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Effectiveness of Three Strains of *Beauveria bassiana* and Tobacco Leaf Extract Against *Plutella xylostella* Larvae (Lepidoptera: Plutellidae)

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ABSTRACT

Diamondback moth, *Plutella xylostella* is the main pest of cabbage crop worldwide. This pest could cause harvest loss around 50% to 100%. This study aimed to investigated the effectiveness of three strains of *B. bassiana* and tobacco leaf extract to control *P. xylostella* larvae. In this study, three strain of *B. basiana* with different spore density (10^4/ml, 10^6/ml, 10^8/ml, and 10^{10}/ml) and tobacco leaf extract with different concentration (20 ml/L, 40 ml/L, 60 ml/L and 80 ml/L.) were used to evaluated third instar larvae of *P. xylostella* mortality and feeding inhibition. In total, this study used 17 treatments consisting of 15 larvae in each treatment and four replications. In this study, The *B. bassiana* strains from Bojonegoro (BJR) and Jatisari (JTR) isolates had the highest percentage mortality of *P. xylostella* larvae. The density of *B. bassiana* at 10^8/ml and 10^{10}/ml showed highest percentage of mortality of *P. xylostella* larvae. Besides, the tobbaco leaf extract with 80 ml/L concentration had the highest mortality. However, the use of tobbaco leaf extract in controlling *P. xylostella* is less effective than *B. bassiana*. The *B. bassiana* and *tobbaco* leaf extract have strong feeding inhibition against *P. xylostella* larvae.

keywords: *Beauveria bassiana*, Feeding inhibition, Mortality, Tobacco leaf extract, *P. xylostella*

INTRODUCTION

Diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) is the main pest of cabbage crop worldwide. The diamondback moth is thought to have originated in Europe, but it can now be found throughout the Southeast Asia, Australia, America and New Zealand (Capinera, 2011). This pest could cause harvest loss around 50% to 100%. In indonesia, (Kristanto et al., 2013) reported that *P. xylostella* can attack on cabbage crop and it could cause yield losses up to 100%.

The common method used by farmers to control *P. xylostella* is chemical pesticides to reduce yield losses of cabbage crop (Hadi et al., 2021). However, the continuous use of chemical pesticides with excessive doses has some adverse effects. In addition to harming the environment, it can also cause pest resistance, pest resurgence, the mortality of natural enemies and harm for human health (Sharma et al., 2019).

The negative impact of excessive chemical pesticides can be overcome by selecting environmentally friendly control techniques. Eco-friendly control can be carried out using entomopathogenic fungi and botanical pesticides. Th entomopathogenic fungi that can be used is *B. bassiana*, this fungi was reported can controling different

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pest in several study (Afandhi et al., 2020, 2021; Saleh et al., 2021)

Plant extracts also have the potential to serve in effective pest control (Isman, 2000). Some secondary metabolites of plant have antifeedant effects and can inhibit growth, development, and reproduction of insect pests (Kubo, 2006; Kraiss and Cullen, 2008). Thus, botanical pesticides represent attractive alternatives to currently used synthetic insecticides, as plants are a rich source of bioactive chemical compounds (Pavela, 2016).

Farmers of the Brejonk Organic Farming Community have used *B. bassiana* to control various types of pests, one of which is *P. xylostella* on cabbage crop. There are three strains of *B. bassiana* isolates commonly used by Brenjonk for propagation: Jatisari, Bojonegoro, and Mojoanyar strains. In addition, the farmer also uses plant-based pesticides from tobacco leaf extract to control pests. Tobacco, being a pyridine alkaloids-bearing plant, has a potential to control agricultural pests (Matsuura and Fett-Neto, 2015).

However, the problem in implementing eco-friendly control for pest at the farm level is entomopathogenic fungi and natural pesticides work slowly. Some farmers return to using chemical pesticides and ignore the negative impacts. To overcome this problem, this study aimed to investigated the effectiveness of three strains of *B. bassiana* and tobacco leaf extract to control *P. xylostella* larvae.

MATERIALS AND METHODS

This research was conducted from October to December 2022 at the Biological Agents Laboratory, Brenjonk organic farm, Mojokerto District and the BPTPH Laboratory (Center for the Protection of Pests and Diseases of Food Plants and Horticulture), Pasuruan District. Tstudy used a randomized block design and consisted of 17 treatments and repeated four times.

Insect cultures

The *P. xylostella* larvae were collected from the fields. The collected larvae was brought to laboratory for rearing purpose. The larvae were kept in plastic jars. Then, the larvae was maintained until pupae and transferred to a mating cage that already contains cabbage leave as a diet.

After emergence of adults, a pair of adults was place separately in rearing cage along 10% honey as a diet. Egg mass were collected and transfered into petri dishes for mass culturing. After hatch, the third instar larvae were used as insect tests (Rahardjo et al., 2014).

The propagation of *B. bassiana*

Three strain of *B. bassiana* isolates were used in this study i.e., JTR (From Jatisari), BJR (from Bojonegoro), and MJR (from Mojoanyar). The propagation medium was made from 200 g of potatoes, 1,000 ml of water, and 20 gr of granulated sugar. The potatoes are first cleaned and cut into small cubes. Then, the potatoes are boiled in 1000 ml of distilled water for 20 minutes (Kurniawan and Panggeso, 2020). Then the extracted potato is filtered and put a gallon. Then, the *B. bassiana* isolate was put into the gallon using a looped needle in an aseptic condition. Then a hose connected to glass wool and KMnO4 was added. Then isolate was incubated for 5–7 days.

Spore density test

Spore density test was measured for *B. bassiana* by diluting the primary liquor up to 10^{-2} and testing it using a haemocytometer. This test was carried out by taking 0.2 ml of the test sample at a 10^{-2} dilution using a pipette. Then slowly drop the conidium suspension on the haemocytometer and observe it under the microscope. The calculation of spore density by using the following formula:

$$S = \frac{x}{(L\ x\ t\ x\ d)}\ 10^3$$

Where:
S : Conidium density/ml
x : Mean number of conidia in boxes a, b, c, d, e
L : The calculating box area of 0.0025 mm^2
T : The depth of field counts 0.1 mm
d : The dilution factor
10^3: The calculated suspension volume.

Dilution of *B. bassiana*

One ml of the primary liquor of *B. bassiana* was taken using a pipette and put it into a tube and filled with 9 ml of distilled water. Then shaken 60 times slowly until the solution was homogeneous. The results of the homogeneous solution were taken 1 ml and put into another tube containing 9 ml of distilled water. This step was repeated until the required dilution stage was 10^4/ml, 10^6/ml, 10^8/ml, and 10^{10}/ml (Riningrum et al., 2020).

Extraction of tobacco leaf

The tobacco leaf extraxtion was carried out by water maceration method (Kheawfu et al., 2021). Tobacco leaf was ground to a powder by using blender. Then, 250 g of tobacco leaf powder was put into the Erlenmeyer and 500 ml of solvent (distilled water) was added. In this study, four concentrations were used i.e., 20 ml/L, 40 ml/L, 60 ml/L and 80 ml/L.

Application of *B. bassiana* and tobacco leaf extract on *P. xylostella* larve

In this study, 15 indivudual larvae were used in each treatment. The application of *B. bassiana* and tobacco leaf extract was carried out using the leaf dip method. The cabbage leaf (5.5 g) dipped into the concentration treatment for about 30 seconds. The leaves that have been dipped then removed and air-dried for about 30–60 sec (Firdaus, 2016).

Mortality test

The mortality of *P. xylostella* larvae was investigated at 24, 48, 78, and 86 hours after application by counting the number of larvae killed. The mortality of larvae was calculated using the following formula (Rosmiati et al., 2018):

$$Mo = \frac{k}{Kn}\ x\ 100\%$$

Where:
Mo : The percentage of mortality (%)
k : The number of larvae dead
Kn : Total number of individual

Feeding inhibition of *P.xylostella* larvae

This test was carried out by weighing the cabbage leaves was treated with *B. bassiana* and tobacco leaf extract. The larvae were put into jars, with each jar containing 15 third-instar larvae. After 24 hours of application, the leaves were weighed. The feeding inhibition of *P. xylostella* larvae was calculate using following formula (de Araújo et al., 2020):

$$FI = \frac{(Bk-Bp)}{(Bk+Bp)}\ x\ 100\%$$

Where:
FI: Feeding inhibition (%)
Bk: Weight of control leaves consumed
Bp: Weight of treated leaves consumed

Data Analysis

The mortality and feeding inhibition of larvae were analyzed using ANOVA with an error rate of 5%. If there is a significant difference between treatments, then the Duncan Multiple Test (DMRT) was performed with an error rate of 5%. All the data were analyzed by using IBM SPSS Statistics 20 and Microsoft Office Excel. In addition, Probit analysis was used to determine the proper time (LT_{50}) to kill 50% of the larvae. The LT_{50} value were analyzed by using Microsoft Office Excel.

RESULTS AND DISCUSSIONS

Mortality of *P. xylostella* larvae

In this study, the percentage mortality of *P. xylostella* increased following the observation time (Table 1). The highest percentage of mortality was found in JTR 10^8 and BJR 10^{10} at 96 hours after treatment

(Table 1). This indicated that differences in spore density had the ability to killed *P. xylostella* larvae. The higher spore density of *B.bassiana* had the highest ability to kill *P. xylostella* larvae. In line with Sianturi et al., (2014), higher spore density had the more conidia in the solution, and will be more fungal conidia attached in *P. xylostella.* In addition, the different strains of *B. bassiana* isolates can also affect insect mortality. Bayu et al., (2021) reported that different strains of fungal isolates would lead to differences in the levels of conidia production and conidia germination which would affect the effectiveness of the fungus. Furthermore, the isolated strain will also affect enzyme production in *B. bassiana*. Enzyme production depends on internal factors such as isolates and nutrients in the growing media during propagation and external factors such as temperature and humidity (Mondal et al., 2016).

***Median Lethal Time* (LT_{50})**

In this study, three *B. bassiana* strain isolates with different spore densities and tobacco leaf extracts with different concentrations had the different LT_{50} values. The result showed that the fastest treatment to kill 50% of the *P. xylostella* larvae were found in the BJR isolate with 10^{10}/ml spore density and tobacco leaf extract at 80 ml/L concentration (Table 2). This indicated that the high spore density and concentration used I this study had the fastest ability to kill 50% of *P. xylostella* larvae. Besides, tobacco leaf extract had the ability to kill 50% of larvae faster than *B. bassiana*. According to Jauharlina and Hendrival (2003), the differences in time that cause insect death depend on various factors, such as pathogen virulence, host resistance, and environmental conditions. Another factor is due to the enzymes produced by *B. bassiana* needing time to be active. According to Liu et al. (2010), enzymes degrading the fungus's integumentary layer will form within 8–10 hours after application. Whereas, the toxic compounds in tobacco leaf extract can directly cause death in *P. xylostella*.

The Effect of *B. bassiana* and Tobacco Leaf Extract on the Feeding Inhibition of *P. xylostella* larvae

The results show that the highest feeding inhibition was found in the JTR 10^8 (Table 3). It could be due to differences in the spore densities used in this study. The treatment with low spore density will produce less toxin (Rosmiati et al., 2018). Altinok et al. (2019) also stated that the toxic compounds in *B. bassiana* will circulate in the hemolymph, affecting pH and disrupting the nervous system. Under these conditions, the insects will lose their appetite to feed. According to Sukar and Abdullah (2021), the secretion of *B. bassiana* metabolites has antifeedant properties such as n-Hexadecanois acid, 12-octadecadienoic acid, methyl esters, hexadecanoic acid, 10-octadecadienoic and tetradecanoic acids. Besides, both *B. bassiana* and tobacco leaf extract had strong antifeedant category with the value was more than 70%. The difference in the concentration of tobacco leaf extract used can affect the feeding inhibition of *P. xylostella*. The tobacco leaf extract may contain antifeedant compounds and can preventing larvae to feed. Harwanto et al. (2012) reported that host was exposed to antifeedant compounds had the possibility to stop temporarily insect to feed or stop permanently.

CONCLUSION

The three strain of *B. bassiana* and tobacco leaf extract had a larvicide effect on P. xylostella third-instar larvae. The *B. bassiana* strains from Bojonegoro (BJR) and Jatisari (JTR) isolates had the highest percentage mortality of *P. xylostella* larvae. The density of *B. bassiana* at 10^8/ml and 10^{10}/ml showed highest percentage of mortality of *P. Xylostella* larvae. Besides, the tobbaco leaf extract with 80 ml/L

concentration had the highest mortality. However, the use of tobbaco leaf extract in controlling *P. xylostella* is less effective than *B. bassiana*. The *B. bassiana* and tobbaco leaf extract have strong feeding inhibition against *P. Xylostella* larvae.

Table 1. The mortality of *P. xylostella* larvae at 24, 48, 72, and 96 hours after treatment

Treatments	Percentage mortality (%)			
	24 HAT (x̄+SD)	48 HAT (x̄+SD)	72 HAT (x̄+SD)	96 HAT (x̄+SD)
JTR 10^4	0.00±3.57 a	5.36±8.91 a	25.41±4.78 ab	36.40±10.30 abc
JTR 10^6	3.57±6.84 abc	21.70±11.98 ab	45.61±8.94 ab	61.81±14.85 cdef
JTR 10^8	5.36±4.12 abc	27.20±5.39 ab	47.25±5.50 ab	83.24±6.74 f
JTR 10^{10}	1.79±0.00 ab	23.63±6.84 ab	43.55±9.03 ab	72.94±5.99 def
BJR 10^4	0.00±0.00 a	5.49±10.27 a	23.49±10.24 ab	52.75±10.12 bcde
BJR 10^6	5.36±5.83 abc	21.98±13.44 ab	43.82±9.34 ab	72.67±13.70 def
BJR 10^8	7.14±6.84 abc	25.14±8.92 ab	52.47±9.50 ab	81.46±9.20 ef
BJR 10^{10}	5.36±0.00 abc	23.63±6.67 ab	56.32±8.63 b	85.44±8.94 f
MJR 10^4	38.19±29.93 d	36.5435.06 ab	25.55±30.88 ab	23.63±27.61 ab
MJR 10^6	31.05±24.57 bcd	49.18±27.66 b	40.11±25.60 ab	25.55±20.68 ab
MJR 10^8	32.83±31.25 cd	27.34±37.62 ab	19.92±34.40 a	16.49± 28.98 a
MJR 10^{10}	38.32± 31.30 d	42.03±24.43 b	29.12±24.53 ab	25.42±24.28 ab
TLE 20 ml/l	29.26±21.38 abcd	23.77±21.54 ab	36.68±23.47 ab	40.25±22.90 abc
TLE 40 ml/l	21.98± 15.8 abcd	29.26±15.91 ab	36.54±21.47 ab	38.46±22.90 abc
TLE 60 ml/l	25.55± 24.53 abcd	38.32±23.90 ab	43.82±28.36 ab	31.18±20.42 ab
TLE 80 ml/l	20.06±9.13 abcd	31.05±12.67 ab	42.04±19.48 ab	49.32±24.93 bcd

Note: Numbers followed by the same letter in the same column are not significantly different at the 5% level on Duncan's test. JTR: Jatisari; BJR: Bojonegoro; MJR: Mojoanyar, and TLE: tobacco leaf extract; HAT: hours after treatment; SD: Standard deviation.

Table 2. LT_{50} value of *B. bassiana* and tobacco leaf extract against *P. xylostella* larvae

Treatment	Regression equation	Value of Regression coefficient	LT_{50}
JTR 10^4	y = 2.927x - 1.0598	R^2 = 0.985	117.57
JTR 10^6	y = 2.3466x + 0.6565	R^2 = 0.8401	70.95
JTR 10^8	y = 3.265x - 0.7705	R^2 = 0.9176	58.53
JTR 10^{10}	y = 2.9867x - 0.4704	R^2 = 0.9682	67.86
BJR 10^4	y = 0.8768x + 2.9448	R^2 = 0.1979	220.79
BJR 10^6	y = 2.7465x - 0.0335	R^2 = 0.9385	68.03
BJR 10^8	y = 3.261x - 0.7586	R^2 = 0.9469	58.33
BJR 10^{10}	y = 3.4334x - 0.9709	R^2 = 0.9599	54.84
MJR 10^4	y = 2.9149x - 0.7639	R^2 = 0.9648	725.50
MJR 10^6	y = 3.0503x - 0.8025	R^2 = 0.9506	79.85
MJR 10^8	y = 3.051x - 0.4787	R^2 = 0.9388	62.48
MJR 10^{10}	y = 2.7211x + 0.1254	R^2 = 0.9198	61.86
TBU 20 ml/l	y = 1.5739x + 1.852	R^2 = 0.6601	100.03
TBU 40 ml/l	y = 1.9056x + 1.2798	R^2 = 0.9765	89.59
TBU 60 ml/l	y = 2.1885x + 1.0368	R^2 = 0.9853	64.70
TBU 80 ml/l	y = 2.9149x - 0.7639	R^2 = 0.9648	28.40

Note: JTR: Jatisari; BJR: Bojonegoro; MJR: Mojoanyar, and TLE: tobacco leaf extract;

Table 3. The feeding inhibition of *P. xylostella* larvae

Treatment	Feeding Inhibition (%)			
	24 HAT (x̄+SD)	48 HAT (x̄+SD)	72 HAT (x̄+SD)	96 HAT (x̄+SD)
JTR 10^4	41.90±7.57 ab	61.5±5.88 abc	71.35±1.91 abcd	77.20±4.0 ab
JTR 10^6	49.69±3.13 cd	74.24±2.94 f	82.05±2.50 ghi	90.03±2.40 ef
JTR 10^8	56.32±1.89 e	69±4.03 def	72.29±1.74 bcde	95.28±1.93 f
JTR 10^{10}	51.56±2.36 de	80±2.08 g	89.80±3.32 j	93.79±3.84 f
BJR 10^4	41.30±3.31 a	58±4.4 ab	57.44±7.89 ab	78.20±3.68 bc
BJR 10^6	49.67±4.76 cd	64±2.89 bcd	70.93±4.88 abcd	92.88±2.30 f
BJR 10^8	50.32±4.18 cd	67.5±4.24 cde	75.17±2.83 def	95.76±2.44 f
BJR 10^{10}	50.87±1.30 d	71±4.08 ef	81.57±2.89 ghi	95.25±5.90 f
MJR 10^4	42.43±2.75 ab	57.5±2.08 a	66.02±3.30 a	86.23±2.86 de
MJR 10^6	43.02±0.58 ab	57.5±2.94 a	73.80±3.38 cdef	91.90±3.32 ef
MJR 10^8	49.71±4.63 cd	65±4.04 cde	82.99±3.11 hi	93.80±3.35 f
MJR 10^{10}	52.05±3.71 de	67.5±3.31 cde	78.10± 2.29 fgh	93.84±2.58 f
TBU 20 ml/l	42.48±2.63 ab	62.5±3.87 abcd	68.41±2.90 abc	71.66±7.58 a
TBU 40 ml/l	44.87±4.24 abc	65±3.74 cde	72.82± 2.03 bcdef	74.42±2.16 ab
TBU 60 ml/l	49.13±2.61 cd	68±3.16 cdef	77.14±2.42 efg	83.46±6.33 cd
TBU 80 ml/l	47.25±3.57 bcd	68±8.04 cdef	86.40±2.74 ij	90.51± 1.64 ef

Note: Numbers followed by the same letter in the same column are not significantly different at the 5% level on Duncan's test. JTR: Jatisari; BJR: Bojonegoro; MJR: Mojoanyar, and TLE: tobacco leaf extract;

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