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Infection of *Ostrinia furnacalis* (Lepidoptera: Pyralidae) by Endophytic *Beauveria bassiana* on Corn

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Abstract: The Asian corn borer, *Ostrinia furnacalis* Gueneé (Lepidoptera: Crambidae), is one of the most important pests of corn. Control against this pest is attempted in several ways, including the biological control with entomopathogenic fungi, *Beauveria bassiana.* Infection of *B. bassiana* in different larval phases can cause differences in insect response. This study aims to examine the effect of different larva's stages when they are inoculated with *B. bassiana* to the growth of *O. furnacalis*. This study was conducted by allowing different larval instars of the pest to feed on corn plants inoculated with different dosages of *B. bassiana* spores. The observed parameters were larval mortality, pupal weight, adult longevity, adult fecundity and number of eggs and larvae infected by *B. bassiana*. The results showed that *B. bassiana* caused mortality to the 1st and 2nd instars of the larvae. The sub-lethal effects of *B. bassiana* on *O. furnacalis* development included reduction in pupal weight, shorter longevity, decreased fecundity of about 50% and reduced number of eggs laid up to 72.3%. *B. bassiana* can be transmitted vertically to the next generation. Up to 88% and 75% of the eggs and larvae were infected by the fungus, respectively. Information provided by this study is very useful for *B. bassiana* conservation efforts in the corn ecosystem and for supporting integrated pest management of *O. furnacalis.*

Keywords: Entomopathogenic Fungi, Endophyte, Larvae, Pupae, Mortality

Introduction

The Asian corn borer, *Ostrinia furnacalis* Gueneé (Lepidoptera: Crambidae) is one of the main pests of corn plant with a wide distribution throughout Southeast Asia, Central Asia, East Asia, and Australasia. The borer attacks all parts and development phases of the corn plants. The pest causes damages on stems, cobs, and flowers of the plant, inflicting yield losses up to 80% (Bato *et al*., 1982). The yield loss occurs the most when the plants are attacked during their reproductive phase of development. The larva is the destructive life stage of *O. furnacalis*. The first instar larvae feed on young leaves and forms small holes in the leaves, the second instar larvae feed on the leaves, especially those that are not yet fully open or developed, the third instar larvae feed on the leaf and stem, and the fourth and fifth instar larvae bore the plant stem (Cook *et al*., 2004; O'Day *et al*., 1998).

Biological controls are alternative management measures that can be developed to control *O. furnacalis*, one of which is using entomopathogenic fungi. The fungus *Beauveria bassiana* has been reported as an endophytic fungus in various plant species and has shown activity against various pests (Bamisile *et al*., 2018). The entomopathogen has been reported effective in controlling many important plant pests, such as *Helicoverpa armigera* on tomato (Qayyum *et al*., 2015), *Hypothenemus hampei* on coffee (Posada *et al*., 2007) and *Lyriomyza huidobrensis* on *Visia faba* and *Phaseolus vilgaris* (Akutse *et al*., 2013). On corn, *B. bassiana* has been reported to control lepidopteran insects such as *Sesamia calamistic* (Cherry *et al*., 2004), *Ostrinia nubilalis* (Bing and Lewis, 1991) and *Spodoptera frugiperda* (Ramírez-Rodríguez and Sanchez-Peña, 2016). There is an evidence that while acting as a plant endophyte, *B. bassiana* can also reduce pest damage (Gurulingappa *et al*., 2010; Akello and Sikora, 2012) by inhibiting insect development and reproduction and may also be antagonistic to plant pathogens (Ownley *et al*., 2008). Another advantage of entomopathogenic fungi is

the ability to attack all stages of insect development including the eggs and pupae encompassing a fairly wide host range (Anand *et al*., 2009).

The existence of *B. bassiana* as an endophyte in insect pest hosts is expected to be sustainable. The ability of *B. bassiana* to be transmitted vertically and horizontally can support the sustainability of *B. bassiana* control in the field. Effects of *B. bassiana* as endophyty to the growth of corn plants and the development of *Rachiplusia nu* (Lepidoptera: Noctuidae) as a corn pest has been investigated by Russo *et al*. (2019). However, the role of endophytic *B. bassiana* in to control and inhibit the growth of *O. furnacalis* has not been demonstrated. This study aims to observe the effect of *B. bassiana* as an endophytic fungus on corn in inhibiting the development of *O. furnacalis* growth and its ability to be transmitted vertically to subsequent offsprings.

Methods

Larva Infection

Test larvae were obtained from eggs laid by adults of *O. furnacalis* which were free from *B. bassiana* infection. Larvae were fed with the American diet. The larvae used in the trial were larval instars 1, 2, 3, 4 and 5. The spores of *B. bassiana* were isolated from homogenized *O. furnacalis* larvae infected by *B. bassiana*. Different dosages of *B. bassiana* spores (0, 100, 200, 400 or 800 spores/mm^2 of the leaf surface) were applied. Stalks of one-week old tested plants were injected with 0.2 mL *B. bassiana* aliquots in H_2O per stalk. One larva was then placed on each of test plants that have been inoculated with *B. bassiana*. Each dosage treatment had five replications of one plant each. The treatment was incubated in a growth chamber with lighting lamps (L:D = 12:12) with a temperature of 27°C and humidity of 70%. After 48 h, the larvae from each treatment were removed from the endophytic stem of *B. bassiana* and transferred individually into a glass bottle (18×65 mm) containing fresh corn leaves that were not inoculated *B. bassiana*. The bottles are then covered with cotton plugs and incubated in an incubator to observe the development of insects until the pupae are formed. Sex of the pupae was determined, and female pupae were weighed and put in insect-care containers.

Mating and Oviposition

The newly formed adult females were allowed to mate with uninfected males. Ten pairs of adults were used in each treatment and each pair was placed in a cup (10 cm in diameter, 5 cm in height). Waxed paper was placed at the top of the cup which served as a substrate for oviposition. Each day the waxed paper was removed and the eggs on it were counted. Observation of the number of eggs laid in each treatment was carried out for 7 days at a temperature of 27°C and humidity of 70%.

Detection of Blastospores in the Eggs and the Larvae

Observation of the blastospore on eggs and larvae began by dividing the waxed paper on which the *O. furnacalis* eggs were laid into 2 equal parts. One part was frozen for observation of blastospore in eggs and the other part was incubated until the eggs hatched. Instar one larvae were frozen for observation of the blastospores in larvae. The presence of the blastospores in the larvae was observed under a compound microscope (400×).

Observation of *B. bassiana* infection in eggs was started by immersing a group of frozen eggs into a 2% trypsin solution in a 0.05 M K_2HPO_4 buffer and 0.05 M KH_2PO_4 (pH = 8). This egg group was then incubated at 36-38°C for 45 min to let egg-mass cement dissolve to form individual eggs. Next, the eggs were washed in the filter funnel, four times with ddH_2O and twice with 70%bethyl alcohol. The rate of *B. bassiana* infection in eggs is estimated by taking 10 eggs chosen at random at each treatment. Then the eggs were observed under a compound microscope (400×).

Experimental Design and Statistical Analysis

The experiment was designed in a randomized complete block with a split-plot arrangement of treatments. The instars were the plots and spore concentrations were the subplots. The experiment was replicated three times. Pupal weight, fecundity and longevity of female moths and blastospore infection rate on eggs and larvae were analyzed using ANOVA and relationships between variables were determined by using contrast coefficients.

Results

Beauveria bassiana infection affected the growth of *O. furnacalis* larvae differently, depending on the larval instar and the spore concentration applied to the corn. The first and second instars of *O. furnacalis* larvae died after feeding on the corn inoculated with *B. bassiana*; while the third, fourth and fifth instars of larvae were able to complete their life cycle. The application of *B. bassiana* on the third, fourth and fifth instar larvae caused a significant decrease in pupal weight relative to the control. The higher dose of *B. bassiana* applied to corn plants tended to cause a greater reduction in pupal weight; although this did not occur in the fourth instar larvae applied with 600 spores/mm^2 of leaf and fifth instar larvae applied with 800 spores/mm^2 of leaf. The weight of the pupae treated with *B. bassiana* decreased by 11% to 28% compared to the control (Table 1).

B. bassiana infection also affected the longevity of *O. furnacalis*. Larvae feeding on *B. bassiana* inoculated plants had a significantly shorter longevity than the control. In general, the higher dose of *B. bassiana* was consumed, the shorter the *O. furnacalis* longevity was. The shortest longevity was found in the treatment of the

third instar larvae fed on plant inoculated with 600 spores /mm^2 of leaf. It was only 8 days or 4.3 days shorter than the control (Table 1).

Adult females on the control treatment produced up to 345 eggs, while females from the larvae fed on *B. bassiana*-corn plants laid maximum 221 eggs. Furthermore, adult females from fifth instar larvae fed on leaf treated with 800 spore/mm^2 produced only 90 eggs. This shows that *B. bassiana* was able to reduce egg production up to 72.3%. In general, the higher the *B. bassiana* dose was used, the lower the *O. furnacalis* fecundity was (Table 1).

Beauveria bassiana infection also affected the number of eggs laid by an adult female of *O. funacalis*. Females from uninfected larvae produced up to 74 eggs as shown on the second day, whereas larvae infected with *B. bassiana* were only able to produce 25 to 37 eggs. The minimum egg laying by *O. furnacalis* infected with *B. bassiana* occurs on the fifth day, which was 3 eggs in the 600 spores/mm^2 leaf treatment. This showed that *B. bassiana* infection reduced the number of eggs produced. The 800 spores/mm^2 treatment had the least eggs produced during the seven days of egg laying period (Fig. 1).

B. bassiana is also transovarial in *O. furnacalis* pest. *B. bassiana* was able to infect eggs from females infected with *B. bassiana*. The percentage of eggs infected with *B. bassiana* increases every day until the seventh day of the egg-laying period. The percentage of *B. bassiana* infected eggs was the highest in *B. bassiana* inoculation of 600 spore/mm^2 of leaf treatment (up to 88%) while the lowest egg infection occurred in *B. bassiana* inoculation of 200 spore/mm^2 leaves treatment (up to 68%) (Fig. 2).

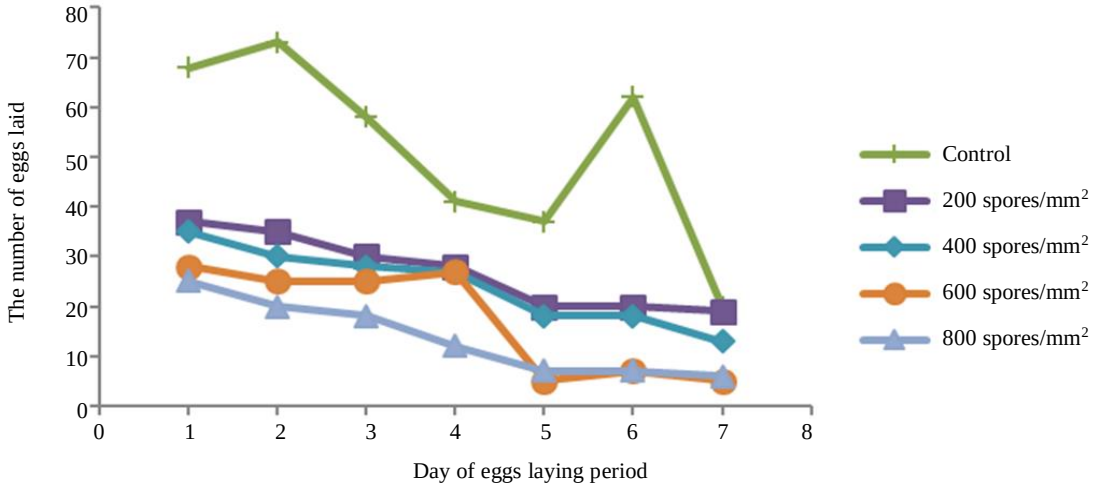

Fig. 1: Effect of different *B. bassiana* dosage treatments on the number of eggs laid by *O. furnacalis* imago

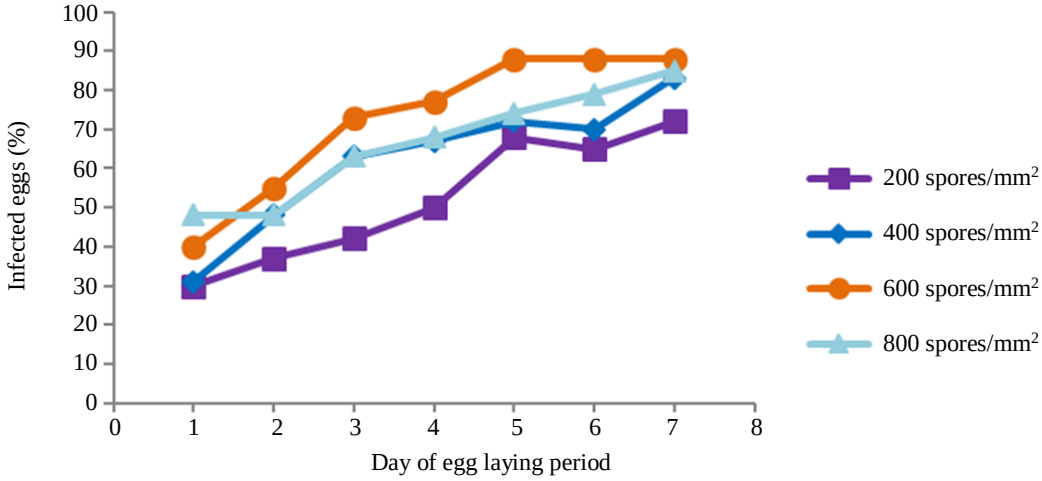

Fig. 2: Percentage of *O. funacalis* eggs infected with *B. bassiana* from imago that larvae fed from corn inoculated with various *B. bassiana* concentrations

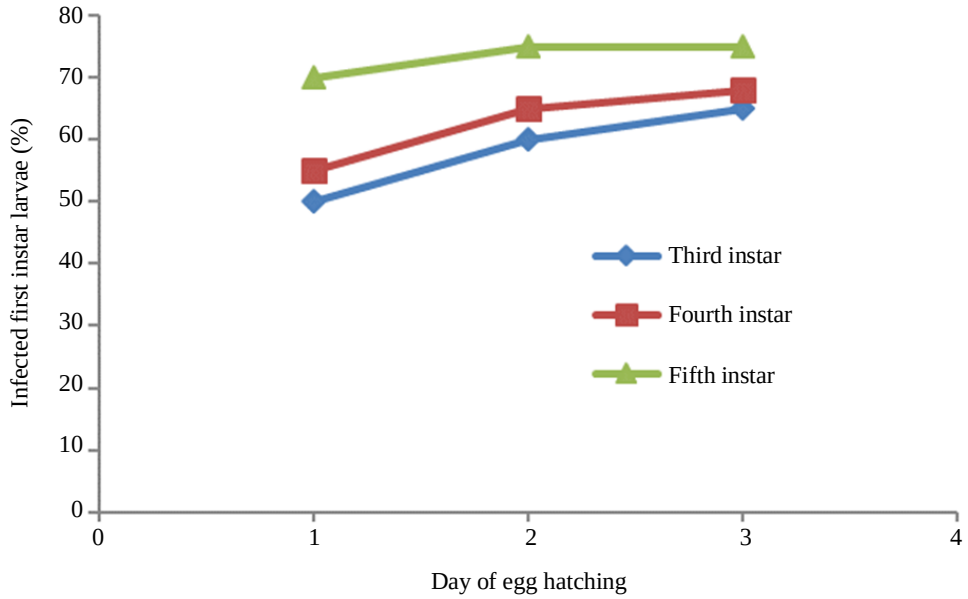

Fig. 3: Percentage of new first instar larvae infected *B. bassiana* with different larval instars fed on corn infected *B. bassiana*

Table 1: Effect of instar larvae fed on corn infected by *B. bassiana* at different doses on pupa weight, longevity and fertility of *O. furnacalis*

	Spores/mm^2 of leaf surface							
Instar	0	200	400	600	800	x	SE	x + SE
Pupa weight, mg								
3	110	98	97	96	88	97.8	7.89	105.69
4	120	100	90	96	90	99.2	12.38	111.58
5	125	99	96	90	90	100	14.51	114.51
x	88.75	124.25	170.75	220.5	267	174.25	71.70	245.95
SE	7.64	1	3.79	3.46	1.15	1.11	3.38	4.49
Longevity, day								
3	12.3	10	8.8	8	8.4	9.5	1.73	11.23
4	13.6	11	10.7	9.2	10.1	10.92	1.65	12.57
5	13	10.2	10.2	9.9	9.4	10.54	1.41	11.95
x	13.0	10.4	9.9	9.0	9.3	10.32	1.57	11.89
SE	0.65	0.53	0.98	0.96	0.85	0.74	0.17	0.90
Fecundity								
3	345	168	117	108	94	166.4	103.67	270.07
4	300	210	217	121	101	189.8	80.48	270.28
5	325	221	117	137	90	178	95.66	273.66
x	323.3	199.7	150.3	122.0	95.0	178.07	89.97	268.04
SE	22.55	27.97	57.74	14.53	5.57	11.70	96.38	108.08

The number of the new first instar larvae infected by *B. bassiana* on the first day was found to be very high from fifth instar larvae that fed from corn infected with *B. bassiana*, then followed by the fourth and third instar larvae which were 65%, 60%, 50%, respectively. *Beauveria bassiana* infection in the first instar larvae was the highest on the third day of observation, reaching 75%. Transovarial transmission appears to be more effective when the last instar larvae fed on the corn infected by *B. bassiana* (Fig. 3).

Discussion

Beauveria bassiana is an entomopathogenic fungus that is effective in controlling insect pests. Generally, this fungus is applied by spraying on the leaves, stems and fruits where the target insects are located, so these spores tend to be exposed to extreme conditions, especially solar radiation. *B. bassiana* which was exposed to UV light at 15 watts with a distance of 30 cm for 1 h, 2 h and 4 h of exposures, showed the ability of spores to germinate by 85.3% to 86.6% but did not cause

death in the test insects. This shows that the pathogenicity of *B. bassiana* is reduced by exposure to UV light, one of which is sun exposure (Daud *et al*., 1998). *Beauveria bassiana* must be protected from direct sun exposure so that its pathogenicity remains stable. Therefore, in this study, *B. bassiana* was applied by injecting it on corn plant stalks upon which the test insects fed. This method is quite effective because *B. bassiana* is also an endophytic fungus. *Beauveria bassiana* is known to endophytically colonize corn (Bing and Lewis, 1991) and banana (Akello *et al*., 2008) and it provides protection to the plants against insect pests.

Beauveria bassiana significantly influences the growth and development of *O. furnacalis*. Younger larvae will be more susceptible to *B. bassiana* infections. In the present study, first and second instar larvae died while eating from *B. bassiana* infected corn. Some reports mention that most entomopathogenic fungi have chitinase enzymes that are large enough to be very toxic in degrading the insect integument wall (Miranpuri and Khachatourian, 1995; Fang *et al*., 2005; Zhu *et al*., 2008). The chitinase of *B. bassiana* CHit1 and CHit2 enzymes are excreted by *B. bassiana* (Fang *et al*., 2005; Holder, 2005) and Fan *et al*. (2007) and highly toxic to integument of Lepidopteran larvae. Larvae instar 1 and 2 have thinner cuticles compared to instar larvae 3, 4 and 5 so that they are more susceptible to *B. bassiana* chitinase degradation.

Effectiveness of *B. bassiana* in controlling insects not only depends on mortality but also on the sub-lethal effects of the fungus on the insect target. The insect instars 3, 4 and 5 fed on corn infected with *B. bassiana* were capable of forming pupae but with lighter body weight compared to the control. Weight reduction of *Costelytra zealandica* larvae fed with pine infected with *B. bassiana* has also been reported by Lefort *et al*. (2016). This weight reduction can be caused by decreased appetite of larvae. Characteristics of *B. bassiana* infected larvae are slow movement, decreased consumption and even stopping eating, gradually becoming quiet and eventually die (Daud *et al*., 1993). Pelizza *et al*. (2017) reported that *B. bassiana* as an endophytic fungus can influence the level of consumption, fertility, and food preference of *Dichroplus maculipennis*. Significant reduction in the food consumption of *D. maculipennis* nymphs in corn plants inoculated with entomopathogenic *B. bassiana* fungi has also been reported (Pelizza *et al*., 2017). In addition, some toxins produced by *B. bassiana* namely beauvericin, bassionolide, cyclodepsi-peptide (Tanada and Rich, 1993) and oosporein (Inglis *et al*., 2001) also play important roles in impeding the development of *O. furnacalis*.

Beauveria bassiana also affects various developmental stages of an insect. The longevity and fecundity of *O. furnacalis* are reduced by the treatment of *B. bassiana* as endophytic fungi in corn. Reduction in the longevity and fecundity of *O. furnacalis* can have an impact on the reduction of population in the next generation. Reduction of longevity due to entomopathogenic infections has also been reported in all phases of development of *Liriomyza huidobrensis* (Akutse *et al*., 2013) and the larval phase of *Tuta absoluta* (Klieber and Rineke, 2016). Decreased longevity of red palm weevil females due to fungal infection has also been reported by Gindin *et al*. (2006). Reduction in longevity and fecundity of *Plutella xylostella* (L.) due to insecticidal treatment has also been documented by Kumar and Chapman (2006). Entomopathogens as endophytes can also slow down reproduction rates (longer time to reproduction and lower fertility rate), reduce feeding and growth insects (Gurulingappa *et al*., 2010). Insect fecundity is directly related to the development of the ovary which produces eggs through oogenesis. Fecundity is affected by the age of the insect when infected. Insect fecundity is lower when *B. bassiana* infection occurs in the younger females when the oogenesis process begins (Sánchez-Roblero *et al*., 2012).

Based on this research, *B. bassiana* can be transmitted vertically. This is due to *B. bassiana* being able to replicate in the host's hemolymph so that the blastospores can survive on the host's body up to the eggs produced by the host. The amount of blastospores increased in the host body due to its ability to multiply rapidly after embryogenesis. Infected larvae of *B. bassiana* have high mortality and a slower rate of development than uninfected larvae. Eggs produced by infected adult insects are contaminated with spores inside of the ovarioles. The level of infection and disease damage caused by the host vary, depending on the stage of development since the spores begin to infect and the number of spores infecting the insects.

Vertical transmission to the next generation provides a great advantage in sustaining the control of *O. furnacalis*. Both vertical and horizontal transmissions affected the effectiveness of *B. bassiana* in controlling *O. furnacalis*. Vertically infected larvae can suffer more severe effects than horizontally infected larvae. However, horizontal transmission is very important to transmit *B. bassiana* spores from infected insects with *B. bassiana* to uninfected insects through copulation. Reports that *B. bassiana* can be transmitted horizontally from infected male imago to uninfected female imago of *Anastrepha ludens* (*Diptera*: *Tephritidae*) (Toledo *et al*., 2007) is an opportunity to spread *B. bassiana* in the field. This is very supportive of biological control of *O. furnacalis*, which is environmentally friendly.

Conclusion

The effectiveness of *B. bassiana* in controlling *O. furnacalis* is influenced by larval instar feeding on

infected corn plants and spore doses applied. First and second instar larvae are very susceptible to the toxicity of *B. bassiana*. Third, fourth and fifth instars of the larvae were able to develop into pupae but with lower weight, and those became adults had reduced fecundity by 50% and shorter life duration. Data shows that spore transfer occurs at each stage of development. Eggs and first instar larvae on the next generation also remain infected by *B. bassiana*. This is very useful for *B. bassiana* conservation efforts in the corn ecosystem and supports integrated pest management of *O. furnacalis*.

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Author's Contributions

Itji Diana Daud: Conceived and designed the study, designed research methodology, manuscript writing and final approval.

Elkawakib: Designed the research methodology and variable assesment method.

Kahar Mustari: Designed research methodology, data analysis, and interpretation.

Aris Baso: Data collection and literature search.

Nuniek Widiayani: Data collection, data analysis and manuscript writing.

Ethics

No potential conflict of interest was reported by the authors.

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