International Journal of Life Science and Agriculture Research
ISSN (Print): 2833-2091, ISSN (Online): 2833-2105
Volume 03 Issue 09 September 2024
DOI: https://doi.org/10.55677/ijlsar/V03I9Y2024-02
Impact Factor: 6.774 , Page No : 733-738

Application of Entomopathogenic Fungus *Beauveria Bassiana* on Shallot Plants Through Soaking the Bulbs and its Effect on the on the Growth of *Spodoptera Exigua* Larvae

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ABSTRACT: *Spodoptera exigua* Hubner is a major pest of shallots. This pest attack can cause a decrease in shallot production. The use of entomopathogenic fungus *Beauveria bassiana* is an environmentally friendly pest control alternative. This study aims to study the development of *S. exigua* larvae in shallot plants applied with *B. bassiana* fungus. This study was arranged in a Completely Randomized Design (CRD) with 6 treatments and 5 replications. Five isolates of *B. bassiana* (BbWS, BbJG, PD114, TD312, PB211) were used in this study. The concentration of *B. bassiana* suspension used was 10^8 conidia/ml. *B. bassiana* was applied to shallots by soaking the bulbs for 15 minutes. The results showed that the mortality of larvae feeding on plants applied with *B. bassiana* reached 100%. The application of B. bassiana also extended the larval period, reducing the percentage of pupae and adults formed. BbWS and TD312 were isolated and were better at inhibiting the growth of *S. exigua* larvae

Published Online: September 13, 2024

KEYWORDS: *Beauveria bassiana*, endophyte, larvae, pupae, *Spodoptera exigua*

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INTRODUCTION

Spodoptera exigua Hubner is a major pest of shallots. This pest attack can cause a decrease in shallot production. *S. exigua* attacks at the beginning of the bulb formation phase can cause red onion yield losses of around 45-47% (Moekasan *et al*., 2012). *S. exigua* attacks can cause yield losses of up to 100% if no control efforts are made (Negara, 2003). Currently, control efforts made by farmers to overcome the problem of *S. exigua* attacks still rely on synthetic pesticides. The unwise use of synthetic pesticides can cause environmental damage, killing non-target insects and natural enemies of the pest. To reduce synthetic pesticides, environmentally friendly control is needed by utilizing biological agents, one of which is the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuill.

Beauveria bassiana, a promising entomopathogenic fungus, is used to control various kinds of pests. According to Tanada and Kaya (1993), *B. bassiana* is a very virulent entomopathogenic fungus (EPF) that mostly affects agricultural pests. Trizelia *et al*. (2017) reported that *B. bassiana* isolated from *Leptocorisa oratorius* and cocoa fruit were virulent against instar II larvae of *S. litura* with a mortality of 80-81.67%. Rosmiati *et al.* (2018) also reported that *B. bassiana* caused a mortality of *S. litura* larvae of 82.50% and the weight of feed eaten by *S. litura* larvae was the lowest at 0.79g. Ihsan *et al*. (2023) reported that the application of *B. bassiana* fungal suspension with a density of 10^9 conidia/ml was able to cause a mortality of *Nilaparvata lugens* of 95%. Hasibuan *et al*. (2024) reported that the application of *B. bassiana* to *Scirpophaga innotata* larvae was able to cause larval mortality of up to 100%,

In addition to acting as an entomopathogen by killing pests directly, *B. bassiana* fungi are also reported to be able to live endophytically on plants, colonize plant tissue, and increase plant resistance to pest attacks. Bagy *et al*. (2018) stated that *B. bassiana* is an entomopathogenic fungus that lives endophytically and colonizes shallot plants cultivated in Egypt. Research by Wei et *al*. (2020) shows that *B. bassiana* can enter tomato tissue randomly with inoculation treatment without causing adverse effects, reducing *Bemisia tabaci* attacks. Trizelia *et al*. (2020) reported that chili plants inoculated with *B. bassiana* through seed soaking were able to suppress the development of the *Myzus persicae* population. The results of Flawerina's (2021) research on *B. bassiana* originating from wheat plants (TD312), chili plants (PD114, PB211), and stink bugs (WS) can live endophytically on tomato plants. They can suppress the development of the *B. tabaci* population; this occurs because of the increase in salicylic acid levels in plants and the density of trichomes on the leaves, which have a negative impact on *B. tabaci*. Hendra *et al* (2023) reported that application of *B.*

bassiana on rice plants through seed soaking increased the mortality of *N. lugens* nymphs and decreased the percentage of adult formation. Sari *et al* (2023) reported that *B. bassiana* and *M. anisopliae* inoculated through seed treatment hurt the growth of *Spodoptera frugiperda* larvae. It appears that the integration of entomopathogenic fungi into integrated pest management systems has great promise. In addition, it might reduce the negative impact that chemical pesticides have on the environment, other biocontrol agents, and helpful microbiological agents. This study aimed to study the development of *S. exigua* larvae in shallot plants that were applied with *B. bassiana* fungi.

MATERIALS AND METHODS

This research was conducted at the Biological Control Laboratory, Department of Plant Protection, Faculty of Agriculture, Universitas Andalas. The research was arranged in a Completely Randomized Design (CRD) with six treatments and five replications. The treatments were:

A= Control (soaking using sterile distilled water)
B= Isolate of B. bassiana from stink bugs (BbWS)
C= Isolate of B. bassiana from corn stem (BbJG)
D= Isolate of B. bassiana from chili leaves (PD114)
E= Isolate of B. bassiana from wheat stem (TD312)
F= Isolate of B. bassiana from chili stem (PB211*)*

Propagation of B. bassiana

The isolate of *B. bassiana* used in this study was a collection of the Biological Control Laboratory, Faculty of Agriculture, Andalas University. *B. bassiana* fungi were propagated on SDAY media by transferring pure cultures of fungi using a cork borer (diameter 0.7 cm) into a petri dish containing SDAY media and incubated at room temperature for 21 days.

Preparation of Shallot

The feed of larva *S. exigua* used was shallot leaves. Shallots were planted using 10 kg polybags. Furthermore, the planting medium used was a mixture of soil and manure with a ratio of 2:1. Care of shallots was carried out by watering, weeding, and mechanical pest control without synthetic pesticides.

Propagation of *S. exigua* Larvae

S. exigua larvae or egg clusters are collected from spring onion/shallot plantations. Furthermore, *S. exigua* larvae are kept in plastic boxes and fed with fresh shallot leaves. Larvae food is replaced after it runs out or is no longer fresh. Larvae are kept until they become adults. All adults are kept en masse in insect cages and given fresh shallot leaves as a place to lay eggs. Honey, with a concentration of 10%, is used as food for adults.

Preparation of *B. bassiana* Fungal Suspension

Conidia of *B. bassiana* fungal isolates were taken at 21 days by adding 10 ml of sterile distilled water and 0.01% tween 80 as a leveling agent into a petri dish. All ingredients were mixed using a medium-sized brush to release the conidia, then put into a test tube and homogenized with a vortex. A serial dilution was carried out up to 10^{-3} to obtain the required conidia, and the concentration used was 10^{8} conidia/ml. The density of conidia was calculated using a Neubauer Improved type hemocytometer.

Application of *B. bassiana*

Shallot bulbs of the Sumbu Marapi variety were prepared with as many as 60 bulbs for each treatment. Before being treated, the shallot bulbs were first surface sterilized by soaking the bulbs in 70% alcohol for 1 minute. Furthermore, the bulbs were washed thrice with sterile distilled water for 1 minute. The bulbs were air-dried in a laminar airflow. The air-dried bulbs were then soaked using a 200 ml *B. bassiana* suspension with a density of 108 conidia/ml for 15 minutes in a 400 ml beaker glass. The bulbs that had been treated were air-dried in a laminar airflow for 60 minutes before being planted. Furthermore, the bulbs were planted in plastic cups containing a mixture of sterile soil and manure in a ratio of 2:1.

Effect of *B. bassiana* on Development of *S. exigua* larvae

The non-choice method was used in this experiment. Shallot plants treated with *B. bassiana* with the bulb soaking method and control plants aged 30 days after planting are used as feed for *S. exigua* larvae. To observe the development of *S. exigua*, 15 first instars were starved for one hour. Shallot leaf pieces inoculated with fungi were given to the larvae, and the feed was changed every day until the larvae became pupae. As a control, the larvae were given shallot leaf feed that did not contain fungi.

RESULTS AND DISCUSSION

Mortality of larvae

Analysis of variance show that soaking shallot bulbs in *B. bassiana* significantly impacted the number of *S. exigua* larvae that died and the length of time they lived as larvae. All *B. bassiana* isolates tested were significantly different from the control but not significantly different between isolates. The larval stage length observations revealed that the BbWS and PB211 isolates had significantly different results from the control. In contrast, the treatments of the TD312, BbJG, and PD114 isolates were not significantly different from the control. Table 1 displays the percentage of larval mortality and the length of the S. exigua larval stage in shallot plants.

Table 1. Mortality of larvae and duration of the larval stage of *S. exigua* on shallot plants applied with the *B. bassiana* fungal isolate

Treatment	Larval mortality (%) ($\bar{x}$ ± SE)	Length of larval stage ($\bar{x}$ ± SE)
BbWS	100,00 ± 0,00 a	18,40 ± 0,40 a
TD312	100,00 ± 0,00 a	17,20 ± 0,49 abc
PB211	98,66 ± 1,33 a	17,40 ± 0,60 ab
BbJG	97,33 ± 2,66 a	16,80 ± 0,58 bc
PD114	97,33 ± 1,63 a	16,60 ± 0,40 bc
Control	43,99 ± 4,98 b	16,00 ± 0,00 c

Numbers followed by the same lowercase letter in the same column are not significantly different according to LSD at the 5% level.

Table 1 demonstrates that feeding *S. exigua* larvae red onion leaves infected with *B. bassiana* fungus resulted in larval mortality ranging from 97.33 to 100%. In contrast, the control treatment yielded larval mortality results of 43.99%. The BbWS isolate had the most extended observation of the larval stage, with a stage length of 18.40 days, compared to the control's 16 days.

When *S. exigua* larvae ate shallot leaves treated with *B. bassiana* fungus and then died, they had white mycelium or conidia on the outside of their bodies. One day after the insect died, the white mycelium began to penetrate the cuticle out of the insect's body, then continued to grow and eventually covered the entire body of the larva (Figure 1.)

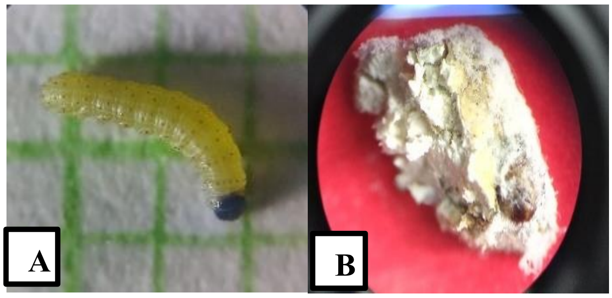

Figure 1. *S. exigua* larvae consuming red onion leaves infected with *B. bassiana*. (A). *S. exigua* larvae (normal), (B). larvae infected with *B. bassiana*

The presence of *B. bassiana* fungus in shallot plant tissue can affect the feeding of S. exigua larvae. The study results showed that inoculation of *B. bassiana* fungus through soaking bulbs in shallot plants used as food for *S. exigua* larvae can cause larval mortality ranging from 97.33 to 100%. The fungus is suspected to live in the host plant tissue and is eaten by the larvae. The entomopathogenic fungus *B. bassiana* can cause death in insects not only through the cuticle but also through the digestive system. Broome *et al.* (1976) reported that oral application of *B. bassiana* caused *Solenopsis richteri* larvae mortality by 84%. This shows that *B. bassiana* infects insects through the digestive tract, and *B. bassiana* fungal spores will germinate 72 hours after infection. Then, the results of Wulandari's (2024) research showed that applying various isolates of entomopathogenic fungi *B. bassiana* through soaking shallot bulbs can cause *S. litura* mortality of 20-48%. Hasibuan *et al.* (2024) reported that applying *B. bassiana* to *S. innotata* larvae can cause up to 100% larval mortality. Infection in the larvae's body causes an increase in blood pH, blood clotting, and cessation of blood circulation, causing the larvae to die and the larvae's body to contain mycelium from *B. bassiana*.

The *B. bassiana* fungus applied by soaking shallot bulbs can also negatively affect the length of the *S. exigua* larval stage, which lasts longer. Therefore,endophytic *B. bassiana* inoculated through bulb treatmen thad a negative impact on the growth of *S. exigua* larvae Fontana *et al.* (2021) revealed that the presence of endophytic fungi during the evolutionary process allows plants to grow better and be more resistant to attacks by herbivorous insects, increase growth, stress resistance, and produce chemical compounds such as enzymes, alkaloids, hormones, and antibiotics. These compounds can cause significant toxicity to herbivorous insects. The mechanisms of endophytic fungi in protecting plants can occur due to changes in chemical nutrition, qualitatively and quantitatively, such as variation in carbohydrate and nitrogen content, including phytosterol composition (Schulz & Boyle 2005)

The percentage of pupa formed

The results of observations on the percentage of pupae formed and the duration of the pupae stage of S. exigua on shallot plants applied with five isolates of *B. bassiana* fungus through bulbs soaking showed that the results of the five isolates tested were significantly different from the control but not significantly different between isolates. The percentage of pupae formed and the duration of the pupae stage of *S. exigua* can be seen in Table 2.

Table 2. The percentage of pupae formed and the duration of the pupal stage of *S. exigua* on shallot plants were applied with the *B. bassiana* fungal isolate.

Treatment	Pupa formed (%) ($\bar{x}$ ± SE)	Duration of pupal stage ($\bar{x}$ ± SE)
Control	55,99 ± 4,98 a	5,60 ± 0,24 b
PB211	1,33 ± 1,33 b	6,00 ± 0,00 a
TD312	0,00 ± 0,00 b	6,00 ± 0,00 a
BbJG	2,66 ± 1,63 b	6,00 ± 0,00 a
PD114	2,66 ± 1,63 b	6,00 ± 0,00 a
BbWS	0,00 ± 0,00 b	6,00 ± 0,00 a

Numbers followed by the same lowercase letter in the same column are not significantly different according to LSD at the 5% level.

In Table 7, it can be seen that the percentage of pupae formed ranges from 1.33 - 55.99%. The five isolates tested showed that the percentage of pupae formed ranged from 0.00 - 2.66%, and isolates TD312 and BbWS did not form pupae. Observation of the duration of the pupae stage in the treatment of all isolates tested was six days, while in the control treatment, it was 5.60 days.

The percentage of Adults formed

The results of observations on the percentage of adult formed and the duration of the adult stage of *S. exigua* on shallot plants applied with five isolates of *B. bassiana* fungus through bulbs soaking showed that the results of the five isolates tested were significantly different from the control but not significantly different between isolates. The percentage of adult formed and the duration of the adult stage of *S. exigua* can be seen in Table 3.

Table 3. Percentage of adult formed and duration of the adult stage of *S. exigua* on shallot plants applied with the *B. bassiana* fungal isolate.

Treatment	Adults formed (%) ($\bar{x}$ ± SE)	Length of adult stage (days)	
		Female	Male
Control	55,99 ± 4,98 a	7,00 ± 0,00	6,00 ± 0,00 a
PB211	1,33 ± 1,33 b	-	1,40 ± 1,4 b
TD312	0,00 ± 0,00 b	-	-
BbJG	2,66 ± 1,63 b	-	1,40 ± 1,4 b
PD114	2,66 ± 1,63 b	-	2,80 ± 1,71 b
BbWS	0,00 ± 0,00 b	-	-

Numbers followed by the same lowercase letter in the same column are not significantly different according to LSD at the 5% level.
Note: (-) Adult is not formed

Table 3 shows that the percentage of adult formed in the isolate treatment ranged from 0.00 - 1.33%, while in the control, it reached 55.99%. In the TD312 and BbWS isolate treatments, no adult was formed. The most extended adult stage observation was in the control with a female imago development period of 7 days. In the five isolates tested, no female adult was formed. The duration of the male adult stage in the control group was six days, while in the treatment group, it ranged from 1.40 to 2.80 days.

Larval mortality is closely related to the percentage of pupae and adult formed; the higher the larval mortality, the lower the percentage of pupae and adult formed. Based on the study's results, it was found that inoculation of *B. bassiana* fungus on bulbs of shallot could inhibit the formation of pupae and adult. The percentage of pupae and adult formed in the control treatment was 56%. In comparison, in the treatment of *B. bassiana* BbJG and PD114 isolates, it was 2.66%, and in the treatment of B. bassiana BbWS and TD312 isolates did not form pupae and adult. This is thought to be because the toxin compound content released by the *B. bassiana* fungus can influence the development process of larvae until they become adult. Mwamburi's (2021) reported that larvae fed on one of the tomato varieties inoculated with *B. bassiana* (BbC1) and *M. anisopliae* (M150) fungi through root immersion did not develop into pupae. This means larval development was delayed due to BbC1 or M150 fungus treatment on tomato plants. At the same time, larvae-fed tomato plants not inoculated with the fungus could pupate successfully after 21 days.

The presence of *B. bassiana* fungus in shallot plant tissue can change the plant's morphology and physiology so that the plant's nutrient content changes and can affect the ability of insects to eat. The observations on the duration of the *S. exigua* adult stage and the adult formed (Table 3) showed that the duration of the female adult stage in the *B. bassiana* fungus isolates treatment was shorter than the control. Then, the percentage of adult formed in the *B. bassiana* fungus treatment was only male adult. It is suspected that the nutrients in the shallot plant are insufficient for developing *S. exigua*, so many male adults are obtained. The effect of *B. bassiana* fungus infection is deadly, affects the growth and development of insects, and reduces their reproductive ability. *S. exigua* larvae that feed on *B. bassiana*-infected shallot leaves also produce abnormal pupae and adult. Abnormal adult causes them difficulty in flying and copulating so it is expected to reduce the population in the field. The results of this study indicate that the application of *B. bassiana* to shallot plants through soaking the bulbs can be used to protect shallot plants from *S. exigua* pest attacks.

CONCLUSION

The results showed that the mortality of larvae feeding on plants applied with *B. bassiana* reached 100%. The application of *B. bassiana* also extended the larval period, reducing the percentage of pupae and adult formed. BbWS and TD312 were better isolated in inhibiting the development of *S. exigua*.

ACKNOWLEDGMENTS

Through this opportunity, the author would like to express his appreciation and gratitude to the Directorate General of Higher Education, Research and Technology, Ministry of Education, Culture, Research, and Technology, by the Letter of Agreement for the Implementation of the Master's Thesis Research Grant No. 130/UN 16.19/PT. 01.03/2023 for the 2023 fiscal year, which has helped fund this research so that it can run smoothly.

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