87 (4.41-5.46)
TD312	64.00 ± 2.45 <sup>a</sup>	5.51 (5.03-6.17)
PB 211	44.00 ± 2.45 <sup>b</sup>	7.23 (6.57-8.62)
PD114	44.00 ± 4.00 <sup>b</sup>	7.34 (6.59-8.81)
PA221	36.00 ± 2.45 <sup>b</sup>	7.80 (6.89-9.82)
Control	00.00 ± 0.00 <sup>c</sup>	

Means followed by the same letter are not significantly different (P<0.05) by Duncan's Multiple Range Test.

Besides being able to infect the eggs, nymphal mortality also occurs in 1<sup>st</sup> nymphs upon hatching from eggs contaminated by *B. bassiana* (Table 2). The nymph mortality varied between isolates (F = 3.55; db = 5, 24; P> 0.0152). WS isolates produced the highest nymph mortality, namely 7.43%, while PA221 isolates only produced nymph mortality of 2.05%. In control, there was no mortality in nymphs.

The results showed that conidia remained active and can infect nymphs successfully emerging from eggs. Occurrence mortality of 1<sup>st</sup> instar nymphs is thought due to contact nymphs emerging from eggs with the conidia attached to the surface of the eggshell and on the leaves. Other researchers<sup>23</sup> also reported that *B. tabaci* eggs had low susceptibility to *B. bassiana*, with >91% nymphs successfully emerging from eggs. However, there is significant mortality of 1<sup>st</sup> and 2<sup>nd</sup> instar nymphs originating from the treated eggs. These results indicated that newly hatched nymphs probably acquired conidia from the eggs soon after hatching or from the leaf surface as secondary exposure. *Trialeurodes vaporariorum* eggs treated with entomopathogenic fungus *Aschersonia aleyrodis* did not become infected, but larvae that hatched from these eggs were infected.<sup>24</sup>

**Mortality of *Bemisia Tabaci* Nymphs**

The results showed that *B. bassiana* isolate had a significant effect on the mortality of 2<sup>nd</sup> instar nymph of *B. tabaci*. WS isolates were the most virulent isolates with the highest mortality namely 70.00% after seven days after the fungal application. PA221 isolate had a low virulence with a mortality of 36.0% (Table 3).

The difference in the ability of *B. bassiana* isolates in killing *B. tabaci* nymphs is thought to be due to differences in viability of conidia or the ability to produce enzymes and toxins. The difference in mortality of *C. pavonana* larvae after *B. bassiana* application was caused by differences in physiological and genetic characteristics of the isolates.<sup>25</sup> Several researchers<sup>26,27,28</sup> stated that the germination of conidia and the ability to produce enzymes and mycotoxins during the infection process in insects affects insect mortality by *B. bassiana*.

This research showed that the mortality of nymphs of *B. tabaci* after application *B. bassiana* was influenced by isolate source. *B. bassiana* isolated from insects belonging to the same taxon of the test insect (WS) was more virulent than that isolated from the plants. Based on the observed values, surveys of *B. bassiana* isolates collected from homopterans seems to be a suitable approach for silver leaf whitefly microbial control programs. Other

researchers also reported that mortality of insect was dependent on the fungal isolates. Isolates or strains of entomopathogenic fungi isolated from the same taxon of the whitefly (Order Hemiptera, suborder Homoptera) were more virulent than the isolates from Lepidoptera, Coleoptera, and Hymenoptera.<sup>23,29,30,31</sup>

There was a difference in  $LT_{50}$  between isolates (Table 3) and  $LT_{50}$  value related to the virulence isolate.  $LT_{50}$  values ranged from 4.87 to 7.80 days. WS isolate has the shortest  $LT_{50}$  value compared to other isolates (4.87 days), and this means that the time needed to kill 50% of 2<sup>nd</sup> instar nymphs

of *B. tabaci* is shorter than other isolates. There are differences in the  $LT_{50}$  value between *B. bassiana* isolates to 2<sup>nd</sup> instar nymphs of *B. tabaci* bio type B. Estimated  $LT_{50}$  values showed that most *B. bassiana* and *I. fumosorosea* isolates killed whiteflies faster (3–5 d) compared with *L. muscarium* isolates.<sup>23</sup>

Mortality of second instar *B. tabaci* nymph due to *B. bassiana* infection began on the second day post-inoculation, and nymph mortality increased after three days. The development of mortality of *B. tabaci* nymphs due to *B. bassiana* infection can be seen in Figure 1.

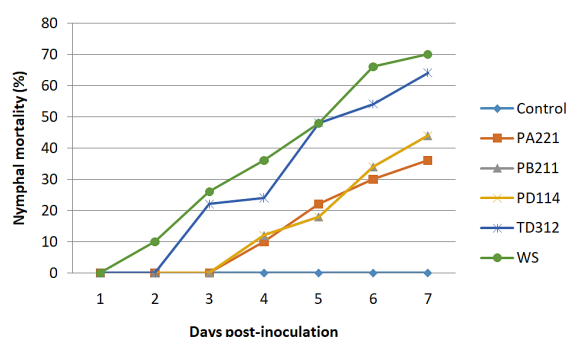


Fig.1: The 2<sup>nd</sup> instar nymph mortality rate of *B. tabaci* after *B. bassiana* application

Table 4: Percentages of emerged adults of *B. tabaci* after application of *B. bassiana*.

Isolates	Percentage of emerged adults ± SE
Control	98.00 ± 9.80 <sup>a</sup>
PA221	60.00 ± 3.16 <sup>b</sup>
PB 211	52.00 ± 2.00 <sup>bc</sup>
PD114	48.00 ± 4.90 <sup>c</sup>
TD312	26.00 ± 2.45 <sup>d</sup>
WS	22.00 ± 2.00 <sup>d</sup>

Means followed by the same letter are not significantly different ( $P < 0.05$ ) by Duncan's Multiple Range Test.

*B. tabaci* nymphs that die from *B. bassiana* infection are characterized by the presence of white mycelia or conidia on the surface of the nymph's body. Nymphs of *B. tabaci* treated with *B. bassiana* dried and presented reddish coloration upon death.<sup>29</sup> Mycelia of *B. bassiana* emerged on the cuticles of the immature insects (*B. tabaci*, *Bactericera cockerelli*, *Frankliniella occidentalis*) 2-3 days after

death, and most conidia were recorded on legs, wings, and thoraces of some adult cadavers.<sup>32</sup> Cherguiet *al.*<sup>33</sup> noted that dead individuals of *Ceratitiscapitata* were covered with a white mycelium characteristic of the fungus *B. bassiana*.

#### Adults Emergence

The results showed that *B. bassiana* applied to 2<sup>nd</sup> instar nymphs of *B. tabaci* had a significant effect on the emerged numbers of *B. tabaci* adults. Percentages of emerged adults under five fungal isolates can be seen in Table 4.

The application of fungus *B. bassiana* to the 2<sup>nd</sup> instar nymph showed a significant effect on adult emergence. In control, the percentage of adult emergence was the highest at 98%, while in the treatment of WS isolates only 22% of nymphs became adult. The low rate of adult emergence was because a lot of some nymphs was killed before becoming an adult. *B. bassiana* applied to *C. capitata* larvae decreased the percentage of pupae formed

and adult emergence.<sup>33</sup> *B. bassiana* can also reduce pupation and adult emergence of *S. litura*. Reduction in pupation and adult emergence is due to phago depression and difficulty in molting.<sup>34</sup>

### Conclusions

The present study showed that *B. bassiana* can infect *B. tabaci* eggs and nymphs. The mortality of *B. tabaci* eggs is only at 2-19%. Isolate WS had the highest virulence, which caused 70.00% mortality of 2<sup>nd</sup> instar nymphs, with an LT<sub>50</sub> of 4.87 days. Isolates of *B. bassiana* have significant effect on the mortality of second instar *B. tabaci*. Nymphs of *B. tabaci* were highly susceptible to *B. bassiana* infection compared with eggs. *B. bassiana* applied to *B. tabaci* nymphs could decrease the percentage of adult emergence.

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### Conflict of Interest

The authors do not have any conflict of interest.

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