International Journal on Advanced Science Engineering Information Technology

Vol.13 (2023) No. 5
ISSN: 2088-5334

A Laboratory Scale: Formulation Test and Bioassay of *Beauveria bassiana* (Bals.) Vuill. Alginate Pellet Carrier Pathogenic to *Spodoptera frugiferda* (J.E.Smith)

Itji Diana Daud [a,*], Melina [a], Muhammad Junaid [a], Mustika Tuwo [b], Otniel Bin Hasri [a], Sri Rahayu [a]

[a] *Department of Plant Pest and Disease, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia*
[b] *Department of Biology, Faculty of Mathematics and Natural Science, Hasanuddin University, Makassar, Indonesia*
*Corresponding author: *itfir@yahoo.com*

***Abstract*— Corn is an important food ingredient because it is the second source of carbohydrates after rice. However, there are obstacles to the attack of the armyworm pest *Spodoptera frugiferda* (J.E.Smith) which is found in corn plantations in Gowa and Takalar Regencies, South Sulawesi. An alternative control solution that can be applied is the use of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. In this study, formulation tests and bioassays of alginate pellet carriers will be carried out against *S. frugiperda* on a laboratory scale. The study was conducted from December 2021 to February 2022. Sampling of test larvae of *S. frugiperda* in corn plantations in Takalar Regency, propagation of the entomopathogenic fungus *B. bassiana*, alginate pellet production, and its application was carried out at the Pest Laboratory, Faculty of Agriculture, Hasanuddin University. The results showed that the alginate pellet formulation of *B. bassiana* could kill *S. frugiperda* larvae with different percentage levels of mortality. The treatment of 50 alginate pellets had a very significant effect with the most influential mortality among other treatments, which was equal to 80%, and had the lowest percentage rate of pupal and imago development. The addition of bran, wheat flour, tapioca flour, and corn flour formulations had an effect on the spore density of the *B. bassiana* alginate pellets. The P3 treatment (wheat flour) had a high spore density of 2.35 x 10^8 spores per ml with high mortality of *S. frugiperda* larvae on days 6-9 days after treatment.**

***Keywords*— Armyworm; corn; entomopathogenic fungi; mortality; spore density.**

Manuscript received 1 Feb. 2023; revised 25 May 2023; accepted 21 Jun. 2023. Date of publication 31 Oct. 2023.

I. INTRODUCTION

Corn is one of the agricultural commodities, the second staple food after rice in Indonesia. Corn production in Indonesia from 2010 to 2018 increased to a peak of 30.25 million tons in 2018, but in 2019, corn production began to decline until the latest data in 2020 noted that corn production fell to 22.5 tons [1]. The obstacle faced in increasing corn productivity is the attack of the corn armyworm pest *Spodoptera frugiperda* (J.E. Smith), a new pest in Indonesia [2]. *Spodoptera frugiperda* is an invasive insect that has become a pest of maize [3], [4]. This insect originates from tropical and subtropical parts of America and has spread to various countries, including Indonesia, which entered mid-2019 [5], [6]. According to [7], in September 2019, this insect was found on corn plants in South Sulawesi Province, particularly in Bontonompo District, Gowa Regency, and Sandrobone District, Takalar Regency. These insects attack plants when the plants are 3-4 weeks old, with an intensity of plant damage of 90%, thereby affecting the economic performance of corn [8], [9]. An alternative solution to control techniques that can be applied is using biological agents of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. was developed as a coinsecticide that can be mass-produced in solid and liquid media [10]-[12].

B. bassiana produces toxins beauvericine, beauverolide, isorolide, dyes, and oxalic acid, which cause aggressive paralysis in insect larvae and imago [13], [14]. The formulation of *B. bassiana* in the form of pellets resulting from mycelium encapsulation is effective for increasing pest mortality and reducing competition with other microbes, thereby increasing the viability of *B. bassiana* [15]-[17]. In utilizing this fungus, efforts are needed to maintain its effectiveness and sustainability through the development of formulations through modification of the fungus's propagation media, carriers, and conidia [18]-[20].

Several carrier materials have been studied for the suitability of the *B. bassiana* pellet formulation, including tapioca flour, rice flour, and corn flour combined with ideal storage temperatures [21], [22]. Tapioca starch at a storage temperature of 5°C effectively maintains the viability of *B. bassiana* conidia for ± 2 months of storage [23]. Corn flour contains lower carbohydrates (73.7 g) than wheat flour (77.3 g) and contains fat, phosphorus, iron, vitamin B1, and beta carotene pigment, which functions as a precursor of vitamin A and higher antioxidants [24]. Wheat flour has a water content of 14%, protein content of 8-12%, ash content of 0.25-1.60%, and wet gluten of 24-36% [25]. So, in this study, formulation tests and bioassays of alginate pellet carriers were carried out against *S. frugiperda* in a laboratory scale.

II. Materials and Method

A. Research Site

The study was conducted from December 2021 to February 2022. Sampling of test larvae of *S. frugiperda* in corn plantations in Takalar Regency, propagation of the entomopathogenic fungus *B. bassiana*, alginate pellet production, and its application to test larvae was carried out at the Plant Pest Laboratory, Faculty of Agriculture, Hasanuddin University Makassar.

B. Propagation of Spodoptera frugiperda Larvae

Test larvae of *S. frugiperda* were obtained from corn plantations in Takalar Regency, South Sulawesi Province. Insect propagation is carried out in the laboratory by feeding baby corn (when it just hatches) and then feeding corn leaves at instars 2 to 6, while at the pupal stage, it is placed in a plastic container filled with moist sand, and the imago is placed in a cage that is the size of 20x20x80 cm^3 by feeding the honey solution to the cotton. The resulting eggs are reared until they reach the third instar larval stage, ready to be tested. During larvae propagation, observations were also made on the larvae, pupae, and imago stages.

C. Rejuvenation and Propagation of the Entomopathogenic Fungus B. bassiana

B. bassiana isolates were obtained from the Laboratory of Plant Pests, Faculty of Agriculture, Hasanuddin University. Isolates were rejuvenated on Potato Dextrose Agar (PDA) media by taking *B. bassiana* hyphae using a needle preparation, then inoculated on sterile PDA media in a petri dish and then incubated for 12-14 days.

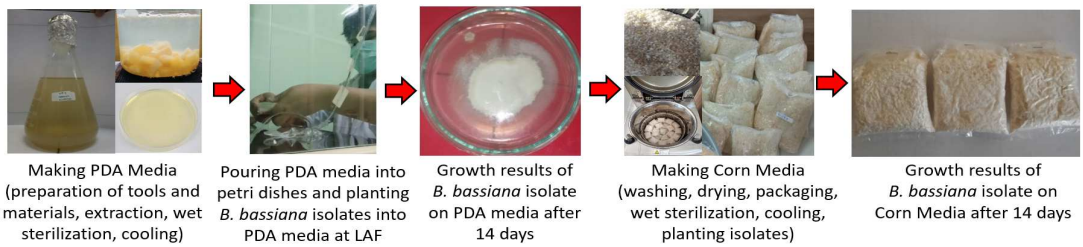

Fig. 1 Rejuvenation and propagation of the fungus *B. bassiana*

B. bassiana isolates were then propagated using corn as media. Corn media was prepared using corn rice which was washed and soaked for 15-30 minutes and then air-dried in a container covered with filter paper for 24 hours. Corn rice is put into clear plastic and then sterilized by autoclaving for 2 hours. The rejuvenated *B. bassiana* isolate was then transferred to a sterilized corn medium and incubated at 25-28^{0}C for 2 weeks.

D. Preparation of Alginate Pellets

Corn rice media incubated and covered with *B. bassiana* spores is dried in the refrigerator at 5-15ºC for ±12 days. After drying, they were crushed using a blender and sifted to form a fine powder containing a lot of spore biomass. Each formulation was mixed in a container to form a dough with the basic composition of the pellet formulation, namely corn rice flour (containing spore biomass) 15 g, 4 g sodium alginate, 2 g nutrients in 100 ml distilled water. Then stir for 5 minutes. After that, pellets were made using a pipette, then put in a 2% $CaCl_2$ solution for 60 minutes to form the pellets. Once formed, the pellets are then dried at 100°C for 24 hours. To evaluate the alginate pellet formulation's carrier material, bran, wheat flour, tapioca flour, and corn flour were used. The process is the same as the control treatment above, only with the addition of media for each treatment, 2 g of bran, wheat flour, tapioca flour, and corn flour.

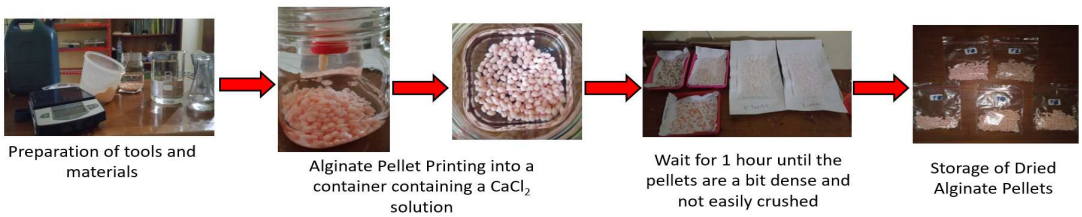

Fig. 2 Making *B. bassiana* alginate pellets

E. Alginate Pellet Application

The alginate pellet formulation test was carried out by taking the finished pellets according to the treatment, namely 30, 40, and 50 alginate pellets. The pellets that have been separated are then crushed using a mortar, and when they are finely ground into powder, they are placed in separate containers. After being mashed, each treatment of 30, 40, and

50 pellets was mixed into 20 ml of distilled water. Each pellet suspension was then homogenized using a vortex for 20 seconds. Each pellet treatment was calculated for its spore density using a hemocytometer. A total of 30 pellets equivalent to 10^6/ml, 40 equivalent to 10^7/ml and 50 alginate pellets equivalent to 10^8/ml. After the suspension has been made, the test larvae are ready to be infected in small containers with different treatments. Each container contains 1 test larva using 5 treatments and 5 replications. Each replicate used 10 test larvae. The method of application for the first treatment was that the larvae of *S. frugiperda* were dipped up to the abdomen of the larvae in sterile water. For the application of the second treatment, *S. frugiperda* larvae were dipped to the larvae's abdomen in the pellet suspension without using *B. bassiana*. Dipping was used with a concentration of 3 g of alginate pellets (50 pellets) dissolved in 20 ml of sterile water. Application of the next treatment, *S. frugiperda* larvae were dipped to the abdomen of the larvae in the *B. bassiana* alginate pellet suspension at concentrations of 30, 40 and 50 alginate pellets for 10 seconds. The application of alginate pellets with carrier material in the alginate pellet formulation was also carried out by immersing the test larvae into the alginate pellet solution with the treatment of bran, wheat flour, tapioca flour, and corn flour. Observations are made every 24 hours.

Preparation of Suspension of *B. bassiana* Alginate Pellets

Application (weighing feed, dipping feed into suspension, and putting feed into container containing test larvae)

Preparation of small containers containing feed and test larvae based on treatment

Fig. 3 Application of *B. bassiana* alginate pellets

F. Experimental Design

This study used a completely randomized design (CRD) with five treatments and five replications. Each replicate used ten test larvae. The larvae used as test larvae were instar 3 larvae. The treatments for the alginate pellet formulation test were as follows:

- P1: Control (sterile water)
- P2: Control (pellets without *B. bassiana*)
- P3: 30 *B. bassiana* alginate pellets (10^6)
- P4: 40 *B. bassiana* alginate pellets (10^7)
- P5: 50 *B. bassiana* alginate pellets (10^8)

Furthermore, the treatment with the carrier material in the alginate pellet formulation is:

- P0: *B. bassiana* (corn rice) +alginate (control)
- P1: *B. bassiana* (corn rice) + alginate + 2 g bran
- P2: *B. bassiana* (corn rice) + alginate + 2 g wheat flour
- P3: *B. bassiana* (corn rice) + alginate + 2 g tapioca flour
- P4: *B. bassiana* (corn rice) + alginate + 2 g corn flour

G. Observation parameters

1) Spore density of Beauveria bassiana: Observation of *B. bassiana* spore density was carried out by dissolving *B. bassiana* alginate pellets with each treatment, namely by taking each suspension using a micron pipette and dropping it on a spore counter haemocytometer and then counting the number of spores. This observation was made only once after one week of making alginate pellets. The density or number of *B. bassiana* spores can be calculated using the [26] formula as follows:

$$C = \frac{t.d}{n \times 0{,}25} \times 10^6 \qquad (1)$$

Description:

- C : Number of spores/ml
- t : The total number of spores in the observed sample box
- n : The number of sample boxes observed
- 0.25: Correction factor for using a small-scale sample box in a hemocytometer

2) Testing the mortality of test larvae of Spodoptera frugiperda: Observation of the mortality of test larvae of *S. frugiperda* was carried out by counting the number of test larvae that died in each treatment starting from 24 hours after application until the seventh day of observation. The percentage of mortality is calculated using a formula based on [27] as follows:

$$P = \frac{n}{N} \times 100\% \qquad (2)$$

Description:

- P : Percentage of mortality
- n : Number of dead larvae
- N : Number of larvae tested

H. Data Analysis

The data obtained was analyzed statistically with variance, then if significantly different data were found, it would be followed by the Least Significant Difference (LSD) test at the 5% level (0.05).

III. Results and Discussion

A. Results

1) Test the alginate pellet formulation: After applying *B. bassiana* alginate pellets for 10 days after application (DAA) and based on the results of analysis of variance Test of Least Significant Difference (LSD) level of 5% (0.05) it is known that the density of conidia of the different *B. bassiana* fungi has a very significant effect on *S. frugiperda* larvae mortality. Table 1 shows the average mortality of *S. frungiperda* larvae for 1 to 10 days after application (DAA) tends to increase. The results of statistical tests showed that on the 1st day after

application, the sterile water control treatment was significantly different from the 30 pellets, 40 pellets and 50 pellets, but not significantly different from the control treatment without B bassiana. Observations on the 2nd day, the treatment of 50 pellets was significantly different from the sterile water control, control without *B. bassiana* and 30 pellets however, not significantly different from the 40 pellets, while the treatment of 30 pellets was significantly different from the other treatments. Observations on the 3rd day with 30 pellets were significantly different from the sterile water control treatment, control without *B. bassaiana*, 40 pellets and 50 pellets, but the control treatment without *B. bassaiana* was not significantly different from the sterile water control.

TABLE I
AVERAGE MORTALITY PERCENTAGE OF S. FRUGIPERDA INSTAR III LARVAE ON OBSERVATION DAY 1 TO DAY 10

Treatments	Observation (Days after application)									
	1	2	3	4	5	6	7	8	9	10
Control (Sterile Water)	0.0[a]	0.0[a]	0.0[a]	0.0[a]	2.0[a]	4.0[a]	8.0[b]	8.0[a]	10.0[a]	10.0[a]
Control (Pellets Without B. bassiana)	0.0[a]	0.0[a]	0.0[a]	2.0[b]	2.0[a]	6.0[b]	6.0[a]	8.0[a]	10.0[a]	10.0[a]
30 B. bassiana alginate pellets	2.0[b]	8.0[b]	14.0[b]	20.0[d]	28.0[c]	34.0[d]	52.0[d]	60.[c]	64.0[c]	66.0[c]
40 B. bassiana alginate pellets	6.0[c]	12.0[c]	16.0[c]	16.0[c]	20.0[b]	28.0[c]	36.0[c]	44.0[b]	54.0[b]	56.0[b]
50 B. bassiana alginate pellets	8.0[d]	12.0[c]	18.0[d]	28.0[e]	38.0[d]	58.0[e]	66.0[e]	74.0[d]	80.0[d]	80.0[d]
Comparison Value of LSD 5%	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.1

Note: Numbers followed by the same letter in the same column are not significantly different in the LSD test level of 0.05

Table 1 shows the average mortality of *S. frungiperda* larvae for 1 to 10 days after application (DAA) tends to increase. The results of statistical tests showed that on the 1st day after application, the sterile water control treatment was significantly different from the 30 pellets, 40 pellets and 50 pellets, but not significantly different from the control treatment without *B. bassiana*. Observations on the 2nd day, the treatment of 50 pellets was significantly different from the sterile water control, control without *B. bassiana* and 30 pellets however, not significantly different from the 40 pellets, while the treatment of 30 pellets was significantly different from the other treatments. Observations on the 3rd day with 30 pellets were significantly different from the sterile water control treatment, control without Bb, 40 pellets and 50 pellets, but the control treatment without *B. bassaiana* was not significantly different from the sterile water control.

TABLE II
DEVELOPMENTAL STAGES AND MORTALITY OF LARVAE, PUPAE AND IMAGO OF S. FRUGIPERDA AFTER APPLICATION OF B. BASSIANA

Treatments	Larvae		Pupa		Imago
	ax	dx	ax	Dx	ax
Control (sterile water)	9	1	9	0	9
Control (pellets without *B. bassiana*)	9	1	9	0	9
30 *B. bassiana* alginate pellets	3	7	2	1	2
40 *B. bassiana* alginate pellets	4	6	3	1	3
50 *B. bassiana* alginate pellets	2	8	1	1	1

Description: ax : The number of living individuals; dx : The number of individuals that died in the age group x

Meanwhile, the control treatment without *B. bassaiana* on the 4th day after application was significantly different from the control treatment with sterile water, 30 pellets, 40 pellets and 50 pellets. Observations on the 5th day, the sterile water control treatment was significantly different from the 30 pellets, 40 pellets and 50 pellets, but not significantly different from the control without *B. bassaiana*, the 40 pellets treatment was significantly different from the other treatments. On the 6th day of DAA observation, the treatment of 50 pellets was significantly different from the treatment of 30 pellets, 40 pellets and the air-sterile control and control without *B. bassaiana*. Observations of the 7 treatments of 30 pellets were significantly different from each other. From the 8th to 10th DAA observations in the treatment of 50 pellets, they were significantly different from each other from the other treatments, but the sterile water control treatment was not significantly different from the control treatment without *B. bassaiana*.

Table 2 shows that the stages of development and mortality of larvae, pupae and imago in the control treatment of sterile water were 1 larvae mortality and 9 individuals that were still alive. In the pupal stage, no pupae died until they changed to imago, resulting in a total of 9 larvae that developed up to imago. In the control treatment without *B. bassaiana*, 1 larvae mortality and 9 individuals lived. Until the imago stage, the number of larvae that developed into imago was 9 tails.

The treatment of 30 pellets showed that the mortality of 7 larvae and 3 of them were still alive. Mortality at the pupal stage was 1 tail and 2 tails that were still alive to become imago. In the treatment of 40 pellets, 6 larvae died and 4 survived, 1 in the pupal stage and 3 that survived to imago. In the treatment of 50 pellets, 8 larvae died and 2 of them survived. The mortality rate of pupae was 1 tail and 1 tail that was still alive to develop into an imago.

2) Bioassay of alginate pellet formulation with carrier: From the results of observations 1 week after the manufacture of *B. bassaiana* alginate pellets, the results are obtained in the following table:

TABLE III
CALCULATION OF THE NUMBER OF OBSERVED SPORES AND SPORE DENSITY IN EACH TREATMENT

Treatments	The number of spores observed (after dilution 10^{-2})	Spore density (/ml)
P0 (control)	164	2.05×10^8
P1 (bran)	107	1.34×10^8
P2 (wheat flour)	188	2.35×10^8
P3 (tapioca flour)	112	1.40×10^8
P4 (corn flour)	115	1.44×10^8

Based on the calculation results above, it is known that P2 had the highest number of spores compared to other treatments, namely 188 spores and had the largest spore density, namely 2.35×10^8, while P1 had the smallest number of spores compared to other treatments, namely 10^7 spores

and also had the smallest spore density was 1.34 x 10^8. Based on the mortality test of *B. bassaiana* against *S. frugiperda* in all treatments for 9 days after application (DAA), the following table shows the results.

TABLE IV
AVERAGE MORTALITY OF INSTAR III S. FRUGIPERDA LARVAE AFTER APPLICATION OF B. BASSIANA AT VARIOUS CONCENTRATIONS

Treatments	Average cumulative mortality (%) of larvae observed 9 days after application							
	2	3	4	5	6	7	8	9
P0 (control)	8^b	24^c	38^b	48^b	56^b	60^{bc}	62^{bc}	62^{bc}
P1 (bran)	0^a	8^a	16^a	20^a	28^a	32^a	34^a	36^a
P2 (wheat flour)	8^b	20^a	36^b	46^b	60^b	68^c	70^c	70^c
P3 (tapioca flour)	2^a	6^a	20^a	26^a	38^a	46^{ab}	48^{ab}	48^{ab}
P4 (corn flour)	0^a	10^{ab}	22^a	30^a	38^a	42^a	42^a	42^a

Note: Numbers followed by the same letter in the same column are not significantly different in the LSD test at the level of 0.05 (5%)

Table 4 above shows the average cumulative mortality of *S. frugiperda* (%) for 9 days after the application of *B. bassiana* began to show its effect since the 2nd day of DAA observation, because on the first day there was no effect on mortality so it is still in the early stages of starting an effect mortality. This can be seen in P0 and P2 showing a mortality of 8% and P3 of 2%, while P1 and P4 have not shown any mortality. P0 and P2 showed significantly different from P1, P3 and P4, but respectively P0 and P2 and P1, P3 and P4 showed no significant difference. On the 3rd day of observation there was an increase in mortality in all treatments, where the highest mortality rate was at P1 which was 24% and mortality had started to appear at P1 by 8% and P4 by 10%. P1, P3 and P4 were not significantly different, but significantly different from P0 and P2, while P2 was not significantly different from P0 and P4. On the 4th and 5th day of observation, the highest mortality rate was at P0, namely 38% (4 DAA) and 48 (5 DAA). P0 and P2 showed significantly different from P1, P3 and P4, but respectively P0 and P2 and P1, P3 and P4 showed no significant difference.

On the 6th day of observation, the highest mortality rate was at P2, which was 60%. This is where the P2 treatment began to have high mortality of *S. frugiperda* larvae since the day of observation after P0 was dominant with high mortality of *S. frugiperda* larvae on the previous day of observation. P0 and P2 showed significantly different from P1, P3 and P4, but respectively P0 and P2 and P1, P3 and P4 showed no significant difference.

On the 7th, 8th, and 9th day of observation, there was an increase in the percentage of mortality, where in P1 there was an increase in mortality for 3 consecutive days but the mortality was still low, while for P0, P2, and P3 there was an increase in mortality for two days and the next day it remained the previous mortality, but P4 did not experience mortality for three consecutive days even though it had experienced an increase in mortality from the 6th day to the 7th day. The P1 and P4 treatments experienced no significant differences but were significantly different from P0 and P2. While P0 experienced no significant difference to P2 and P3.

From the research results that have been obtained from day 5 or starting from the formation of pupae, the development of *S. frugiperda* larvae into pupae and imago has different population levels. In the pupal phase/stadia, the percentage of the pupal population in the P0 treatment was 38%. Meanwhile, the P1 treatment had a pupal population percentage of 64%, which was the highest percentage of all treatments. The P2 treatment had a pupal population percentage of 30%, which was the lowest percentage of all treatments. Then the P3 treatment had a pupal population percentage of 52%. And the P4 treatment had a pupal population percentage of 58%. Treatments P1 and P4 had no significant difference, but were significantly different from P0 and P2, while P0 had no significant difference to P2 and P3.

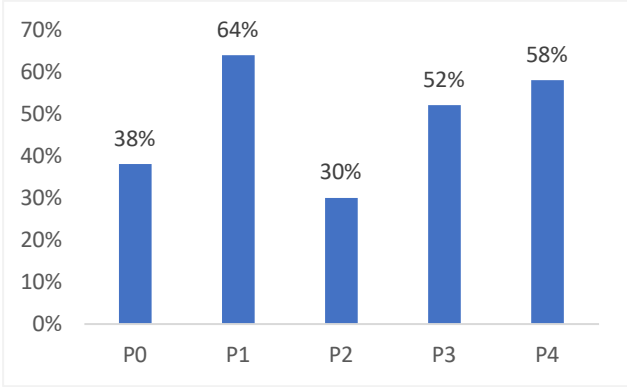

Fig. 4 The average percentage of larvae developing into pupae after being infected with *B. bassiana* on third instar larvae

At the time of entering the imago phase, the results obtained were in the P0 treatment. The percentage of the imago population was 30%. In treatment P1, the percentage of the imago population was 54%, which was the highest percentage of all treatments. In the P2 treatment, the percentage of the imago population was 24%, which was the lowest percentage of all treatments. Then in the P3 treatment, the percentage of the imago population was 42%. And in the P4 treatment, the percentage of the imago population was 48%. Treatments P1 and P4 had no significant difference, but were significantly different from P0 and P2, while P0 had no significant difference to P2 and P3.

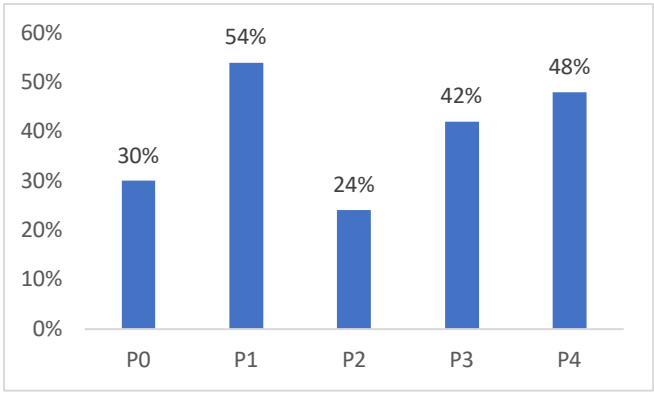

Fig. 5 The average percentage of pupae developing into imago after being infected with *B. bassiana* in third instar larvae

The results obtained from the percentage mortality of *S. frugiperda* larvae, pupae and imago after all treatments accumulated showed that the percentage of death or mortality in larvae was 52% or a total of 129 individuals who died. The percentage of death or mortality in pupae was 9% or a total of 22 individuals who died. While the percentage of survival

from third instar larvae to imago was 39% or a total of 99 individuals who survived to imago.

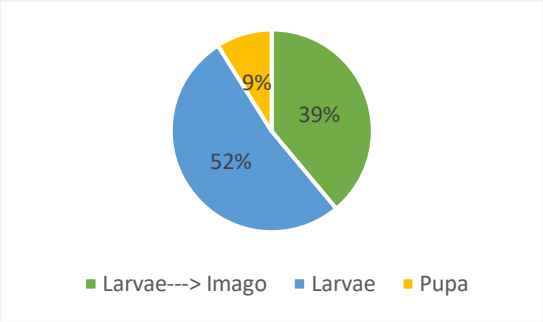

Fig. 6 Percentage of mortality of *S. frugiperda* larvae, pupae to imago if accumulated in all treatments

B. Discussion

The alginate pellet formulation applied to *S. frugiperda* larvae showed differences in mortality in each treatment. The highest mortality rate was found in the treatment of 50 pellets of 80% on the 10th day of observation and the sterile water control treatment of 10% was the same as the control without Bb of 10% on the 10th day of observation which was the lowest percentage of mortality compared to other treatments (Table 1).

The percentage of mortality of *S. frugiperda* infected by *B. bassiana* in the treatment of 50, 40 and 30 pellets were able to kill more than 50% of the larvae on the 10th day of observation. This shows that in this experiment the application of *B. bassiana* alginate pellets effectively killed *S. frugiperda* larvae because the high spore density resulted in denser spores so that larvae infected with *B. bassiana* had a higher chance of mortality, this was in accordance with the opinion of [28]-[30] which states that the higher the spore density, the more toxins the fungus produces, thereby accelerating the process of death of the larvae.

In the treatment of 40 pellets, it appears that the increase in concentration was not followed by an increase in the mortality of the test insects, this was seen in the treatment of 30 pellets, the mortality of larvae was higher compared to the treatment of 40 pellets. This is presumably because the concentration of spores in the treatment of 40 pellets experienced an uneven distribution of spores on the bodies of the test insects. This is supported by the statement of [31], [32] that the growth medium, the level of virulence, viability and pathogenicity of entomopathogenic fungi greatly determine the success of the fungus in the process of infecting the host.

Table 1 shows that the percentage of larval mortality in the treatment of 50 pellets, 40 pellets and 30 seeds tended to increase in mortality every day. It is suspected that the fungus takes a long time to infect and develop in the bodies of the larvae which will result in more and more larvae dying every day. This is in accordance with the opinion of [33], which states that pest mortality increases after a few days of application. reproduce the mycelium in the insect's body until the insect dies. This is reinforced by the opinion of [34], who said that the infection mechanism of *B. bassiana* begins with contact between the conidia of the fungus and the cuticle of the insect, then the conidia germinate, then the hyphae penetrate the integument and produce the enzymes chitinase, protease and lipase to soften the cuticle.

The spores multiply within the insect's body for 36 to 72 hours and begin to damage the host's internal structures and then the host insect dies between 4 and 10 days after infection. The color of the insects will also turn black after being infected with the fungus *B. bassiana* and there are white hyphae on the surface of the larvae's body. This is corroborated by the statement of [35] that the spores of the fungus *B. bassiana* were able to cause death on the fourth day of the test insects and on the seventh day they began to show symptoms. The time of death depends on the species, the age of the insect and the number of conidia attached to the insect's body [34], [36], [37].

In this study, *B. bassiana* had not shown effectiveness of up to 100% larval mortality in controlling *S. frugiperda* larvae populations until 3 days after application. The success of entomopathogenic fungi in controlling target pests is highly dependent on the diversity of isolate species, conidial density, quality of growing media, target pest species, pest stage, time of application and environmental factors including temperature and humidity [10], [38], [39]. The humidity obtained during the study ranged from 81% and a temperature of 27ºC. While the fungus *B. bassiana* can grow optimally on the insect's body with the appropriate humidity of 92% and will increase at low temperatures. Temperature is one of the factors that most influence the development of the *B. bassiana* fungus [34], [40], [41].

The stadia of the test larvae also greatly affect the resulting mortality percentage level. The younger the instars used as test insects, the higher the mortality rate of the larvae and vice versa. According to [27] stated that the younger the instars used, the higher the mortality rate of the larvae. It can also be said that the effectiveness of the applied fungus and the increased mortality affect the age of the instar larvae of an insect. This is also supported by the opinion of [42] that the effectiveness of the fungus is not only influenced by the growth media, the level of virulence and the frequency of application, it is also largely determined by the age of the insect instars, especially the entomopathogenic fungi which generally penetrate the integument in the part between the head capsule and the thorax and between the vertebrae of the limbs [43], [44].

In the application of *B. bassiana* alginate pellets, it can be seen that the treatment of 50 pellets showed the best percentage of mortality, reaching 80% compared to other treatments. This can be seen in the test larvae, which show symptoms of less active insects, reduced appetite, so that over time they will die and can also be seen on the insect's body overgrown with white hyphae. This is in accordance with the opinion of [45] which states that in host insects that have been infected with *B. bassiana*, the hyphae of the fungus will grow into the cells of the insect's body and absorb the insect's body fluids resulting in the insect dying in a hard body condition such as mummy.

Based on observations of *S. frugiperda* that developed into pupae and imago after the application of *B. bassiana* alginate pellets, it was shown that the larvae that developed into pupae had different mortality and length of life (Table 2). Pupae mortality in the treatment of 30 seeds, 40 seeds, and 50 pellets was 1 tail and in the sterile water control treatment and the control without Bb there was no pupal mortality. While the number of pupae that were still alive in the sterile water

control treatment and the control without *B. bassiana* was 9 tails and was the highest number of pupae among the other treatments. treatment of 50 pellets the number of pupae that were still alive was 1 tail and was the lowest number of pupae among the other treatments. It is suspected that the highest concentration level is still able to inhibit the larvae from developing into pupae. In accordance with the opinion of [46] which states that the high concentration of spores results in a higher number of spore densities resulting in a relatively large toxic content produced from *B. bassiana* [47], [48].

After a 7-day pupal stage, on the 16th day the observations showed that the pupae that developed into imago had different mortality and length of life (Table 2). The number of imagoes that lived in the sterile water control treatment and the control without *B. bassiana* was 9 individuals and was the highest number of imago among all treatments and the least number of living imago was in the treatment of 50 pellet seeds as much as 1 tail. The decrease in the percentage of imago in the treatment of 50 pellets proved that the toxicity of the alginate pellets of *B. bassiana* still inhibited the development into imago. The less conidia of the fungus spores are applied, the percentage of development to imago tends to increase. In accordance with the results of a study by [49] which stated that the higher the density of conidia of the fungus *B. bassiana*, the greater the number of conidia of the fungus attached to the body of the larvae and the greater the amount of poison produced [50].

The results of observations on the carrier material in the alginate pellet formulation showed that the P2 treatment (*B. bassiana* (corn rice) + alginate + wheat flour) had the number of spores observed in the haemocytometer and a high spore density compared to other treatments. The nutritional ingredients contained in wheat flour such as protein, starch, vitamins, fats/oils and the most important substances have a large percentage of content so that the *B. bassiana* spores formed in pellets can retain their nutrition so that the spores remain active. In addition, room temperature and humidity also affect the resistance of the spores in the pellets. The ideal room temperature for research is 24-28ºC and humidity is around 70-90%. [51] stated that the media or material used for the growth of entamopagenic fungi determines the rate of formation of new colonies/ isolates and the number of conidia or spores during the growth of the fungus. This is often influenced by the nutritional conditions contained in each media material. So that it can be said that nutrition can affect the growth of entomopathogenic fungi.

From the results of the *B. bassiana* mortality test on the test larvae of *S. frugiperda* in all treatments, it was shown that there was the highest mortality percentage of all treatments. Treatment P0 (control) had the highest percentage of mortality from day 3 to day 5, the highest mortality was 48%. Furthermore, from day 6 to day 9, treatment P2 (*B. bassiana* (corn rice) + alginate + wheat flour) had the highest percentage of mortality, the highest mortality was 70%. This happened at P0 from the start which showed a very high mortality, possibly because the spore density was still high so that the test larvae died faster. Then at P2 from the 6th day it showed a very high mortality because the treatment of the test material required a process to give the test material toxin to the test larvae so that at that time there was a very large mortality rate. Treatments P0 and P2 can be said to have a high spore density and have a high average mortality compared to other treatments and these two treatments every day after application have a high mortality and are almost close in mortality value. [52] states that the density of spores of fungal diseases that are applied determines the mortality of insects. The higher the spore density, the higher the mortality rate of the tested insects. Optimal spore density for pest control depends on the type of insect to be controlled. [53] also stated that the closeness of the insect relationship where *B. bassiana* was isolated from the target insect had an effect on mortality. In addition, the high and low mortality of insect-potent fungi is influenced by several factors, namely spore production, spore growth and spore viability.

Mortality testing from giving each treatment to the test larvae was carried out using the dip method, meaning that the feed given was dipped into pellets that had been mixed with distilled water in each treatment and then given to the test larvae of *S. frugiperda*. This method is said to be quite successful because the larvae eat feed mixed with a solution containing *B. bassiana* isolate and soon the larvae become limp and then die with mortality stages every day of observation. In a study conducted by [54] the method used in his research was the leaf residue or feed dip method which causes pesticide residues (or entomopathogenic fungi) to stick to leaves or feed. After that, leaves or feed with toxic residues attached (or spores of entomopathogenic fungi) will be given to the larvae to be tested as a form of application for the mortality of the test larvae.

The behavior of the test larvae of *S. frugiperda* was highly visible after the administration of the test material in each treatment. When after being treated, the larvae eat feed that has been mixed with a pellet solution containing *B. bassiana* spores and then the spores that have entered the larva's body will grow into hyphae threads throughout the larva's body tissues so that they can damage internal organs, nerves. nerves, parts of the digestive organs and so on. As a result, the larvae have a lack of appetite, appear weak, lethargic, stiff, usually the body lies down, walking slows down somewhat, the larval body's metabolism is disrupted, and damage to important nerves that inhibits appetite and inhibits the growth and metamorphosis (life cycle) of the larvae. [14] stated that *B. bassiana* produces poisons (toxins) such as beauvericine, beauverolide, isorolide and dyes and oxalic acid which can cause aggressive paralysis of insect larvae and imago. Symptoms of attack on insects that are attacked or infected by *B. bassiana* are larval movements that start to slow down, decreased appetite, weakness, stiffness and hardness. Research conducted by [55], shows that the time required for insect mortality from *Beauveria* spp. seems varied. The variation in the time of mortality was probably caused by differences in virulence between isolates and the response of the host insects. Insect mortality time can be influenced by the dose of application treatment and the virulence of the isolate.

The color and physical changes of the test larvae of *S. frugiperda* occurred after the test larvae died due to the toxicity of *B. bassiana*. After death, the color of the insect's body changes from yellow brown to black and a few white spots appear, which are the mycelium of *B. bassiana*. The body of the larva is stiff and wrinkled and the weight of the larva is getting lighter and drier. Research conducted by [56] found that the fungus *Beauveria* spp. which results in the

death of the larvae, marked by the larvae being hardened or stiff and usually blackish brown in color and then over time they turn white. This white color occurs because the mycelium of *Beauveria* spp. covered the insect's entire body. [18] stated that entomopathogenic fungi need time to grow, starting from spore germination which takes 24-48 hours. After 72 hours, fungal hyphae had begun to appear on the integumentary cuticle of the host insect. If the hyphae have developed inside the insect's body, the fungus mycelium will appear on the surface of the insect's body.

The test larvae of *S. frugiperda* used in this study were instar 3 larvae, which had the characteristics of being 7-8 days old, starting to change color to brownish green, the head was smaller than the body of the larvae. This is because when in the field, usually instar 2 larvae are still inside the corn plants which still eat the corn leaf meristems, so they rarely come out of the corn plants. By the time the 3rd instar larvae have started to come out of the corn plants (usually in the afternoon) so that it is easy to apply the entomopathogenic fungus (*B. bassiana*) test material to the larvae. Also, when applied in the laboratory, using instar 3 larvae will be more effective in observation and require a process of *B. bassiana* toxicity in the body of the larvae. Compared to using instar 2 larvae, they will die faster and are not effective in research because the larvae's bodies are vulnerable to water and are still small. Research conducted by [34] that his research used instar larvae 3, 4, and 5, and where the most effective mortality was instar 3 larvae because their mortality was higher compared to instar 4 and 5 larvae. His research proved that the level of stadia (instar) larvae affects the difference in larval mortality tested, the lower the larval instar, the higher the larval mortality rate. [52] states that apart from being influenced by the growing medium, the level of virulence and the dosage or dosage of the application, the effectiveness of the fungus is also largely determined by the age of the insect instars tested.

The *B. bassiana* alginate pellets in this study had various shapes, some were round, oval and some were flat. There are also small and large pellet sizes. This is because at the time of printing they still use conventional tools (drinking bottles are perforated using a large pipette) so that the shape changes. However, the materials used in the manufacture of alginate pellets are complete enough so that the manufacturing process is easier, and the pellets can be formed properly. In addition, these alginate pellets can also retain the conidia (spores) of *B. bassiana* in them so they can last a long time (up to 4 months) and be stored in an effective place.

This also supports research conducted by [57] which provides evidence that *B. bassiana* alginate pellets can significantly reduce the population of *S. invicta* or other test insects and can be used as an effective control agent in the field. The delivery system developed in this study can effectively introduce pathogens into the test insect colonies. Baited pellets target dominant soil-dwelling fauna, while protecting fungal pathogens from degradation due to exposure to UV light or other environmental factors. [58] states that there are several steps that can be taken in an effort to obtain good quality pellets, namely crushing, mixing, conditioning, molding, cooling and drying.

The percentage of *S. frugiperda* larvae that developed into pupae, with the highest pupal population was found in treatment P2 of 64%, while the lowest pupal population was found in treatment P3 of 30%, and the two treatments were significantly different from each other. Likewise with the percentage of *S. frugiperda* pupae that developed into imago, where the highest imago population was in treatment P2 at 54%, while the lowest imago population was in treatment P3 at 24%, and the two treatments were also significantly different from each other. The treatment that had the highest pupal and imago populations was due to the low mortality of *S. frugiperda* larvae and the effect of the material being tested was still susceptible to the test larvae. In contrast, the treatment that had the lowest pupal and imago populations was due to the very high mortality of *S. frugiperda* larvae and the toxicity effect of the test material on the test larvae.

Based on the data obtained in this study, the mortality percentage of *S. frugiperda* larvae in all treatments did not reach 100%. Likewise with the percentage of mortality of larvae (52%) and pupae (9%) if the total is only 61% (Figure 6). This is due to the temperature and humidity factors that are not supportive in maintaining the quality of *B. bassiana* alginate pellets so that the spores in it gradually no longer survive. Research conducted by [34] found that the percentage of insect mortality did not reach 100% due to environmental factors (humidity and temperature) which could not be tolerated for the growth of the fungus *B. bassiana*. Humidity obtained during research usually ranges from 70.6 - 79.3 and the temperature is between 28.6 - 32ºC. [59] stated that different concentrations of conidia will result in different mortality, the higher the concentration of conidia, the higher the mortality (and can reach 100%) depending on the formulation used.

IV. Conclusion

B. bassiana was able to kill *S. frugiperda* larvae with different percentage levels of mortality, in the treatment of 50 alginate pellets it had a very significant effect among all treatments and resulted in the most influential mortality among other treatments of 80%. The treatment of 50 alginate pellets was also the treatment that had the lowest percentage of pupal and imago development, only 1 animal each. The addition of bran, wheat flour, tapioca flour and corn flour formulations had an effect on the spore density of the *B. bassiana* alginate pellets. The P3 treatment (wheat flour) had a high spore density of 2.35×10^8 spores per ml with high mortality of *S. frugiperda* larvae on day 6-9 DAA respectively of 60%, 68% and 70%.

Acknowledgment

We thank the Ministry of Research and Technology through the 2021 National Bureau Research and Innovation for funding this research on the *Skim Penelitian Dasar* (Basic Research Scheme).

References

[1] Food and Agriculture Organization (FAO). *World Food and Agriculture - Statistical Yearbook*. Food and Agriculture Organization of the United Nations. (2021). doi:10.4060/cb4477en.

[2] A. Hirdapina, A. Hudoyo, and A. Soelaiman, "Pengaruh program upaya khusus (upsus) terhadap produktivitas, biaya pokok produksi, dan pendapatan usahatani jagung di Kabupaten Lampung Timur," *Jurnal Ilmu-Ilmu Agribisnis*., vol. 8, no. 4, pp. 617–624, 2020.

[3] B. R. Ahissou, W. M. Sawadogo, A. H. Sawadogo, A. H. B. Ganta, , I. Somda, M. P. Kestemont, and F. J. Verheggen, "Baseline toxicity data of different insecticides against the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) and control failure likelihood estimation in Burkina Faso," *African Entomology.*, vol. 29, no. 2, 2021.

[4] G. Hailu, S. Niassy, T. Bässler, N. Ochatum, C. Studer, D. Salifu, M. K. Agbodzavu, Z. R. Khan, C. Midega, and S. Subramanian, "Could fall armyworm, *Spodoptera frugiperda* (J. E. Smith) invasion in Africa contribute to the displacement of cereal stemborers in maize and sorghum cropping systems," *International Journal of Tropical Insect Science.*, vol. 41, no. 1, pp. 1753–1762, 2021.

[5] N. Nelly, E.C. Lina, H. Hamid, and Yunisman. "Distribution and genetic diversity of *Spodoptera frugiperda* J. E. Smith (Noctuidae: Lepidoptera) on maize in West Sumatra, Indonesia," *Journal of Biological Diversity.*, vol. 22, no. 5, pp. 2504–2511, 2021.

[6] B. M. Prasanna, A. Bruce, Y. Beyene, D. Makumbi, M. Gowda, M. Asim, S. Martinelli, G. P. Head, and S. Parimi, "Host plant resistance for fall armyworm management in maize: relevance, status and prospects in Africa and Asia, "*Theoretical and Applied Genetics.*, vol. 1, no. 1, pp. 1–20, 2022.

[7] Balai Besar Permalan Organisme Pengganggu Tanaman (BBPOPT). *Peta Sebaran OPT Pada Tanaman Jagung Berdasarkan pengamatan keadaan lapangan Petugas BBPOPT.* (2019). Available: http://bbpopt.tanamanpangan.pertanian.go.id.

[8] F. Assefa, D. Ayalew, and M. T. Moral, "Status and control measures of fall armyworm (*Spodoptera frugiperda*) infestations in maize fields in Ethiopia: A review," *Cogent Food & Agriculture.*, vol. 5, no. 1, pp. 1–16, 2019

[9] A. A. Saldi, "Toksisitas Beauveria bassiana (Bals.) Vuil. berbagai konsentrasi terhadap larva *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) di laboratorium," S.P. thesis, Dept. Plant Pest and Disease., Universitas Hasanuddin., Indonesia, 2020.

[10] T. B. Saputro, Y. Prayogo, F. L. Rohman, and N. H. Alami, "The virulence improvement of *Beauveria bassiana* in infecting *Cyclas formicarius* modulated by various chitin based compounds," *Biodiversitas*., vol. 20, no. 9, pp. 2486-2493, 2019.

[11] A. C. da S. Santos, R. da S. Lopes, L. G. de. Oliveira, A. G. Diniz, M. Shakeel, E. A. de L. A, Lima, A. F. da. Costa, and V. L. de M. Lima, "Entomopathogenic Fungi: Current Status and Prospects," in *New and Future Development in Biopesticide Research: Biotechnological Exploration,* Springer Nature, 2022, pp. 55–91.

[12] R. Wasuwan, N. Phosrithong, B. Promdonkoy, D. Sangsrakru, C. Sonthirod, S. Tangphatsornruang, and S. Likhitrattanapisal, "The fungus *Metarhizium* sp. BCC 4849 is an effective and safe mycoinsecticide for the management of spider mites and other insect pests," *Insects.*, vol. 13, no. 42, pp. 1–18, 2022.

[13] I. D. Daud, A. Nurriaty, and Rasmi, "Inventory of arthropoda in endophytic hybrid corn plants (Variety Mr14 x Variety Nei9008)," in *IOP Conference Series: Earth and Environmental Science*, 2020, pp. 1–7, doi:10.1088/1755-1315/486/1/012143. 2020.

[14] D. G. P. De Oliveira, R. B. Lopes, J. M. Rezende, and I. Delalibera, "Increased tolerance of *Beauveria bassiana* and *Metarhizium anisopliae* conidia to high temperature provided by oil-based formulations," *Journal of Invertebrate Pathology.,* vol. 151, pp. 151-157, 2018.

[15] C. Cafarchia, R. Pellegrino, V. Romano, M. Friuli, C. Demitri, M. Pombi, G. Benelli, and D. Otranto, "Delivery and effectiveness of entomopathogenic fungi for mosquito and tick control: current knowledge and research challenges," *Acta Tropica.*, vol. 234, pp.1-11, 2022.

[16] S. Shah, G. J. Ash, and B. A. L. Wilson, "Resporulation of *Metarhizium anisopliae* granules on soil and mortality of *Tenebrio molitor*: implications for wireworm management in sweet potato," *Annals of Applied Biology.*, vol. 1, no. 1, pp. 1–12, 2022.

[17] H. E. White, *Alginate pellet formulation of Beauveria bassiana pathogenic to the red imported fire ant*. Lubbock, TX, 1995

[18] A. Idrees, A. Afzal, Z. A. Qadir, and J. Li, "Bioassays of *Beauveria bassiana* isolates against the fall armyworm *Spodoptera frugiperda*," *J. Fungi*., vol. 8, no. 717, pp. 1-16, 2022.

[19] W. Islam, M. Adnan, A. Shabbir, H. Naveed, Y. S. Abubakar, M. Qasim and M. Tayyabi, "Insect-fungal-interactions: A detailed review on entomopathogenic fungi pathogenicity to combat insect pests," *Microbial Pathogenesis.*, vol. 159, pp. 1–8, 2021.

[20] S. G. Jeong, H. M. Kim, J. Kim, J. S. Kim, and H. W. Park, "Effect of storage conditions on the shelf-life extension of fungus-colonized substrates based on *Metarhizium anisopliae* using modified atmosphere packaging," *Scientific Reports.*, vol. 12, no. 423, pp. 1–8, 2022.

[21] Y. Fitriana, R. Suharjo, I. G. Swibawa, *et al,* "*Aspergillus oryzae* and *Beauveria bassiana* as entomopathogenic fungi of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) infesting corn in Lampung, Indonesia," *Egypt J Biol Pest Control.,* vol. 31, no. 127, 2021.

[22] D. Stephan, T. Bernhardt, M. Buranjadze, C. Seib, J. Schafer, N. Maguire, and J. Pelz, "Development of a fluid-bed coating process for soil-granule-based formulations of *Metarhizium brunneum*, *Cordyceps fumosorosea* or *Beauveria bassiana*," *Journal of Applied Microbiology.*, vol. 131, no. 1, pp. 307–320, 2020.

[23] S. Sukamto, and K. Yuliantoro, "Pengaruh suhu penyimpanan terhadap viabilitas *Beauveria bassiana* (Bals.) Vuill. dalam beberapa pembawa," *Pelita Perkebunan*., vol. 22, no. 1, pp. 40–57, 2006.

[24] Hardiyanti, Kadirman, and M. Rais, "Pengaruh substitusi tepung jagung (*Zea mays* L.) dalam pembuatan cookies," *Jurnal Pendidikan Teknologi Pertanian.*, vol. 2, no. 2, pp. 123–128, 2016.

[25] S. A. Matz, *Bakery Technologu and Engineering (Third Edit).* Springer New York, 1991.

[26] Gabriel, and Riyatno. *Metarhizium Anisopliae Taksonomi, Patology, Produksi dan Aplikasinya. Proyek Perkembangan Perlindungan Pertanaman Perkebunan.* (1989). Direktorat Perlindungan Tanaman Perkebunan.

[27] I. Masyitah, S. F. Sitepu, and I. Safni, "Potensi jamur entomopatogen untuk mengendalikan ulat grayak *Spodoptera litura* F. pada tanaman tembakau in vivo," *Jurnal Online Agroekoteknologi.*, vol. 3, no. 3, pp. 484–493, 2017.

[28] Y. Mardiana, D. Salbiah, and J. H. Laoh, "penggunaan beberapa konsentrasi *Beauveria bassiana* Vuillemin lokal Untuk mengendalikan maruca testulalis geyer pada tanaman kacang panjang (*Vigna sinensis* L.)," *Jurnal Online Mahasiswa Fakultas Pertanian Universitas Riau*., vol. 2, no. 1, pp. 1–11, 2015.

[29] C. Tong, J. Wei, G. Pan, C. Li, and Z. Zhou, "Study of pathogenesis using fluorescent strain of *Cordyceps farinosa* revealed infection of *Thitarodes armoricanus* larvae via digestive tract," *Insects*., vol. 13, no. 11, pp. 1–23, 2021.

[30] P. Vivekanandhan, K. Swathy, L. Alford, S. Pittarate, S. P. Subala, S. Mekchay, D. Elangovan, and P. Krutmuang, "Toxicity of *Metarhizium flavoviride* conidia virulence against *Spodoptera litura* (Lepidoptera: Noctuidae) and its impact on physiological and biochemical activities," *Scientific Reports.*, vol. 12, pp. 1–10, 2022.

[31] Suhairiyah, Isnawati, and E. Ratnasari, "Pengaruh pemberian cendawan *Lecanicillium lecanii* terhadap mortalitas ulat grayak (*Spodoptera litura*) secara in vitro," *Lentera Bio*., vol. 2, no. 3, pp. 253–257, 2013.

[32] B. S. Bamisile, J. A. Siddiqui, K. S. Akutse, L. C. R. Aguila, and Y. Xu, "General limitations to endophytic entomopathogenic fungi use as plant growth promoters, pests and pathogens biocontrol agents," *Plants.*, vol. 10, pp. 1–23, 2021.

[33] U. C. Sitompul, and Lazuardi, "Pengaruh cendawan beauveria bassiana sebagai pengendalian hayati terhadap mortalitas hama ulat katung (*Metisa plana* Walker)," *Prosiding Seminar Nasional Biologi dan Pembelajarannya*, pp. 1–10, 2014.

[34] N. R. Simanjuntak, "Patogenisitas *Beauveria bassiana* (Bals.) Terhadap larva *Chilo sacchariphagus* Boj. (Lepidoptera: Crambidae) di laboratorium," S.P. thesis, Fakultas Pertanian, Universitas Sumatera Utara Medan,, Indonesia. 2017.

[35] S. Herlinda, N. Octariati, Suwandi, and Hasbi, "Exploring entomopathogenic fungi from South Sumatra (Indonesia) soil and their pathogenicity against a new invasive maize pest, *Spodoptera frugiperda," Journal of Biological Diversity.*, vol. 21, no. 7, pp. 2955–2965, 2020.

[36] I. Piandari, S. Ulpah, T. E. Sabli, and S. Salman, "Inventory of termite species in the area of islam riau university and efficiency of fungus *Metarhizium anisopliae* against terms in the laboratory," *Jurnal Agronomi Tanaman Tropika.*, vol. 4, no. 2, pp. 1–17, 2022.

[37] Z. Zhang, C. Zheng, N. O. Keyhani, Y. Gao, and J. Wang, "Infection of the western flower thrips, *Frankliniella occidentalis,* by the insect pathogenic fungus *Beauveria bassiana*," *Agronomy*., vol. 11, no. 10, pp. 1–15, 2021.

[38] S. B. Sudarmadji, Handoyo, and Suhardi. *Procedures for the Analysis of Food and Agriculture*. Liberty, 1997.

[39] R. Bava, F. Castagna, C. Piras, Y. Musolino, C. Lupia, E. Palma, D. Britti, V. Musella, "Entomopathogenic fungi for pests and predators control in beekeeping," *Vet Sci.,* vol. 9, no. 2, 2022.

[40] S. Mishra, P. Kumar, A. Malik, "Effect of temperature and humidity on pathogenicity of native *Beauveria bassiana* isolate against *Musca domestica* L.," *J Parasit Dis.,* vol. 39, no. 4, pp. 697-704, 2015.

[41] I. O. Ozdemir, C. Tuncer, I. Erper, and R. Kushiyev, "Efficacy of the entomopathogenic fungi; *Beauveria bassiana* and *Metarhizium anisopliae* against the cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae: Bruchinae)," *Egyptian Journal of Biological Pest Control.*, vol. 30, no. 24, pp. 1–5, 2020.

[42] A. Sala, A. Artola, A. Sánchez, and R. Barrena, "Rice husk as a source for fungal biopesticide production by solid-state fermentation using *B. bassiana* and *T. harzianum*," *Bioresource Technology.*, vol. 296, no. 1, pp. 1–9, 2020.

[43] Y. Prayogo, "Upaya mempertahankan keefektifan cendawan entomopatogen untuk mengendalikan hama tanaman pangan," *Jurnal Litbang Pertanian.*, vol. 24, no. 1, pp. 19-26. 2006.

[44] A. Shourie, R. Singh, and A. Singh, "Histopathologic diagnosis of fungal infections of lab animals," *Fungal Diseases in Animals.*, vol. 1, no. 1, pp. 73–91, 2021.

[45] D. F. Q. Smith, E. Camacho, R. Thakur, A. J. Barron, Y. Dong, and G. Dimopoulus, "Glyphosate inhibits melanization and increases susceptibility to infection in insects," *Plos Biology.*, vol. 1, no. 1, pp. 1–35, 2021.

[46] A. Rosmiati, C. Hidayat, E. Firmansyah, and Y. Setiati, "Potensi *Beauveria bassiana* sebagai agens hayati *Spodoptera litura* Fabr. pada tanaman kedelai," *Jurnal Agrikultura.*, vol. 29, no. 1, pp. 43–47, 2018.

[47] H. Liu, X. Zhao, M. Guo, H. Liu, Z. Zheng, "Growth and metabolism of *Beauveria bassiana* spores and mycelia," *BMC Microbiol*., vol. 15. 2015.

[48] M. Abuhena, M. G. Kabir, M. F. Azim, J. Al-Rashid, and N. M. Rasul, "A stressing method for producing high-density *Trichoderma* spores in a dual-layer by utilizing a starch-based medium in a reconditioning approach," *Bioresource Technology Reports.*, vol. 19, no. 1, pp. 1–13, 2022.

[49] B. Poulin, G. Lefebvre, S. Hilaire, and L. Despres, "Long-term persistence and recycling of *Bacillus thuringiensis* israelensis spores in wetlands sprayed for mosquito control," *Ecotoxicology and Environmental Safety.*, vol. 243, no. 1, pp. 1–14, 2022.

[50] A. S. Budi, A. Afandhi, and R. D. Puspitarini, "Patogenesitas cendawan entomopatogen *Beauveria bassiana* Balsamo (Deuteromycetes: Moniliales) pada larva *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae)," *Jurnal Hama Penyakit Tumbuhan.*, vol. 1, no. 1, pp. 57–65, 2013.

[51] J. B. Molina-Hernandez et al., "Cold Atmospheric plasma treatments trigger changes in sun-dried tomatoes mycobiota by modifying the spore surface structure and hydrophobicity," Food Control, vol. 145, p. 109453, Mar. 2023, doi: 10.1016/j.foodcont.2022.109453.

[52] Fitrah, Z., Suriyanti, S., & Syam, N. Uji Pertumbuhan Jamur Beauveria Bassiana Pada Beberapa Media Pertumbuhan. *Jurnal Agrotekmas*, *2*(1), 18–23, 2021.

[53] S. M. N. Islam, M. Z. H. Chowdhury, M. F. Mim, M. B. Momtaz, T. Islam, "Biocontrol potential of native isolates of *Beauveria bassiana* against cotton leafworm *Spodoptera litura* (Fabricius)," *Sci Rep.*, vol. 13, no. 1, 2023.

[54] P. Siahaan, J. Wongkar, S. Wowiling, and R. Mangais, "Patogenitas *Beauveria bassiana* (Bals.) yang diisolasi dari beberapa jenis inang terhadap kepik hijau *Nezara viridula* L. (Hemiptera: Pentatomidae)," *Jurnali Ilmiah Sains.*, vol. 21, no. 1, pp. 26–33, 2021.

[55] M. Gustianingtyas, S. Herlinda, S. Suwandi, "The endophytic fungi from South Sumatra (Indonesia) and their pathogenecity against the new invasive fall armyworm, *Spodoptera frugiperda*," *Biodiversitas.,* vol. 22, no. 2, pp. 1051-1062, 2021.

[56] E. Quesada-Moraga, E. A. A. Maranhao, P. V. Garcia, and C. S. Alvarez, "Selection of *Beauveria bassiana* isolates for control of the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum* on the basis of their virulence, thermal requirements, and toxicogenic activity," *Biological Control.*, vol. 36, no. 3, pp. 274–287, 2016.

[57] M. Gustisningtyas, S. Herlinda, and Suwandi, "The endophytic fungi from South Sumatra (Indonesia) and their pathogenecity against the new invasive fall armyworm, *Spodoptera frugiperda*," *Biodiversitas.*, vol. 22, no. 2, pp. 1051–1062.

[58] B. R. Bextine, and H. G. Thorvilson, "Field applications of bait-formulated *Beauveria bassiana* alginate pellets for biological control of the red imported fire ant (Hymenoptera: Formicidae)," *Environmental Entomology.*, vol. 31, no. 4, pp. 746–752, 2002.

[59] C. J. Lei, R. H. I. R. Ahmad, N. A. Halim, N. Asib, A. Zakaria, W. A. Azmi, "Bioefficacy of an oil-emulsion formulation of entomopathogenic fungus, *Metarhizium anisopliae* against adult red palm weevil, *Rhynchophorus ferrugineus*," *Insects*., vol. 14, no. 5, 2023.