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The Use of *Beauveria bassiana* as Biological Control against the Pepper Aphid Insect Pest *Myzus persicae* Sulz

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Abstract. This research is motivated by chillies, which are one of the plant products that become raw materials for daily needs and are therefore classified as secondary food requirements. Insect pests are a factor affecting chilli productivity, use of fungi as biological controllers, to determine the use of the fungus *Beauveria bassiana* as a biological control against the insect pest of chilli aphids *Myzus persicae* Sulz, the research method used in this study is an experimental method using the complete randomized design (CRD) method with 5 treatments and 3 replications, where each plot has 5 samples, the treatment doses were Control, P1 (6 g/500 ml), P2 (7 g/500 ml), P3 (8 g/500 ml), P4 (9 g/500 ml) and Lethal Dose 50, the results of this study showed that the application of *B. bassiana* had a significant effect on the mortality of *M. persicae* Sulz with the mortality obtained was 0.75% of the total number of samples 120 outside the control treatment, so the higher the dose, the higher the mortality, the LD_{50} result was 6.025 g/ml, showing that the toxicity of *B. bassiana* falls into the category of mild toxins. It can therefore be concluded that the fungus *B. bassiana* can be used as a biological control agent against the insect pest *M. persicae* Sulz.

Keywords: *Beauveria bassiana*, lethal dose 50, mortality, *Myzus persicae* Sulz

I. INTRODUCTION

The chilli pepper is a type of vegetable that is the main ingredient in daily requirements, so it is included in non-essential consumption needs. Chillies belong to the shrubby plants of the Solanaceae species, native to the American continent, in particular the Peruvian region, and extending to countries in America, Europe and Asia, including our country [1]. Chillies are important horticultural products for daily consumption, which means they are an additional basic need.

Based on the census data of the Central Statistics Agency 2023 of the province of North Sulawesi in 2019-2021 related to the production of vegetable crops, in particular the production of chilli in 2019 was 433.00 ku, increased to 1012.00 ku in 2020 and decreased again to 681.00 ku in 2021. The fall in chilli production in 2021 at national level is the first in the last ten years. The drop in chilli production was caused by pests and diseases.

Thrips, fruit flies and *Myzus persicae* Sulz aphids are the most common pests to attack chillies [2]. In general, *M. persicae* Sulz aphids on chillies can cause leaf curling, shoot curling, wilting and can interfere with growth and even cause plant death [3]. The chilli aphid *M. persicae* Sulz is a serious pest as it can disrupt plant growth and development and have an impact on reducing chilli production.

One of the pest and disease control technologies that can reduce the occurrence of resistance and recurrence is biological control through the use of natural enemies [4]. *Beauveria bassiana* is one of the natural enemies in the form of entomopathogenic fungi that form part of biological control agents. *B. bassiana* is an entomopathogenic fungus that has been widely used for biological control of insect pests [5]. *B. bassiana* is one of the entomopathogenic fungi widely used to control various agricultural crop pests [6].

Based on previous research according to Zuriyati (2018) managed to identify the insect pests that often attack chilli plants during his field research, namely whitefly, caterpillars and aphids [7]. The reason for the appearance of insect pests is due to the attraction of pests to chilli plants, as well as being a breeding ground, the leaves of chilli plants are also food for aphids and caterpillars. Other research results according to Rahayu *et al*, 2021 show that the application of the fungus *Beauveria bassiana* is effective in controlling insects of the order Coleoptera with a mortality of up to 77.5% at 7 hectare of observation, so it has the potential to be used as a biological control agent [8].

B. bassiana is capable of attacking different types of insects in both the larval and adult stages [9]. Rosmiati *et al* (2018) stated that the higher the spore density of the fungal isolate of *B. bassiana* applied, the more spores are attached to the body of larvae or insects, the more enzymes and toxins are produced, accelerating the death of larvae or insects [10].

On the basis of the above explanation, the researcher would like to carry out research into 'the use of the fungus *B. bassiana* as a means of biological control of the insect pest *M. persicae* Sulz*'*. This research should provide a new alternative and can be developed again in agriculture to enrich chilli plants so that they are resistant to pests and diseases.

II. METHODS

Research Time and Location

The research method used in this study is an experimental method using the complete random design (CRD) method with 5 treatments and 3 replications, where each plot has 10 samples.

The treatment doses were as follows: Control, P1 (treatment 1) = 6 g/500 ml water, P2 (treatment 2) = 7 g/500 ml water, P3 (treatment 3) = 8 g/500 ml water, P4 (treatment 4) = 9 g/500 ml water. This research was carried out in the laboratory of the Biology Department of the Faculty of Mathematics, Natural Sciences and Earth Sciences at Manado State University. It lasted 4 months, from December 2023 to March 2024.

The instruments in this study include the tools and materials used to propagate isolates for application of *B.* bassiana on *M. persicae* Sulz. The tools used in this study are: containers, mushroom propagating plastic, spraying equipment, hand counter, measuring cup, elementary, refrigerator, spritus, bunsen, balance, lebel paper, spoon, cooker, tray, matches, sieve and cotton. The materials used in this study were pure isolates of *B. bassiana*, rice and distilled water.

Search Procedures

Investigation of the Fungal Host B. bassiana on Coffee Fruits

Exploration of coffee fruits showing signs of infection by the fungus *B. bassiana* following spraying of coffee plants with *B. bassiana* against borer insects, which are then used as pure isolates.

Propagation of the B. bassiana Fungus

The rice used as a carrier was washed and soaked in water for about 24 hours, then drained and dried. The clean, dry rice was placed in a plastic bag at a rate of 100g per bag, then steam sterilised for 2 to 3 hours. After steaming, it is cooled and placed in a clean container.

Pure isolates were taken as a starter of *B. bassiana* up to 2 coffees and put them in the rice medium in a plastic bag, making sure that the rice medium and the fungus starter are mixed and sealed with a stapler so that they are not contaminated. Then label each medium with the name of the fungus and the date of propagation [6]. The media are then incubated at room temperature in the dark for less than 2 weeks until the mycelium develops on the surface of the rice.

Preparing the Insecticide and Applying B. bassiana

For the manufacture and application of insecticides, the incubated *B. bassiana* is then weighed according to the dose of each treatment and mixed with 500 ml of water, then placed in a container to be shaken to dissolve it and finally placed in a sprayer.

The application method consists of spraying the insect pests placed in a Petri dish covered with gauze and placing 10 *M. persicae* Sulz insects and a chilli leaf inside. The insect pests were sprayed three times in each treatment plot to ensure that the entire surface and the insect pests were exposed to the fungal isolate of *B. bassiana* applied.

Microscopic Observation of Insect Pests

The insect pests that have been infected for approximately 4 days are then observed under the microscope to determine the benefits of the *B. bassiana* fungus that has infected the host or insect pest.

Data Collection

Data were collected by observing percentage mortality and lethal dose (LD) 50 directly after application using Weil's method. Mortality was calculated using the following formula:

$$M = Y/X \times 100\%$$

M = Mortality
X = Number of insects tested
Y = Number of dead insects
Calculation of the lethal dose 50 (LD) Weil's method, the formula used is as follows:

$$\text{Log (LD)}_{50} = \text{Log D} + d\,(f + 1)$$

D = smallest dose used
d = logarithmic multiple
f = factor obtained from the table
Data obtained from the number of insects tested that died within 24 hours at each dose.

Data Analysis Techniques

Data analysis techniques are obtained both quantitatively and qualitatively. Quantitative analysis uses mortality observations which will be made daily for approximately 4 days after treatment, while control benefits are indicated by percentage mortality and lethal dose 50 (LD).

Data were obtained using analysis of variance (ANOVA) with a true level of 5% and additional tests were performed on the BNT to see any differences between treatments. Qualitative analysis was carried out on the basis of observations and documentation relating to each treatment and each observation variable.

III. RESULTS AND DISCUSSION

Results

The results of the *M. persicae* Sulz chilli aphid mortality data caused by the fungus *B. bassiana*, show a difference in the average number of *M. persicae* Sulz chilli aphid mortalities in each treatment dose listed in Figure 1.

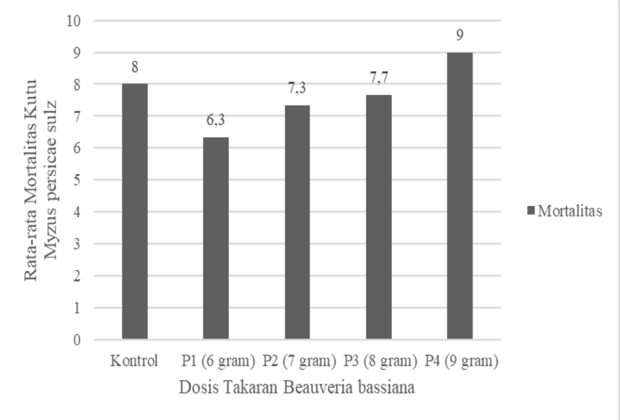

Fig. 1. Average *M. persicae* Sulz Aphid Mortality with *B. bassiana*

TABLE 1.
DATA ON DEAD *M. persicae* Sulz APHIDS AFTER TREATMENT WITH *B. bassiana*

Treatment	**Repetition**			**Total**
	U1 (500 ml)	U2 (500 ml)	U3 (500 ml)	
Control	0	0	0	0
P1 6 g	6	7	6	19
P2 7 g	7	7	8	22
P3 8 g	7	8	8	23
P4 9 g	9	8	10	27
Total	29	30	32	**91**

Figure 3 above shows that *B. bassiana* from pure isolates effectively killed *M. persicae* Sulz with average mortality in each of the different treatments. The highest average mortality occurred with a dose of 9 g/500 ml of water with 9 ticks, followed by the control (Curacorn) with 8 ticks, a dose of 8 g/500 ml of water with 7.7 ticks, a dose of 7 g/500 ml of water with 7.3 ticks, and finally a dose of 6 g/500 ml of water with 6.3 ticks.

These results show that the higher the dose of *B. bassiana*, the greater the number of *M. persicae* Sulz deaths. The application of *B. bassiana* for 4 days of observation resulted in *M. persicae* Sulz mortality of 0.75% of the total number of samples 120 outside the control treatment.

The treatment of *B. bassiana* on *M. persicae* Sulz aphids showed a significant effect. On the basis of the calculation and the analysis of variance, it was established that the dose of *B. bassiana* administered to the *M. persicae* Sulz aphids had a real effect on *M. persicae* Sulz mortality, and the real difference test was therefore recalculated.

TABLE 2.
ANALYSIS OF VARIANCE OF THE EFFECT OF *B. bassiana* APPLICATION ON *M. persicae* Sulz APHID MORTALITY.

	Sum of Squares	Df	Mean Sqaure	F	Sig
Between Groups	11.333	4	2.833	7.083**	0.000
Within Groups	4.000	10	0.400		
Total	15.333	14			

(**) Significantly affected
If F Count 7.083 > 0.05 then there is a very real difference **

TABLE 3.
SCORING OF THE TEST FOR THE SMALLEST SIGNIFICANT DIFFERENCE NUMBER OF DEAD APHIDS *M. persicae* Sulz AFTER THE APPLICATION OF *B. bassiana*

Treatment	Average	Average LSD	Notation LSD
P1	6.3	7.451	a
P2	7.3	8.452	ab
P3	7.7	8.852	bc
Control	8	9.152	bcd
P4	9	10.152	d

Tables 4 show that treatment P1 (6 g/500 ml of water) was significantly different from treatments P3 and P4. Treatment P2 (7 g/500 ml water) was significantly different from P4. Treatment P3 (8 g/500 ml water) was significantly different from P1 and P4. Treatment P4 (9 g/500 ml water) was significantly different from P1, P2 and P3.

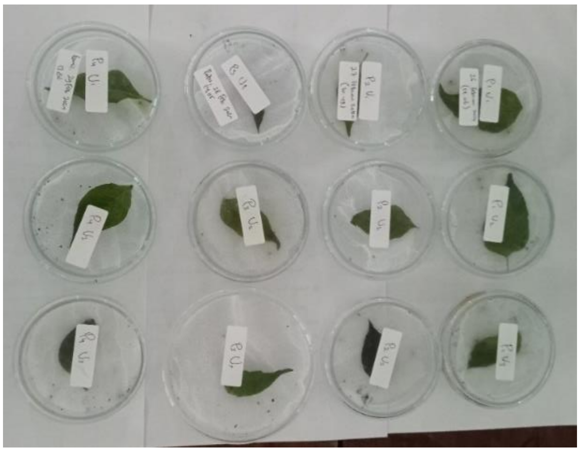

Fig. 2. Mortality of *M. persicae* Sulz Aphids after Application of *B. bassiana*

The lethal dose or LD_{50} is defined as the dose or concentration administered once or several times over 24 hours of a substance that is statistically expected to kill 50% of the animals tested.

TABLE 4.
LETHAL DOSE 50 CALCULATION DATA

Treatment	Doses	Number of animals	Death (r)
P1	6 g	10	1
P2	7 g	10	2
P3	8 g	10	2
P4	9 g	10	2

Calculation of the LD_{50} gave a result of 6.025 g/ml (light toxin), which shows that the toxicity of *B. bassiana* falls into the category of light toxins, meaning that the dose tested is not yet effective enough to kill 50% of the animals tested.

Discussion

Observations of the incidence of *M. persicae* Sulz aphids after treatment with the fungus *B. bassiana* showed that there were changes in the behaviour and activity of the insect pests. After 24 hours of application, some insect pests were found to be dead after treatment. The death of the insect pests within 24 hours is entered as data in order to be able to calculate the value of the lethal dose (LD) 50 on the insect pests. The data relating to the calculation of the lethal dose (LD) 50 are shown in Table 5, which shows that the toxicity of the dose of *B. bassiana* administered as a treatment to *M. persicae* Sulz is included in the mild toxicity category on the basis of the toxicity table according to the LD_{50} category.

Changes in insect pest behaviour and activity are influenced by the effects of the infection obtained after treatment, as well as by the high dose, which results in a high percentage of pest mortality. This is due to the high dose affecting multiplication and accelerating the toxicity of fungal spores to develop on pests. Rosmiati *et al*, (2018), stated that the higher the spore density of the fungal isolate of *B. bassiana* applied, the more the spores are attached to the body of the larvae or insects, the more enzymes and toxins are produced so as to accelerate the death of the larvae or insects [10]. Changes in the activities of insect pests that have been infected are generally slow to respond, stop feeding, become weakened and die.

Fig.3. Observations on Infected *M. persicae* Sulz Pepper Aphids

According to Soetopo and Indrayani (2007), insects infected with *B. bassiana* physically show swelling accompanied by hardening of the insect body [11]. This is consistent with the results of the study, which showed that *M. persicae* Sulz infected with *B. bassiana* exhibited a brownish body colour change to blackish, followed by a hardening and stiffening of the insect body, as shown in Figure 3. Observations form Figure 3 indicate that insects infected with the fungal pathogen undergo a noticeable alteration in cuticle coloration, characterized by a dark brown pigmentation, concurrent with the emergence of fungal spores that exploit the host's nutritional resources. The insects that die are caused by insect tissues that have been completely infected. However, in this study, most infected *M. persicae* Sulz showed fungal mycelium developing on the surface of the insect body. Soetopo and Indrayani (2007) reported that dead insects are not always accompanied by symptoms of spore growth [11]. Humidity, temperature and environmental factors are very important in the germination and conidial production process.

The results of the research carried out show that the dose used has an effect on insect pest mortality, which is in line with the assertion put forward (Gargita *et al*, 2017) that the higher the concentration of the *B. bassiana* formulation, the greater the spore density, and therefore the more likely the insect is to be infected [12]. The process of insect infection by *B. bassiana* begins when the conidia attach to the insect. The attached conidia develop and germinate to enter the body of the host or insect. In addition, the hyphae of the fungus develop and penetrate the blood vessels [13]. In the next stage, *B. bassiana* produces several toxins, including baevericin, beaverolide, bassianin and cyclosporine, which circulate in the insect's bloodstream, causing an increase in the pH of the insect's blood and disrupting the nervous system, resulting in the insect losing its appetite and eventually dying [4]. In addition to spore density, the effectiveness of entomopathogenic fungi in controlling target pests also depends on the age of the insect, developmental stages and cuticle surface area [13]. The use of pure isolates and the use of rice-growing media also prove that the use of *B. bassiana* is relatively effective. This is supported by research results (Fitrah *et al*, 2021) indicating that the best medium for *B. bassiana* growth on spore density and viability is rice + coconut pulp with 6.44 x 109 spores / g and 76.125% respectively [14].

In treatment P4 (9 g/500 ml water), *B. bassiana* showed the best results with a mortality rate of 0.9% and an average of 9 dead insects, which can be explained by the fact that this dose is the highest of all the treatments, so the higher it is, the greater the density of *B. bassiana* spores on *M. persicae* Sulz aphids will be able to infect. Thus, the results that can be obtained from this study are the treatment dose of *Beauveria bassiana* that is most effective in causing mortality is at a treatment dose of 9 g / 500 ml of water given the mortality rate of *M. persicae* Sulz aphids the most and also the dose criterion Lethal Dose (LD) 50 with a calculation of 6.025 g/ml which is included in the category of mild toxicity according to the toxicity category of Lethal Dose 50. The low density of the fungi does not allow the chitin and fat layers of the insect cuticle to be broken down, so penetration and infection take a long time [15]. Another factor that may explain the long period of death of *M. persicae* Sulz pepper aphids due to *B. bassiana* infection is that the fungus needs several stages to infect and kill the insects. Insect death generally occurs 3 to 4 days after application. Temperature and humidity are other factors that play a role in the successful infection of insect bodies by the fungus [4].

IV. CONCLUSION

Based on the results of the research conducted, it can be concluded that the treatment dose of *Beauveria bassiana* effectively causes the mortality of pepper aphids *M. persicae* Sulz at a dose of 9 g / 500 ml with an average of 9 dead insects and a percentage of 0.9%, and the category of the treatment dose of *B. bassiana* is 6.025 g / ml which falls into the category of mild toxicity according to the toxicity of Lethal Dose 50.

Recommendations: Field research is required to determine the efficacy of the treatment dose of B. bassiana when tested directly on chilli plants. It is hoped that further research will be carried out with treatment rates above 10 g/500 ml, or even higher, for insecticides used for biological control of pure isolates of B. bassiana. Further research is required into the use of B. bassiana as a biological control agent against other insect pests, as well as other horticultural plants.

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