Testing The Effectiveness Of Entamopathogenic Fungi Beauveria Bassiana To Overcome Peach Peater Pest(*Myzus Persicae* Sulz) On Chili Plants

Heny Munawaroh[1*], Rizka Rohmawati[2], Sharfina Mutia Syarifah[1], Ika Afiffah Nugraheni[1]

[1]Departement of Biotechnology, Faculty of Sains and Technology, Universitas 'Aisyiyah Yogyakarta[2]Research and Development, PT.Biotek Cipta Kreasi, Sleman, Yogyakarta

*Email: Heny190602@gmail.com

Abstract

Purpose: This research aims to determine alternative media that are suitable for the entamopathogenic fungus *Beauveria bassiana* and the effectiveness of the entamopathogenic fungus *Beauveria bassiana* in dealing with *Myzus persicae* S. onchili plants. This research was carried out in September-December 2023 in the PT laboratory. Biotek Cipta Kreasi Yogyakarta province.

Method: This research used a completely randomized design (CRD) method consisting of 3 treatments, namely (Beauveria with a density of 10 8, 10 7, 10 6) with different application times (1 day, 2 days, 3 days). The parameters observed were mortality and symptoms of peach aphids *Myzus persicae* which were calculated manually using the mortality calculation formula.

Result: Based on the results of research using the *Beauveria bassiana*fungus, it was concluded that this fungus is effective in dealing with peach aphid pest populations. As well as the alternative media used for cilembu tubers which issuitable for the growth of the *Beauveria bassiana* fungus.

Key words: *Beuveria bassiana,* Chili, *Myzus persicae* Sulz

Introduction

Chili (Capsicum annuum, L.) is one of the important vegetable commodities and has high economic value in Indonesia. Chili plants are developed both in the lowlands and highlands[1]. Chili fruit is widely used by people as a spice and cooking seasoning. Along with rapid population growth and the development of the food industry, the need for chilies in Indonesia has also increased[2]. Agriculture is a major activity, with chili (*Capsicum annuum* L.) being a significant crop due to its high economic value and widespread use in cooking and industry. Chili contains essential nutrients such as protein, fat, carbohydrates, calcium, phosphorus, iron, and vitamins C, A, and E, as well as beneficial alkaloids like capsaicin[3].The demand for chilies is growing due to population growth and the food industry's expansion [2]. However, continuous planting without regard for environmental factors has led to declining production, caused by low soil fertility, high evaporation rates, and pest attacks [4].

OPT attacks are one of the factors that hinder the smooth cultivation of chilies. One of the pests that attacks chili plants is peach aphids (Myzus persicae Sulz). This pest is a type of pest that attacks all types of cultivated plants (polyphages), especially those belonging to the Solanaceae family. This pest is often found on the underside of young chili leaves [5]. This pest can be a vector for more than 100 types of viruses, losses due to attacks by peach aphids range between 10-30% and during the dry season the losses incurred can be even greater, namely up to 40% if no control measures are taken [6]. Due to the high number of peach aphid attacks, farmers can experience quite high crop failures, so farmers make efforts to overcome them, one of which is by using pesticides to eradicate these pests. The use of pesticides and insecticides with active chemicals that

are difficult to decompose causes various negative impacts on the environment such as loss of biodiversity, decreasing the population of organisms around plants which are useful as natural enemies, and has dangerous impacts on health when used. in contact with the body[7].

To reduce the impact caused by insecticides, pest control techniques such as the use of entamopathogenic fungi are needed. The use of entamopathogenic fungi in controlling plant pests is quite effective because they have high pathogenicity against target pests and can suppress pest populations in the long term, are relatively cheap, and are environmentally friendly compared to using chemical pesticides. Entamopathogenic fungi are heterotrophic fungi. Because of their heterotrophic nature, entamopathogenic fungi live as parasites on insects [8]. The use of entamopathogenic fungi to control insects has the advantage of high production capacity, a relatively short cycle of entamopathogenic fungi and the ability to form spores that are resistant to adverse environmental conditions. Beauveria bassiana is a fungus that has great potential as a biological control agent because Beauveria bassiana causes white muscardine disease in pests. Beauveria bassiana has white mycelium and conidia or spores[9].
Beauveria bassiana is a type of entomopathogenic fungus which has been widely developed as a biological agent to control various types of pests and diseases[10]. The success of biological control using this fungus is able to kill all stages of insects up to 96% and has a fairly wide host range including the orders Homoptera, Hemiptera, Ortoptera, Coleoptera, Lepidoptera, Diptera, Isoptera and Hymenoptera and does not cause resistance. on target insects [11]. Apart from that, this fungus also produces secondary metabolites which are effective in reducing the intensity of disease caused by soil-borne pathogens by up to 99%[12].

Methods

This research employs an experimental method. This research was carried out in the research and development division at PT. Biotek Cipta Kreasi whose address is Kyai Samiyoredjo street, Jetis, Donolayan, Donoharjo, Ngaglik Subdistrict, Sleman Regency, Special Region of Yogyakarta.

Materi and tools

The tools used are digital scales, laminar air flow (LAF), hot plate, vortex, petridish, autoclave, Erlenmeyer, shaker, test tube, bunsen, pipette, microscope, hemocytometer, measuring cup, scissors, brush number 00, micropipette, camera , cotton wool, glass jar, tissue paper, tweezers, label paper, object glass, deck glass, ose needle, aluminum foil,

spray bottle, rubber nipple, knife, ruler, and stationery and other tools that support this research. The material used is Beauveria bassiana fungus isolate collected from PT. BCK, PDA (Potato Dextrose Agar) and PDB (Potato Dextrose Broth) media, sterile Reverse Osmosis (RO) water, peach aphids (Myzus persicae S), young leaves of chili plants and leaves of chili plants infected with peach aphids taken from Green house PT. Biotech Cipta Kreasi. This research used a completely randomized design (RAl) method with 9 treatment doses of B. bassiana mushrooms. The treatments were carried out without repetition so that 9 experimental units were obtained. The experiment was carried out in a jar with a height of 10 cm, then each unit had 1 young chili leaf with a sample of 20 peach aphids.

Creation of media and alternative media for growth

Making media begins by making PDA media so that it tilts the test tube and petri tube. Each tube contains 6 ml of media while each petri tube contains 10 ml of media. In making PDA media as much as 39 gr/L media. Then on a hot plate and stir using a magnetic stirrer. After boiling and mixing, it is then sterilized using an autoclave for 15 minutes [13].

Making alternative media from cilembu tubers. 600 grams of Cilembu sweet potatoes are peeled and cut into cubes, then washed until clean and boiled for 30 minutes using a pan with 2 liters of sterile RO water. After that, the essence or boiled water from the Cilembu sweet potatoes is filtered using a cloth filter. And put 600 ml into 2 Erlenmeyer flasks, then add 30 grams of agar powder and 20 grams of dextrose and 20 grams of dextrose then put on a hotplate until homogeneous. Then sterilized in an autoclave for 1 hour at a temperature of 121℃ and a pressure of 1 atm, then cooled. Next, the sweet potato media with the addition of agar powder and dextrose was divided into 2 (300 ml) with 1 Erlenmeyer's jar given ciprofloxacin (1ml/1L) and 1 Erlenmeyer's jar with nothing added [14].

Inoculation of the Beauveria bassiana fungus into the media

After making agar media (PDA) and making alternative agar media (cilembu tuber media), inoculation is carried out by preparing tools and materials, preparing LAF by sterilizing it using 70% alcohol and using UV. Then after lighting the Bunsen, sterilize the LAF using 70% alcohol. Then burn the tip of the tube needle and the lip of the test tube and the lip of the petridish on Bunsen before use, then take 1 tube of isolate and put it directly on the Petridish agar medium, the tip of the petridish is closed and burned with Bunsen then wrapped in cling wrap and given a label containing the name and date of inoculation. After that, it is incubated at room temperature for approximately 3-5 days and growth is observed. inoculation from agar media to liquid media (harvesting), harvesting is done by pouring 5ml of sterile RO on the agar media petridish then scratching the fungus and pouring it into the prepared liquid media then vortexing until homogeneous and incubating for 1 week, transfer is carried out aseptically inside the LAF [13].

Spore density calculation

Calculations of spore density in the Beauveria bassiana fungus were carried out starting on the 7th day of incubation, growth was observed and then spore density was calculated with the help of a Haemocytometer. The calculation was carried out by taking 0.5 µl of the solution with the help of a micropipette, after which it was placed in the hemocytometer, 1 drop for the bottom column and 1 drop for the top column. Calculation of spore density using the calculation formula:[15]

$$C = \frac{t}{n \times 0{,}25} \times 10^6$$

Ket C = kerapatan spora per ml larutan,

t = jumlah total spora dalam kotak sampel yang diamati

n = jumlah kotak sampel

0,25 = faktor koreksi penggunaan kotak sampel skala kecil pada haemocytometer.

Creation of the experimental arena and sampling

The chili leaves used in this research were taken from the chili plant greenhouse at PT. Biotech Cipta Kreasi. The chili leaves used are young chili leaves because peach aphid attacks generally start from young leaves, young stems, lower leaf surfaces, plant shoots, flower buds and young stems [13]. The chili leaves that had been taken were then placed in the experimental arena. The experimental arena used was using a jar containing cotton which had previously been moistened with water to prevent aphids from leaving and to maintain a constant temperature, then the jar was covered with tissue paper and tied using a rubber nipple. The peach aphids used were caught in the green house area of PT's chili plants. Biotech Cipta Kreasi. The peach aphids used in this experiment were 20 individuals/jar. The peach aphids are then placed on the chili leaves in the jar using a 00 size brush and a magnifying glass.

Treatment and treatment applications

P 1 (1): Beauveria bassiana fungus with a density of 10^6, 1 spray, every day

P 2 (1): Beauveria bassiana fungus with a density of 10^6, 1 spray, every 2 days

P 3 (1): Beauveria bassiana fungus with a density of 10^6, 1 spray, once every 3 days

P 1 (2): Beauveria bassiana fungus with a density of 10^7, 1 spray, every day

P 2 (2): Beauveria bassiana fungus with a density of 10^7, 1 spray, every 2 days

P 3 (2): Beauveria bassiana fungus with a density of 10^7, 1 spray, once every 3 days

P 1 (3): Beauveria bassiana fungus with a density of 10^8, 1 spray, every day

P 2 (3): Beauveria bassiana fungus with a density of 10^8, 1 spray, every 2 days

P 3 (3): Beauveria bassiana fungus with a density of 10^8, 1 spray, every 3 days

Pest observation population

Population observations were carried out in each jar on all plants. Pest observations were carried out every day for a week. Observations of symptoms of aphids were observed every day for a week and recorded.
Observation of symptoms of peach aphids infected with entomopathogenic fungi. Observations are made every day. Peach aphids infected by the fungus will experience loss of appetite and the Beauveria bassiana fungus will grow on their bodies. Peach aphids were then inoculated on agar media to identify the fungus attached to their bodies.
Observation of peach aphid mortality. The mortality percentage of peach aphids is calculated using the following formula[15]:

$$P = \frac{n}{N} X\ 100\%$$

Ket :

P = Mortalitas (%)

n = Jumlah imago yang mati

N = Jumlah seluruh imago

Results

Tabel 1. Aphid pest mortality data

No	Treatment	Mortality
1	P1(1)	61,9%
2	P2(1)	60%
3	P3(1)	45%
4	P1(2)	50%
5	P2(2)	52%
6	P3(2)	43,4%
7	P1(3)	91%
8	P2(3)	86%
9	P3(3)	87,5%

The research results showed that Beauveria bassiana was able to kill peach aphids above 10 individuals/treatment within 5 days and had an effect on the death of aphids. The highest death rate for aphids due to the influence of Beauveria bassiana was in treatment P1(3) with a density of 10^8 and a daily application interval (Tabel 1), which caused approximately 91% death of peach aphids and the lowest death rate for peach aphids due to the influence of Beauveria bassiana was treatment P3(2) with a density of 10^7 and an application interval of every 2 days, which causes around 43.4% death of peach aphids.

(A) (B)

Figure 1. Peach aphid. A) peach aphid before applying Beauveria bassiana. B) peach aphid after application of Beauveria Bassiana

Discussion

Beauveria bassiana fungus is known to produce many secondary metabolites such as beauvericin, beauverolides, bassianolides, oosporein, cyclosporin A, and oxalic acid with antibacterial, antifungal, cytotoxic and insecticidal activities.The results of mortality data from the Beauveria bassiana fungus show that the most effective treatment for killing aphids is treatment p1(3) using a spore density of $10^8$and with an application interval every day with one spray (0.6 ml) at a distance of 10 cm from the plant leaves. chilli. This research also shows that the Beauveria bassiana fungus tested on peach aphids had a significant effect on the mortality rate of peach aphids. Beauveria bassiana is able to kill more than 10 peach aphids/treatment in 5 days. The results of the study also showed that Beauveria bassiana had an influence on aphid mortality, the highest level of death of aphids due to the influence of Beauveria bassiana was the P1(3) treatment with a density of 10^8 and an application interval every day, which caused approximately 91% death of peach aphids and the death rate The lowest level of peach aphids due to the influence of Beauveria bassiana was treatment P3(2) with a density of 10^7 and an application interval of every 2 days, which caused approximately 43.4% death of peach aphids. High percentage of death mortality. Spraying is carried out in 3 intervals, namely spraying every day, spraying once every 2 days and spraying once every 3 days. Mechanism of infection. Beauveria bassiana fungus against insects, carried out directly by infecting the outer skin layer. The spores begin to germinate to form hyphae and continue to grow while producing chitinase and protease enzymes which destroy the cuticle. Due to damage to the cuticle, the hyphae will penetrate and develop inside the insect's body. The pest will die because the entire body is filled with Beauveria bassiana mycelium. At an advanced level, the hyphae will penetrate and grow on the outside of the insect's body, and produce conidia which will be spread into the environment and infect other insects[13]. The death of aphids due to the influence of Beauveria bassiana occurs gradually. Based on observations of peach aphids infected with the Beauveria bassiana fungus (figure 1), they generally show the same initial symptoms, namely lack of appetite, slowed movements, stiff body, hardening, and drying out like a mummy (mummification)[6]. The process of attack by entomopathogenic fungi causing the host to die is as follows: the conidia contact the insect's integument, then attach and germinate and penetrate by forming a sprout tube (appressorium)[16]. After entering the hemocoel, the fungus forms blastospores which circulate in the hemolymph and form secondary

hyphae to attack other tissues such as the nervous system, trachea and digestive tract. The occurrence of nutritional deficiencies and the presence of toxins produced by fungi, as well as tissue damage in the insect's body will cause paralysis and death of the insect. Peach aphids infected with Beauveria bassiana die and are blackish brown in color. Fungal infections on peach aphids began to occur 3 days after application. The bodies of dead aphids infected with the Beauveria bassiana fungus appear dry and hard, this happens because all the fluids in the aphids' bodies are used for growth by the Beauveria bassiana fungus. In accordance with the statement of jauharlina and Hendrival (2003) in [17] states that in general all networks and the insect's body fluids are used up by the entomopathogenic fungus for its growth and development, as a result the insect dies with its body hardening like a mummy. If conditions are favorable, the fungus will penetrate outside the insect's body, especially at the articulation of the body appendage and mouthparts. Behavioral changes that occurred in the larvae 5 days after application showed that movements began to slow down, feeding activity began to decrease. Changes in pests infected with the toxin were rupture and blackening of the tissue in the abdomen, the pests shrank, turned pale yellow then changed again to blackish brown, shape the pest's body shrinks, stiffens and hardens. This is in accordance with research by Boucias and Pandland (1998)[8] stating that the black color change that occurs on the insect's body is caused by the melanization process which is a form of defense of the insect's body against pathogens. Mycelium initially appears between the segments of the pest's body on the abdomen.

that due to the enzymatic and chemical mechanism of insect infection, it will cause an increase in blood pH, blood clots and cessation of blood circulation in insects, which will cause death and dead pests will turn wrinkled and black[12]. .Each entomopathogenic fungus produces metabolite compounds that act as toxins or cuticle degrading compounds, this greatly influences the effectiveness of entomopathogenic fungi in infecting host insects [17].

Conclusion

The conclusion obtained from the results of research using the Beauveria bassiana fungus is that this fungus is effective for the peach aphid pest population. The body of dead aphids infected with the Beauveria bassiana fungus looks dry and hard, because all the fluid in the aphid's body is used for growth by the Beauveria bassiana fungus. As a result, the insect dies with its body hardened like a mummy. If conditions are favorable, the fungus will penetrate outside the insect's body, especially at the articulation of the body appendage and mouthparts. As well as the alternative media used for cilembu tubers which is suitable for the growth of the Beauveria bassiana fungus.Summarize the main findings and contributions of the research.

Acknowledgment

This research is part of an internship activity which is a program of the Ministry of Education, Culture, Research and Technology which is carried out at PT. Biotek Cipta Kreasi supported by the Biotechnology study program at Aisyiyah University, Yogyakarta in 2023.

References

[1] M. Syukur and R. Yunianti, "Pemanfaatan Sumber Daya Genetik Lokal dalam Perakitan Varietas Unggul Cabai (Capsicum annuum) Tahan Terhadap Penyakit Antraknosa yang Disebabkan oleh Colletotrichum sp (Local Genetic Resources Utilization in Assembling Superior Chili Varieties (Capsic," *J. Ilmu Pertan.*, vol. 18, no. 2, pp. 67–72, 2013.

[2] V. Soelaiman and A. Ernawati, "Pertumbuhan dan Perkembangan Cabai Keriting (Capsicum annuum L.) secara In Vitro pada beberapa Konsentrasi BAP dan IAA," *Bul. Agrohorti*, vol. 1, no. 1, p. 62, 2013, doi: 10.29244/agrob.1.1.62-66.

[3] R. Zuhra, "EFEKTIVITAS BAKTERI ENDOFIT SEBAGAI PUPUK HAYATI TERHADAP PERTUMBUHAN DAN PRODUKSI CABAI (Capsicum annuum, L.)," *J. Pertan. Trop.*, vol. 4, no. 1, pp. 65–74, 2017, doi: 10.32734/jpt.v4i1.3071.

[4] M. G. M. Polii, T. D. Sondakh, J. S. M. Raintung, B. Doodoh, and T. Titah, "Kajian Teknik Budidaya Tanaman Cabai (Capsicum annuum L .) Kabupaten Minahasa Tenggara," *Eugenia*, vol. 25, no. 3, pp. 73–77, 2019.

[5] Trizelia, E. Sulyanti, and R. Saputra, "Kemampuan Kolonisasi Cendawan Endofit Trichoderma sp dan Beauveria bassiana Pada Tanaman Cabai dan Pengaruhnya Terhadap Populasi Kutu Daun Myzus persicae," *Pros. Semin. Nas.*, pp. 188–198, 2020.

M. M. Nasution, M. Sayuthi, H. Hasnah, J. P. Tanaman, and F. Pertanian, "Patogenisitas Cendawan Entomopatogen Beauveria bassiana terhadap Serangga Nezara viridula (L.) pada Stadia yang Berbeda (Pathogenicity of Entomopathogenic Fungus Beauveria bassiana to Insect Nezara viridula (L.) at Different Stages)," *J. Ilm. Mhs. Pertan.*, vol. 8, no. 1, p. 2023, 2023, [Online]. Available: www.jim.usk.ac.id/JFP

[6] R. Rustam, J. H. Laoh, J. Agroteknologi, F. Pertanian, and U. Riau, "Riyanto Tamba 1".

[7] M. A. Permadi, R. A. Lubis, and I. Kinarang, "Studi Keragaman Cendawan Entomopatogen Dari Berbagai Rizosfer Tanaman Hortikultura Di Kota Padangsidimpuan," *EKSAKTA J. Penelit. dan Pembelajaran MIPA*, vol. 4, no. 1, p. 1, 2019, doi: 10.31604/eksakta.v4i1.1-9.

[8] A. Rosmiati, C. Hidayat, E. Firmansyah, and Y. Setiati, "Potensi Beauveria bassiana sebagai Agens Hayati Spodoptera litura Fabr. pada Tanaman Kedelai," *Agrikultura*, vol. 29, no. 1, p. 43, 2018, doi: 10.24198/agrikultura.v29i1.16925.

[9] T. Ramakuwela *et al.*, "Establishment of Beauveria bassiana as a fungal endophyte in pecan (Carya illinoinensis) seedlings and its virulence against pecan insect pests," *Biol. Control*, vol. 140, no. September 2019, 2020, doi: 10.1016/j.biocontrol.2019.104102.

[10] G. M. Mascarin and S. T. Jaronski, "The production and uses of Beauveria bassiana as a microbial insecticide," *World J. Microbiol. Biotechnol.*, vol. 32, no. 11, 2016, doi: 10.1007/s11274-016-2131-3.

[11] M. S. Y. I. Bayu, Y. Prayogo, and S. W. Indiati, "Beauveria bassiana: Biopestisida Ramah Lingkungan dan Efektif untuk Mengendalikan Hama dan Penyakit Tanaman," *Bul. Palawija*, vol. 19, no. 1, p. 41, 2021, doi: 10.21082/bulpa.v19n1.2021.p41-63.

[12] Y. Harun, A. K. Parawansa, and A. Haris, "KAJIAN PATOGENISITAS Beauveria bassiana dan Metarhizium sp TERHADAP LARVA ULAT GRAYAK (Spodoptera frugiperda) PADA TANAMAN JAGUNG," *AGROTEK J. Ilm. Ilmu*

Pertan., vol. 6, no. 2, pp. 81–93, 2022, doi: 10.33096/agrotek.v6i2.238.

[13] I. F. N. Kholifah and L. Y. Romli, "Ubi Jalar (Ipomoea batatas L.) Cilembu Sebagai Media Alternatif Untuk Diagnostik Pertumbuhan Candida Albicans," *STIKes ICME JOMBANG*, 2021, [Online]. Available: https://scholar.google.com/citations?view_op=view_citation&hl=en&user=lNOniTUAAAAJ&pagesize=100&citation_for_view=lNOniTUAAAAJ:ldfaerwXgEUC"Muhammad Ihsan_2.pdf."

[14] R. Hasibuan, D. Retnosari, N. Yasin, P. Purnomo, and L. Wibowo, "Pengaruh Beberapa Teknik Pengendalian Terhadap Populasi Wereng Jagung Di Kecamatan Natar Kabupaten Lampung Selatan," *J. Agrotek Trop.*, vol. 9, no. 1, p. 61, 2021, doi: 10.23960/jat.v9i1.4799.

[15] H. N. Wildan, E. Firmansyah, and S. Nurhidayah, "Keefektifan Lecanicillium lecanii Mengendalikan Crocidolomia pavonana Pada Skala Laboratorium," *Agro Wiralodra*, vol. 5, no. 1, pp. 15–19, 2022, doi: 10.31943/agrowiralodra.v5i1.63.