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Viability and role of Beauveria Bassiana as endofit in Corn Bima 11 Tammu Tammu varieties against Aphids sp.

Viability and role of Beauveria Bassiana as endofit in Corn Bima 11 Tammu Tammu varieties against Aphids sp.

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Abstract. Corn production can be increased by the use of superior varieties both free-range (non-hybrid) and hybrid corn. Hybrid corn can produce higher yields compared to free-roasted corn. Bima-11 Tammu Tammu variety is corn seed from the breeding of B11-126 (female), Mr-15 (male) varieties. One of the pests commonly found in corn plantations is aphids *Aphids* spp. One of the control alternatives that can be used is applying the entomopathogenic of *Beauveria bassiana*. The effectiveness of *B. bassiana* as a control for a number of insect pests has been proven. Damage due to *Aphids* sp. can be more severe, because these pests can transmit viral diseases to corn plants. This research begins with the preparation of Bima 11 Tammu Tammu varieties, seed viability test, preparation *B. bassiana*, re-isolation of plant tissue to detect the presence of *B. bassiana*, and Preparation of *Aphids* sp. The results showed that 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, and *Aphids* sp. mortality in endophytic corn plants and in corn plants sprayed with *B. bassiana* is no different.

1. Introduction

Corn plants (*Zea mays* L.) are annual crops that are widely cultivated in Indonesia and are important food commodities after rice. Corn is also used as animal feed, industrial raw materials, cake flour and drinks, so that the national corn demand is increasing [1], [2]. Corn is a source of carbohydrates and proteins that can be used as food and animal feed ingredients, and raw materials for industry. Lately, the development of corn in Indonesia has been increasingly rapid in line with the increasing demand for feed, especially for animal feed [3]. Of the total raw materials needed for the manufacture of poultry feed, the portion of corn is around 50% [4]. The use of quality seeds can reduce the risk of cultivation failure because it is free from pests and diseases that can damage while eliminating the potential for existing results up to 80%.

Corn pests can attack in all phases of growth, both vegetative and generative. One of the pests commonly found in corn plantations is *Aphids* sp. *Aphids* form large colonies in leaves which include females producing parthenogenesis (without mating). A female with no wings can bear an average of

68.2 nymphs, while a female with 49 nymphs. The life span of imago is 4-12 days. Generally, the nymph stage consists of four instars. The nymph stage occurs for 16 days at 15^0C, nine days at 20^0C, and five days at 30^0C. These insects prefer to be in warmer temperatures than in cold temperatures. Imago is more active in the field at temperatures of 17^0C and 27^0C [3].

Maize production can be increased by the use of superior varieties both free-range (non-hybrid) and hybrid corn. Hybrid corn can produce higher yields compared to free-roasted corn. Bima-11 variety is corn seed from the breeding of B11-126 (female), Mr-15 (male) varieties [5]. One of the control alternatives that can be used is applying the entomopathogen *Beauveria bassiana*. The effectiveness of *B. bassiana* as a control for a number of insect pests has been proven. Damage due to *Aphids* sp. can be more severe, because these pests can transmit viral diseases to corn plants. Besides that *Aphids* sp. causing dwarf plants, curly leaves, rolling and mosaic. In extreme cases colonized aphids can drop leaves and fruit [6].

B.bassiana is one of the entomopathogenic fungi that is widely used to control various agricultural pests. When *B.bassiana* is applied to one of these plants and infects *Aphids* sp. indicates a change in body color from green to brown and then black. The activity of these insects was reduced marked by the *Aphids* sp. become slow. Insects infected with *B. bassiana* fungus are characterized by white mycelia. Fungus growth occurs in the body of insects and hardened dead insects due to the influence of toxins released by the fungus *B. bassiana* [7]. According to [8], states that in the body of insects *B. bassiana* multiplies and produces beauvericin toxins which can cause disruption in the function of hemolimph and insect cells resulting in swelling accompanied by hardening of infected insects.

Daud [9] study explained that soaking seeds with a solution of *B. bassiana* showed that these fungi could enter into plant tissues, roots, stems and leaves. *B. bassiana* is in plant tissue, evidenced by the toxicity of leaves and stems against larvae. The presence of *B. bassiana* fungi in plant tissue does not interfere with the growth and development of corn plants.Weakness of *B. bassiana* spores is that it is very easy to lose their lethal power if exposed to ultra violet in the sun. [9] shows that laboratory experiments in *B. bassiana* spores exposed to ultraviolet light will decrease their viability by damaging tissue toxins and made observations about *B. bassiana* as endophytes in corn plant tissue. The seeds come from soaking female parent seeds (B11-126) according to the density of predetermined spores for 24 hours in a jar and using male elders (Mr-15) seeds. Then the seeds are planted in a plot measuring 4.4 meters x 4.95 meters with a spacing of 75 cm x 20 cm. Planting the seeds is done using the 3: 1 method. Taking male flowers on female plants is carried out before the male panicles come out (while still wrapped in flag leaves). Next, the harvest is done manually. The harvest criteria are when the leaves and corn husks are brown, blackish corn hair and hard corn seeds. Seed threshing and corn seed drying are done manually. The results of breeding or corn seed production are given the name "Bima 11 Tammu-Tammu".

As a breeding seed, the viability of Bima-11 Tammu-Tammu corn seed product is needed, the presence of *B. bassiana* in endophytic corn plants, and the ability of *B. bassiana* to kill *Aphids* sp.

2. Materials and Methods

2.1. Preparation of corn seeds

Corn Bima 11 Variety Tammu - Tammu has undergone a *B. bassiana* immersion treatment and then crossed between males (MR) x Females (NR) then produces Bima 11 Tammu-Tammu which has endophytes. Corn Bima 11 Maros taken from Cereal Crops Research Institute and has undergone the process of crossing the male and female from the mother and then producing the Bima 11 variety.

2.2. Planting and viability of seeds

Planting seeds using cotton media placed in aqua glass. Corn seeds are soaked for 24 hours, then prepare aqua glass. Place the planting media in the form of cotton / tissue into an aqua glass, solidify it until it reaches a thickness of 2 cm, then spray using a spray that has been filled with water, then place 1 seed of corn seed that has been soaked in aqua glass.

Observations were made once a week, observations were made from the first day of planting until all the seeds were grown. The number of plants observed at each observation was 120 plant samples per observation. Taking plant samples is done randomly. To calculate the viability of corn seeds using the formula:

$$\text{Ability to Grow (\%)} = \frac{Number of\ seeds\ that\ grow}{Number\ of\ seeds} x100\%$$

2.3. *Preparation of B. bassiana*

B. bassiana isolate used in the study was a collection isolate from Itji Diana Daud originating from the *Ostrinia furnacalis* larva and has been multiplied in the laboratory. Pure isolates were made by pure culture by transferring using a cock borer to the new PDA media.

B. bassiana which has been placed on PDA media is given distilled water, then taken and placed into a test tube as much as 1 ml which has been given 9 ml of distilled water and mixed using a vortex device. Then put it into a microscope and count the number of spores using the formula:

$$K = \frac{t\ x\ d}{n\ x\ 0{,}25} x10^6$$

Note :
K : spore density per ml of solution
t : total number of spores in the number of sample boxes observed
d : the number of beauveria that will be taken
n : number of sample boxes (5 large boxes x 16 small boxes)
0,25 : correction factor using small-scale sample boxes on hemasitometers

The test tube which contained *B. bassiana* with a density of 10^8/10ml of water was mixed. Then poured into a hand sprayer to be applied and each one plant tube in BTTS treatment, application only once.

2.4. *Re-isolaton of plant tissue to detect the presence of B. bassiana*

Parts of plants that want to be isolated (roots, stems, leaves) washed with water, sterilizing the surface of the plant is done gradually. Roots, stems, and leaves are cut 6 mm and soaked in a tube containing 1% NaOCl for 3 minutes. After that it is transferred in 70% alcohol for 1 minute and distilled for 30 seconds 3 times. After that, dry it on filter paper for 1 minute. Sterile samples are then planted on PDA (Potato Dextrosa Agar) media. The root part of the plant taken for observation is the meristem area, for the stem to be taken in the middle, while for the leaves the lower leaves are taken, the leaves in the middle of the leaves are close to the shoots.

Plants grown on PDA media were isolated as many as 3 pieces/petri dish for roots and leaves while for stems only 1/petri dish so that the number of each part of the isolated plant was 18 petri dish. The parameter of observation is the percentage of endophytic fungi in each part of the Bima-11 Tammu-Tammu varieties corn plant.

2.5. *Preparation of Aphids sp.*

Maintenance of *Aphids* sp. taken from corn plants or the same host plant attacked, then maintained on corn plants that have been provided and propagated for the testing process of every 1 plant around 10 *Aphids* sp. *Aphids* sp. which has been multiplied is put back on 2 weeks old corn plants, because the most active process of *Aphids* sp. that is in the age of 1 month in corn plants.

2.6. Procedure

The corn seeds of Bima 11 Maros (control), Bima 11 Tammu-Tammu (endophytic) and Bima 11 Tammu-Tammu (endophytic) plus *B. bassiana* which have been soaked for 24 hours. Then after that it was planted in a 0.5 kg polybag. Each polybag contains 1 plant with 15 plants. After the corn plants all treatments were 2 DAP. Placed *Aphids* sp. 10 tails/plants. A week later, the application was sprayed with $10x10^8$/ml *B. bassiana* on the treatment of three Bima 11 Tammu-Tammu varieties. Furthermore, observing mortality of Aphids sp. done on 3 weeks old plants for all treatments. This observation is carried out every day for 7 days. When all test insects in the BTT and BTTS treatments die, the observation is stopped. Then followed by observation of *Aphids* sp. cadaver to find out the cause of the death of the test insect. Continued re-isolation of endophytes in BTT and BTTS treatment plants.

3. Results and Discussion

3.1. Percentage of ability to grow corn varieties of Bima varieties 11 Tammu-Tammu

Based on observations, the percentage of the ability to grow Bima-11 Tammu-Tammu corn seeds, can be seen in table 1.

Table 1. Percentage of the growth ability of Bima 11 and Bima 11 Tammu-Tammu seeds

Treatment	Percentage grows at the time of observation	
	7 Hst	15 Hst
Bima 11 (Control)	95 %	100 %
Bima 11 Tammu-Tammu (Endophytic)	96 %	100 %

Based on the results in table 1, the ability to grow the seeds of Bima 11 Tammu-Tammu and Bima 11 were not different. Starting from the observation of 7 days all seeds to the second treatment reached 95-96%, and at 15 days, 100% of the seeds in the 2 treatments had grown. So even at 24 DAP (Day After Planting) the ability to grow seeds reaches 100%. Seeds containing *B. bassiana* do not affect seed germination, the seeds germinate well, this is no different from seeds that do not contain *B. bassiana*. Based on the results show that the tested seeds have high seed viability and are in very good condition. This is reinforced by the opinion of [10], who said that high-quality seeds have a viability of more than 90%. With the condition of 90% seeds, plants are able to grow normally in the physical condition of the seeds so that their growing power or germination can be maintained without any deviations from their quality.

The percentage of seed germination is the amount of proportion of seeds that have resulted in germination in certain conditions and time periods. According to the Steinbauer-Sadjad concept, the first period of viability is the period in which seeds form and develop until the seeds reach physiological cooking. Viability of seed potential in the first period increases sigmoidally and reaches its maximum point when the seeds reach physiological maturity [11].

3.2. Presence of B. bassiana in plant tissue of Bima 11 Tammu Tammu

Based on the observation of the presence of *B. bassiana* in the Bima 11 Tammu-Tammu of corn plant tissue, it can be seen in table 2.

Table 2. Percentage of presence of endophytic *B. bassiana* fungi in plant tissues

Sample	Bima 11 Tammu-Tammu		
	7 Hst	15 Hst	24 Hst
Root	33 %	33 %	83 %
Stem	33 %	50 %	50 %
Leaves	16 %	33 %	50 %

Based on the results in table 2, percentage of the presence of *Beauveria bassiana* at 7 days showed that *B. bassiana* was in the roots, stems, and leaves. From the observations of roots and stems showed the same number of presence, namely 33%, whereas in leaves only 16% of the samples were isolated. At 15 days the roots and leaves showed the same number of presence, namely 33%, while in the stem 50%. At 24 days the stem and leaves show the same presence of 50% and at the root of 83%.
Isolation of parts of endophytic plants *B. bassiana* has characteristics of overgrown white mycelium. This shows that from the seeds planted there are endophytic fungi in the corn plant tissue. The presence of *B. bassiana* fungus, evidenced by growth in PDA medium for 7 DAP, 15 DAP, and 24 DAP and white muskardine are clearly visible.

This is in accordance with [12], that the presence of endophytic fungi is found in plant tissues through seeds. [13] stated that starch is found in roots, stems, leaves and seeds, which through the metabolic process produce carbohydrates. The presence of *Beauveria bassiana* in plant tissue was demonstrated through re-isolation of the plant tissue. [14] show that fungus is generally found on the second leaf and stem of the armpit of leaves, but this fungus is not stable inside plant tissues during plant growth. The presence of endophytic fungi *Beauveria bassiana* in root, stem and leaf tissue of corn plants can be seen in Figure 1.

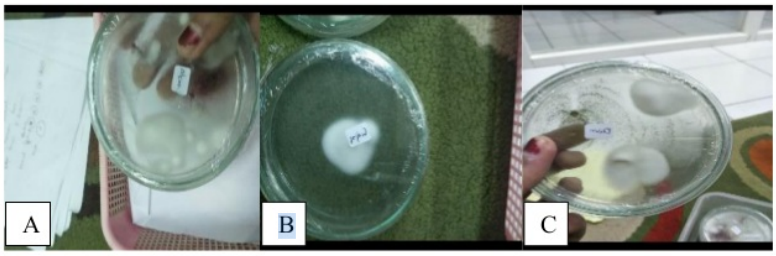

Figure 1. The part of the corn plant containing *Beauveria bassiana*

3.3. Percentage of mortality Aphids sp.

From the observation chart the mortality percentage at day 1 has not seen the occurrence of mortality and on the second day of treatment 2 (HTT) and treatment 3 (HTTS) experienced an increase in the number of mortality until the 7th day observation. Because of the conidia of *B. bassiana* that attach to the body parts of *Aphids* sp. *B. bassiana* can penetrate through the cuticles and segments of the insect's limbs. The penetration mechanism begins with the growth of conidia in the infected epicuticle of insects, followed by the formation of bodies such as apresoria. Penetration lasts 12-24 hours with the help of the enzyme khitinase, lipase, and protease released by hyphae. Inside the epidermis, mycelia grows radially from the center of infection and will reach the haemocoel in 1-2 days. Furthermore, mycelia will grow to all body tissues, penetrate the body surface, and form conidia.

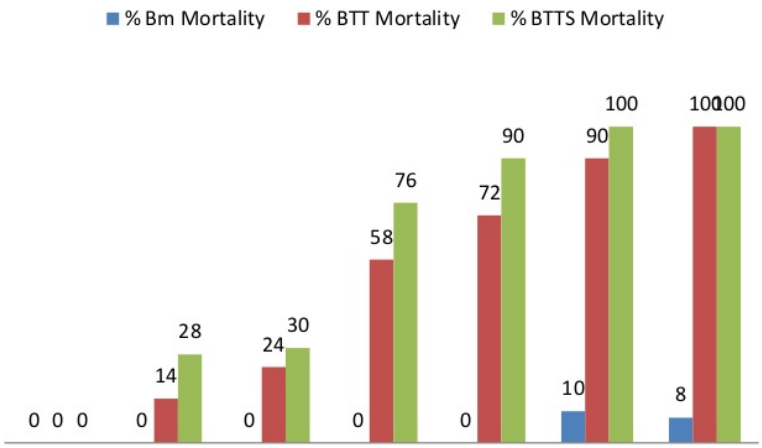

Figure 2. Percentage of *Aphid* sp. mortality

3.4. Presence of B. bassiana

The observation of the presence of *B. bassiana* fungus in maize plant tissue is presented in table 1. It shows that *B. bassiana* fungi began to appear when the corn plants were three weeks after application by re-isolating on PDA media.

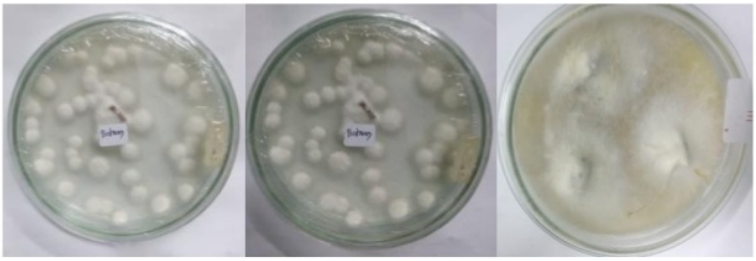

Figure 3. Results of the endophytic test of *B. bassiana* on corn plants (macroscopic form)

From table 3 shows that *B. bassiana* fungus appears to be present in corn plant tissue, especially in roots at week 1 after being applied, as well as in stem tissue of corn plants. Whereas the leaf part is seen at week 3. The presence of *B.bassiana* in corn plant tissue proves the occurrence of endophytic relationships between corn plants and *B. bassiana* fungi and requires 4 weeks after application. After *B.bassiana* enters the plant root tissue, the next process is spread to the stem tissue and leaves of endophytic maize plants by spraying it differently on plant tissue. When spread to food tissues, the fungus continues to germinate or multiply. Factors that can influence the development of fungi in plant tissues are the number of fungi applied, temperature, and humidity. [9] states that temperature and humidity are very important in the infection and sporulation of entomopathogenic fungi.

Table 3. Results of Observation of the Existence of B. Bassiana in Endophytic Corn Plants

Plant tissue	Week After Treatment			
	1	2	3	4
Root	+	+	+	+
Stem	+	+	+	+
Leaf	-	-	+	+

Description: (+) there is *B. bassiana*, (-) Not there *B. bassiana*

3.5. Symptoms of an attack of Beauveria bassiana against Aphids sp.

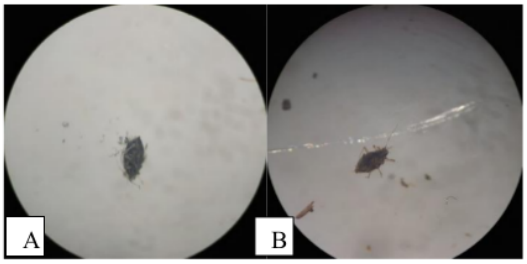

Figure 4. *Aphids* sp. infected with *B. Bassiana* fungus. (A) BTT; (B) BMS

Table 4. Mortality of Aphids sp.

Treatments	N	Duncan [a] Subset for alpha = 0.05		Notation
		1	2	
BM	5	1,40		A
BTT	5		10,00	B
BTTS	5		10,00	B
Sig.		1,000	1,000	

Description: The average value followed by the same letter means not significantly different at α = 0.05 with the duncan test

The results of the study on statistical analysis through the Duncan test at a significant level of 0.05 showed significantly different in the control with the second treatment. While the second treatment with the third treatment was not significantly different. This is due to the maize plant which was not applied with *B. bassiana*, the population of *Aphids* sp. fixed and no mortality. In contrast to corn plants that are applied by spraying *B. bassiana* can cause mortalis faster. The success of *B. bassiana* fungus in infecting host insects is largely determined by the concentration of conidia in contact with the host's body. [15] states that the more conidia attached to the target host will infect the target host faster. Mortality of *Aphids* sp. very high is caused by *B.bassiana* so that when applied to optimal conditions and a supportive environment will increase its ability to infect test insects.

16] states that setting the application time and monitoring the proper stage, controlling pest insects with insect pathogens will be more effective. Conidia attached to the body of the nymph begins to form a sprout tube at the fastest after 8 hours. This occurs only if the humidity around the conidia is

above 90%. The process of germination of conidia and penetration into the integument of insects takes 12-24 hours after conidia comes into contact with the surface of the host's body [17]. Entomopathogenic *B.bassiana* produces beauvericin which results in a disruption in the function of haemolymph and the host cell nucleus. Like most fungi, *B. bassiana* infects host insects through physical contact, ie by attaching conidia to the integument. Conidia germination occurs within 1-2 days and grows its micellar in the host's body. Infected insects will usually stop eating, causing their immunity to decrease, 3 to 5 days later to die with a marked increase in integument conidia [18].

After the application of *B. bassiana*, cadaveric *Aphids* sp. the re-isolates have swelling, harden and there are no hyphae due to the toxins that work so that *Aphids* sp. die. [8] states that in the body of insects *B. bassiana* multiplies and produces beauvericin toxins which can cause disruption in the function of hemolimfa and insect cells resulting in swelling accompanied by hardening of infected insects. In this study most of the infected *Aphids* sp. is not found in fungal mycelium which grew on the surface of the infected insect body. Pathogenic fungi can kill insects through a series of processes, one of which is the production of toxins.

Production of toxin has been studied in *B. bassiana* where toxin compounds can weaken the host after attacking insect organs and damaging the hemolymph so that the metabolic processes in the insect's body are inhibited. With the spread of insect organs and hemolymph, the activity of insects infected with the *B. bassiana* fungus will usually stop eating, so that it becomes weak so that it speeds to death. [8] states that dead insects are not always accompanied by symptoms of spore growth. Conidia attached to the body of the nymph begins to form a sprout tube at the fastest after 8 hours. This occurs only if the humidity around the conidia is above 90%. Process of germination of conidia and penetration into the integument of insects takes 12-24 hours after conidia comes into contact with the surface of the host's body [17].

Corn plant applied by *B. bassiana* by spraying showed the highest percentage of 40%-60% in corn plants indicating that the value of the mortality percentage of larvae was categorized as mortality in accordance with the report conducted by [19], stating that the mortality rate between 40% and 60% are categorized as moderate mortality rates.

4. Conclusion

Viability Bima 11 Tammu-Tammu grows well because at 95-96% the seeds grow no different from Bima 11. Bima 11 Tammu-Tammu during growth there is *Beauveria bassiana*. Isolation of parts of corn (roots, stems, leaves) endophytic *B. bassiana* has characteristics of overgrown white mycelium. The application of *B.bassiana* to corn plants proves that the presence of *B. bassiana* in corn plants is three weeks after application. The mortality of *Aphids* sp. in corn plants is endophytic and in sprayed corn plants with *B. bassiana* are no different.

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