EXPLORATION, IDENTIFICATION AND MULTIPLICATION OF ANTAGONISTIC BACTERIA *Bacillus* sp. AS BIOLOGICAL CONTROL AGENT IN UPT PTPH JAWA TIMUR

KULIAH KERJA PROFESI REPORT

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FOREWORD

Alhamdulillah, all praise and gratitude to Allah SWT, because thanks to His grace, the author can complete the Kuliah Kerja Profesi (KKP) report entitled "Exploration, Identification and Propagation of Antagonistic Bacteria *Bacillus* sp. as Biological Control Agents at UPT PTPH Jawa Timur". This report was prepared after completing a series of Kuliah Kerja Profesi activities at the East Java Food Crops and Horticulture Protection Unit on January 03 to February 03, 2022.

In writing this report, the author has received a lot of help, both morally and materially. For this reason, on this occasion the author expresses his gratitude to::

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This report is written based on practice and observation in the field and supported by the results of literature studies on *Bacillus* sp. The author is looking forward to constructive criticism and suggestions. As an evaluation material for this report. Hopefully this report can provide information related to science and technology.

Surabaya, 22 Februari 2022

Author

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I. INTRODUCTION

1.1 Background

Crops as foodstuffs are basic human needs for daily survival. Farmers are constantly improving good and correct cultivation techniques to increase crop yields in accordance with the needs of the community. Agricultural techniques are being improved for all types of crops, including food crops and horticulture. Plant cultivation is susceptible to attack by diseases and pests so that it requires plant protection efforts. Plant protection will minimize disease and pest attacks so as not to cause losses in quality and quantity.

Unit Pelaksana Teknis Proteksi Tanaman Pangan dan Hortikultura (UPT PTPH) Jawa Timur is a Unit Pelaksana Teknis under the auspice Dinas Pertanian dan Ketahanan Pangan Provinsi Jawa Timur which handles food security control projects. The main task of UPT PTPH Jawa Timur is to carry out some of the duties of the Office in the field of observation, forecasting, and application of Plant Disturbing Organism control techniques, administration and community services. One of the activities carried out by UPT PTPH East Java is biological control using Biological Control Agents (BCA). *Bacillus* sp. is one of the BCA that is usually used for biological control.

Kuliah Kerja Profesi (KKP) activities aim to get to know the world of work in accordance with the majors taken during college. The main task of UPT PTPH Jawa Timur, which is engaged in plant protection, is in line with the author's specialization, namely pests and plant diseases. This is also the reason why the author chose UPT PTPH Jawa Timur as a place to carry out KKP because of the facilities and work programs that support learning in terms of plant protection. In addition, this KKP activity can be a provision for the author in carrying out research for the final project.

Biological control as a key component of integrated pest control basically consists of controlling pest populations by using and utilizing natural enemies (parasitoids, predators, and antagonistic pathogens). Biological Control Agent (BCA) is any organism in the form of fungi, bacteria, viruses, nematodes, insects, and other animals that can be used to control pests and plant diseases. Although

these BCA are readily available in nature, their presence is not balanced and their population needs to be increased in the field.

There are many advantages of using BCA in its utilization to overcome plant diseases. BCA serves to suppress pathogen populations resulting in improved plant growth. BCA in plant roots is unique because of its association with root exudates.

In the soil environment, the position of BCA is a balance between plants and pathogens. BCA affects plants, pathogens and the environment. The effect of BCA on plants is the ability to protect plants or support plant growth through one of its mechanisms, namely supporting plant growth. Meanwhile, plants provide nutrients for biological control agents in the form of root exudates, which are indispensable for their growth. Meanwhile, the effect of BCA on pathogens is very clear, namely suppressing the resistance and growth of pathogens. This suppression will cause a decrease.

Biological control agents (BCA) play an important role in the process towards stable agro-ecosystem conditions. This role is indicated by the ability of biological control agents to reduce the population density of target pests above the economic threshold to below the economic threshold, and regulate the pest population to remain below the economic threshold (Sopialena, 2018). Thus, biological control is included in the main component of Integrated Pest and Disease Management (IPM).

Biological Control Agents can be parasitoids, predators, or antagonistic pathogens. BCA that are often used to control plant diseases are antagonistic pathogens. Biocontrol agents from the genus *Bacillus* have been widely used to control pests and diseases in plants (Jacobsen et al., 2004).

Bacteria of the *Bacillus* genus live in the soil of plant roots or rhizosphere. *Bacillus* can secrete antimicrobial compounds consisting of bacitracin, bacillin, bacillomycin, diphidine, oxidificidin, lecithinase, subtilisin. In addition, it also produces the compound fengymycin which is known as an antifungal, and many other antibiotic peptide compounds produced by *Bacillus* sp. (Abidin, et al., 2015). Bacteria of this genus are known to be able to secrete protease, chitinase and lipopeptide enzymes that have the potential to lyse bacterial cell walls and inhibit their growth (Rodas-Junco et al., 2009).

The effectiveness of *Bacillus* sp. in inhibiting pathogenic bacteria and fungi has been proven by several researchers. In addition, the use of *Bacillus* sp. is also environmentally friendly because it leaves no residue and does not cause negative effects on the environment. With the Integrated Pest Management (IPM) program, the use of *Bacillus* sp. is required in large quantities.

1.2 General Purpose

The general purpose of implementing the Kuliah Kerja Profesi (KKP) is to fulfill the compulsory curriculum set by the Faculty of Agriculture Agrotechnology Study Program Universitas Pembangunan Nasional “Veteran” Jawa Timur.

1.3 Special Purpose

The special purpose of the Kuliah Kerja Profesi (KKP) is to learn and practice directly about the exploration, identification and propagation of *Bacillus* sp. as an environmentally friendly biological control agent at UPT PTPH Jawa Timur.

II. LITERATURE REVIEW

2.1 Biological Control

Control using natural or biological enemies has been known since before 1945 using the larval parasitoid *Microbracon* to control stem borers. Starting in 2000, the toxin *Bacillus thuringiensis* protein was transformed into rice plants to obtain varieties resistant to stem borers. The basis of ecological biological control is to keep pest populations as low as possible and optimize the role of natural enemies. Biological control needs to be carried out in a sustainable manner and supported by the provision of Biological Control Agents (BCA) that are ready for use in the field (Kartohardjono, 2011).

Biological control is a major component of Integrated Pest Management (IPM). Biological control optimizes the role of natural enemies in pest population management efforts, where natural enemies are part of the chain in the agroecosystem. The goal of biological control is to suppress pathogens by reducing the population of pathogen inoculum, reducing infection of host plants by pathogens. To minimize the occurrence of infection can be done by suppressing as low as possible the population or quantity and quality of the source of infection (Sopialena, 2018).

Biological control can occur not only through the introduction of APH, but can also occur through other activities that can indirectly increase the number of antagonists or antagonist activity so as to suppress pathogen attacks, so that certain technical culture activities, plant breeding, certain chemicals can be said and included as part of biological control, such as technical culture control and the use of certain chemicals (Muslim, 2019).

The advantages of biological control include being safe for the environment, humans, and useful animals; effective against target pests; long-term efficiency; relatively cheap and very profitable; and compatible or can be combined with other control methods. Due to a number of advantages of biological control, it implies that farmers should try to transform gradually to chemical control (Sopialena, 2018).

2.2 Antagonistic Bacteria

Antagonists are biological agents that have the potential to disrupt the growth and development process of pathogens. Antagonists include all classes of organisms: fungi, bacteria, nematodes, protozoa, viruses, viroids, and plant seeds as trap plants. These antagonists can be said to be natural enemies such as in entomology (parasites, predators, and pathogens). Usually antagonists will be found in an area inoculated with pathogens, but the disease does not appear, or the incidence of plant disease decreases, or the disease cannot develop in susceptible plants, rather than areas where the disease appears (Muslim, 2019).

Antagonistic microbes can function as pathogen control agents through competition, antibiosis, parasitism or induced resistance mechanisms. The use of antagonistic bacteria to increase crop yields and protect plants from plant pest organisms is a promising approach in modern agricultural systems (Kuswinanti 2014). Antagonistic bacteria can induce plant resistance to pathogens by activating signaling pathways and involving plant hormones jasmoic acid and ethylene. In addition, antagonistic bacteria, especially rhizobacteria, can increase plant growth (Nurhayati 2011).

Biological control mechanisms can occur through competition for space and food, antibiosis, hyperparasites, and induction of resistance and cross-protection.

a) Competition is a mechanism for suppressing pathogen activity by BCA against limited resources such as organic substances, inorganic substances, space and other growth factors (Nurhayati, 2011).
b) Antibiosis is an antagonistic mechanism by producing secondary metabolites in the form of antibiotics or antibiotic-like compounds such as lyase enzymes, volatile compounds, siderophores, and other toxic substances (Haggag & Mohamed, 2007).
c) Hyperparasitism occurs when antagonistic microbes parasitize pathogenic microbes and take nutrients from them. Hyperparasitism is also the destruction of pathogenic microbes by compounds or substances produced by BCA such as chitinase, cellulase, glucanase, lyase enzymes and others. (Nurhayati, 2011).

d) Induction of resistance is a process to activate the natural resistance of the host plant through the provision of certain stimulants without the introduction of new genes (Walters *et al*., 2007).

e) Cross-protection is plant disease inhibition resulting from pre-inoculation with the same or closely related pathogen that is inoculated simultaneously after the first inoculation. Protection may occur through competition and induction of resistance (Muslim, 2019).

2.3 *Bacillus* sp.

Bacillus sp. is an antagonistic bacterium that can suppress several diseases in plants. *Bacillus* bacteria are characterized by rod-shaped, gram-positive, can be obtained from soil, water, air, and decomposed plant material. *Bacillus* is 0.3-22 x 1.27-7 πm in size, some are motile (able to move), their mobility is caused by flagellum, if heated it will form endospores, which is a dormant form of vegetative cells as a form of self-defense that appears during extreme conditions (Graumann, 2007).

The *Bacillus* genus is one of the antagonistic bacteria that can control several types of plant pathogens. *Bacillus* sp. is able to compete with pathogens, able to produce several secondary metabolites, such as antibiotics, siderophores, bacteriocins, and extracellular enzymes. These bacteria are also able to induce plant resistance compounds and can act as Plant Growth Promoting Rhizobacteria (PGPR) (Zalila- Kolsi et al., 2016).

Bacillus sp. is a Gram-positive bacterium with a short rod to a single rod with a single arrangement (Sofyan et al., 2009). *Bacillus* sp. colonies have general characteristics that have a whitish beige color and a round and irregular colony shape (Corbin, 2004). *Bacillus* sp. bacteria have various kinds of flat and uneven colony edges, the surface is rough and not slimy, some even tend to be dry and powdery, large colonies and not shiny (Hatmanti, 2000).

Bacillus sp. is widely distributed and well used as Plant Growth Promoting Rhizobacteria (PGPR), and are commonly present in the rhizosphere. *Bacillus* sp. promote plant growth by eliminating or controlling pathogens with unique antimicrobial activities including producing antibiotics (Awais et al., 2007) and toxins (Mukry et al., 2010), as well as hydrolysis enzymes such as α-amylase and

β-galactosidase that can be increased on substrates such as corn, wheat, and rice compared to commercial medium (Konsoula & Liakopoulou-Kyriakides, 2007).).

Amylase enzymes are enzymes that can be obtained from plants, animals and microbes that play a role in carbohydrate metabolism. Among microbes, *Bacillus* sp. is widely used because it produces α-amylase and is thermostable (Sankaran & Ravikumar, 2011). The function of amylase enzyme in plant disease control is as an inhibitor, which is abundant in vegetables, tubers and cereals and efficiently increases plant resistance to pathogens such as fungi (Rekha & Padmaja, 2002).

The genus *Bacillus* has the ability to synthesize several compounds that are useful in agriculture and industry. Several secondary metabolites are produced by some *Bacillus* species and strains that exhibit antibacterial and antifungal activities against plant pathogens (Ongena and Jacques, 2008). One of the suppression mechanisms by strains of *Bacillus* genus members is antibiosis, which is indicated by the formation of an inhibition zone in *Bacillus* sp. cultures grown on medium in layers with pathogenic bacteria (Prihatiningsih et al., 2013).

Bacillus sp. bacteria are classified as bacteria that have antagonistic mechanisms in the form of antibiosis (Abidin et al., 2015). *Bacillus* species can protect plants from pathogens through several mechanisms, including the induction of systemic resistance (Kloepper et al., 2004), competition for space and nutrients and by the secretion of antifungal compounds, such as lipopeptide antibiotics, surfactin, iturin and fengycin (Kumar et al., 2011).

The mechanism of systemic induction of resistance by *Bacillus* sp. is through ultrastructural changes during infection by pathogens and cytochemical changes. Observation of signal transduction pathways, similar to those caused by Pseudomonas spp. biological agents, is not dependent on salicylic acid but depends on jasmonic acid, ethylene, and the role of the NPR1 gene. Some *Bacillus* sp. is also dependent on salicylic acid and independent of jasmonic acid and NPR1, depending on the *Bacillus* sp. applied (Muslim, 2019).

2.4 Bacterial Exploration and Identification

Exploration is collecting potential BCA that can be taken from the rhizosphere and plant parts that do not show symptoms of disease (soil samples or

plant material) (Sopialena, 2018). The rhizosphere is the soil that covers the surface of plant roots that are still influenced by root activity. The rhizosphere is an excellent habitat for the growth of insect pathogens, because plant roots can provide a variety of organic materials that generally stimulate the growth of microorganisms (Prasetyawati, 2009).

The standard procedure for soil microbial sampling involves taking a soil sample, suspending the soil sample with water and placing it on agar media to detect microbial growth. Plant roots represent one of the main sources of energy for soil microbes and their influence on soil microflora (Fitter and Hay, 1998). The method used in sampling is the composite sampling method. Each location was taken 5 sampling points randomly. (Hyde, et al., 2009).

Samples that have been homogenized, taken and weighed as much as 1 gram and given 10 ml of sterile distilled water. After that, 1 ml was taken for dilution from 10^{-1} to 10^{-9}. Furthermore, the diluted suspension was taken with a micropipette of 0.1 ml each at dilutions of 10^{-7}, 10^{-8}, and 10^{-9}. The suspension that has been taken is dripped on solid NA media. The method used was the spread plate method (Luthfiyyah, 2016).

Spread plate is one of the bacterial breeding methods carried out by spreading bacterial suspensions on the surface of solid media. This method aims to obtain a single colony of bacteria contained in the sample. In this spread plate method, the volume of culture spread is less than 0.5 ml on the plate and leveled using a tool called a glass spreader. Then the plate is incubated for 24-48 hours at 37°C (Alhabsyi et al., 2016).

Several studies on the exploration of bacteria in soil obtained varying results of bacterial characterization. Research conducted by Khaeruni et al. (2010) on the rhizosphere in ultisol land obtained bacterial isolates that have the character can secrete extracellular enzymes chitinase, cellulase and protease, IAA producers, phosphate solvents and nitrogen fixation freely, and able to inhibit the growth of pathogenic fungi in vitro and spur plant growth.

The identification of bacteria found from the exploration was guided by Schaad et al. (2001). Here are some methods used for:

a. Hypersensitive Test

Hypersensitivity test is a test conducted to determine whether the tested bacterial isolate is a pathogenic bacterium or not. Testing is done by inoculating bacterial suspensions on tobacco plant tissue. A positive reaction will be indicated by wilting or even death of tobacco plant tissue on the inoculated part of the bacteria.

b. Bacterial Gram Test

- Gram staining

Testing is done by taking a bacterial isolate with an ose needle and placed on a sterilized object glass and dried over a bunsen. Isolates are dripped with 5% Crystal violet solution as much as 2-3 drops for 1 minute, then rinsed with running water and dried. Subsequently, it was dripped with iodine as much as 1-2 drops and allowed to stand for 1 minute. Then rinsed with 70% alcohol and allowed to stand for 20 seconds which was then rinsed with running water and dried. The last stage is that the isolate is dabbed with 0.15 safranin solution and allowed to stand for 20 seconds then rinsed with running water. Isolates are ready to be observed with a microscope. Gram-positive bacteria will show a purple color, while negative bacteria will be red in color.

- Bacterial Gram Test (KOH 3%)

The bacterial isolate to be tested is placed on a glass object that has been dripped with 3% KOH. Then the bacterial suspension is pulled using an ose needle quickly and repeatedly. Gram-negative bacteria will appear mucus when removed, while positive bacteria will remain dilute or show no reaction. 3% KOH treatment of Gram-negative bacterial mass will cause damage to the bacterial cell wall and release DNA which is a viscid or mucus-like component. (Lay, 1994).

c. Spore staining

This spore staining aims to determine whether the test bacteria have spores or not. Testing is done by placing the bacteria on a glass object that has been sterilized and dried on a Bunsen. Then it is dripped with malachite green while placed on Bunsen and allowed to stand for 2-3 minutes which is then rinsed with sterile distilled water and dried. Then the bacteria were stained with safranin and allowed to stand for 30 seconds which was then rinsed with sterile distilled water and dried. Bacteria were observed with a microscope at 1000x magnification with

the help of immersion oil. If the test bacteria are able to form spores, the spores will appear green and the vegetative cells will appear red.

d. Oxidative-Fermentative (OF) Test

The materials needed for 1 L of OF test media are: peptone 2g; NaCl 3g; K2HPO4 0.3g; agar 3 g and bromotymol blue 1% 3 ml. The ingredients were dissolved and set at pH 7.1. Then the media was poured into 13 mm diameter test tubes as much as 4.5 ml per tube. The media was sterilized at 121 OC for 20 minutes. When sterilized, each tube was added with 0.5 ml of 10% glucose solution.

The test was carried out by inserting bacteria by poking on 2 media, namely media without covered with liquid paraffin and not covered with liquid paraffin. Then incubated at room temperature. Observations were made on the color change that occurred from blue to yellow. If the media covered with paraffin changes color then the change indicates a fermentative reaction, if the change is found in the tube without paraffin then the change indicates an oxidative reaction.

e. Catalase Test

This test is done by placing the bacteria on a sterile glass object and then dripped with 3% H2O2. A positive reaction is characterized by the presence of air bubbles. The air bubbles are formed because the tested bacteria have a catalase enzyme that is able to convert H_2O_2 into water and oxygen.

f. *Fluorescens* pigment

This test is done by growing bacteria on King's B selective media and incubated for 24-48 hours. The composition of King's B media consists of 20 g peptone protease; 1.5 g K_2HPO_4; 1.5 g $MgSO_4.7H_2O$; 15 ml glycerol and 15 g agar. The grown bacteria were observed under UV light. If the bacteria appear fluorescent then the bacteria are able to produce fluorescent pigments and vice versa. In fluorescent bacterial colonies, the bacteria can be included in the genus *Pseudomonas*.

g. YDC selective media

This test aims to select whether the test bacteria enter the genus *Xylophilus* and *Xanthomonas* or not. Testing is done by growing bacteria on YDC selective media. The composition of YDC media is yeast 10 g; glucose 20 g; $CaCO_3$ 20 g and agar 15 g in 1 liter of distilled water. If the test bacteria grown are yellow after

incubation, the bacteria can be classified into the genus *Xylophilus* and *Xanthomonas*.

III. GENERAL DESCRIPTION OF UPT PTPH JAWA TIMUR

3.1 Brief History

At the beginning of its establishment, this institution had the name UPT of the Directorate General of Agriculture for Food Crops under the name Balai Proteksi Tanaman Pangan (BPTP) VI Surabaya. In accordance with the Decree of the Minister of Agriculture Number 530/KPTS/UM/1978 dated August 24, 1978, BPTP VI Surabaya was officially established on March 8, 1982. Then on June 9, 1994, in accordance with the Decree of the Minister of Agriculture Number 5469/KPTS/OT.201/6/94, BPTP VI Surabaya changed its name to Balai Proteksi Tanaman Pangan dan Hortikultura (BPTP) VI Surabaya. Through East Java Provincial Regulation No. 9/2008 and East Java Governor Regulation No. 128/2008, the name of BPTPH VI Surabaya was changed to the Unit Pelaksana Teknis (UPT) Proteksi Tanaman Pangan dan Hortikultura Jawa Timur. UPT Proteksi Tanaman Pangan dan Hortikultura Jawa Timur is equipped with available facilities including Pesticide and Fertilizer Testing Laboratory, Biological Agents Laboratory, Musholla, and many more.

Figure 3. 1 UPT PTPH Jawa Timur Office

3.2 Geographical Location

UPT Proteksi Tanaman Pangan dan Hortikultura is located at Jl. Pagesangan II No. 58, Pagesangan Village, Jambangan District, Surabaya City, East Java Province, Indonesia. Geographically Pagesangan Village is located at 7º - 20º N and 112º - 42º E and has an area of 98,081 m2. The administrative boundaries of Pagesangan Village include:

Northern Boundary: Wonokromo Sub-district

Eastern boundary: Kebonsari sub-district

Southern boundary: Sidoarjo City

Western boundary: Karangpilang sub-district

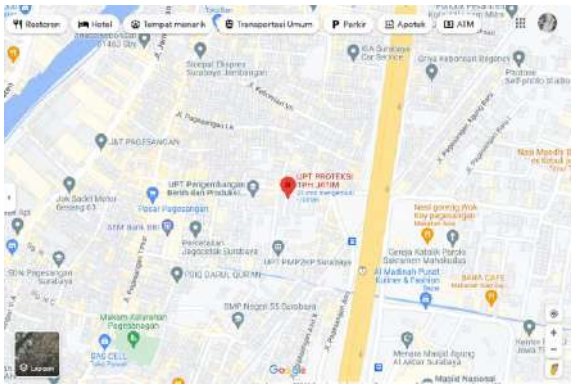


Figure 3. 2 Location of UPT PTPH Jawa Timur

3.3 Vision and Mission

3.3.1 Vision

The realization of steady, safe and sustainable food crop and horticulture production.

3.3.2 Mission

a. Improve farmers' knowledge, skills and abilities on integrated pest and plant disease management;
b. Reducing the risk of yield loss due to pest and DPI attacks;
c. Minimizing the risk of the impact of the use of protection facilities;
d. Implementing environmentally sound agroecosystem management with the MTS strategy.

3.4 Main Tasks and Functions of UPT PTPH Jawa Timur

3.4.1 Main Task

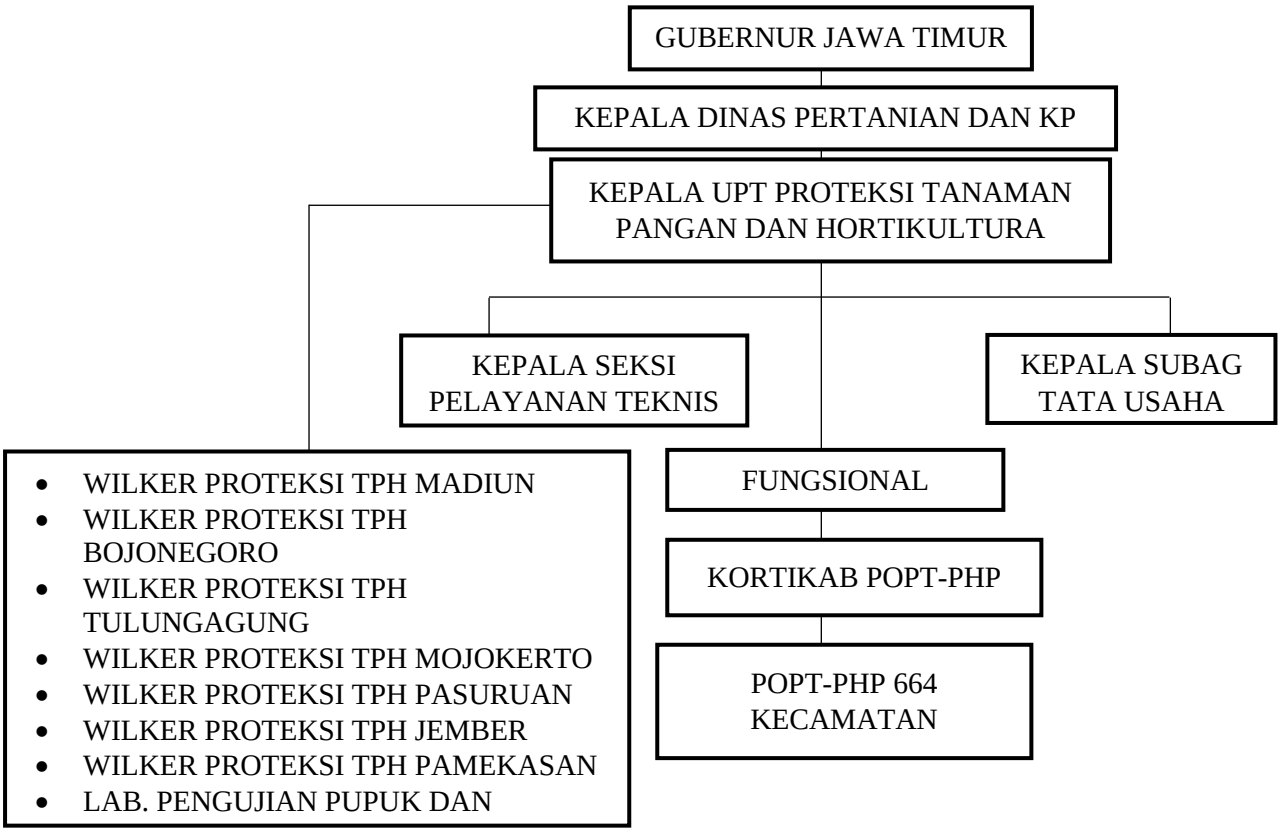
The main task carried out by UPT Proteksi Tanaman Pangan dan Hortikultura is to carry out some of the duties of the Service in the fields of observation, forecasting, and application of plant pest organism control techniques, administration and community services.

3.4.2 Function

The functions of the UPT Proteksi Tanaman Pangan dan Hortikultura Jawa Timur are as follows:

a. Preparation of program planning and UPT activities;
b. Implementation of observation, diagnosis and dissemination of information on Plant Disturbing Organisms (OPT), and climate factors;
c. Implementation of location-specific OPT forecasting in order to provide recommendations for OPT control;
d. Implementation of location-specific pest control technology assessment;
e. Implementation of providing technical considerations/early warnings of incidental pest control;
f. Implementation of technical assistance and pest control movements;
g. Implementation of fertilizer and pesticide testing;
h. Implementation of administration and community services;
i. Implementation of monitoring, evaluation and reporting; and
j. Implementation of other tasks assigned by the Head of Service.

3.5 Organization Structure



IV. IMPLEMENTATION METHOD KULIAH KERJA PROFESI

4.1 Time and Place

The implementation of Kuliah Kerja Profesi (KKP) starts on January 03, 2022 - February 03, 2022. KKP activity time every Monday to Saturday at 08.00-16.00. However, if there is still work that has not been completed, such as preparing media for testing, then the activity time can be carried out until 19.00. KKP activities are located at the Biological Agents Laboratory of the Food Plant and Horticulture Protection UPT, East Java Provincial Agriculture and Food Security Office, Jl. Pagesangan II No. 58, Jambangan District, Surabaya City.

4.2 Data Collection Methods

Data collection is done through primary data collection, secondary data collection and documentation.

4.2.1 Primary Data Collection

Primary data collection is carried out by various methods including::

a. Observation

The observation method is carried out by direct observation and documentation of the object to be studied. The observations carried out consist of a series of exploration activities as follows:

1. Soil sampling of the rhizosphere of bamboo and tomato plants
2. Isolation of rhizosphere bacteria from soil samples
3. Identification of bacteria found through various tests

b. Discussions and Interviews

Discussions and interviews were conducted with field supervisors and local farmers. These discussions and interviews aim to obtain complete information about the samples taken and the bacteria that have been obtained.

c. Direct practice in the laboratory

Direct practice is carried out in the biological agent laboratory in accordance with the object to be studied. Practical activities in the laboratory include bacterial isolation and bacterial identification. In

carrying out practical activities according to the direction of the field supervisor.

4.2.2 Secondary Data Collection

Secondary data is data obtained from literature and other written sources. Literature and information sources obtained can be from journals, theses, theses, literature books and regulations related to the utilization of antagonistic bacteria *Bacillus* sp. as environmentally friendly biological control agents.

4.2.3 Dokumentation

The next data collection method is documentation. Documentation is data in the form of images of each activity carried out. Documentation is done during the activity.

4.3 Data Analysis Method

Data obtained during the KKP activities from each activity starting from rhizosphere soil exploration, isolation of rhizosphere bacteria from soil samples and identification of bacteria found through various tests. Furthermore, data analysis will be presented in the form of descriptive explanations in the form of narratives, tables, and figures.

4.4 Report Preparation

Report preparation is carried out after carrying out a series of CTF activities and obtaining the required data. The report writing is adjusted to the format of the KKP report writing of the Faculty of Agriculture, Universitas Pembangunan Nasional “Veteran” Jawa Timur.

V. IMPLEMENTATION KULIAH KERJA PROFESI

5.1 Sterilization of Tools and Materials

Sterilization is a method or technique used to obtain a condition free of microorganisms or any process carried out either physically, chemically, and mechanically to kill all forms of life, especially microorganisms. In the field of microbiology, whether in research or practicum work, a sterile state is the main condition for the success or failure of work in the laboratory. Based on this, every process (both physical, chemical and mechanical) that kills all forms of life, especially microorganisms, is called sterilization (Luklukyah et al., 2019).

Sterilization carried out at the Biological Agents Laboratory UPT PTPH uses a wet sterilization technique using a manual autoclave. All tools and materials that will be used for the exploration and identification of *Bacillus* sp. must be sterilized. Tools to be sterilized include media bottles, test tubes, erlenmeyers and petri dishes. While materials to be sterilized such as NA media, PDA, Kelman's, and King's B.

The initial step of sterilization is to wrap the media bottle caps, test tubes and erlenmeyers using cotton until tight and re-coated with aluminum foil to avoid contamination. Next, wrap the entire tool with paper. The media sterilization step is to put the media into a sterilized bottle and then close it using cotton and aluminum foil..

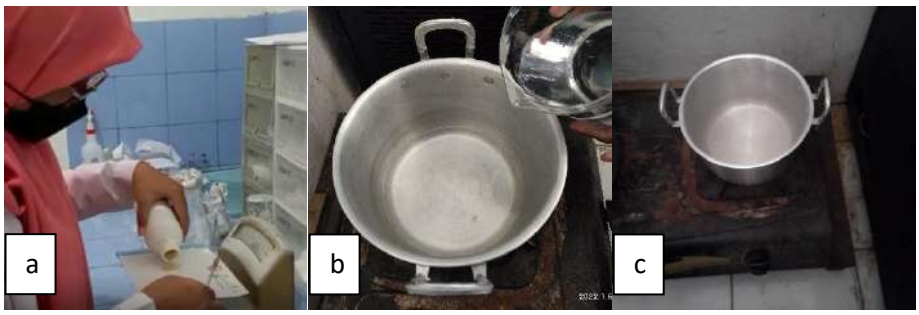
The next step is to spray alcohol on the autoclave pan room and coat the bottom of the autoclave pan room with newspaper. Then put all the tools and materials to be sterilized. The stove flame is turned on. Control until the parameter needle shows the number 12 which means the pressure is 1 atm and the temperature is 1210 C. After 30 minutes the stove is turned off. The total sterilization time of tools and materials is about 45-60 minutes.

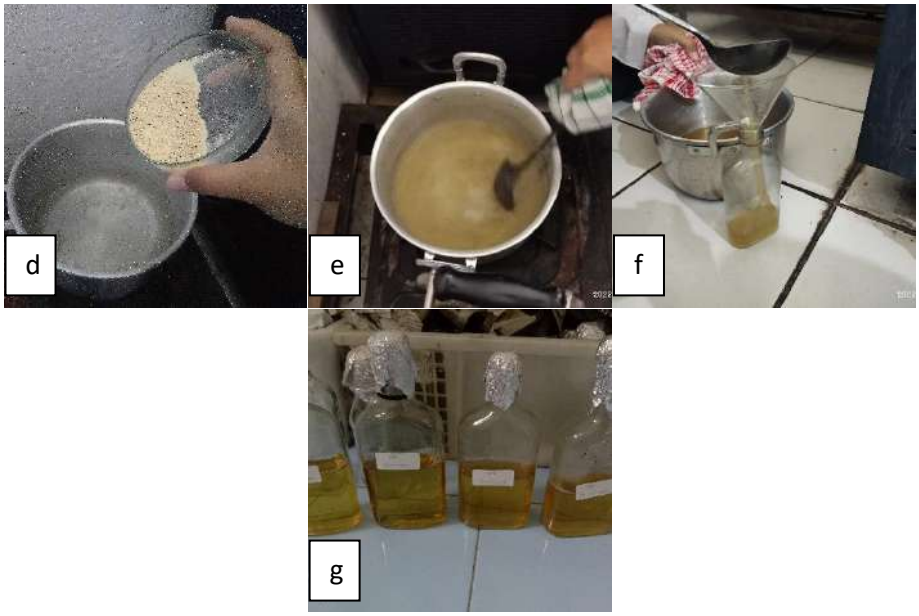
Figure 5. 1 Sterilization of Tools and Materials, (a) Wrapping petri dishes, (b) Petri dishes that have been wrapped, (c) Equipment to be sterilized, (d) Media to be sterilized. (Source: Personal documentation).

5.2 Preparation of Growth Media and Test Media

5.2.1 Nutrient Agar (NA) Media

NA media is a medium used for bacterial growth. The materials for making NA media are 32 grams of Nutrient Agar and 1000 ml of distilled water. The initial step in making NA media is to weigh the ingredients and boil the distilled water. Then put the NA powder into the boiling distilled water and stirred until dissolved with water. When it has dissolved, it is immediately put into a special glass bottle for media. The bottle must be immediately closed using cotton and aluminium foil.

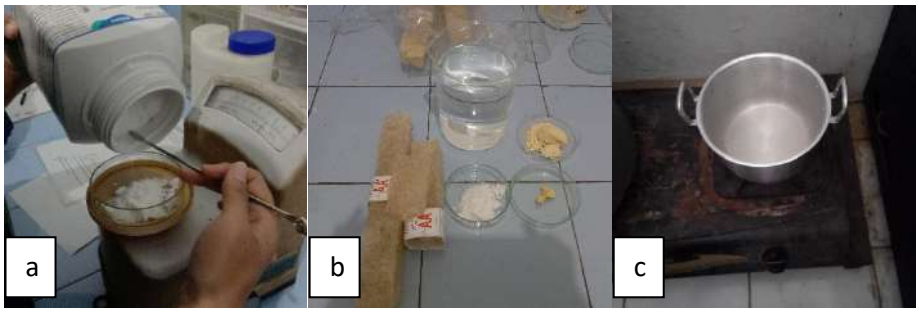




Fogure 5. 2 Making NA Media, (a) Weighing NA media, (b) Putting distilled water into a saucepan, (c) Boiling distilled water, (d) Adding media, (e) Stirring the media until it is all dissolved, (f) Putting the media into bottles, (g) NA media that has been sterilized. (Source: Personal documentation).

5.2.2 Kelman's Media

Kelman's media is a selective medium for the growth of bacteria of the genus Pseudomonas. The materials for making Kelman's media are Peptone 10 grams, Glucose 10 grams, Casein Hydrolysate 1 gram, agar 20 grams, and distilled water 1000 ml. The first step in making Kelman's media is to weigh the ingredients and boil the distilled water. Then put all the ingredients into the boiling distilled water and stirred until dissolved with water. When it has dissolved, it is immediately put into a special glass bottle for the media. The bottle must be immediately closed using cotton and aluminum foil.



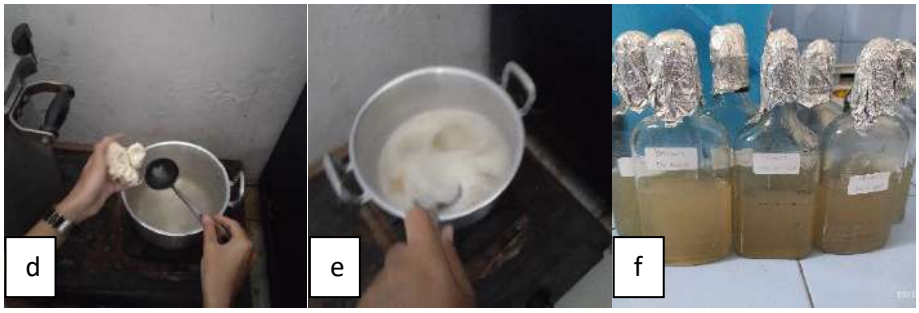


Figure 5. 3 Making Kelman's Media, (a) Weighing all Kelman's media materials, (b) Kelman's media materials, (c) Boiling distilled water, (d) Adding media materials, (e) Stirring the media until all dissolved, (f) Kelman's media that has been sterilized. (Source: Personal documentation).

5.2.3 King's B Media

King's B media is a selective media for pigmentation test. The ingredients of King's B media are Peptone 10 grams, Proteose Peptone no.3 20 grams, $MgSO_4.7H_2O$ 1.5 grams, K_2HPO_4 1.5 grams, Glycerin 10 ml, agar 20 grams and distilled water 1000 ml. The first step in making King's B media is to weigh the ingredients and boil the distilled water. Then put all the ingredients into the boiling distilled water and stirred until dissolved with water. When it has dissolved, it is immediately put into a special glass bottle for the media. The bottle must be immediately closed using cotton and aluminum foil.

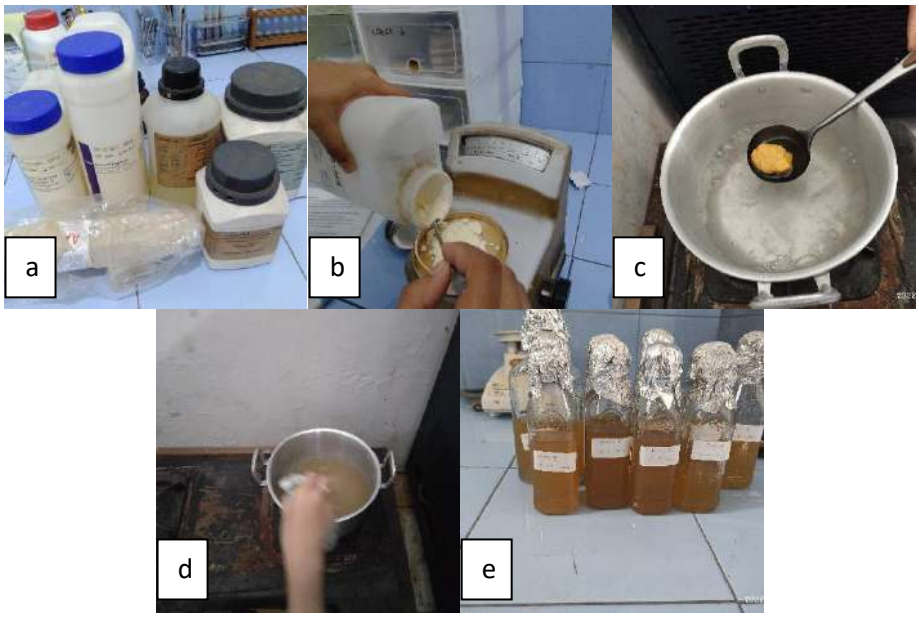


Figure 5. 4 Making King's B Media, (a) King's B media materials, (b) Weighing King's B media materials, (c) Putting media materials into distilled

water, (d) Stirring the media until all dissolved, (e) King's B media that has been sterilized. (Source: Personal documentation).

5.2.4 TZC Media

TZC media is a medium for bacterial virulence tests. The materials for making TZC media are 1% TZC and Kelman's media. The first step in making TZC media is to dilute 100 ml Kelman's media. If the Kelman's media is liquid and warm, 0.5 ml of 1% TZC is added to the liquid Kelman's media. The media and TZC are then homogenized. Next, TZC media is poured into petri dishes and allowed to solidify.

5.2.5 Potato Dextrose Agar (PDA) Media

PDA media is a medium used for fungal growth. The materials for making PDA media are 20 grams of sugar, 20 grams of agar sticks, 250 grams of potatoes, 1,000 ml of distilled water. The first step is to weigh all the ingredients and cut the potatoes into cubes. Then boil the distilled water. After boiling, all ingredients were added and stirred until dissolved. The media is then put into a special glass bottle for the media. The bottle must be immediately closed using cotton and aluminum foil.

Figure 5. 5 Making PDA Media, (a) Cutting potatoes, (b) Weighing potatoes, (c) Weighing agar, (d) Entering the media and stirring until all dissolved, (e) Putting PDA media into bottles, (f) PDA media that has been sterilized. (Source: Personal documentation).

5.3 Field Exploration

Exploration in the field aims to find bacteria and antagonistic fungi and insects. Exploration was carried out in Made Village, Sambikerep Subdistrict, Surabaya City. Exploration carried out is looking for soil samples of healthy plants and exploration of diseased plants. Soil samples and symptomatic plants were then taken to the laboratory for observation.

5.3.1 Exploration of rhizosphere soil

Soil samples were taken from the roots of healthy plants among diseased plants. This was because the soil was suspected to contain antagonistic microbes. Soil samples were taken compositely at 5 different points. Exploration to find soil samples was taken around the roots of bamboo, corn, and tomatoes. The tomato plants were on Mr. Kartono's land. Corn plants are on Mr. Jumeneng's land. Both are in the same farmer group, Sumber Rejeki. Meanwhile, the bamboo rooting soil was taken from Mr. Karnoto's land in Sendang Biru farmer group. The results of these soil samples will then be isolated to look for microbes that have the potential as Biological Control Agents (BCA).

Figure 5. 6 Rizosphere soil exploration, (a) tomato soil exploration, (b) bamboo soil exploration, (c) corn soil exploration. (Source: Personal documentation).

5.3.2 Exploration of diseased plants

Diseased plant samples were taken from chili and rice plants. The chili plants were suspected to be attacked by anthracnose caused by the fungus *Colletotrichum acutatum*. Rice plants are suspected to be attacked by leaf blight caused by *Helminthosporium oryzae*. The plants are from Mr. Kartono's land. Samples of diseased plants aim to determine the development of pathogenic microbes on plants.

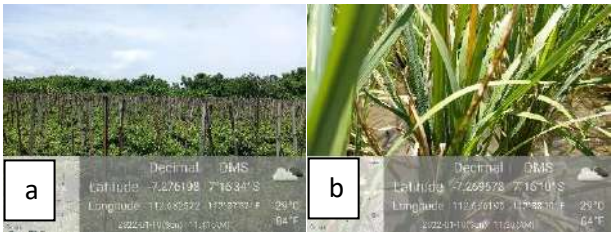
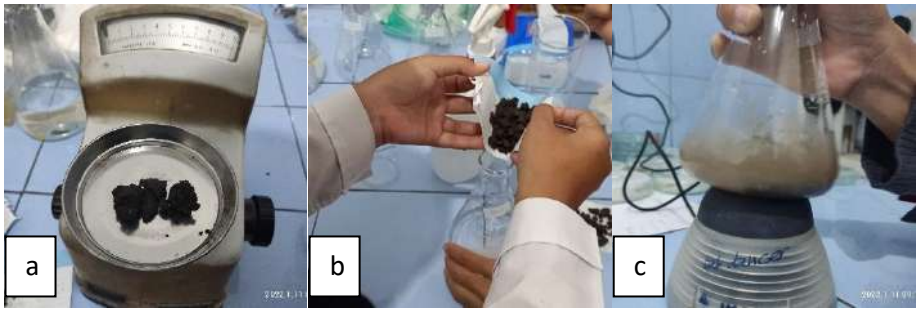


Figure 5. 7 Exploration of diseased plants, (a) Exploration of tomato plant diseases, (b) Exploration of rice plant diseases. (Source: Personal documentation).

5.4 Stratified Soil Dilution

Rhizosphere soil samples obtained from exploration will be diluted using a multistage dilution technique. According to Wasteson and Hornes (2009), the purpose of multilevel dilution is to minimize or reduce the number of microbes suspended in liquid. Determination of the amount or number of dilution levels depends on the estimated number of microbes in the sample. A ratio of 1:9 was used for the sample and the first and subsequent dilutions, so that the next dilution contained 1/10 of the microorganism cells from the previous dilution.

The rhizosphere soil samples that have been taken are then diluted using the multilevel dilution method as done by Juwita et al. (2013) with slight modifications. A total of 10 g of soil sample was homogenized with 90 ml of sterile water, then shaken. This soil suspension is a 10^{-1} dilution. A total of 1 ml of soil sample suspension was put into a test tube containing 9 ml of sterile water to obtain the 10^{-2} dilution level, and so on up to dilutions 10^{-4} and 10^{-8}.



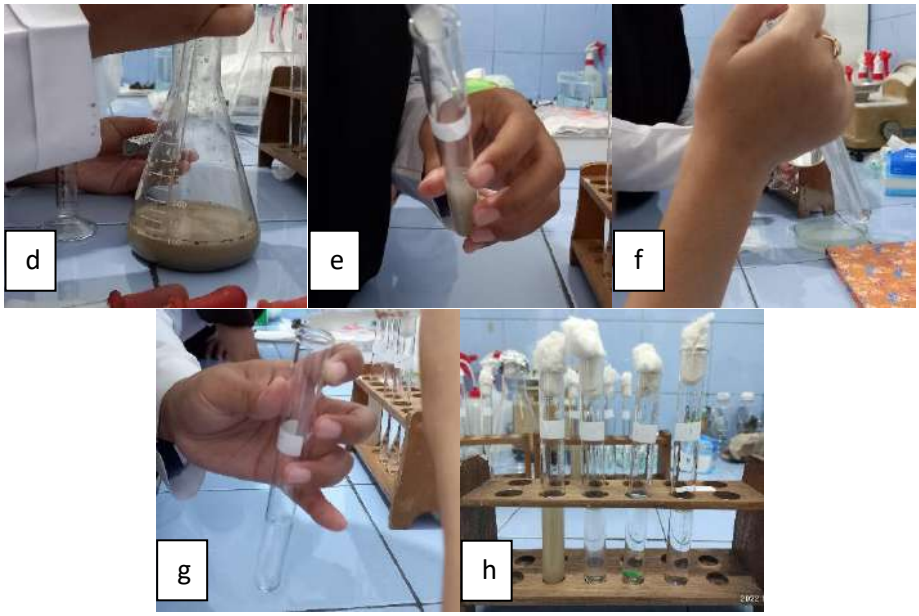


Figure 5. 8 Stratified Soil Dilution, (a) Weighing the soil sample, (b) Putting the soil sample into 90 ml distilled water, (c) Homogenizing the soil, (d) Taking 1 ml of soil dilution 100, (e) Putting 1 ml of soil 100 into tube 10^{-1}, (f) Homogenizing the soil and distilled water, (g) Taking 1 ml of soil dilution 10^{-1} into tube 10^{-2}, (h) Diluting the soil to 10^{-4}. (Source: Personal documentation).

5.5 Isolation of Bacteria in Rhizosphere Soil

Isolation of bacteria aims to select bacteria from rhizosphere soil that have the potential as APH. Isolation of bacteria in rhizosphere soil samples or root soil is carried out by taking the results of a multilevel dilution of 1 ml and then cultured into a petri dish that contains NA and Kelman's media by means of a spread plate. The results of the culture will be incubated for 18 hours at room temperature.

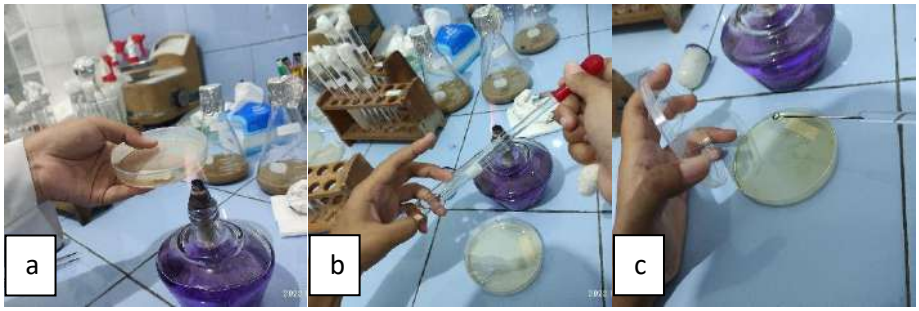


Figure 5. 9 solation of Bacteria in Rizosphere Soil (a) Heating the petri dish near the bunsen, (b) Taking a 10^{-4} dilution of soil, (c) Leveling by spreading. (Source: Personal documentation).

5.6 Preparation of Test Solution

5.6.1 KOH 3% Solution

3% KOH solution will be used for bacterial gram test. Materials that need to be prepared are 0.3 grams of KOH solids and 10 ml of sterile distilled water. KOH solids are then transferred into a bottle and distilled water is added. Then the solution is stirred until completely dissolved.

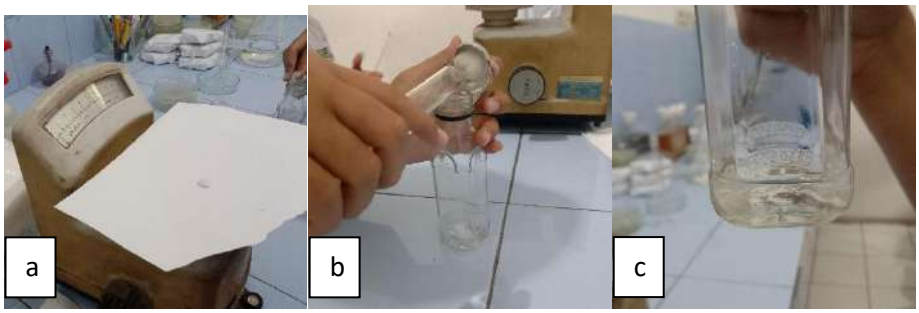


Figure 5. 10 Preparation of 3% KOH Solution, (a) Weighing KOH powder, (b) Putting distilled water into the bottle, (c) Homogenizing KOH and distilled water. (Source: Personal documentation)

5.6.2 1% TZC Solution

TZC solution will be used for the bacterial virulence test. Materials that need to be prepared are 0.5 grams of TZC and 50 ml of sterile distilled water. TZC is then transferred into a bottle and distilled water is added. Then the solution is stirred until completely dissolved. The TZC solution is then sterilized with a 1 ATM autoclave at 121°C for 7 minutes.

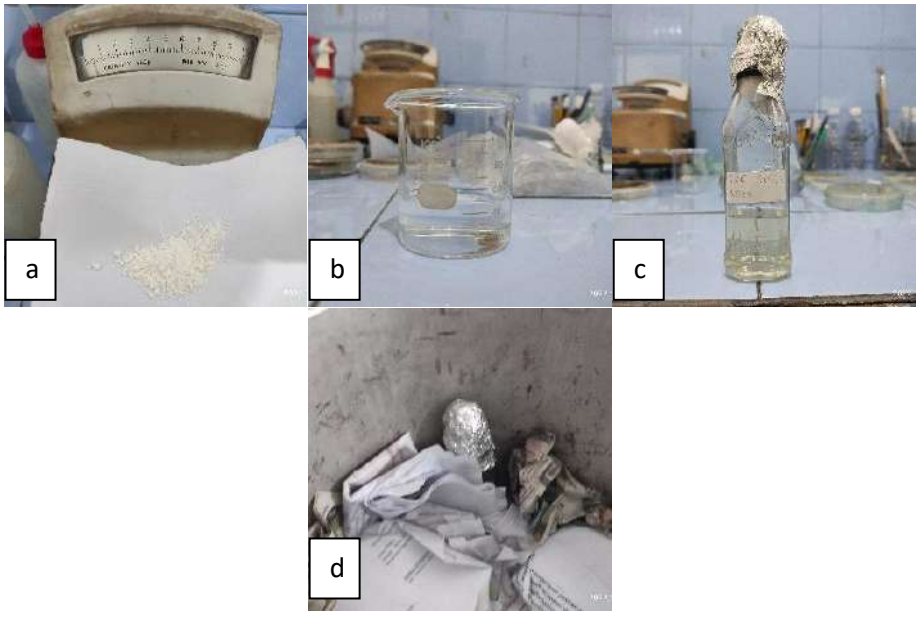


Figure 5. 11 Preparation of 1% TZC Solution, (a) Weighing TZC powder, (b) Preparing distilled water, (c) Homogeneous TZC solution, (d) Sterilized TZC solution. (Source: Personal documentation)

5.7 Bacterial Identification

The identification of bacteria found from the exploration was guided by Bergey's Determinative Bacteriology book (Holt et al., 1994) and Schaad et al. (2001). The methods used are selective media test, bacterial gram test (KOH 3%), bacterial virulence test, pigmentation test, soft rot test and hypersensitivity test. In addition, observations were also made on the morphology of bacterial colonies.

Selective media test was conducted on Kelman's media which is a selective media of the genus Pseudomonas. Bacteria that have grown during isolation will be re-grown on Kelman's media. If it grows, then it is suspected that the bacteria are Pseudomonas bacteria. Bacterial gram test (3% KOH) to determine the gram of a bacterium. The bacterial isolate to be tested is placed on a glass object that has been dripped with 3% KOH. Then the bacterial suspension is pulled using an ose needle quickly and repeatedly. Gram-negative bacteria will appear mucus when removed, while positive bacteria will remain dilute or show no reaction (Luthfiyyah, 2016).

The bacterial virulence test uses Kelman's media that has been given 1% TZC. If the bacterial isolate that grows has a reddish color, the bacteria have low virulence and do not have the potential to cause disease. While bacterial isolates that grow bluish red in color, the bacteria have high virulence and are suspected of being pathogenic. The pigmentation test is carried out by growing bacteria on King's B selective media and incubated for 24-48 hours. The bacteria that have been grown are observed under UV light. If the bacteria appear fluorescent then the bacteria are able to produce fluorescent pigments and vice versa. In fluorescent bacterial colonies, the bacteria can be included in the genus Pseudomonas (Luthfiyyah, 2016).

The soft rot test is a test to determine the nature of parasitic or saprophytic bacteria and is carried out using potatoes. Antagonistic microbes are usually obligate saprophytes so that they can only live on dead tissue. If there is decay in potatoes after being given a bacterial isolate, it is suspected that the bacteria are parasitic. Hypersensitivity test is a test conducted to determine whether the tested bacterial isolates are pathogenic bacteria or not. This test is only carried out on

isolates BS 2 and BS 4. The test is carried out by inoculating the bacterial suspension on tobacco plant tissue. A positive reaction will be indicated by wilting or even death of tobacco plant tissue on the inoculated part of the bacteria (Luthfiyyah, 2016).

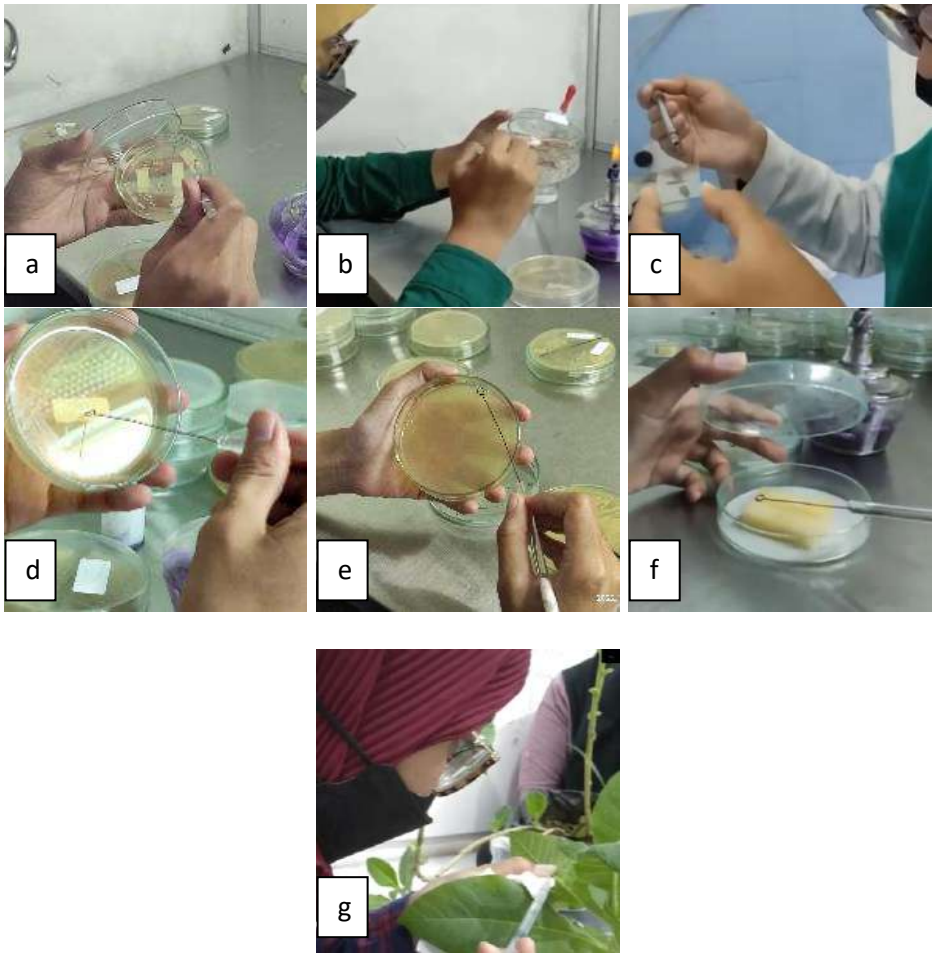


Figure 5. 12 Bacterial Identification, (a) Kelman's selective media test, (b) NA selective media test, (c) KOH test, (d) Virulence test, (e) Pigmentation test, (f) Soft rot test, (g) Hypersensitive test. (Source: Personal documentation).

5.8 Preparation and Sterilization of Soy Liquid Media

Soy liquid media is a medium for bacterial propagation. The materials for this media are 600 grams of soybeans, 90 grams of sugar, and 3 liters of sterile water. The first step in making soybean liquid media is to boil water until it boils. Then put 600 grams of well-washed soybeans into the boiling water. After boiling for approximately 5-6 minutes, the soybeans are drained from the water. Soybean

juice is then added with sugar and stirred to boil for 5-10 minutes. The medium was then transferred into an Erlenmeyer flask. The media was sterilized by autoclaving at 1 ATM pressure 121ºC for 30 minutes.

Figure 5. 13 Preparation and Sterilization of Soy Liquid Media, (a) Weighing soybeans, (b) Washing soybeans, (c) Boiling soybeans, (d) Draining soybeans, (e) Boiling soybean juice, (f) Measuring soy liquid media, (g) Putting liquid media into the flask. (Source: Personal documentation).

5.9 Propagation of Bacteria on Soy Liquid Media

Propagation of bacteria on soy liquid media aims to multiply bacterial colonies. The initial step of bacterial propagation is to dilute the bacterial isolate with sterile water and put the suspension into soybean liquid media. The media containing the bacterial suspension is then installed in the fermenter. Then the fermenter is turned on and wait for the results after 2 days.

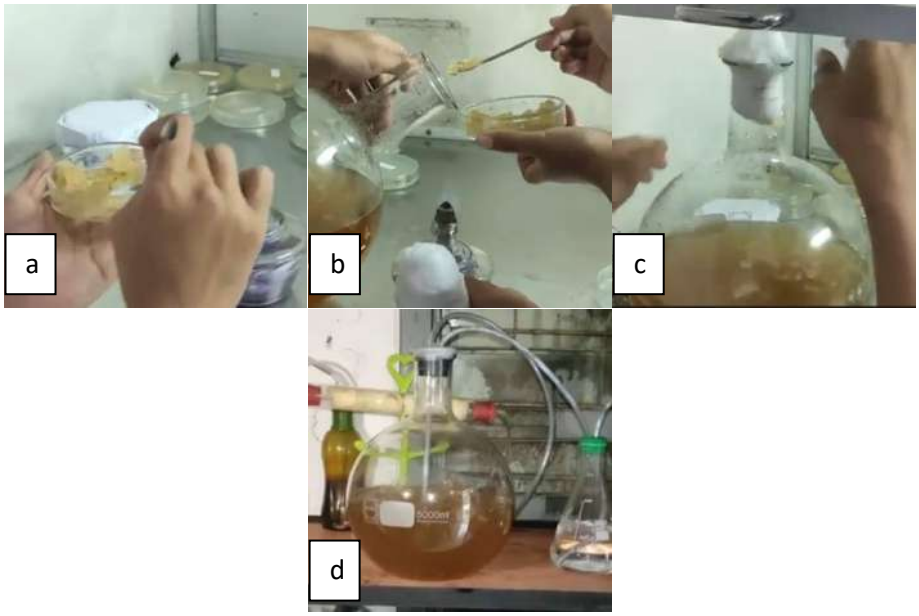


Figure 5. 14 Propagation of Bacteria on Soy Liquid Media. (a) Diluting the bacterial isolate, (b) Putting the isolate into the soybean liquid medium, (c) Closing the flask, (d) Installing the fermenter for propagation. (Source: Personal documentation).

5.10 Other Activities

5.10.1 Fungus Isolation

a. Isolation from Rhizosphere Soil

Isolation of fungi aims to select fungi from rhizosphere soil that have the potential as APH. Isolation of fungi in rhizosphere soil samples or root soil is carried out by taking the results of multilevel dilutions of 1 ml and then cultured into petri dishes containing PDA media by means of spread plates and pour plates. The results of the culture will be incubated for 3 days at room temperature.

b. Fungal Baiting

This fungus baiting is used to look for the presence of entomopathogenic fungi in corn soil samples. Entomopathogenic fungi are a type of bioinsecticide that can infect insects by entering the host insect's body through the skin, digestive tract, spiracles and other holes. The fungal inoculum attached to the host insect's body will germinate and develop to form a sprout tube, then penetrate through the skin of the body. The penetration is done mechanically and or chemically by releasing enzymes or toxins. The fungus will develop in the host body and attack all body tissues, causing the insect to die (Herdatiarni et al., 2014).

Isolation of insect pathogenic fungi was carried out using the baiting technique as done by Agastya et al. (2018). Soil samples from the field were placed evenly on a Petri dish. The soil on top of the Petri dish was moistened then the larvae of *Tenebrio molitor* were placed to incubate for 48 hours. After 48 hours the dead larvae will have the mark of the insect pathogenic fungus in the form of white hyphae on the surface of the body of *T. mollitor*. The marked larvae were then cultured on PDA media for purification.

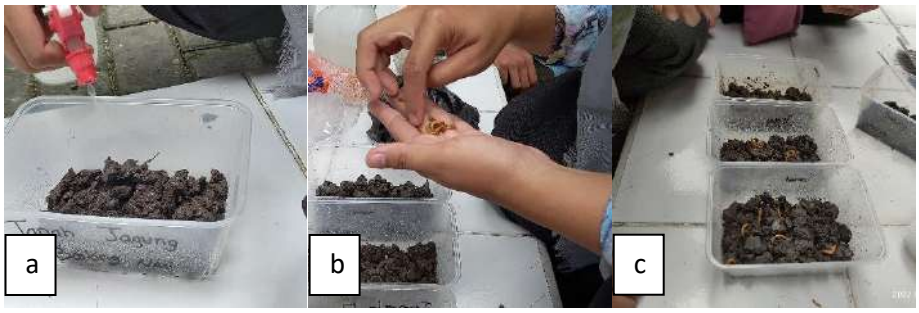


Figure 5. 15 Baiting the fungus, (a) Wetting the soil sample, (b) Inserting the Hong Kong worm, (c) Soil sample that has been treated with Hong Kong worm. (Source: Personal documentation).

5.10.2 Identification Fungus

Identification of fungi through macroscopic and microscopic morphological identification. Macroscopic morphology is seen based on the color of the mycelium while microscopic morphology is carried out using a microscope by observing spores to fruiting bodies.

Figure 5. 16 *Aspergillus niger* fungus. (Source: Personal documentation).

5.10.3 Fungus Propagation

a. Preparation of Corn Media

The solid media used is corn media. This media will be used for fungus propagation. The first step is to steam the washed corn rice until half cooked. After cooling the corn is given sugar and shrimp powder. When it is evenly distributed, the corn is put into heat-resistant plastic. The corn is then put into an autoclave for sterilization.

Figure 5. 17 Making Corn Media, (a) Washing corn rice, (b) Steaming corn rice, (c) Corn rice is given sugar and shrimp powder, (d) Corn rice is put into plastic. (Source: Personal documentation).

b. Fungal Propagation on Solid Media

The fungi propagated on corn media were *Trichoderma* sp., *Metharizium* sp., and *Lecanicillium lecanii*. The things that were prepared were fungal isolates and sterilized corn solid media. The prepared materials are trays that have been cleaned using 70% alcohol and fungal isolates that have been mashed. Next, the corn solid media was put into the tray and leveled. Corn solid media is mixed with fungal isolates. The last step is to cover the tray with plastic tightly and label the date on the tray.

Figure 5. 18 Fungus Propagation on Solid Media, (a) Diluting the fungus isolate, (b) Putting the isolate dilution into a bottle, (c) Homogenizing the isolate dilution, (d) Preparing corn rice media into a plastic tray, (e) Mixing the isolate dilution into the corn rice media. (Source: Personal documentation).

c. Propagation of Fungi on PDA Media

Propagation on PDA media is carried out in petri dishes and test tubes. Things that are prepared are fungal isolates, Petri dishes and test tubes containing PDA media. The fungi propagated on corn media were *Trichoderma* sp., *Metharizium* sp., *Lecanicillium lecanii*, *Beauveria bassiana*, *Aspergillus niger*, and *Gliocladium* sp. The fungal isolates propagated on petri dishes were taken five ounces of fungal isolates and placed at five different points. As for propagation on PDA media in test tubes, it is done by taking one ose of fungal isolate and spreading it in a streak plate.

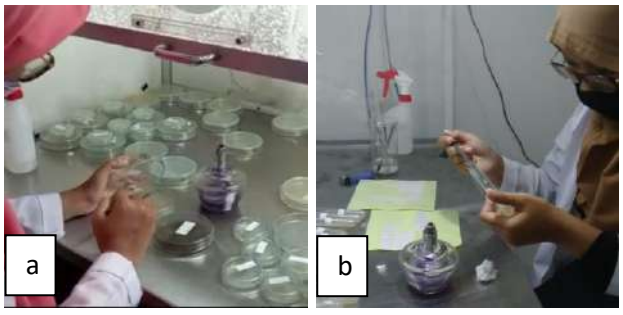


Figure 5. 19 Propagation of Fungi on PDA Media, (a) PDA media propagation in petri dishes, (b) PDA media propagation in test tubes (tilted media). (Source: Personal documentation).

5.10.4 Sporulation of fungal pathogens

a. Sporulation on PDA media

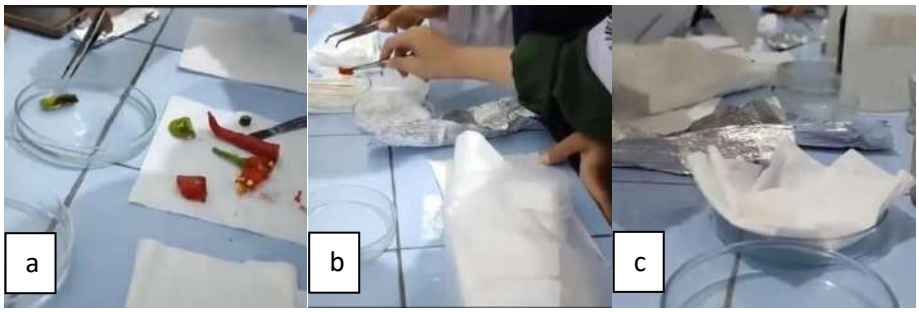
Sporulation is carried out using symptomatic plant samples. Plant diseases that have been found are anthracnose on chili peppers and brown spot on rice plants. The materials needed are PDA media in Petri dishes, sterile tissue, sterile water, anthracnose samples on chili plants, and *Helminthosporium oryzae* samples on rice plants.

The work step starts with cutting the rice and chili samples into smaller pieces. Then cleaned using sterile water and alcohol. The sample is then dried using tissue. The next sample is placed on a Petri dish that contains PDA media. All of these activities must be done aspetically.

b. Sporulation with the Toothpick Method

Sporulation with the toothpick method aims to induce the pathogenic fungus to sporulate towards the outside of its host. The materials needed are sterile tissue, sterile toothpicks, sterile water, anthracnose samples on chili plants, and *Helminthosporium oryzae* samples on rice plants.

The work step starts with cutting the sample into smaller pieces. Then coat the surface of the Petri dish with sterile tissue on the top and bottom sides and give sterile water to the tissue until moist. On top of the tissue is given a toothpick that has been arranged and the sample is placed on the toothpick. Then incubated for 24 hours at room temperature.



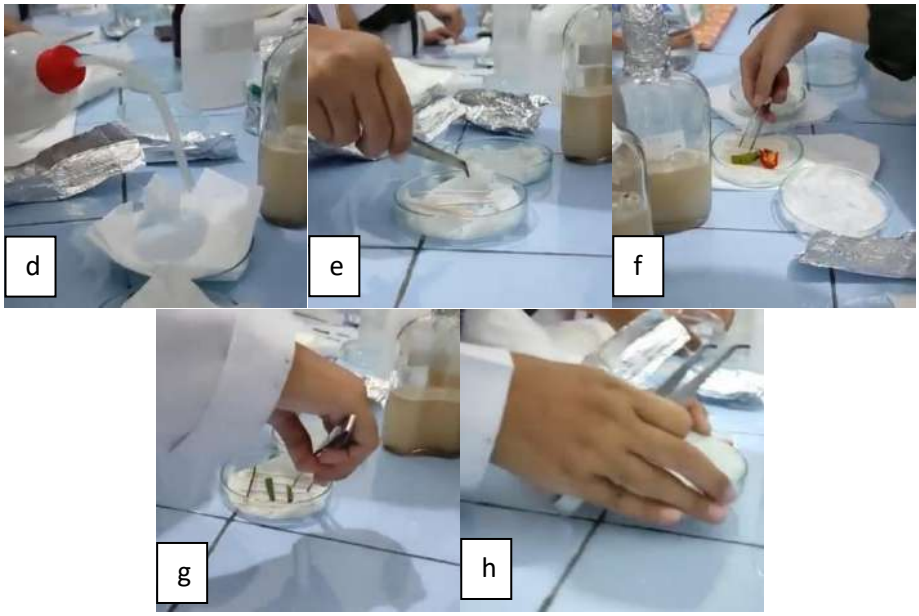


Figure 5. 20 Sporulation by Toothpick Method, (a) Cutting the disease sample, (b) Washing the sample in alcohol and water, (c) Preparing a tissue in a petri dish, (d) Wetting the tissue, (e) Placing a toothpick on the tissue, (f) Placing a chili anthracnose sample on the toothpick, (g) Placing a rice brown spot sample on the toothpick, (h) Closing the petri dish. (Source: Personal documentation).

5.10.5 Fruit Fly Population Observation

Fruit crop cultivation often experiences a drastic decline in production. This happens because it is attacked by the fruit fly Bactrocera sp. (Diptera: Tephritidae). Fruit flies are one of the most important insect pests of horticultural crops in the tropics and subtropics. Fruit flies lay their eggs under the skin of the fruit, hatch into larvae and then consume the fruit flesh. Fruit attacked by fruit flies will become rotten faster and fall from the tree prematurely (Susanto et al., 2017).

One of the important steps that must be taken in order to control fruit flies is to set traps to a.) attract fruit flies to be killed in the trap, b.) disrupt fruit fly mating so as to reduce the number of fruit flies. Fruit fly traps can be made simply from plastic bottles. Antractants lure fruit flies into the trap. Antractants are compounds containing methyl eugenol that attract male fruit flies. (Susanto et al., 2019). There are several types of attractants ranging from chemical substances, namely petrogenol to those derived from natural ingredients such as selsaih leaves and basil. The three attractants have different effectiveness in attracting fruit flies to enter the trap, for this reason, a comparison test will be conducted on the

population of fruit flies trapped with 3 types of attractants, namely methyl eugenol, basil leaves, and basil leaves.

a. Making and setting fruit fly traps

Fruit fly traps were made from 600 ml plastic aqua bottles and attractant. The aqua bottle was cut off at the top quarter. Then the piece is inserted into the bottle in an inverted position. The lid of the bottle was perforated to allow fruit flies to enter. The tool is then given a wire so that it can be tied with a rope and hung. Furthermore, the tool was given an attractant in the form of methyl eugenol, basil leaves, and basil leaves. The number of traps based on the attractant is methyl eugenol 5 bottles of traps, basil leaves 5 bottles of traps, basil leaves 5 bottles of traps. The finished fruit fly traps were then installed on tree branches around UPT PTPH Jawa Timur.

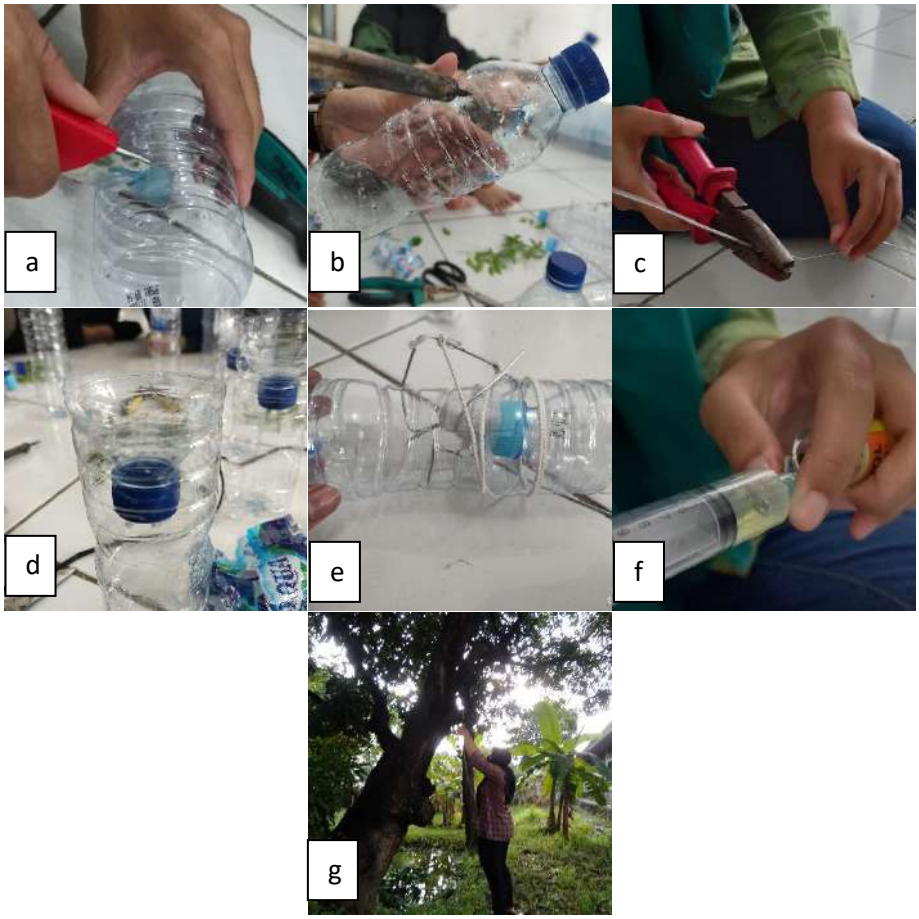


Figure 5. 21 Making and Setting Fruit Fly Traps, (a) Cutting plastic bottles, (b) Punching holes in plastic bottles, (c) Cutting wire, (d) Stringing bottle pieces, (e) Attaching wire and rope, (f) Inserting methyl eugenol

attractant, (g) Placing fruit fly traps on tree branches. (Source: Personal documentation).

b. Fruit Fly Observations

Fruit fly observations were made on traps that had been set on mango plants. The purpose of the observation was to determine the number of fruit flies that had been trapped in each type of trap. Observations of the dynamics of fruit flies trapped every day at 08.00 and 12.00 for 3 weeks, then counted, recorded, and collected in plastic containers.

Figure 5. 22 Fruit Fly Counting (Source: Personal documentation).

5.10.6 Horticultural Plant Cultivation

Plant cultivation is a farming activity whose main purpose is to produce plant products, in the form of tubers, stems, sap, flowers, leaves, and fruits/seeds. In this KKP activity, there are three types of plants that are cultivated, namely kale, mustard greens, spinach, and batik spinach. Before the seeds are planted, it is necessary to prepare the seeds first. First, the seeds of kale, mustard greens, and batik spinach are placed in their respective containers and then soaked with water. Then the floating seeds are discarded because their quality has decreased.

Next, the seeds were soaked at 40ºC for 5 minutes to break the dormancy. The treatment of breaking dormancy by soaking seeds at high temperatures aims to soften the seed coat so as to facilitate the process of water absorption, with the incoming water the physiological processes to germinate can take place. Seeds soaked in water at high temperatures allow the breakdown of tannin and lignin content contained in the seed coat so that the seeds become softer so that imbibition easily occurs (Melasari et al., 2018).

After soaking, the seeds are then matured for 12-24 hours. Tamping the seeds is done by placing wet newspaper at the bottom of the container and then

spreading the seeds on the wet newspaper. Then cover the seeds with wet newspaper. Seed soaking is a technique that connects hydration until the germination process occurs, but no growth occurs (Marjenah, 2021). After soaking, kale plants were spread on polybags and mustard, spinach, and batik spinach plants were planted by making planting holes. Then the plants are watered using water and observed for growth.

Figure 5. 23 Cultivation of Horticultural Plants, (a) Pulling out old plants, (b) Inserting mustard seeds, (c) Inserting spinach seeds, (d) Inserting batik spinach seeds, (e) Covering the planting hole again. (Source: Personal documentation).

5.10.7 Making Local Microorganisms (MOL)

Making MOL aims to utilize organic waste to be useful for plants. The MOL made will then be used as liquid organic fertilizer, decomposer and vegetable pesticide. The MOL made included banana stump mole, tape mole and bamboo shoot mole.

a. Banana Stem Mol

Banana plants have many benefits, one of which is banana pith. Banana pith contains microbes that decompose organic matter. The decomposing microbes are located on the outside and inside of the banana stem (Suhastyo, 2011). The types of microbes that have been identified in banana pseudostem MOL include *Bacillus*

sp., *Aeromonas* sp., and *Aspergillus niger*. These microbes usually decompose organic matter (Kesumaningwati, 2015).

Banana stump mole is a mol that has a very high nutrient content and microbial richness. It also contains growth hormones that can increase nutrients in organic fertilizers. Therefore, the success of making compost can be influenced by the addition of banana pomace mole as a decomposer. It is the local micro-organisms found in the banana pomace that function as decomposers to break down organic matter in making organic fertilizer. Mole serves as a decomposer that breaks down organic matter in the manufacture of organic fertilizer. Banana stalk mole not only functions as an organic material, but also contains NPK nutrients as well as auxin, gibberellin and cytokinin hormones that are good for plant growth (Salma and Purnomo, 2015).

The basic ingredients for making banana bark mole are 2.5 kg of banana bark, 500 grams of brown sugar, and 5 liters of rice water. The first step in making mole is to chop the banana stem and brown sugar into small pieces. Then mix the ingredients with rice water in a jar and stir until well mixed. The jar is then tightly closed and insulated at the edges. The result of the mole was waited for 7 days.

Figure 5. 24 Making Banana Stem Mol, (a) Cutting banana stems, (b) Cutting coconut sugar, (c) Putting in rice water, (d) Putting coconut sugar into rice water, (e) Putting in banana stems pieces, (f) Closing the jar. (Source: Personal documentation).

b. Mol Tape

The addition of Mol Tape activator to compost materials can affect composting time, because Mol solution contains macro and micro nutrients and also contains bacteria that have the potential to break down organic matter, stimulate growth and control pests and plant diseases (Panjaitan et al., 2014).

The basic ingredients for making mole tape are 1 kg of cassava tape, 200 grams of brown sugar, and 5 liters of coconut water. The first step in making mole is to chop the cassava tape and brown sugar into small pieces. Then mix the ingredients with coconut water in a jar and stir until well mixed. The jar is then tightly closed and insulated at the edges. The result of the mole waited for 7 days.

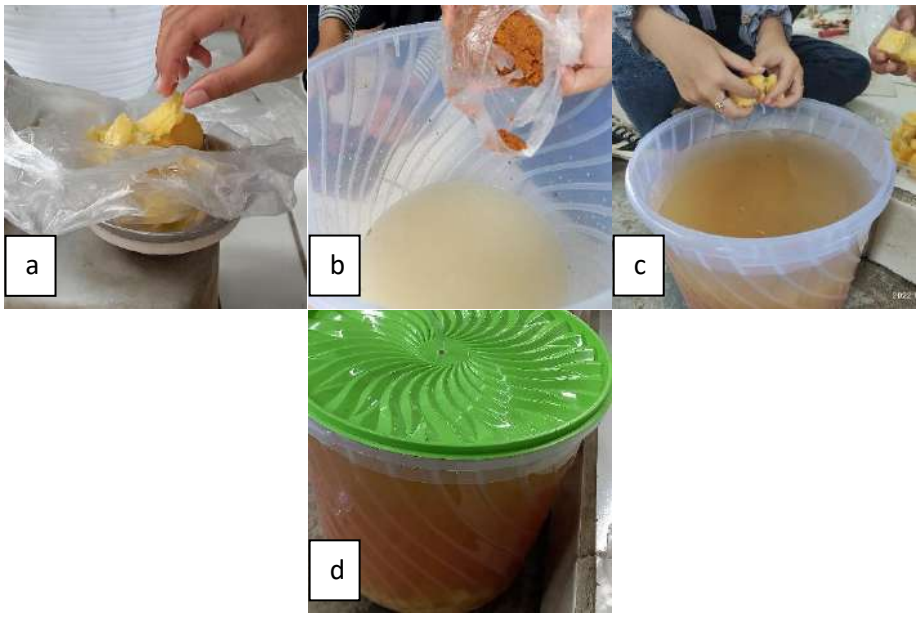


Figure 5. 25 Making Mol Tape, (a) Weighing and cutting the tape, (b) Putting coconut sugar into coconut water, (c) Putting the tape, (d) Closing the jar. (Source: Personal documentation).

c. Mol Bamboo Shoots

Bamboo shoots are one type of plant that has the potential to be extracted into mol because of its high content of growth regulators. Local microorganisms contain substances that can stimulate plant growth and substances that can encourage plant development such as gibberellin, cytokinin, auxin and inhibitors. Bamboo shoots contain the hormone Gibberellin so that the extract can be used to spur seedling growth (Samosir and Gusniwati, 2014).

Mol bamboo shoots contain Phosphorus 59 mg, Calcium 13 mg, Iron 0.50 mg, Potassium 20.15 mg (Nugroho, 2013). Giving bamboo shoot extract 20 ml / seedling has a significant effect on the increase in stem diameter of sengon seedlings while at a dose of 50 ml / seedling bamboo shoot mole affects the growth of height and wet weight of sengon seedling shoots (Maretza, 2009).

The basic ingredients for making bamboo shoot mole are 3.5 kg of bamboo shoots, 200 grams of brown sugar, and 5 liters of rice water. The first step in making mole is to chop the bamboo shoots and brown sugar into small pieces. Then mix the ingredients with rice water in a jar and stir until well mixed. The jar is then tightly closed and insulated at the edges. The result of the mole waited for 7 days.

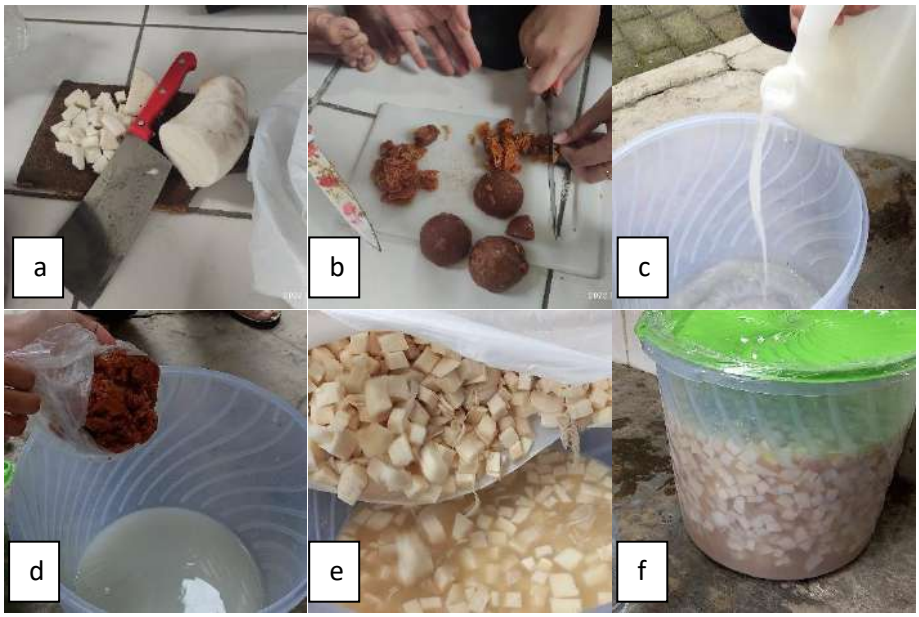


Figure 5. 26 Pembuatan Mol Rebung, (a) Memotong rebung, (b) Memotong gula jawa, (c) Memasukkan air beras, (d) Memasukkan gula jawa. (e) Memasukkan rebung, (f) Menutup toples. (Sumber: Dokumentasi pribadi).

5.10.8 Pelatihan Agens Hayati Kilat (PELAGIAT)

Pelatihan agens hayati kilat (PELAGIAT is a bioagent training activity conducted by representatives from the protection PTPH laboratory each working area throughout East Java. UPN Veteran Jawa Timur Student acted as assistants and presenters in these activities. The activity was carried out from 24-27 January 2022 which included material on antagonistic BCAand entomopathogenic fungi,

material on preparation and sterilization of equipment and materials and good and correct isolation, material on baiting Hong Kong caterpillars and rice baiting, practice of site-specific soil dilution, pour plate method, isolation of bacteria into Kelman's and NA media, identification of fungi using a microscope, sporulation of fungi using the toothpick method, selective media test, 3% koh test, pigmentation test, bacterial virulence test, soft rot test, and hypersensitivity test.

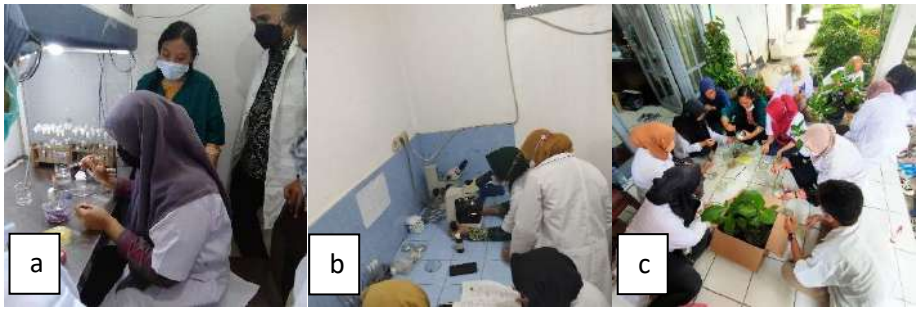


Figure 5. 27 Acara Pelatihan Agens Hayati Kilat, (a) Guiding the spread plate method, (b) Guiding the morphological observation of fungi, (c) Guiding the hypersensitivity test. (Source: Personal documentation).

VI. DISCUSSION

6.1 Isolation Results of Antagonistic Bacteria in Rizosphere Soil

Isolation of antagonistic bacteria using soil samples from exploration. Rhizosphere soil samples of bamboo and tomato roots were taken from land in Made Village. Samples were taken compositely at five different points. The exploration results were 750 grams of bamboo rhizosphere soil samples and 750 grams of tomato rhizosphere soil samples. The soil samples were then dried and diluted. The results of the dilution were then isolated on NA media and incubated for 24 hours. According to Putri and Kusdiyanti (2018) microbial isolation activities aim to separate one type of microbe from another from a variety of microbial mixtures with the aim of obtaining pure culture.

Figure 6. 1 Results of Rizosphere Soil Isolation. (Source: Personal documentation).

The results of isolation on NA media found various bacterial colonies suspected of being *Bacillus* sp. According to Hatmanti (2000) *Bacillus* spp. bacteria have various kinds of flat and uneven colony edges, the surface is rough and not slimy, some even tend to be dry and powdery, large colonies and not shiny. Based on the literature, six colonies were found that were similar to the morphology of *Bacillus* bacteria colonies. The colonies were then developed as one isolate. The six bacterial isolates are BS 1, BS 2, BS 3, BS 4, BS 5, and BS 6. Each isolate is then propagated on NA media before being identified with various tests.

6.2 Observation Results of Colony Morphology Characterization of Antagonistic Bacterial Isolates

The results of the isolation of antagonistic bacteria were then identified by the morphological characteristics of the colonies. Macroscopic morphological observations were made by observing the morphology of colonies formed from bacteria, including the number of colonies, color, colony shape and colony edges. The purpose of this morphological observation is to determine the morphological characteristics of an isolate (Murtiyaningsih and Hazmi, 2017). Observation of colony morphology includes the shape, size, edge, color, and surface of the colony. The observed bacteria were grown on NA media and aged 48 hours.

The isolation results that have been grown again on NA media were then re-observed for colony morphology under a stereo microscope. Of the six colonies, only BS 3 colony could not develop. Colony BS 1 has a circular colony shape, jagged colony edges (rhizoid), flat colony elevation, and dull colony appearance. Colony BS 2 has a circular colony shape, flat colony edge (entire), flat colony elevation, and dull colony appearance. Colony BS 4 has a circular colony shape, jagged colony edges (rhizoid), flat colony elevation, and dull colony appearance. Colony BS 5 has an irregular colony shape, jagged colony edges (rhizoid), flat colony elevation, and dull colony appearance. Colony BS 6 has a circular colony shape, jagged colony edges (rhizoid), flat colony elevation, and dull colony appearance. Several studies have shown that morphological characteristics within colonies vary greatly depending on the strain used. In addition, it is likely that variations in gene content and differences in regulation can explain the differences in phenotype (Matsushita et al., 2005).

All isolates have a colony shape in accordance with the general form of *Bacillus* sp. According to Corbin (2004), *Bacillus* sp. colonies have general characteristics that have a whitish beige color and a round and irregular colony shape. *Bacillus* sp. bacteria have various kinds of flat and uneven colony edges, the surface is rough and not slimy, some even tend to be dry and powdery, large colonies and not shiny (Hatmanti, 2000).

Table 6. 1 Description of Colony Morphology of Antagonistic Bacterial Isolates in the Rhizosphere Layer of Bamboo and Tomato Plants

No	Isolate Code	Morphological Characteristics of Colonies			
		Colony Shape	Colony Edge	Colony Elevation	Colony Appearance
1.	BS 1	Circular	Rhizoid	Flat	Dull
2.	BS 2	Circular	Entire	Flat	Dull
3.	BS 4	Circular	Rhizoid	Flat	Dull
4.	BS 5	Irregular	Rhizoid	Flat	Dull
5.	BS 6	Circular	Rhizoid	Flat	Dull

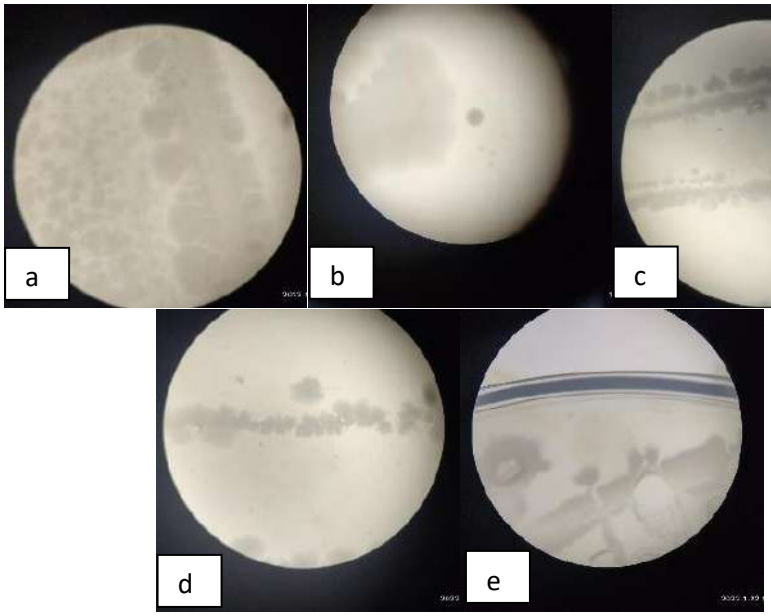


Figure 6. 2 Colony Morphology Observation Results, (a) Observation of colony BS 1, (b) Observation of colony BS 2, (c) Observation of colony BS 4, (d) Observation of colony BS 5, (e) Observation of colony BS 6. (Source: Personal documentation).

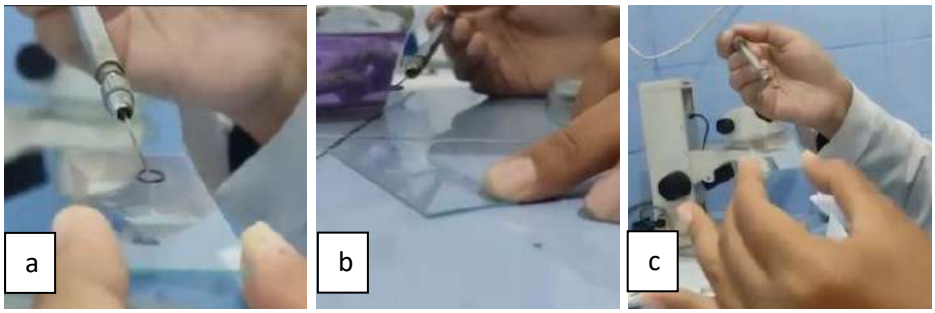
6.3 Observation Results of Physiological and Biochemical Characterization of Antagonistic Bacterial Isolates

Table 6. 2 Results of Physiological and Biochemical Characteristics of Antagonistic Bacterial Isolates

No.	Isolate Code	Morphological Characteristics of Colonies		
		Gram test (KOH 3%)	Soft Rot Test	Hypersensitive Test
1.	BS 1	+	-	
2.	BS 2	-	-	-
3.	BS 4	-	-	-
4.	BS 5	+	-	
5.	BS 6	-	+	

6.3.1 Bacterial Gram Test Observation Results (3% KOH)

Bacterial gram test aims to determine the gram of a bacterium. Based on the cell wall, bacteria can be divided into two groups, namely, gram-positive bacteria and gram-negative bacteria. Bacterial gram test is carried out using 3% KOH. This test was repeated twice. The results of the bacterial gram test on BS 1 and BS 5 showed a positive reaction indicated by the presence of mucus formed after the bacterial isolate was reacted with 3% KOH. Then BS 1 and BS 5 are gram-negative bacteria. Meanwhile, BS 2, BS 4, and BS 6 showed a negative reaction because there was no mucus when reacted. Then BS 2, BS 4, and BS 6 are gram-positive bacteria. According to Soesanto (2008) *Bacillus* sp. is a gram-positive, rod-shaped, one-celled bacteria. So that in accordance with the characteristics of *Bacillus* sp. are isolates BS 2, BS 4, and BS 6.



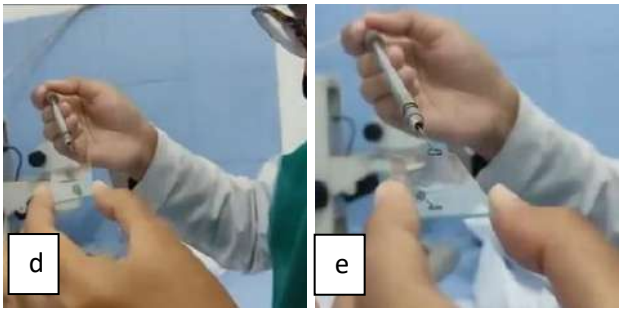


Figure 6. 3 Gram test results, (a) Gram test BS 1, (b) Gram test BS 2, (c) Gram test BS 4, (d) Gram test BS 5, (e) Gram test BS 6 (Source: Personal documentation).

6.3.2 Soft Rot Test Observation Results

The soft rot test aims to determine the pathogenic properties of a bacterial isolate. The soft rot test carried out on potato media is carried out to determine the parasitic nature of the bacteria. In isolates BS 1, BS 2, BS 4, and BS 5 there is no necrosis on potatoes. While in isolate BS 6 there is necrosis on potatoes. This indicates that isolates BS 1, BS 2, BS 4, and BS 5 have saprophytes because these isolates cannot develop in living tissue. While isolate BS 6 is suspected as a parasite because it causes necrosis on potatoes. Bacteria that are soft rot are characterized by the decay of the center of the potato that is scratched by the bacteria. Soft rot bacteria are a group of bacteria that cause soft rot on plants and are pathogenic (Oviana et al., 2015).

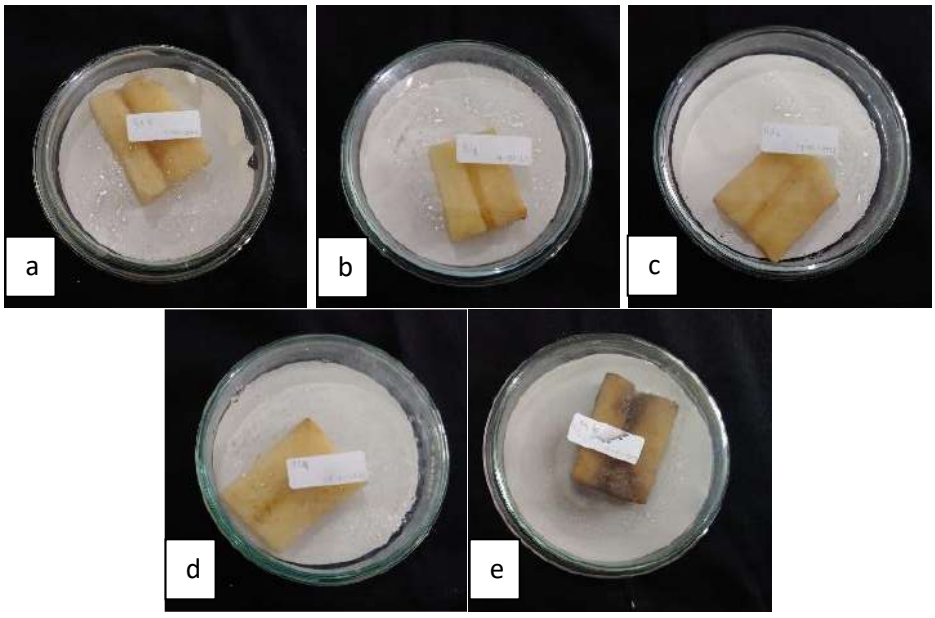


Figure 6. 4 Soft Rot Test Results, (a) Soft rot test BS 1, (b) Soft rot test BS 2, (c) Soft rot test BS 4, (d) Soft rot test BS 5, (e) Soft rot test BS 6 (Source: Personal documentation).

6.3.3 Hypersensitivity Test Observation Results

Hypersensitivity test aims to determine whether the bacterial isolates found are plant pathogenic bacteria or not. Hypersensitivity test is done by infiltrating bacterial suspensions into the leaves of tobacco plants. Symptoms of hypersensitivity are seen if the infiltrated part of the bacterial suspension occurs necrosis. Isolates BS 2 and BS 4 tested on tobacco plants did not show symptoms of necrosis. So it can be assumed that isolates BS 2 and BS 4 do not act as pathogens and have the potential as Biological Control Agents (APH). This is in accordance with the opinion of Marsaoli et al. (2019) that a negative reaction in the hypersensitive test indicates that a bacterium does not have the potential as a plant pathogen. This can be seen in the presence or absence of necrosis reactions on the leaves of tobacco plants that have been injected with bacterial suspensions BS 2 and BS 4.

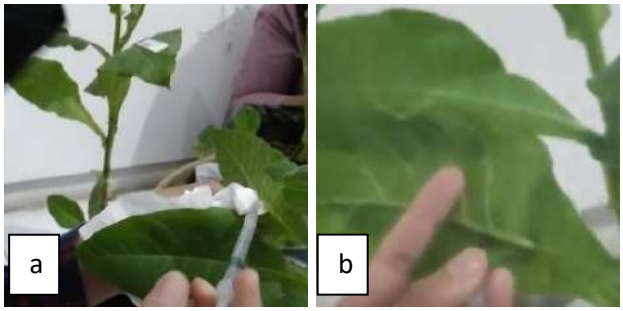


Figure 6. 5 Hypersensitivity Test Results, (a) Hypersensitivity test of isolate BS 2 on tobacco plants (b) Hypersensitivity test of isolate BS 4 on tobacco plants. (Source: Personal documentation).

6.4 Multiplication of *Bacillus* sp.

The biological control agent bacterium *Bacillus* sp. has the potential to be mass-produced as a biological fertilizer. To meet the demands of high production volume, it is necessary to propagate. Propagation was carried out using soybean liquid media using a fermenter as a bioreactor. Bacterial isolates that have been carried out various tests, then multiplied on soybean liquid media as much as 3 liters. Of the five bacterial isolates that have developed, isolates BS 2 and BS 4 are

isolates suspected of being *Bacillus* sp. The isolates were then put into soybean liquid media, and multiplied with a fermenter for 48 hours. According to Sari & Rahmawati, (2020) soybean cooking water has better content than soybean soaking water. Soybean cooking water contains protein, fat, carbohydrates, water and ash. The content in soybean cooking water is the content needed by bacteria to survive. The results of propagation will be applied to plants either mixed with fertilizer or applied directly to the soil..

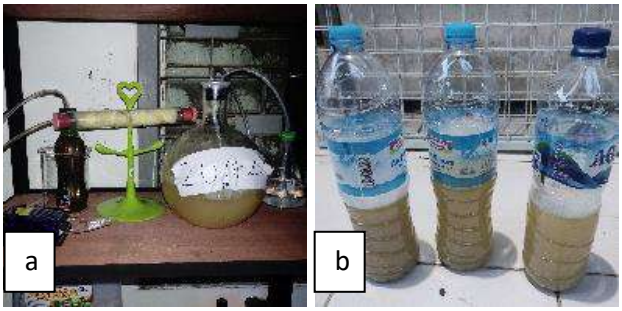


Figure 6. 6. Results of *Bacillus* sp. bacteria propagation, a) Results of propagation in fermenters, b) *Bacillus* sp. bacteria harvest results (Source: Personal documentation).

6.5 Results of Fruit Fly Population Observations

Observations of the trapped fruit fly population were observed every day at 08.00 and 12.00 for 2 weeks. Trapped flies were then counted, recorded, and collected in plastic containers. The results of observations of the accumulated fruit fly population are presented in tabular form.

Table 6. 3 Results of Fruit Fly Population Observations

Date	Observation Time	Methyl Eugenol					Basil					Selasih				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
05/01/2022	08.00	221	270	93	133	102	0	0	0	0	0	4	35	10	0	13
	12.00	52	81	30	47	87	0	0	0	0	0	2	13	3	0	20
06/01/2022	08.00	10	60	24	16	94	0	0	0	0	0	0	1	0	0	3
	12.00	19	12	9	35	13	0	0	0	0	0	0	0	0	0	0
07/01/2022	08.00	47	8	11	66	52	0	0	0	0	0	8	4	0	0	0
	12.00	29	17	2	20	8	0	0	0	0	0	0	0	0	0	0
08/01/2022	08.00	41	5	48	29	99	0	0	0	0	0	0	0	0	0	0
	12.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11/01/2022	08.00	105	87	20	89	155	0	0	0	0	0	0	0	0	0	0

	12.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12/01/2022	08.00	24	52	48	6	65	0	0	0	0	0	0	0	0	0	0
	12.00	7	6	6	17	24	0	0	0	0	0	0	0	0	0	0
13/01/2022	08.00	5	2	3	1	23	0	0	0	0	0	0	0	0	0	0
	12.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14/01/2022	08.00	9	4	2	6	20	0	0	0	0	0	0	0	0	0	0
	12.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15/01/2022	08.00	5	2	3	19	19	0	0	0	0	0	0	0	0	0	0
	12.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17/01/2022	08.00	13	3	21	23	10	0	0	0	0	0	0	0	0	0	0
	12.00	7	0	7	4	7	0	0	0	0	0	0	0	0	0	0
18/01/2022	08.00	4	3	3	11	38	1	0	0	0	0	0	0	0	0	0
	12.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19/01/2022	08.00	8	36	7	16	63	0	0	0	0	0	0	0	0	0	0
	12.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20/01/2022	08.00	2	4	38	10	48	0	0	0	0	0	0	0	0	0	0
	12.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total tiap jenis				3110					0				116			

The methyl eugenol attractant trap trapped many fruit flies at the beginning of the observation, but towards the end of the observation there was a decrease in the number of trapped populations. This trap was the trap that attracted the most fruit flies with a total of 3110 fruit flies. This is because the range of methyl eugenol is quite far reaching hundreds of meters, the strength of the aroma produced by methyl eugenol is very strong and easily captured by fruit fly sensors. This opinion is in accordance with Algifani et al. (2021) that the range of methyl eugenol is hundreds or even thousands of meters because it is volatile. The use of methyl eugenol as a fruit fly trap is one way to control environmentally friendly, methyl eugenol leaves no residue.

The basil attractant-based fruit fly trap had a total of 116 fruit flies trapped. The basil traps were only effective at the beginning of the observation. In the middle to the end of the observation, the trapped population decreased and even no fruit flies were trapped. Basil is one of the plants that has a methyl eugenol compound, basil leaves (*Ocimum basilicum* L.) have a methyl eugenol compound content of 77.9% (Kardinan et al., 2009). The low content of methyl eugenol and also the not

too strong aroma of the leaves can be the cause of the lack of effectiveness of basil traps.

Traps based on basil attractant based on the observation results there were no fruit flies trapped. Basil has a kinship with basil so that basil leaves can also produce compounds that are attractants. According to Singkhornart et al. (2009) the amount of methyl eugenol content in basil leaves is 52.27%, this compound functions as an attractant for fruit flies to enter the trap. The content of methyl eugenol in basil leaves is lower than that of basil leaves, so the ability to trap fruit flies is also lower than that of basil leaves.

VII. CONCLUSIONS AND SUGGESTIONS

7.1 CONCLUSIONS

Based on the Kuliah Kerja Profesi (KKP) activities that have been carried out at the Laboratory of Biological Agents UPT PTPH East Java with the main study "Exploration, Identification and Propagation of Antagonistic Bacteria *Bacillus* sp. as Biological Control Agents at UPT PTPH East Java". can be drawn conclusions.:

1. *Bacillus* sp. bacteria can be found in the rhizosphere soil of bamboo and tomatoes in Made Village, Sambikerep District, Surabaya City on January 10, 2022.
2. Biological Control Agents (BCA) *Bacillus* sp. can act as a biological control agent and also Plant Growth Promotion Rhizobacteria (PGPR).
3. Characteristics of *Bacillus* sp. bacteria seen from colony morphology have a circular colony shape, jagged colony edges (rhizoid), flat colony elevations, and dull colony appearance.
4. Of the five isolates suspected as *Bacillus* sp. are isolates BS 2 and BS 4

7.2 Suggestion

The need for several tests that must be done in order to know the genus and species of isolates precisely. In every process, sterilization must be maintained to prevent contamination and facilitate the identification process.

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ATTACHMENT

ATTACHMENT 1. Certificate of Carrying Out KKP Activities

PEMERINTAH PROVINSI JAWA TIMUR
DINAS PERTANIAN DAN KETAHANAN PANGAN
UPT PROTEKSI TANAMAN PANGAN DAN HORTIKULTURA
Jl. Pagesangan II No. 58 Telp. Fax 031- 8282970 Email : uptproteksi.jatim@gmail.com
Website : www.proteksijatim.com
SURABAYA
Kode Pos 60233

SURAT KETERANGAN TELAH SELESAI MELAKSANAKAN KEGIATAN KULIAH KERJA PROFESI
NOMOR : 009/98 /110.64/2022

Yang bertanda tangan dibawah ini :

Nama	: Denny Kurniawan, SP, MM
Jabatan	: Kepala UPT. Proteksi Tanaman Pangan Dan Hortikultura

Menerangkan bahwa,

Nama	: Fatimah Lailatus Sa'ádah
NPM	: 19025010083
Fakultas/Jurusan	: Pertanian/Agroteknologi
Universitas	: Universitas Pembangunan Nasional "Veteran" Jawa Timur

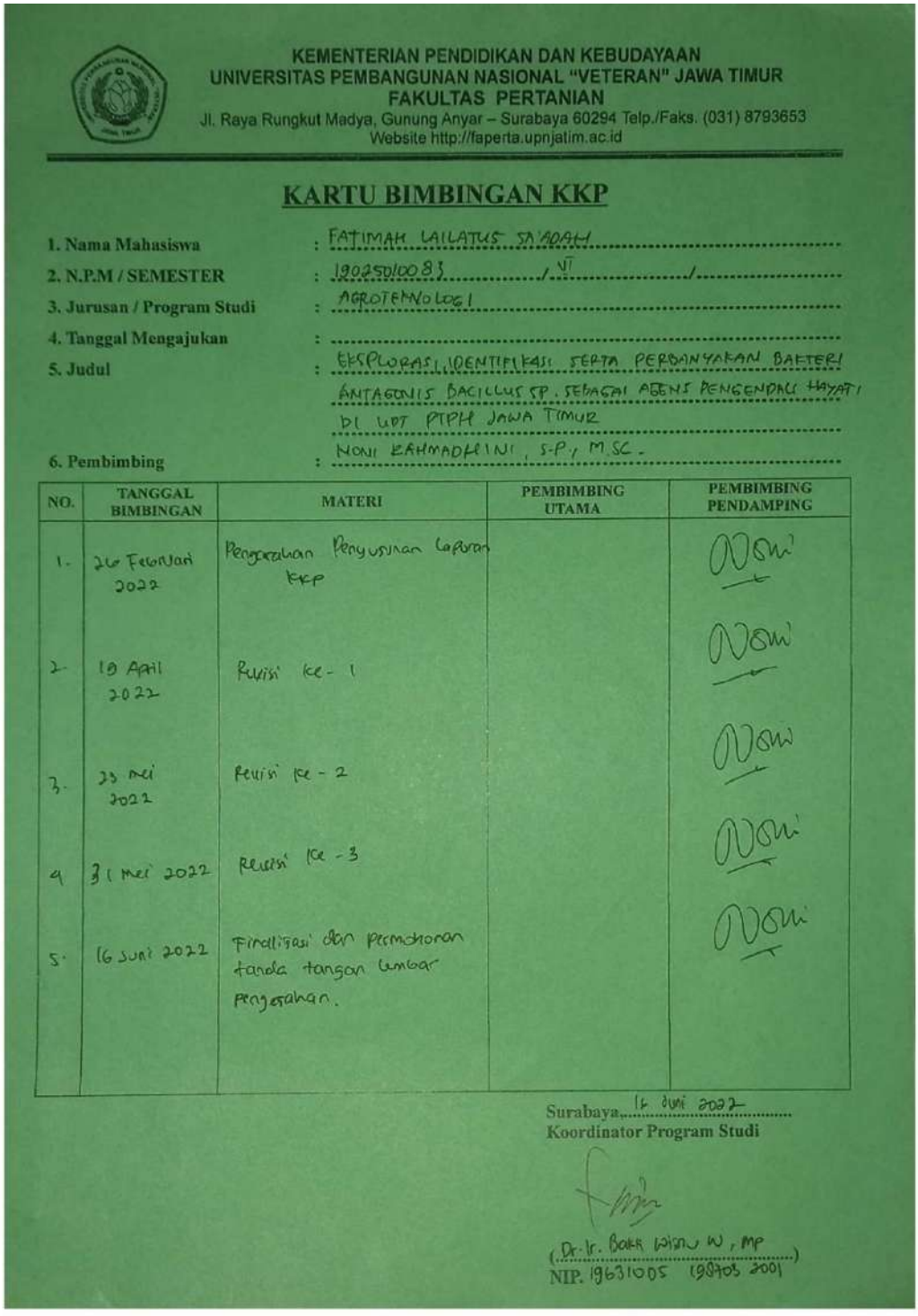
Telah menyelesaikan kegiatan Kuliah Kerja Profesi dengan jenis pekerjaan pengembangan agensi hayati yang dilaksanakan sejak tanggal 3 Januari 2022 sampai dengan 3 Februari 2022

Surabaya, 06 Juni 2022
KEPALA UPT PROTEKSI TANAMAN PANGAN DAN HORTIKULTURA

DENNY KURNIAWAN, SP . MM
Pembina
NIP. 19730922 199903 1 006

Dipindai dengan CamScanner

ATTACHMENT 2. KKP Guidance Card



KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN
UNIVERSITAS PEMBANGUNAN NASIONAL "VETERAN" JAWA TIMUR
FAKULTAS PERTANIAN
Jl. Raya Rungkut Madya, Gunung Anyar – Surabaya 60294 Telp./Faks. (031) 8793653
Website http://faperta.upnjatim.ac.id

KARTU BIMBINGAN KKP

1. Nama Mahasiswa : FATIMAH LAILATUS SHOLAH
2. N.P.M / SEMESTER : 19025010083 / VI
3. Jurusan / Program Studi : AGROTEKNOLOGI
4. Tanggal Mengajukan :
5. Judul : EKSPLORASI, IDENTIFIKASI SERTA PERSENYAWAAN BAKTERI ANTAGONIS BACILLUS SP. SEBAGAI AGENS PENGENDALI HAYATI DI UPT PTPH JAWA TIMUR
6. Pembimbing : NONI RAHMADHINI, S.P., M.SC.

NO.	TANGGAL BIMBINGAN	MATERI	PEMBIMBING UTAMA	PEMBIMBING PENDAMPING
1.	26 Februari 2022	Pengarahan Penyusunan Laporan KKP		Noni
2.	19 April 2022	Revisi ke-1		Noni
3.	23 Mei 2022	Revisi ke-2		Noni
4.	31 Mei 2022	Revisi ke-3		Noni
5.	16 Juni 2022	Finalisasi dan permohonan tanda tangan lembar pengesahan.		Noni

Surabaya, 16 Juni 2022
Koordinator Program Studi

(Dr. Ir. Bakti Wisnu W., MP)
NIP. 19631005 199103 2001

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ATTACHMENT 3. Monitoring and Guidance Card for KKP Students

KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN
UNIVERSITAS PEMBANGUNAN NASIONAL "VETERAN" JAWA TIMUR
FAKULTAS PERTANIAN
Jl. Raya Rungkut Madya, Gunung Anyar – Surabaya 60294 Telp./Faks. (031) 8793653
Website http://faperta.upnjatim.ac.id

KARTU MONITORING DAN EVALUASI KEAKTIFAN MAHASISWA
KULIAH KERJA PROFESI / KKP (MAGANG)

1. Nama Mahasiswa / NPM : FATIMAH LAILATUS SA'ADAH / 1902501008
2. Program Studi / SMT : AGROTEKNOLOGI / VII /
3. Instansi / Perusahaan : UPT PROTEKSI TANAMAN PANGAN DAN HORTIKULTURA JAWA TIMUR
4. Judul KKP : EKSPLORASI, IDENTIFIKASI, SERTA PERBANYAKAN BAKTERI ANTAGONIS BACILLUS SUBTILIS SEBAGAI AGENS PENGENDALI HAYATI DI UPT PTPH.
5. Dosen Pembimbing : NONI RAHMADHINI, S.P., M.Sc.

No.	Tanggal	Kegiatan	Tanda Tangan	Nilai
1.	03/01/22	orientasi dan teknik sterilisasi		9,2
2.	04/01/22	Sterilisasi bahan dan perangkap lalat buah		9,3
3.	05/01/22	Pembuatan dan sterilisasi media PDA organik & PDA		9,1
4.	06/01/22	Pembuatan dan sterilisasi media Kelman's, NA dan Kings' B		9,4
5.	07/01/22	pembuatan dan sterilisasi media PDA organik, dan budi daya tanaman		9,1
6.	08/01/22	Budidaya tanaman, mengencerkan dan menuang media ke cawan petri		9,3
7.	10/01/22	Eksplorasi tanah, sporulasi media tusuk gigi dan PDA, mengeringanginkan tanah		9,2
8.	11/01/22	Dinamika populasi lalat buah, pengenceran sampel tanah metode streak plate dan pour plate.		9,3
9.	12/01/22	Pembuatan media TZC 1%, memperbanyak isolat jamur, dan pengenceran bakteri		9,3
10.	13/01/22	pengenceran media dan pengenceran bakteri		9,5
11.	14/01/22	uji KOH 3%, uji virulensi, uji pigmentasi, uji soft rot		9,5
12.	15/01/22	materi Training of Trainer dan membuat MOL rebung, bonggol pisang dan tape		9,1
13.	17/01/22	membuat MOL rebung, baiting ulat hongkong, baiting nasi, identifikasi hasil uji bakteri		9,2
14.	18/01/22	maintenance ulat hongkong dan uji hipersensitif pada tanaman tembakau		9,1
15.	19/01/22	Identifikasi bakteri dan jamur, membuat media padat beras jagung, membuat media cair kedelai		9,4

Pembimbing lapang menanda tangani serta menilai (score 1 s/d 10), dan memberi saran :
* Tingkatkan semangat belajar karena dengan ilmu segala cita cita akan mudah diwujudkan
* Banggalah dengan diri ini sendiri dengan mengolah multitalenta

Surabaya, 03 Februari 2022

Koordinator Program Studi

Dr. Ir. Bakti Wisnu, W. MP.
NIP. 19651005 198703 2001

Pembimbing Lapang
(Instansi/Perusahaan)

(SUHARTO SP, MMA)

Dipindai dengan CamScanner

KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN
UNIVERSITAS PEMBANGUNAN NASIONAL "VETERAN" JAWA TIMUR
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Website http://faperta.upnjatim.ac.id

KARTU MONITORING DAN EVALUASI KEAKTIFAN MAHASISWA KULIAH KERJA PROFESI / KKP (MAGANG)

1. Nama Mahasiswa / NPM : FATIMAH LAILATUS SA'ADAH / 19025010083
2. Program Studi / SMT : AGROTEKNOLOGI / VI
3. Instansi / Perusahaan : UPT PROTEKSI TANAMAN PANGAN DAN HORTIKULTURA JAWA TIMUR
4. Judul KKP : EKSPLORASI, IDENTIFIKASI, SERTA PERBANYAKAN BAKTERI ANTAGONIS BACILLUS SUBTILIS SEBAGAI AGENS PENGENDALI HAYATI DI UPT PTPH
5. Dosen Pembimbing : NONI RAHMADHINI, S.P., M. Sc.

No.	Tanggal	Kegiatan	Tanda Tangan	Nilai
1.	20/01/22	Perbanyakan Trichoderma pada media padat jagung dan perbanyakan PF pada media cair kedelai		9,2
2.	21/01/22	Pembuatan dan sterilisasi media PDA, NA, King's B		9,4
3.	24/01/22	Pembukaan acara PELAGIAT 2022. Pre-test, materi APH dan Materi bakteri antagonis		9,3
4.	25/01/22	Isolasi bakteri dan identifikasi cendawan		9,3
5.	26/01/22	Uji Media selektif, uji KOH 3%, Uji Virulensi, uji soft rot, dan uji hipersensitif.		9,5
6.	27/01/22	Materi bakteri ular hongkong, pengamatan hasil pengujian bakteri		9,4
7.	28/01/22	Distasi umum, penutupan acara PELAGIAT 2022		9,2
8.	31/01/22	Eksplorasi sendiri.		9,2
9.	02/02/22	Panen hasil bakteri jamur, Antagonisme PF dan BS, Identifikasi jamur.		9,2
10.	03/02/22	Materi kewirausahaan, memberesi alat laboratorium.		9,3
11.				
12.				
13.				
14.				
15.				

Pembimbing lapang menanda tangani serta menilai (score 1 s/d 10),dan memberi saran :

.....

.....

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Surabaya, 03 Februari 2022

Koordinator Program Studi

(Dr. Ir. Bain Wiarti, W. MP)
NIP. 19631505 198705 2001

Pembimbing Lapang
(Instansi/Perusahaan)

(SUHARTO SP, MMA)