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Efficacy of biological insecticides against *Helicoverpa armigera* in sweet corn crop (*Zea mays saccharata*)

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Abstract

Helicoverpa armigera Hubner (*H. armigera*) is a significant pest to corn crops and constant problem for farmers. The objective of this research was to obtain a more effective insecticide for suppressing the population and intensity of *H. Armigera,* as well as providing high economic value for sweet corn crops. This experiment used a randomized block design, consisting of 4 treatments and 6 replications. The treatments consisted of a control (P0 = without insecticides), P1 = biological insecticide (*Beauveria bassiana* 5 g.l^{-1}), P2 = botanic insecticide (pandan wangi leaf extracts 5 ml.l^{-1}), and P3 = chemical insecticide (Deltamethrin synthetic 3 ml.l^{-1}). The results showed that the application of various types of insecticides has a significant effect on population density of larvae, intensity of *H. armigera* presence and sweet-corn crop yield. All types of insecticides applied are highly effective but the effectiveness of *Beauveria bassiana* 5 g.l^{-1} tends to be greater in comparison to others. The highest cost-benefit ratio was obtained from the application of the biological insecticide followed by the botanic insecticide and the chemical insecticide.

Keywords: *Beauveria bassiana*, deltamethrin, pandan wangi, economical.
Abbreviations: H_ Helicoverpa; B_ Beauveria; DAC_days after cropping.

Introduction

Sweet corn (*Zea mays saccharata*) is a crop grown for human consumption and industrially processed food ingredients worldwide. Sweet corn is different to other corns, mainly in the expression of genes that determine the carbohydrate content of endosperm and affect the corn growth (Znidarcic, 2012). In general, sweet corn is white-haired, whereas regular corn is red-haired. The sweet corn life cycle endures between 60-70 days (Kasryno, 2007). Sweet corn was entered to commercial cultivation in Indonesia in the 1980s, although on a small scale. This corn is known for its sweeter taste (Sugito et al., 1991). As well, it is an important source of fiber, minerals, and certain vitamins (Lertrat and Pulam, 2007).

Among the various obstacles of production, insects are most impactful, as sweet corn is affected by more than 50 species of insects. The main loss-causing pest is *Helicoverpa armigera* Hubner (*H. armigera*). This insect has a variety of host crops, such as green beans, tomatoes, beans, potatoes, cotton, and corns (Zalucki et al., 1986; Topper 1987; Fitt 1989). *H. armigera* is generally regarded as the most serious sweet corn pest worldwide and responsible for significant losses (Archer and Bynum 1994; Wiseman and Widstrom, 1992). As a result of its many host crops, feeding behavior, and long-range mobility, *H. armigera* is considered a formidable threat to agriculture (Guerrero et al., 2014).

H. armigera attack is first noticeable when it affects the formation of flower buds, flowers, and, young fruit. Larvae enter the young fruit, eating the corn seeds inside.

Occasionally, they move into young crop stems and buds of crop leaves, especially when the larvae population is high (Sudarmo, 1988). An *H. armigera* attack causes the number of corn seeds to decrease. Most significantly, if the larvae feed on the tuna hair, they interfere with the pollination process and formation of corn beans.

In an effort to control *H. armigera*, farmers in Sigi Regency Indonesia rely on the use of synthetic chemical insecticides. The application of insecticides is unscheduled and uses non-recommended doses. This activity raises concerns regarding chemical residues on sweet corn, soil, and water, as well as other potential human and environmental hazards (Pimentel 2005; Konradsen 2007; Sam et al., 2008; Adeogun and Agbongiarhuoyi, 2009; Hou and Wu, 2010; Adejumo et al., 2014).

Farmers constantly take risks regarding the harmful effects of chemical materials. WHO and UNEP reported that pesticide use is responsible for 3 million acute poisonings and causes about 20,000 deaths annually among agricultural workers. Exposure to pesticides has long-term effects on thyroid function, causing low sperm counts in men, congenital disabilities, testicular cancer, reproductive and immune disorders, endocrine disorders, dermatitis,

behavioral changes, cancer, immunotoxicity, and neurobehavioral and developmental disorders (Mesnage et al., 2010; Tanner et al 2011; Cocco et al. 2013; Gill and Garg 2014). Furthermore, Ntow (2006) and Gill and Garg (2014) reported other short-term effects, such as a headache, body aches, skin or eye irritation, respiratory problems, weakness, dizziness, visual impairment, and nausea.

To suppress *H. armigera* and reduce the problem of residues, research focuses on control alternatives, such as botanic and biologic insecticides. One botanic insecticide in this study is derived from pandan wangi leaf extract, *Pandanus amaryllifolius* (*P. amaryllifolius*). According to Kardinan (2002), pandan wangi leaf extract has an effective toxicity to kill imago insect *Sitophilus oryzae* (*S. oryzae*). The pandan wangi leaf contains alkaloids, saponins, flavonoids, tannins, polyphenols, and dyes. Saponin is a sugar compound that binds to hydrophobic aglucone, in the form of steroids or triterpenes. It can bind free sterols, the precursor to the hormone ecdyson, during digestion. In turn, decreasing the number of free sterols interferes with the insect molting process.

The superiority of botanic insecticides is noted by Grainge and Achmed (1988). They state that insects do not readily become resistant to botanic insecticides containing certain active ingredients; as well, insects simultaneously form a system of resistance to several different compounds. The pandan wangi leaf extract was tested on *Sitophilus oryzae* at the concentration of 5 ml with 20% active ingredient. Using this extract the insect mortality reached 94% (Yunus et al., 2016).

The fungus *Beauveria bassiana* (*B. bassiana*) is a biological agent used in controlling populations of insect pests. Some orders of insects that host *B. bassiana* are Lepidoptera, Coleoptera, and Homoptera (Ahmad et al., 2008). *B. bassiana* produces toxins to control pests in a variety of crop commodities (Wraight et al. 2000). As well, it is naturally present in the soil as a saprophyte fungus and can live on insect tissue. Therefore, it is highly effective in suppressing the development of Lepidoptera larvae (Herlinda et al., 2006). This study aims to discern the most effective type of insecticide in controlling *H. armigera* and provide a high economic value for sweet corn crops.

Results and Discussion

Population density of H. armigera

ANOVA analyses show a significant effect of insecticide treatment type (α = 5%) on the population density of *H. armigera* larvae, attack intensity, and sweet corn cob production. Tukey test results (α = 5%) show that the lowest population density of *H. armigera* larvae is found from the treatment of *B. bassiana* 5 g l^{-1}. This yields a 0.35 tail per crop, which is not significantly different from the treatment of pandan wangi leaf extract 5ml l^{-1} and Deltamethrin 3ml l^{-1} (Table 1).

The results show that the application of insecticide has a significant effect on the population density of *H. armigera* larvae. The declining population density of *H. armigera* larvae is a result of the effective toxicity of each insecticide type. The population density of *H. armigera* larvae tends to be lowest (0.35 tail per crop) with *B. bassiana* application of 5g l^{-1}, compared to other treatments. This is a result of the *B. bassiana* attaching and growing on the young corn cob. When the larvae enter the cob, *B. bassiana* infects the insects' skin and digestive system, causing the larvae to die.

When the fungus *B. bassiana* is in contact with the outer skin of the insect, spores form a sprout tube to penetrate the skin and body fat and reach the hemocoel (Jauharlina, 2000). Next conidia grew in the infected insect epicycles around parts of the body, such as appressoria. Penetration lasts for 12-24 hours, with the help of enzymes secreted by hyphae, such as chitinases, lipases, and proteases. In the epidermis, the mycelia grow radically from the center of the infection and reach the hemocoel within 1-2 days. Furthermore, the mycelia grow throughout the body tissues, penetrate the body surface, and form conidia (Cheung and Grula 1982; Suntoro, 1991).

B. bassiana produces toxins in the form of beauvericin compounds, consisting of four bassianolide molecules, cyclodepsipeptides I-N methylleaucyl-dhidroxysovalerik. These compounds poison mosquito larvae, saltwater prawns and bacteria, causing disruption during the exchange of substances between cells at Luecophase maderae (Tanada and Harry, 1993). *B. bassiana* also produces beauverolite, bassianolite, isorolite, pigment and oxalic acid. It causes increased blood pH, blood clots and cessation of blood circulation in insects.

Furthermore, the fungus also causes mechanical damage to the hemocoel in tissues and organs, such as the respiratory tract, muscles, nervous system, and respiratory system. The consequences of this whole process lead to insect death (Cheung and Grula 1982). Once the insect dies, the entire body is covered with hyphae and hardens. If colored white, the spore flour growth enveloping the cadaver is suspected to be an infection of *B. bassiana* (Riyatno and Santoso, 1991; Patulak, 1995). The use of *B. bassiana* does not pollute the environment and is not harmful to the health of farmers and consumers.

An alternative control is possible by using pandan wangi leaf extracts of 5ml l^{-1}. Its effectiveness in suppressing population density of *H. armigera* larvae, at 45 tails per 100 crops, which is not significantly different to the that of *B. bassiana* (Table 1). Flavonoid compounds have insecticidal properties that cause nerve poisoning in some vital organs, causing insect death. The products of pandan wangi leaf extraction, using ethyl acetate, are terpenoid compounds and steroids that can kill *S. oryzae* insects (Elimam et al. 2009; Sukandar et al., 2016).

Attack intensity

Tukey test results (α = 5%) show that the lowest intensity of *H. armigera* is resultant of the *B. bassiana* 5g l^{-1} treatment, which is 22.38% per plot. This is not significantly different from the pandan wangi leaf extract 5ml l^{-1} and Deltamethrin 3ml l^{-1} treatments (Table 2).

The results show that the intensity of *H. armigera* at age 70 DAC ranges from 22.38-40.89% (Table 2). Application of *B. bassiana* causes the lowest intensity of *H. armigera* larvae attack of 22.38%. The intensity of *H. armigera* on sweet corn cobs is closely related to the population density of *H. armigera* larvae. The regression analysis gives a slope of $Y = 52.49X + 7.46$, $R^2 = 0.949$ (Figure 1). Therefore, a higher larvae population density is associated with a higher attack intensity.

Table 1. Average population density of *H. armigera* larvae on sweet corn cobs.

Treatments	Density average (tail per crop)*
Control (without insecticide)	0.68[a]
Fungus *B. bassiana* of 5 g l^{-1}	0.35[b]
Liquid extracts of pandan wangi leaf of 5 ml l^{-1}	0.45[b]
Deltamethrin chemical insecticide of 3 ml l^{-1}	0.37[b]

***** The different letters were significant at α = 5% (p < 0.05).

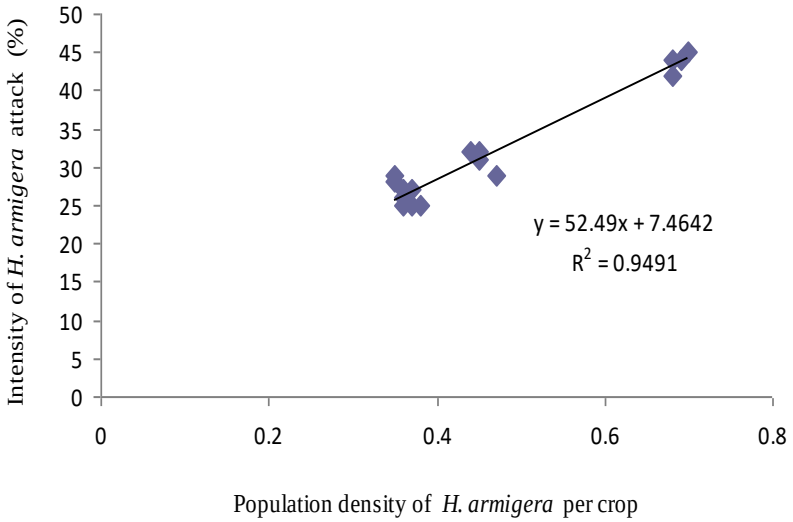

Fig 1. Relation between larvae population density and intensity of *H. armigera* attack.

Table 2. Average intensity of *H. armigera* attacks (%) on sweet corn cobs.

Treatments	Average of attack intensity (%)*
Control (without insecticide)	40.89[a]
Fungus *B. bassiana* of 5 g l^{-1}	22.38[b]
Liquid extracts of pandan wangi leaf of 5 ml l^{-1}	25.49[b]
Deltamethrin chemical insecticide of 3 ml l^{-1}	25.88[b]

***** The different letters were significant at α = 5% (p < 0.05).

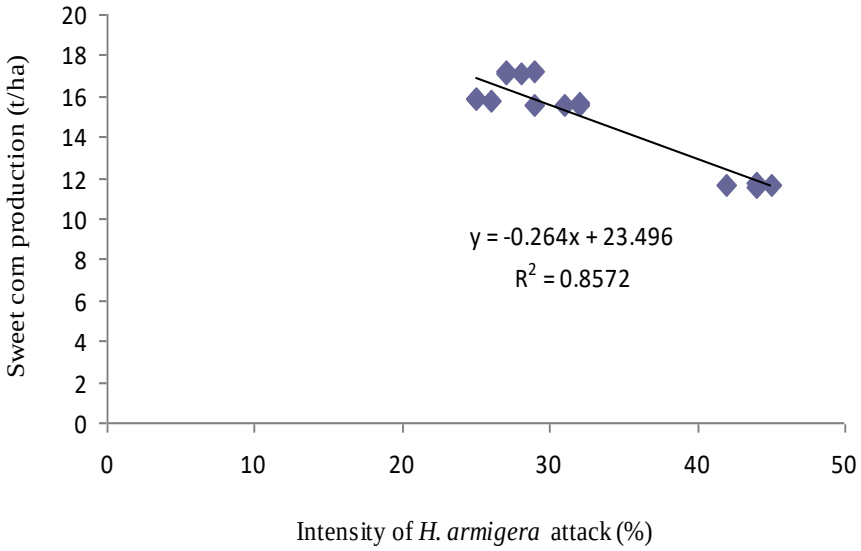

Fig 2. Relation between attack intensity with production of sweet corn cobs.

Table 3. Production of sweet corn cobs.

Treatments	Average of sweet corn cobs production (ton ha^{-1})*
Control (without insecticide)	11.64[a]
Fungus *B. bassiana* of 5 g l^{-1}	17.1[b]
Liquid extracts of pandan wangi leaf of 5 ml l^{-1}	15.67[b]
Deltamethrin chemical insecticide of 3 ml l^{-1}	15.84[b]

***** The different letters were significant at $\alpha = 5\%$ ($p < 0.05$).

Table 4. The analysis results of cost-benefit ratio of insecticide application benefits in sweet corn.

Treatments	Cost ha^{-1} (IDR)	Yield (ton ha^{-1})	Increase in yield over control (ton)	Benefit (IDR)	Cost: Benefit
Biological insecticide of *B. bassiana*	1,800,000	17.10	5.46	10,920,000	1 : 6.07
chemical insecticide of Deltamethrin synthetic	2,340,000	15.84	4.20	8,400,000	1 : 3.59
botanic insecticide of pandan wangi leaf extracts	1,530,000	15.67	4.03	8,060,000	1 : 5.27
Without insecticide (control)	-	11.64			

Note: IDR 13,881 = 1 USD as on May 2018.

Sweet corn production

The Tukey test ($\alpha = 5\%$) shows the highest production of sweet corn cobs is associated with *B. bassiana* 5g L^{-1}, at 17.1 t ha^{-1}. This is not significantly different from pandan wangi leaf extract 5ml l^{-1} and Deltamethrin 3ml l^{-1} (Table 3).
Statistical analysis shows the application of various types of insecticides has a significant effect on the production of sweet corn cob per hectare. Production is closely related to a higher intensity of *H. armigera*. This relation is shown in the regression equation $Y = -0.264X + 23.50$, $R^2 = 0.857$ (Figure 2). Therefore, higher intensity of *H. armigera* attack is associated with a lower production of sweet corn cobs at harvest time.

Cost-benefit ratio analysis

The results of cost-benefit ratio of insecticide to sweet corn are shown in Table 4.
The economic analysis of various applications of insecticides shows the biologic insecticide of solid formulations *B. bassiana* 5g l^{-1} gives the greatest cost-benefit ratio and is more economically profitable, as compared to *H. armigera*. This treatment results in a sweet corn yield greater than that of the control treatment and shows a maximum cost-benefit ratio of 1:6.07. Therefore, the application of biologic insecticide of *B. bassiana* provides higher economic value in controlling *H. armigera* in sweet corn cultivation in Sigi Regency Indonesia.

Materials and Methods

Field sites and Treatments

This study is conducted in Sidera Village, Sigi Biromaru Subdistrict, Sigi Regency, Central Sulawesi Province Indonesia, at the geographical location $119^0$56' East longitude, $01^0$01' South latitude, and at an altitude of 176 m above sea level. It takes place from September to December 2015. The research is conducted using Randomized Block Design, consisting of 4 treatments and 6 replications. The treatments are as follows: P_0 = without insecticides (control), P_1 = biological insecticide (*B. bassiana* 5 g l^{-1} water), P_2 = botanic insecticide (pandan wangi leaf extracts 5 ml l^{-1}), and P_3 = chemical insecticide (Deltamethrin synthetic 3 ml l^{-1}). The biological, botanic and chemical insecticides were dissolved in water. In general, farmers of Sigi Regency control pests by spraying insecticides containing the active ingredient Deltamethrin (Decis 2.5 EC). Deltamethrin affects a large variety of pests, from orders such as Lepidoptera, Homoptera, Coleoptera, Orthoptera, Diptera, and Thysanoptera. The recommended dose is placed in 1.5-3 ml l^{-1} of water in EC formulation (Bayer, 2015).

Treatments

Source B. Bassiana

Milled corn media preparation. Milled Corn, as raw material for making media, was washed, drained, and then steamed until half-cooked. After steaming, it is lifted, cooled, and packed in 100g per plastic bag. The packaged milled corn was further sterilized using an autoclave at 121^0C and 15 Psi, for 60 minutes. After sterilisation, the milled corn medium was removed from the autoclave and cooled (Kusnadi and Sanjaya, 2003).

Pure isolate inoculation in corn medium. The inoculum source was obtained from the *B. bassiana* starter collection of the Crop Pest Disease Laboratory of Agriculture Faculty UNTAD. Entkas was first sprayed with alcohol, and left for 10-20 minutes. Inoculation of pure *B. Bassiana*, isolated in a sterile corn medium, was carried out in the aseptic

receptacle. Plastic was inserted where the corn medium was folded, inflated and tied at the edges. After re-packaging, it was shaken and labelled with the date of inoculation. Inoculated milled corn medium was used as a starter and incubated at room temperature for 10-12 days in the dark. The starter was then ready to be used for propagation at the farm level (Kusnadi and Sanjaya, 2003).

Preparation of Pandan Wangi leaf extracts

Munawaroh and Handayani (2010) described the preparation of pandan wangi leaf extract with the following steps: (1) 3 pounds of pandan wangi leaves were dried under sunlight for 2 days, then cut into small pieces; (2) dried pandan wangi leaves were blended into powder, weighing 10 grams, wrapped with filter paper and inserted in soxhlet set; (3) the pandan wangi leaves in soxhlet were extracted with 100 ml of n-hexane solvent at 72-86^{0}C heating temperature, until the solvent color returned to its original state. The extract of pandan wangi leaves then was purified with a soxhlet extractor at 81-96^{0}C, until the solvent no longer dripped. The extract produced from the soxhlet set was then rotated with an evaporator tool to separate the extract and solvent. The temperature used during separation is 62^{0}C, with a rotary speed of 5 to 8 rpm. The resulting extract is then dissolved in a concentration, according to the treatment protocol. It is further diluted with distilled water, using the following dilution formula:

$$V_1.M_1 = V_2.M_2 \quad (1)$$

Where;
V1 = Volume before dilution
V2 = Volume after dilution
M1 = Concentration before dilution
M2 = Concentration after dilution

Implementation of research

Soil processing and preparation of cropping plot. The soil was processed using a plow and then was reared, cleaned, and flattened. The plots were 4 x 3.5m, 20cm tall, 0.5m apart, and 1m between replications.

Cropping. The corn seeds were planted 3cm into the soil, with two seeds in each hole. Thinning was done at 3 weeks after cropping and leaves one plant in each hole, with 70 x 20cm spacing. There is a total of 100 plants in a single crop plot.

Fertilization. Planting used 50g of manure per plot, placed down when the plot was made. Inorganic fertilizer was given twice, with Urea 360g, TSP 120g, and KCL 60g per plot. The first fertilization was conducted at cropping time. A 1/3 of Urea fertilizer was given, while TSP and KCL were given entirely. The second fertilization was applied when the crops were about 35 days after cropping (DAC), together with 2/3 of Urea. Fertilizer was applied in a given array to the side of the crop.

Maintenance. Crop maintenance includes weeding and irrigation. Watering was done every morning and afternoon, at the beginning of growth, and then done three times a week, by draining water into the plot until the field reaches capacity.

Applications of Insecticides

The insecticides were made in a suspension, according to the treatment protocol, and then inserted into the hand sprayer. Spraying is done three times, targeting corncobs attached to the flower or fully formed cobs, aged around 42 DAC. The next application was done according to a seven-day interval until the time of approaching harvest.

Observation variables

Identify test insects. Insect larvae had a length of 1.44 mm, with a yellowish-white body and a black head. The color of larvae varies between green, purple, yellowish, and dark brown. On the body, there were warts and hair and there was a wave line across the side. The fully grown larvae was 34.5 mm. The larval stage ranged from 17-24 days. The pupa is reddish or bright brown, fat and shiny. Male imago of *H. armigera* is brightly and darkly colored, while the female is only bright brown. The male front wing has round dark spots, located at the center of the wing, and a wingspan of 30-40 mm (Kalshoven, 1981).

Population density of *H. armigera* larvae. The observation of *H. armigera* larvae population density was conducted by observing the corn cob directly and counting the number of larvae present in each sample crop. A sample of 10 crops was observed. The observation was done 3 times, starting at 7 days after application and with a 1 week interval for sweet corn crop at age of 49 DAC, 56 DAC, and 63 DAC.

Attack Intensity. The attack intensity was calculated at the time of harvest with the following absolute formula (Antralina and Santoso, 2015):

$$P = \frac{n}{N}.100\% \quad (2)$$

Where;
P = Attack Intensity (%)
n = Number of attacked corn cobs (fruit)
N = Number of observed corn cobs (fruit)

Crop Production. Production of cob per hectare (measured by ton) is observed after the young harvest (fresh cob form), at a crop age of about 70 days. A total of 25 fresh cobs (weighed in grams) were sampled from each crop plot, weighed, and then averaged. Furthermore, the obtained data was converted to cob production per hectare, with the following formula (Subandi et al., 1998):

$$production(ton/ha) = \frac{10{,}000(m^2)}{a} x \frac{b}{1{,}000(kg)} \quad (3)$$

a = Area Size of Plot (m^2)
b = Cob Production / Plot (kg)

Data analysis

Observation data is tested using Analysis of Variance (ANOVA) or Fisher test (F). If there is the effect of treatment, then the Tukey's different test was used at 5% alpha level.

Conclusion

Biorational insecticide application had the significant effect on the population density of larvae and intensity of *H. armigera* attack and sweet corn cob yield. Biological insecticide of *B. bassiana* at 5 g l^{-1} tended to be more effective than other insecticides, in which population density of *H. armigera* larvae was reached to 0.35 tail per crop, attack intensity of 25.88%, and sweet corn cob yield reached 17.1 t ha^{-1}. Biologic insecticide of *B. bassiana* at 5 g l^{-1} gave the maximum result of cost-benefit ratio of 1: 6.07. *H. armigera* control should use a biological agent of fungus *B. bassiana* with a dose of 5g l^{-1} because it had a more environmentally friendly nature and tended to be more economical.

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