Arthropod diversity and corn stem borer *Ostrinia furnacalis* Guenee population in corn endophytes

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**Abstract.** Corn (*Zea mays* L.) is a strategic commodity that plays an important role in the development of agriculture and the Indonesian economy because corn has a multipurpose function that can be used for food and feed purposes. Obstacles in corn cultivation that cause low corn productivity include the attack of plant pests. The use of entomopathogenic fungi as endophytes is an alternative to chemical insecticides but can also be used to decrease the use of fertilizers. The research has been carried out at the Department of Food Crops and Horticulture Agriculture, Bontomarannu District, Gowa Regency, South Sulawesi, and the Laboratorium of Plant Pests and Diseases, Faculty of Agriculture, Hasanuddin University. Observation of larval population of *O. furnacalis* was started when the plant was 35 days after planting (DAP) by observing the presence of grinding marks on the leaves and stalks of corn. The larvae found in the field are reared in the laboratory. Arthropod exploration in the field used 4 methods, namely visual observation, pitfall traps, yellow traps and using nets. The results obtained in this study were the arthropods found in the land of endophytic corn using the pitfall trap method, found as many as 48 insects. Using a net, 150 insects were found. Using the yellow trap, 243 insects were found and with visual observation found as many as 95 insects. The number of larvae of *O. furnacalis* infected with the fungus *B. bassiana* in corn female parents was 20 with an infection percentage of 83.3% and 11 male parents with an infection percentage of 91.7%.

1. **Introduction**

The development of national corn productivity in the period 1980-2000 shows a decreasing trend, although the level of productivity is still increasing. This is an illustration of the increasingly limited development and application of agricultural technology, both because of the increasingly limited potential trends and due to a lack of attention and support for the development of productivity. Furthermore, it is stated that the indication of a decrease in the productivity of national corn is more emphasized by the condition which shows that there has been a decrease in the productivity of land and agricultural labor for 30 years (1967-2001) [1].

The corn stem borer (*Ostrinia furnacalis* Guenee) or asian corn borer is one of the main pests that attack all phases of growth of the corn plant. The larval attack that occurs in the vegetative phase causes the leaves and stems to be damaged so that the photosynthesis process is inhibited, the stems become weak and break in the internodes. *O. furnacalis* is a corn stem borer that is widespread in corn plantations in Asia and Australia. *O. furnacalis* completely metamorphosed with life cycle stages: egg - larva - pupa - imago (adult insect in the form of moth) [2]. In South Sulawesi, *O. furnacalis* has attacked many corn crops in the regency of Gowa, Sidrap, Wajo and Luwu. The attack of corn stem borer pests began to...
appear on corn plants from 3-4 weeks of maturity and ended until the cobs were ripe. The limit of tolerance for population density in determining control strategies is the discovery of one group of newly hatched eggs per 30 plants [3]. The use of entomopathogenic fungi as endophytes is an alternative to chemical insecticides but can also be used to increase or decrease the use of fertilizers. The advantages of biological control are relatively safe and economical [4].

The microorganism can act as biological agents to promote plant growth as well as improve plant health because they are free from pest attacks (plant pest organisms). *Streptomyces* sp. as biological control of plant diseases combined with *Bradyrhizobium japonicum* in biological fertilizers has the best influence on germination, wet weight, and dry weight of rice seeds [5]. There are isolates of bacterial that can provide resistance to disease attacks on plants. *Paenbacillus polymixa* isolate is one of the antagonistic bacterial isolates that can control several types of plant diseases, especially crackle and blast disease in rice plants, each of which is caused by *Pyricularia oryzae* (Blast Disease) and *Xanthomonas oryzae* pv. *oryzae* (Bacterial Leaf Blight) [6]. Extracellular polysaccharides (EPS) obtained from *Ralstonia zyzigii* subsp. *celebensis* can also produce the highest shoot number of banana plantlets var. Barangan *Musa acuminata* L. in vivo [7].

Infection of *B. bassiana* in different larval phases can cause differences in insect response. *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 a 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 [8]. *Beauveria bassiana* Vuill is a fungal species that is often used to control insects. *Beauveria bassiana* is applied in the form of conidia which can infect insects through the cuticles, mouth and segments found in the insect body. This fungus has a wide spectrum and can control many insect species as plant pests. Endophytic seeds had high viability and *B. bassiana* as endophytes in corn plants, application of *B. bassiana* to corn plants proves that it appears that the presence of *B. bassiana* in corn plants is three weeks after application [9].

*B. bassiana* still exists in F1 seeds that grow as endophytic and the ability to infect stem borer on two corn varieties, Lamuru and Batara. F1 Corn containing *B. bassiana* in Batara varieties was still effective on *O. furnacalis* [10]. Arthropods have an important role in maintaining ecosystems and determining the stability of agroecosystems on agricultural land, a stable ecosystem illustrates population stability between arthropods that damage plants or pests and their natural enemies which result in reduced crop damage [11]. According to [12], based on their trophic level, arthropods in agriculture are divided into three, namely herbivorous arthropods, carnivorous arthropods and decomposer arthropods. Herbivorous arthropods are a group that eats plants and the presence of their population causes damage to cultivated plants, which are called pests. Carnivorous arthropods consist of all species that prey on herbivorous arthropods including predators, parasitoids which act as natural enemies of herbivorous arthropods. Decomposer arthropods are organisms that function as decomposers that can help restore soil fertility and besides that arthropods are also pollinators in plants [13].

Based on the information previously mentioned and considering the potential of entomopathogenic fungi as endophytes in plants, it is deemed necessary to conduct research on the diversity of arthropods and endophytic of corn male parents Mr14 and female parents Nei9008 on mortality of *O. furnacalis* larvae.

2. Materials and methods

2.1. Selection of endophytic seeds

The endophytic corn seeds used were the male parent variety Mr14 and the female parent variety Nei9008. Endophytic corn seeds are ready to be planted on land in Bontomarannu District, Gowa Regency, South Sulawesi.
2.2. Preparation of Beauveria bassiana isolate
Cultivating isolates as a source of inoculum from insects that died due to Beauveria bassiana with a per liter composition of medium glucose 10 g, 20 g agar, and 4 g instant yeast on petri dishes and incubated at room temperature supplemented with corn obtained by extracting corn extracts.

2.3. Soaking corn seeds
Soaking is done the day before planting. The seeds were soaked for 24 hours using B. Bassiana isolate with a concentration of 10^7.

2.4. Land preparation
The land used in the research area was made a hole where 2-3 corn seeds were planted with a spacing of 70 cm x 20 cm, 3 rows of females and 1 row of males.

2.5. Observation method
Arthropod exploration in the field used 4 methods, namely visual observation, pitfall traps, yellow traps and using nets.

2.6. Visual observation
Visual observations are made by taking insects hiding in the leaves or stems of corn plants. These observations are made on selected plant samples, namely 4 plants each in a diagonal shape. The results of the insects can then be put into a preparation bottle that already contains 70% alcohol and counts the population of each plant sample and takes to the laboratory to be observed under a microscope. Visual observations are made at each observation.

2.6.1 Pitfall traps. Pitfall traps are devices used to catch arthropods that are active at ground level. This trap is made of plastic glass. The glass trap is filled with water and detergent as much as 1/3 of the volume of the glass then planted with a position parallel to the soil surface. The number of traps installed is 20 with the position of the traps evenly distributed in the observation location. Traps are planted at 08.00 am for one 24 hour period with the aim that arthropods on the soil surface that are active during the day and at night can enter the trap. After the arthropods entered the trap and then put them in the sample box filled with alcohol, the arthropods obtained were then observed and matched using the Kalshoven reference book, their numbers were counted and recorded.

2.6.2 Using nets. The method using a net is carried out five times a swing at 5 points in a diagonal form, the arthropods can be inserted into the sample box filled with 75% alcohol and then observed. The arthropods obtained and matched using the Kalshoven reference book were counted and recorded.

2.6.3 Yellow traps. Observations of arthropods were carried out using yellow manila paper and then smeared with mouse glue, then attached to 5 points in the plant area, namely a diagonal shape. Then observed until trapped pests. Observations of natural enemies were carried out at 06.00 am then taken the next morning and then counted the population of each arthropod caught.

2.7. Observation parameters
The number of corn plants observed every 3 days using 20 samples of each parent variety so that the total plants observed per observation amounted to 40 plants. Sampling was done diagonally. The observed parameters observed were:

2.7.1. Observation of O. furnacalis population. Observation of the population of O. furnacalis larvae and pupae was carried out on corn leaves by observing the presence of cross-cutting marks on the stem, young corn cobs hair (pale green), inside the cobs and old corn cobs hair (dark brown). Larvae and pupae of O. furnacalis inside the stem were examined by carefully cutting and splitting the stem segments so as not to damage the plant.
2.7.2. Maintenance of larva O. furnacalis. The larvae of O. furnacalis found in the sample plants were taken and then maintained and fed with corn stalks. It is hoped that the O. furnacalis larva cadaver when it dies will be overgrown by the fungus B. bassiana because it has eaten the corn stalks containing the entomopathogenic fungi endophyte.

2.7.3. Isolation of the endophytic fungus B. bassiana. Isolation of the fungus on the larva cadaver of O. furnacalis and identifying the fungus based on [14].

2.8. Arthropod observations
The parameters of the observation were counting the number of populations and the types of arthropods obtained, the observations were made 8 times and carried out once a week, then observed and matched using the Kalshoven reference book, then the number was counted and recorded.

3. Results and discussion

3.1. The existence of arthropods in each observation method
In the corn fields of Mr.14 and Nei9008 varieties, 19 species of arthropod families were found, starting from week 1 to week 8 of observation. With the pitfall trap method, 2 families were found, namely Forficulidae and Formicidae with a total of 48 species. Using a net, found 13 families with a total number of species of 150 insects. By using yellow traps, 8 families were found with a total of 243 insects. Visual observations found as many as 7 families with a total number of species of 95 insects. The method with the most total insects is the yellow trap because insects prefer contrasting colors. The way insects see color is not the way we see it, just as for the insect green leaves are yellow and blue separately, considering that green is a combination of blue and yellow. Insects that are attracted to this color are usually pests that attack the leaves. Insects also like colors that are ultraviolet-biased, insects that are attracted to colors like red or blue are usually bees. Therefore the color traps used to catch insect pests are mostly yellow because insect pests usually attack the leaves the most. The yellow color for insects also indicates that the fruit is ripe, therefore the yellow color attracts the most insects to perch. Color traps can be maximized to focus on capturing certain insects. One of the trait of insects is to have an interest in light, in traditional practice this has long been applied, for example using petromax lights to catch moths, catching fruit flies in yellow, catching flies with striking colors and catching mosquitoes using ultraviolet light. Even in Malaysia, a limited number of applications have also been applied in agriculture.

Based on the calculation of the Diversity Index ($H'$), it shows that the diversity index of the Mr.14 and Nei9008 varieties as a whole is 2.5341 and is categorized in the moderate diversity index. The criteria for the diversity value of Shannon $H'$, namely $2 < H < 3$, are a moderate level of diversity. The number of arthropods that attack corn plants has existed from the first observation to the last observation. This is following the opinion of [15] which states that every arthropod in corn is known to attack all phases of plant growth, both vegetative and generative. Some of the arthropods obtained using the pitfall trap method, visual observation, using nets and yellow traps on corn plants with endophytic B. bassiana varieties Mr.14 and Nei9008, namely Coccinella septempunctata (Linnaeus, 1758); Valanga nigricornis (H. Burmeister, 1838); Ophimia phaseoli sp.; Tetragnata (Latreille, 1804); Forficula auricularia (Linnaeus, 1758); Solenopsis (K. B. presl, 1836); Odontoponera (Mayr, 1862); Oxya chinensis (Thunberg, 1815); Leptocoriza acuta (Thunberg, 1783); Culex sp.; Apis (Linnaeus, 1758); Ostrinia furnacalis (Guenée, 1854); Helicoverpa armigera (Hubner, 1808).
Based on the graph in figure 1, the abundance of pest arthropods from the first observation continued to increase until the third observation, the same thing happened to predatory arthropods. In the sixth observation, pest arthropods decreased as well as predatory arthropods. This shows that an increase in the population of pest arthropods is followed by an increase in the number of predator populations. Each component has a different niche and function and is related to one another. As long as these components carry out their functions and work well together, the order of the ecosystem will be maintained. From the graph in figure 1 shows that insect attack has decreased in accordance with the opinion of [16], suggesting that corn plants containing endophytes can be used as a strategy to protect plants from *O. furnacalis* attacks while improving the quality of plant growth. The same thing was also stated by [17] that infected insects will usually stop eating, causing their immunity to decrease, 3-5 days later they die, marked by conidia growth on the integument. Beauveria bassiana can live as an endophyte without causing symptoms of damage to its host plants.

From table 1 it can be seen that the ecosystem is still in a balanced state. This can be seen from the variety of insects caught (heterogeneous). In a stable ecosystem, the population of an organism is always in a state of balance with other populations in its community [18]. Based on table 1, the insects found in the corn area were very diverse, both as plant pests, predators, parasitoids, useful insects and other insects which, although present, did not have a negative impact on plants. Not all types of insects in agro-ecosystem are dangerous insects or pests, in fact, most of the types of insects we encounter are non-pest insects that can be natural enemies of pests (predators and parasitoids) or useful insects, such as flower pollinators and insects that destroy organic debris [18].

### 3.2. Population of *Ostrinia furnacalis* larvae in endophytic corn plants

Corn plants are a source of staple food for a small proportion of the population who live in eastern Indonesia. *O. furnacalis* or asian corn borer is a corn stem borer insect that is very dangerous because its larvae are very destructive and have the potential to kill plants. During the observation, no *O. furnacalis* pupae were found in the corn fields. The results of observing the number of larvae of *O. furnacalis* in two parents of corn plants with *B. bassiana* endophytes are presented in figure 2 below:
Figure 2. Number of *O. furnacalis* larvae in two elder varieties of corn.

Figure 2 shows the results of observing the number of *O. furnacalis* larvae at each observation per 20 corn plants. The highest number of *O. furnacalis* larvae was found in female parent corn plants at 38 DAP (5 tail) and 41 DAP (5 tail) then continued to decrease until it reached 56 DAP with a total of 24 larvae found bore. The highest number of *O. furnacalis* larvae in male parents occurred at 38 DAP (3 tail) and 41 DAP (3 tail) then continued to decrease until 56 DAP with a total of 12 *O. furnacalis* larvae. It is suspected that this occurs because the age of the plant continues to increase along with the hardening of the stems of the plant so that the larvae of *O. furnacalis*, which have just hatched from the eggs, have difficulty penetrating because the plant stems contain less water. [19] suggested that generally the early instar larvae of *O. furnacalis* like plant stems that contain lots of water (succulents). This is reinforced by the results of research by [20] which states that the stems of corn plants are cylindrical, dense fiber with a thickness of about 2 - 4 cm depending on the variety. Genetic factors have a strong influence on plant vigor. Generally, corn cobs undergo physiological maturity ranging from 100 - 105 days so that it can be said that during the generative phase of the larvae of *O. furnacalis*, the plant penetrates the plant due to the hardening of the stems which are its food source.

3.3. Percentage of *ostrinia furnacalis* larvae infected with the fungus *B. bassiana*

*B. bassiana* is an entomopathogenic fungus that attacks insect pests mainly from the orders Lepidoptera, Coleoptera, Orthoptera, Homoptera and others. The impact of the presence of the endophytic fungus *B. bassiana* on *O. furnacalis* larvae in corn is presented in table 2 below:

| Corn varieties tested | The impact of the endophytic fungus *B. bassiana* on *O. furnacalis* larvae in corn |
|-----------------------|-----------------------------------------------------------------------------------|
|                       | Infected  (%) | Not Infected  (%) |
| Elder Female          | 20  83.3     | 4  16.7          |
| Elder Male            | 11  91.7     | 1  8.3           |

The results of collecting *O. furnacalis* larvae from the two types of corn parents found 24 larvae in female parents and 12 larvae in male parents. Based on table 2 it can be seen that the percentage of larvae of *O. furnacalis* infected with *B. bassiana* found in female parents was 83.3% and uninfected larvae were 16.7%. The larvae of *O. furnacalis* were infected with the endophytic fungus *B. bassiana*, which was found in 91.7% of male parents, while 8.3% were not infected. It is suspected that the lack of *O. furnacalis* larvae in male parent corn because the *O. furnacalis* moth when laying eggs could detect through its antennae the presence of secondary compounds produced by plants containing endophytic fungi. This is supported by [21] who found that aphids do not colonize host plants containing endophytes.
because they can detect the presence of terpenoid compounds which can act as anti-feeding of insect pests.

Conidia are the most important part of the development and distribution of entomopathogenic fungi. There are two ways B. bassiana infects insect pests, namely conidia attaches to insect integument (acting as contact poison) and food containing conidia enters the mouth and digestive tract (digestive poison) [22]. Because the digestive pH of insects is acidic, it is easy for conidia to breed in them. Furthermore, [23] stated that the fungus B. bassiana produces a toxin called beauvericin. This compound is an antibiotic that causes disturbances in the function of hemolymph and insect cells, resulting in swelling accompanied by a hardening of the infected insects. Apart from contact, B. bassiana can also infect insects through inoculation or feed contamination, for example about 37% of B. bassiana conidia mixed into Helicoverpa armigera's feed germinate in the digestive tract of its host within 72 hours, while B. bassiana hyphae penetrate the intestinal wall between 60-72 hours. In the host body, B. bassiana rapidly multiply until the entire insect network is infected. Generally, insects that have been infected with B. bassiana will stop eating, so they become weak and their death can be faster. Dead insects are not always accompanied by symptoms of conidial growth [21]. For example, aphids infected with B. bassiana only experience swelling without changing color.

Insect mortality due to B. bassiana infection was caused by overall tissue damage and the presence of toxins produced by fungi [16]. White muscardine disease that attacks the digestive tract of Plutella xylostella results in nutritional disturbances and causes death [22]. The bodies of adults and larvae/ nymphs infected with B. bassiana will be white because they are overgrown with B. bassiana conidia. The number of conidia an insect can produce is determined by the size of the insect. Any insects infected with B. bassiana will be an effective source of infection for the surrounding healthy insects. Like other fungi, B. bassiana growth is also largely determined by environmental humidity [24]. However, B. bassiana has a resistant phase which can maintain its ability to infect the host in dry conditions. Its epizootic presence in nature causes B. bassiana to rapidly infect insect populations and cause death. The high penetration ability of B. bassiana in the insect body causes this fungus to easily infect sucking insects, namely aphids which are difficult to infect by bacteria and viruses.

4. Conclusion
Arthropods found in corn fields with endophytes using the pitfall trap method were found as many as 48 insects. Using a net, 150 insects were found. Using the yellow trap, 243 insects were found and with visual observation found as many as 95 insects. The number of larvae of O. furnacalis infected with the fungus B. bassiana in corn female parents were 20 with an infection percentage of 83.3% and 11 male parents with an infection percentage of 91.7%.

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