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Profile and Bioactivity of Bioactive Compounds of *Beauveria bassiana* Fungi Entomopathogens of Endophytes as Plant Growth Boosters

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Abstract

The purpose of this study was to identify bioactive compounds of Beauveria bassiana entomopathogenic fungi derived from insect isolates of Leptocorisa oratorius and endophytic fungi B. bassiana from cocoa plants that have bioactivity as growth boosters. The research was conducted in the laboratory of Biological Control, Faculty of Agriculture, Andalas University. The study was conducted for 4 months, using the GCMS method. From the results of the study, bioactive compounds identified as growth boosters of B. bassiana endofyt fungi from cocoa plants are compounds Acetic acid, Ethanoic acid, Ethylic acid, Glacial acetic acid, CH_3COOH; n-Hexadecanoic acid Hexadecanoic acid, Palmitic acid; 9-Octadecenoic acid (2)-(CAS), Oleic acid, Red oil, Oelsaure; octadecanoic acid (CAS) Stearic acid, n-Octadecanoicacid, vanilla; Ergosta-5,7,22-trien-3-ol, (3.,beta.,22 E) – (CAS), Ergosterol (CAS); while from the entomopathogenic B. bassiana fungus from L. oratorius insects are Acetic acid (CAS), Ethylic acid, vinegar acid, Ethanoic acid, CH3COOH, Dianhydromannitol; Isosorbide, D-Glucitol,1,4-3,6-dianhydro-(+)-D-Isosorbide Devicoran; Hexadecanoic acid, methyl ester (CAS), Methyl palmitate, Uniphat A60; n-Hexadecanoic acid Hexadecanoic acid, n- Hexadecanoic acid, Palmitic acid; 9-octadecenoic acid, methyl ester, (E)-elaidic acid; 9-octadecanoic acid (Z)-(CAS) , oleic acid, Red oil, Oelsauere; octadecanoic acid, stearic acid, -n-octadecanoic acid, Humko Industrene R; Ergosta-5,7,22-trien-3-ol, (3.beta.,22E)-CAS, Ergosterol (CAS)

Keywords: *Bioactivity, B.bassiana fungi, endophyte, entomopathogen.*

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INTRODUCTION

One of the microbes producing bioactive compounds is endophytic fungi which are fungi that grow and colonize in host plant tissues, especially in the roots, stems and leaves. Microbes (fungi and bacteria) that are present in healthy plant tissue without causing the plant to get sick are called endophytic microbes. Generally, endophytic microbes infect plants without causing symptoms, microbes colonize host tissues, and can be isolated by surface sterilization (Stone *et al*., 2000). Endophytic entomopathogenic fungi can actively or passively spur plant growth through the role of secondary metabolite compounds produced by fungi or host plants. Endophytic fungi isolated from a plant can produce the same metabolites as their host plant even in greater quantities (Radji, 2005). Utilization of endophytic fungal metabolites for seed treatment can improve seed quality and reduce the incidence of disease in the field (Suryanarayanan *et al*., 2009). *Beauveria bassiana* fungus is one of the entomopathogenic fungi that can be used to control plant disturbing organisms both in the rhizosphere and in plant tissues. As an endophyte, the entomopathogenic fungus *B.bassiana* is able to spur the growth of *Phaseolus vulgaris* plants (Affandi *et al.,* 2019). *B. bassiana. B.brongniartii* and *M.brunneanum can improve the plant height,* root wet weight and shoots of *Vicia faba* by foliar spraying

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(Jaber dan Enkerli, 2017). Research Qayyum *et al*. (2015), *B. bassiana* effectively controls *H. armigera* pests and is able to settle as endophytes and spur the growth of tomato plants through foliar spraying inoculation. Inoculation of *B. bassiana* against corn plants can increase height, number of leaves, seed weight, germination percentage compared to control (Russo *et al*., 2019). B. bassiana and *M. brunneum* fungi have the ability of 74.19 percent as seed growth boosters and are able to increase the growth of red chili using fungal conidia suspension (Jaber and Araj, 2018). Endophytic fungi can produce phytochemical compounds that have important biological functions including defending attacks from predators such as insects, fungi and the interference of competitors, protection from pollution, stress and drought**.** One source of bioactive compounds that are becoming popular today is those derived from microbes. This is thought to be because endophytic fungi undergo coevolution of genetic transfer from their hosts. The ability of endophytic microbes to produce bioactive compounds is very potential to be developed into growth hormones, insecticides, herbicides and herbal medicines, this is because endophytic microbes are microorganisms that are easy to grow, have a short life cycle and can produce large amounts of bioactive compounds by fermentation methods. *B. bassiana is a* very widespread entomopathogenic fungus that lives or resides in healthy plant tissues without causing disease or damage to its host (Van Bael *et al*., 2005). This fungus can be isolated from several types of plants and can be introduced in several other plants such as banana *(Musa paradisiaca* L.) (Akello et al. 2007), coffee (*Coffea arabica* L.) (Posada *et al* .2007). Research on the ability of the entomopathogenic fungus *B. bassiana* obtained from the insect walang sangit (*L. oratorius*) which is an endophytic fungus in chili plants that can spur germination and plant growth through seed soaking techniques and the composition of chemical compounds found in the entomopathogenic fungus *B. bassiana* Walang sangit isolate and compounds found in chili root roots after application with endophytic entomopathogenic *B. bassiana* have never been carried out in West Sumatra, so the author intends to conduct research on the profile of chemical compounds found in the entomopathogenic fungus *B. basiana* and chemical compounds found in chili roots after application of *B. bassiana* fungus which is able to spur the germination and growth of chili plants.

MATERIAL AND METHODS

Material and Equipment

The ingredients used in this study include chili plant roots, PDA medium, GDP medium, chloramphenicol, methanol, aquades, 70% ethanol, NaOCl, filter paper, chloroform, DPPH spray reagent, H_2O_2 3%, Lieberman, dragendrof.

Tools used in this study include: oven, autoclave, laminar air flow, rotary shaker, refrigerator, incubator, microscope, centrifuse, centrifuge tube, digital scale, electric stove, funnel, erlenmeyer flask, beaker, petri dish, bunsen burner, measuring cup, aluminum foil, plastic wrapper, volumetric pipette, swab, micropipette, scissors, spatula, vortex mixer, newspaper, sterile knife, tweezers, plastic, chamber, cotton, ose, ruler, label paper, ruler and tissue.

Research Procedure

Samples of chili plant roots came from the results of research on the effect of the application method of soaking chili seeds with endophytic entomopathogenic fungus *B. bassiana* on the germination and growth of chili seedlings. Chili roots are taken in the phase of entering the generative phase, which is when chili plants begin to flower, namely at the age of 8-10 MST Isolate of *B. bassiana* fungi obtained from the collection of the biological control laboratory of the Faculty of Agriculture, Andalas University which has been purified.

Making Fermentation of *B.bassiana* Fungi

Pure isolate of B. bassiana fungus is an endofyt fungus from cocoa fruit skin, entomopathogenic fungus *B. bassiana* from Walang Sangit insect (*Leptocorisa oratorius*). Fermentation of *B. bassiana* fungi is carried out by liquid fermentation using PDB media (Potato Dextrosa Broth). Pure colonies of *B. bassiana* fungus on PDA petri dishes that have been incubated for 5-7 days, then using round ose 3 pieces of mushroom culture measuring 1x1 cm were taken. The fungus pieces were then inoculated into 50 ml PDB liquid media in a 100 ml erlenmeyer flask and incubated for 3-5 days. Then 20 ml was taken and put into the erlenmeyer containing 250 ml PDB medium, then rocking fermentation was carried out using a 150 rpm rotary shaker at room temperature for 14 days. From the results of the fermented

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culture is put into a 15 ml centrifuge tube that has previously been sterilized first, then centrifuged at a speed of 3000 rpm for 20 minutes. The supernatant is taken and then filtered using filter paper. The settled mycelia is then taken and dried in the oven at 40^0 C for 3 hours and then soaked with 10 % methanol for 2 days. Next it is filtered, then the mycelial extract of endophytic fungi is dried in a waterbath and the extract obtained is then ready to be tested.

Analysis of Bioactive Compounds with GC-MS

The bioactive compound content of endophytic *B. bassiana* fungi from cocoa plants and entomopathogenic fungi from *L. oratorius* insects were analyzed with GC-MS at the Indonesian National Police Forensic laboratory center (PUSLABFOR POLRI) in Jakarta. Chromatograms and mass spectra were evaluated using the MASSLAB program (Roessner *et al*, 2000). The obtained data is then entered into Microsoft Excel. Analysis of compounds identified using software pubchem (https://pubchem.ncbi.nlm.nih.gov).

RESULTS AND DISCUSSION

Gas Chromatography Mass Spectrophotometry (GCMS) is one of the best techniques for identifying constituents of volatile substances, long chains, branched chain hydrocarbons, alcohols, acids, esters, etc. This method is simple, sensitive and effective in separating the components of a mixture (Marston 2007; Chaman dan Verma, 2006; De-Fatima *et al*, 2006; Kaushik *et al*, 2002). In addition, GCMS is a tool for reliable identification of bioactive compounds (Jhonson *et al*, 2011). GCMS analysis of cocoa fruit peels showed the presence of 13 (thirteen) compounds and from isolates of the entomopathogenic fungus *B.bassiana* 27 (twenty-seven) compounds. The compounds shown in Table-1 and Table-2 are those that come close to the same similarity index.

Table 1. Composition of Bioactive Compounds of *Methanol Extract* of *B.Bassiana* Mushroom Root From Cocoa Plants

Peak No.	Retention Time (minutes)	Compound name	Similarity Index	Percentage (%)
1	1.772	Acetic acid, Ethanoic acid, Ethylic acid, Glacial acetic acid, CH_3COOH	80	4,02
2	10.013	n- Hexadecanoic acid Hexadecanoic acid, Palmitic acid	99	13.30
3	11.116	9-Octadecenoic acid (2)-(CAS), Oleic acid, Red oil, Oelsaure	98	20.42
4	11.236	octadecanoic acid (CAS) Stearic acid, n-Octadecanoicacid, vanicol	91	9.55
5	17.194	Ergosta-5,7,22-trien-3-ol, (3.,beta.,22 E) – (CAS), Ergosterol (CAS)	95	26.03

Based on the results obtained through GC-MS, it is known that methanol extract of cocoa fruit peel has various compound content, shown through peaks in GC spectra with different retention times (Table-1). The identified chemical compounds are primary and secondary metabolites. The compounds obtained consist of fatty acid groups, namely Acetic acid, Ethanoic acid, Ethylic acid, Glacial acetic acid, CH3COOH; n- Hexadecanoic acid Hexadecanoic acid, Palmitic acid; 9-Octadecenoic acid (2)-(CAS), Oleic acid, Red oil, Oelsaure; octadecanoic acid (CAS) Stearic acid, n-Octadecanoicacid, vanicol; Ergosta-5,7,22-trien-3-ol, (3.,beta.,22 E) – (CAS), Ergosterol (CAS). Fatty acids are primary metabolite compounds that are used as energy for plant growth. Ringbom *et al.* (2001) states that compounds are included in the fatty acid group such as Hexadecanoic acid, methyl ester (CAS) Methyl palmitate, Uniphat A60, n-Hexadecanoic acid, Hexadecanoic acid, n-Hexadecoic acid, Palmitic acid It is widely found in plants as an energy substrate for cells. Acetic acid compounds, Ethanoic acid, Ethylic acid, Glacial acetic acid, CH_3COOH compounds from the carboxylic acid group that act as regulators of cell enlargement and trigger cell elongation in the back area of the meristem end. This compound acts as an important hormone in plant growth so that it can be used to spur the speed of plant growth in intensive plant cultivation fungi.

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Table 2. Composition of Bioactive Compounds of *B.bassiana* Mushroom Methanol Extract

No.	Retention Time (minutes)	Compound name	Similarity Index	Percentage (%)
1	1.875	Acetic acid (CAS) $$ Ethylic acid, Vinegar acid, Ethanoic acid, CH_3COOH	90	7,76
2	4.551	Dianhydromannitol	90	35,22
3	5.277	Isosorbide, D-Glucitol, 1,4:3,6-dianhydro-, (+)-D-Isosorbide, Devicoran	97	2,25
4	9.706	Hexadecanoic acid, methyl ester (CAS), Methyl palmitate, Uniphat A60	96	0,96
5	10.030	n-Hexadecanoic acid, Hexadecanoic Acid, n-Hexadecanoc acid, Palmitic acid	99	2,28
6	10.817	9-Octadecenoic acid, methyl ester, (E)-Elaidic acid, methyl ester	99	1,90
7	11.125	9-Octadecenoic acid (Z)- (CAS), Oleic acid, Red oil, Oelsauere	98	3,03
8	11.236	Octadecanoic acid, Stearic acid, n-Octadecanoic acid, Humko Industrene R	95	0,62
9	17.203	Ergosta-5,7,22-trien-3-ol, (3.beta .,22E)-(CAS) , Ergosterol (CAS)	97	2,48

Based on the results obtained through GC-MS, it is known that methanol extracts of the entomopathogenic fungus *B. bassiana* contain various compounds, shown through peaks in GC spectra with different retention times (Table-2). The chemical compounds found in *B. bassiana* fungus isolate walang sangit consist of fatty acid groups, namely Hexadecanoic acid, methyl ester (CAS) Methyl palmitate Uniphat A60, n-Hexadecanoic acid Hexadecanoic n-Hexadecanoic acid Palmitic acid, 9-Octadecenoic acid, methyl ester, (E)- Elaidic acid, methyl ester, 9-Octadecenoic acid (Z)- (CAS) Oleic acid. Red oil, Oelsauere, Octadecanoic acid, Stearic acid, n-Octadecanoic acid, Humko Industrene R**.** Acetic acid (CAS), Ethylic acid, Vinegar acid, Ethanoic acid CH_3COOH is a compound from the carboxylic acid group (carboxylic acid*)* which acts as a regulator of cell enlargement and spurs cell elongation in the back area of the meristem end. This compound acts as a hormone that is important in plant growth so that it can be used to spur the speed of plant growth in intensive plant cultivation. Ergosta-5,7,22-trien-3-ol, (3.beta., 22E)-(CAS), Ergosterol (CAS) compounds are a group of sterol compounds that act as plant growth boosters (Widyati, 2017).

Fatty acids are primary metabolite compounds that are used as energy for growth, so they are not inhibiting. Ringbom *et al.* (2001) states that compounds belonging to the fatty acid group are found in plants as energy substrates for cells. This fatty acid compound is also present in chili roots treated with soaking application of seeds of B. *bassiana* fungus isolate from *L. oratorius* insects. The next compound such as dianhydromannitol is a group of sugar compounds, which act as root lubricants to penetrate the soil, protect from pathogen attacks, chemical traps (*chemoattractants);* microbial growth boosters **(**Widiaty**,** 2017).

Associated with the results of observations on the ability of *B. bassiana* fungi from *L. oratorius* through seed soaking methods, it can be seen that *B. bassiana* fungi are able to settle as endophytic fungi and colonize roots, stems and chili leaves and spur germination and growth of chili plants, visible from plant height and number of leaves that differ markedly with controls, Liang – Dong *et al*. (2008) reports that endophytic fungi are able to settle in plant tissues adapted to suitable conditions within parts of plant tissues. The presence of metabolite compounds produced by endophytic fungi in plants can increase plant growth. Indole acetic acid compounds from *Fusarium tricinctum* and *A. alternata* enhance plant growth (Khan *et al.* 2015).

The success of this fungus as an endophyte through artificial inoculation of chili plants gives hope for more extensive research on chili plants. The results of Saragih *et al*., (2018), the effect of the application of entomopathogenic *B. bassiana* fungi and endophytic fungi from cocoa plants can increase plant height compared to controls, while the height of chili plants between *B. bassiana* fungi from cocoa is not real from *B*. *bassiana* fungi from *L. insects,* oratory. Similarly, the average leaf count data from the treatment of isolates of entomopathogenic *B. bassiana* fungi and endophytic fungi from cocoa were significantly different from controls. The application of *B. bassiana* fungus is given through

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seed soaking inoculation when observed in inoculation perkuan through seed soaking compared to soil drenching or foliar spraying.

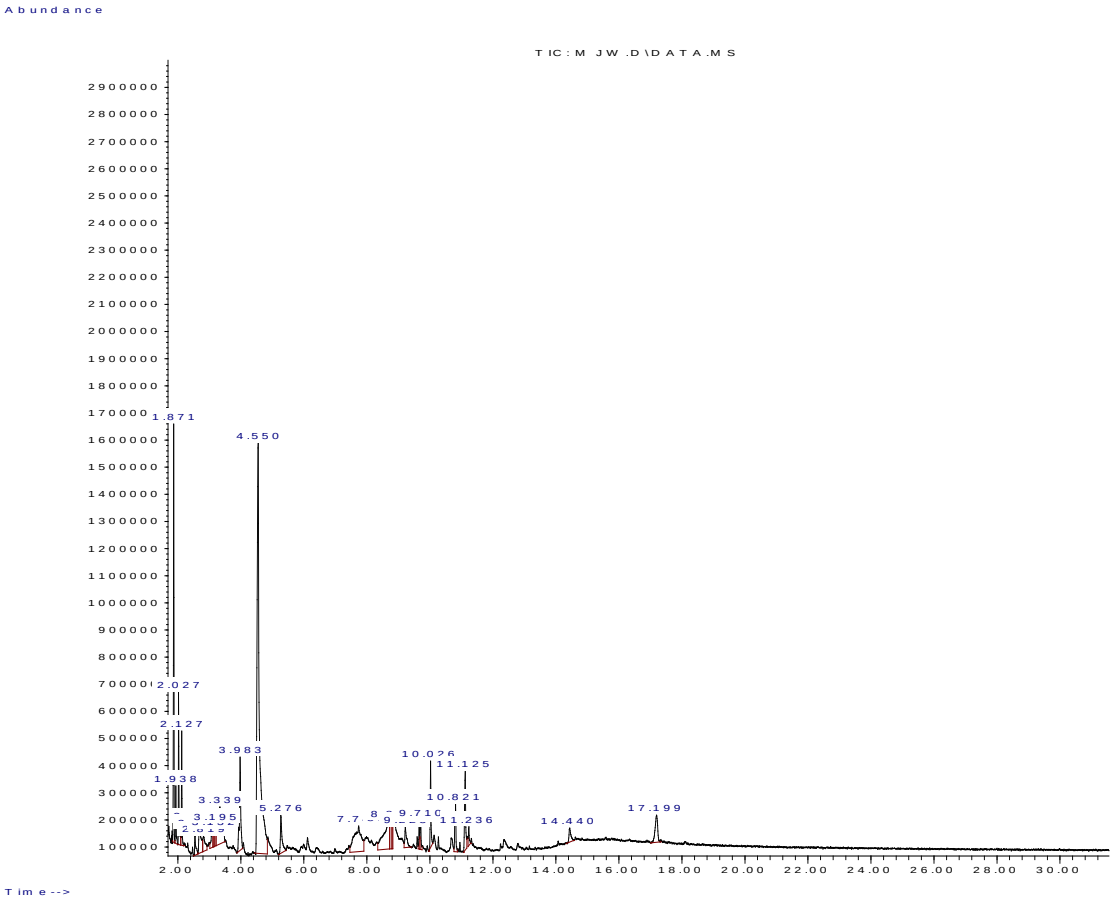

Figure-1. Chromatogram of *B.bassiana* Fungus Chemical Compound

The results of observations of *B. bassiana* inoculation treatment through soaking seeds on the number of chili leaves showed a real difference between walang sangit isolate and cocoa isolate against the control. Here it can be seen that the ability of entomopathogenic fungi is able to colonize chili plants and settle as endofyt fungi. The influence of chemical compounds found in *B. bassiana* isolates on *L. oratorius* can spur the formation of chili plants. The results of GC-MS analysis of clam compounds found in *B. bassiana* fungi from walang sangit and chili plant roots contain fatty acid derivative compounds that act as plant growth boosters by carrying out cell enlargement activities at the ends of cell meristems, while dianhydromannitol compounds are a group of sugar compounds that are able to act as plant growth boosters.

According to Rodrigues (2009), endofyt fungi are able to transmit horizontally and colonize roots, stems and leaves, increasing root biomass and root shoots. Jaber and Vidal (2010), explain that inoculation with some endophytic fungi can increase plant growth, tolerance to stress factors and induce plant resistance to herbivorous insects and to pathogens that cause plant diseases.

CONCLUSION

Based on the analysis of methanol extract compounds contained in the entomopathogenic fungus *B.bassiana* there are 27 (twenty-seven) active compounds, and compounds that play a role in spurring plant cell growth there are 6 compounds, in cocoa fruit as many as 13 (thirteen) compounds, there are 5 (five) compounds that act as plant growth boosters consisting of fatty acid compound groups, carboxylic acid compound groups and sugar compound groups Compounds produced by Entomopathogenic fungi and endophytic fungi *B. bassiana,* have bioactivity as growth promoters tested on chili plants.

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