Moler Disease Control in Shallots using Botanical pesticides Jengkol Peel powder and Its Impact on Microbial Biodiversity in Peatlands

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Moler Disease Control in Shallots using Botanical pesticides Jengkol Peel powder and Its Impact on Microbial Biodiversity in Peatlands

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ABSTRACT. Chemical pesticides are still used to control Moler disease on shallots. The impact has the potential to kill macro species as well as non-target bacteria. Several prior research has shown that botanical pesticides can suppress plant-disturbing organisms, but data on their impact on beneficial microbes is still limited. Hence, the impact of applying jengkol peel to control the primary disease of shallots and its impact on non-target organisms in peatlands was investigated in this study. The study was carried out in Landasan Ulin, South Kalimantan, from April to November 2021. Treatments were t0 (negative control), t1 (positive control, fungicide), t2 (0.125 kg/ha jengkol peel powder), t3 (0.25 kg/ha jengkol peel powder), and t4 (0.375 kg/ha jengkol peel powder). The parameters observed were the intensity of moler disease, the components of shallot production, species diversity, species richness, the evenness of microbial species, and the dominance index. The results showed that the application of jengkol peel powder could suppress the attack of moler disease on shallots. Microbial diversity in shallot plantations treated with botanical pesticides and those not treated with botanical pesticides was similar, in the moderate range, as in shallot plantations treated with chemical pesticides. The species richness index, dominance index, and balance index had low-status values. The types of microbes found were *Trichoderma* sp., *Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Aeromonas* sp., *Corynebacterium* sp., *Enterobacter* sp. *Sphingomonas* sp., and *Penicillium* sp. The microbial population was affected by pesticide application. Plants that were not applied with botanical pesticides or chemical pesticides had fewer microbes. The application of botanical pesticides produced various impacts, the higher the dose, the lower the microbes in the shallot rhizosphere.

Keywords: species diversity, species richness, evenness of species, jengkol peel, microbes.

INTRODUCTION

Given the numerous negative consequences of synthetic pesticide application, including environmental degradation and ecosystem imbalances that might result in human poisoning, an environmentally benign method of controlling plant pests and diseases is required. Certain pesticides derived from plants have a detrimental effect on natural enemies and pollinators (Sharma *et al*., 2012; Lebuhn *et al*., 2013). This is because botanical pesticides contain active ingredients similar to those found in synthetic pesticides and thus have a detrimental effect on natural enemies and non-target pollinators. Some botanical pesticides are non-specific and toxic. Several studies have found that the application of three types of botanical pesticides (kepayang fruit extract, galam leaf, and *Chromolaena odorata*) has an effect on the diversity of arthropods (Meiyana *et al*., 2021) and microbes (Salamiah and Aidawati, 2021) in shallot plantations. The maximum diversity index of 2.03 was obtained when botanicalpesticides were combined with 1 ml/L kepayang fruit extract. In comparison, *Chromolaena odorata* fruit extract and galam leaf extract significantly reduced the population of microbes by 80.44 % and 75.26 %, respectively. Chirinyuh increased the population by 36.60% (Salamiah and Aidawati, 2021). For example, the nicotine from tobacco plant extracts is categorized by WHO as a group Ib toxin, which is very dangerous. Rotenone from Derris and Tephrosia species is classified as Class II. The natural rotenone and pyrethrum of chrysanthemum are highly toxic (Isman, 2013). Synthetic pesticides, also when applied, can kill other non-target organisms such as natural predators and parasites from pests and organisms that are beneficial to the health and balance of the ecosystem (McMichael, 2003; Zacharia, 2011). In Africa, pesticide use rates are very low compared to global markets (FAOSTAT, 2005).

The limited understanding of the impact of botanical pesticides on natural enemies and microorganisms makes botanical pesticides still recommended as an environmentally friendly alternative for controlling plant pests and diseases. However, there is insufficient information about the type, dose, and time interval for the proper administration of botanical pesticides. This highlights the importance of collecting information on the impacts of botanical pesticides on beneficial arthropods, particularly natural enemies and beneficial microorganisms in agriculture.

One of the natural materials that can be used as a botanical pesticides to control pests and plant diseases is jengkol peel waste. According (Nurussakinah, 2010), the chemical substances identified in jengkol peel include terpenoids, saponins, phenolic acids, and alkaloids. These substances are classified as secondary metabolites and have the potential to protect plants from pests and diseases. The tannin and flavonoid components of jengkol peel are just as protective against pests and illnesses as tannins found in woody plants and herbs. Jengkol peel has the potential to be utilized as a biopesticide due to the presence of these tannins (Nurussakinah, 2010). Therefore, this study will

investigate the effects of jengkol peel in suppressing primary shallot infections and their effect on non-target species (beneficial microorganisms) in shallot plantations on peatlands.

RESEARCH MATERIALS AND METHOD

The research was carried out from April to November 2021 at the Phytopathology Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru, and Tegal Arum Village, Landasan Ulin, Banjarbaru, South Kalimantan, Indonesia.

Research Method

The study used an environmental design, randomized block design with one factor: the administration of 3 doses of jengkol peel powder(0.125 kg/ha, 0.25 kg/ha, and 0.375 kg/ha) plus two controls (positive and negative controls). *Fusarium oxysporum* was inoculated at a density of 10^6 spora/m. The factors tested were: t0 (negative control) = *Fusarium oxysporum* inoculation; t1 (positive control) = Inoculation of *Fusarium oxysporum* + Fungicide (Benomil); t2 = Jengkol peel powder0.125 kg/ha + *Fusarium oxysporum* inoculation; t3 = Jengkol peel powder0.25 kg/ha + *Fusarium oxysporum* inoculation; and t4 = Jengkol peel powder0.375 kg/ha + *Fusarium oxysporum* inoculation. The shallot seeds used were the Bima Brebes variety tubers planted at a spacing of 20 cm x 20 cm.

Botanical pesticides were applied three weeks after planting (WAP). Subsequent applications were made at weekly intervals for a total of seven. Maintenance of plants referred to applying basic NPK fertilizer, irrigation, and mechanical removal of nuisance plants.

Microbial Isolation and Purification

Isolation of Non Heat Resistant Bacteria

A total of 10 g of soil was taken from the rhizosphere of shallot plantations and put into an Erlenmeyer containing 90 ml of distilled water, and then shaken for 15 minutes at 150 rpm. The solution was diluted with distilled water to 10^{-7}. A total of 0.5 ml of the 10^{-7} dilution was spread on King's B medium, incubated, and purified .

Isolastion of Heat Resistance Bacteria

Isolation of heat-resistant bacteria was carried out by shaking the soil obtained from the rhizosphere of shallot plantations as much as 10 g, which had been dissolved in 90 ml of distilled water for 15 minutes, then diluted to 10^{-7}. The 10^{-7} dilution results were put into a glass bottle and heated at 80°C for 30 minutes, after which 0.5 ml was taken and spread on NA media. The NA medium containing the bacterial suspension was incubated at 27° C for 48 hours and then purified (Schaad N *et al*., 2000)

Isolation of fungi from shallot rhizosphere

The soil sample in the rhizosphere of shallot plantations was weighed as much as 10 g, then suspended in 90 ml of distilled water and shaken for 15 minutes at 150 rpm. Following that, 1 ml of the suspension was added to 9 ml of distilled water and homogenized using a vortex. The dilution was carried out until 10^{-5} dilutions were obtained. A total of 0.5 ml of the dilution results was transferred to a PDA medium, cultured, and purified.

Microbial Identification

Fungus Identification

Identification used pure isolates of fungi from healthy shallot plants and sterile water. The PDA media was cut into a rectangle using a spatula and placed on a glass slide. The isolates to be identified were taken using an ent needle and placed at the end of the rectangular piece of PDA media under the slide glass and then covered with a cover glass. The tissue under the slide glass was moistened in a petri dish using a dropper. Then the petri dish was closed and wrapped with a cling wrap. The growth of spores was observed under a microscope and identified morphologically.

Bacteria Identification

Bacterial identification was performed by evaluating the colony's shape, optical characteristics, colony color, colony size, the shape of the colony's edge/periphery, and the gram of bacteria. Gram bacteria were identified using pure bacterial isolates from healthy shallots, sterile water, and 3% potassium hydroxide (KOH). Gram testing is classified into two major groups, namely gram-positive and gram-negative. The test was conducted by staking one ose of bacterial isolate in a sterile manner and placing it on a glass slide that had been treated with one drop of 3% KOH solution. The bacterial mass was mixed, and the changes were observed. The mucus formation indicated that the bacteria were Gram-negative, whereas the mass of bacteria that did not form mucus indicated that the bacteria were Gram-positive.

Following gram staining, a test was conducted to classify the bacteria into fluorescent and non-fluorescent bacteria. The incubated media was inspected under UV light to observe the bacteria's luminescence. Colonies on each medium were observed, photographed, and identified by comparing the existing literature from books and other sources related to identification guides.

Observation

1. Intensity of Moler Disease

The disease intensity was observed every week from the appearance of symptoms until before harvest. Based on the systemic nature of the disease, the intensity of the disease was calculated by the formula (Wiyatiningsih *et al*., 2009):

$$\mathbf{I = \frac{a}{b} x\ 100\%}$$

Notes: I: Disease intensity

a: Number of diseased plants

b: Total number of plants

2. Types of microbes. Microbial species identification was carried out to the microbes found in the rhizosphere of shallot plantations treated with botanical pesticides.

3. Microbial Population. The number of colonies was calculated using a colony counter. The petri dish was placed upside down, or the petri dish lid was opened and placed on the colony counter. Calculations were carried out with the help of thick lines on a checkerboard patterned base. The total population was determined by counting the colonies in the top row, then in the bottom row from left to right, and so on. The formula used is as follows:

$$Cc = \frac{Colony\ number}{FX\ diluttion\ .\ ml\ suspension}$$

4. Diversity, richness, dominance, and evenness index of species. The diversity of species was calculated based on Shannon-Wiener diversity index (H'). The richness of species was calculated based on Margalef indeks ($\boldsymbol{R}$) (1958). The dominance was calculated based on Simpson dominance index ($\boldsymbol{D}$).The type evenness was calculated based on Shannon-Wiener's evenness Index ($\boldsymbol{E}$) (Krebs 1999). The index equation is as follows:

$$H' = -\sum_{i=1}^{s}(pi)(ln\,pi) \qquad R = \frac{(S-1)}{In\ N} \qquad \boldsymbol{D} = \sum_{i=1}^{s}(\boldsymbol{ni}/\boldsymbol{N})^2 \qquad E = \frac{H'}{ln\ S}$$

Note:
$pi =$ ni/N
ni = the i-th individuals in the species
N = The total number of individuals of all types of species
S = The number of types of species

Data Analysis

Data collection and identification were processed using Microsoft Office Excel 2007. Next, the data was calculated based on the Shannon-Wiener species diversity index (Megurran, 1988), the Margalef species richness index (1958), the Simpson species dominance index and the Pielou species evenness index (1975).

RESULTS AND DISCUSSION

Chemical and botanical pesticides have varying effects on the intensity of moler disease infections, productivity, and microorganism populations in the rhizosphere of shallot plantations (Table 1).

Table 1. The correlation between the presence of microbes, the attack of moler disease, and the production of shallot in peatland

Treatments	Species	disease intensity (%)	the yield components			
			Σ bulbs/ clump	bulbs diameter (cm)	wet weight (g)	dry weight (g)
t0	*Trichoderma* sp. *Aspergillus* sp. *Trichoderma* sp. *Aeromonas* sp *Sphingomonas* sp	87.5[b]	6.8[a]	1.61[a]	25.45[a]	15.08[a]
t1	*Aspergillus* sp. *Fusarium* sp. *Trichoderma* sp.	56.3[a]	55.5[b]	2.20[a]	719.85[b]	336.00[b]
t2	*Fusarium* sp. *Trichoderma* sp. *Corynebacterium* sp *Enterobacter* sp. *Sphingomonas* sp	86.3[b]	8.8[a]	1.64[a]	57.13[a]	26.98[ab]
t3	*Aspergillus* sp. *Trichoderma* sp. *Aeromonas* sp *Corynebacterium* sp	70.0[ab]	15.0[a]	2.05[a]	94.65[a]	58.50[a]
t4	*Aspergillus* sp. *Penicillium* sp. *Fusarium* sp. *Scopulariopsis* sp. *Curvularia* sp. *Aeromonas* sp *Corynebacterium* sp	60.0[a]	11.8[a]	1.73[a]	70.78[a]	39.88[a]

Description: t = treatment code, t0 = Negative Control (*F. oxysporum* inoculation), t1 = Positive control (*F. oxysporum* + Benomyl fungicide), t2 = Jengkol Skin Powder 0, 125 kg/ha + *F. oxysporum* inoculation, t3 = Jengkol peel powder 0.25 kg/ha + *F. oxysporum* inoculation, t4 = Jengkol peel powder 0.375 kg/ha + *F. oxysporum* inoculation, The average value which has the same letter shows no significant difference according to the LSD test at a significant level of 5 %.

Table 1 shows that the dose of botanical pesticides given has an effect on the microbes in the rhizosphere of shallot plantations. The types of microbes found varied, but no single species dominated in each experimental plot.

The provision of botanical pesticides could reduce the intensity of moler disease. The higher the dose, the lower the moler disease attack. Applying 0.375 kg/ha of botanical pesticides resulted in the lowest moler attack intensity of 60.0 % (40% inhibition). Meanwhile, chemical pesticide application resulted in low intensity of moler attack, 56.3 %. It indicates that the secondary metabolites present in jengkol peel powderare equivalent to chemical pesticides. The phytochemical test conducted by (Nurussakinah, 2010) found that jengkol peel powdercontains terpenoids, saponins, phenolic acids, and alkaloids.

Siswandi (Siswandi *et al*., 2020) discovered that jengkol bark extract at a concentration of 90% inhibited *Fusarium oxysporum* at a rate of 78.43 %. Compared to the 10% jengkol peel powdertreatment and negative control, it had a very significant effect. It was discovered that jengkol bark extract was more effective than jengkol peel extract. However, fruit peel powderis better than

bark extract because fruit peel is a waste while the bark is not a waste. If excessive exploration is carried out, it will damage plant stems.

Shallot plants that were given botanical pesticides at a dose of 0.125 kg/ha and 0.25 kg/ha did not give a significantly different effect from plants that were not given pesticides (control plants). The types of microbes explored in the rhizosphere of shallot plantations were not positively correlated with a decrease in moler disease intensity and shallots production (Table 1). The application of botanical pesticides derived from jengkol peel extract, in addition to affecting the intensity of moler disease, also affects the yield components of shallots on the number of bulbs per clump, wet weight, and dry weight of shallots.

Soil is inhabited by various microscopic life forms such as bacteria, fungi, actinomycetes, protozoa, and algae. Bacteria are the most prevalent of these microbes, accounting for 95% of them. It has long been recognized that soil has a high concentration of bacteria, ranging from approximately 10^8 to 10^9 cells per gram of soil (Schoenborn *et al*., 2004). The number and types of bacteria found in different soils are influenced by soil conditions, including temperature, humidity, presence of salts and other chemicals, and the number and types of plants found in the soil. Additionally, bacteria are not equally distributed throughout the soil. It means that the concentration of bacteria found around plant roots (rhizosphere) is usually much higher than in other parts of the soil. It is due to the presence of nutrients including sugars, amino acids, organic acids, and other small molecules from plant root exudates which can account for up to one-third of the carbon fixed by plants (Glick, 2012) .

Most fungi-type microbes function as decomposers in peat soil because the microbes secrete extracellular enzymes such as cellulases, hemicellulases, and lignocellulose that can decompose organic matter. Microbial activity that decomposes organic matter determines the maturity level of peat because the saprophytic microbial properties depend on the environment and the organic matter of the substrate. (Rosita *et al*. 2014) found that on fibrous peatlands, six pure microbial isolates were found, namely *Aspergillus niger*, *Fusarium* sp., *Paecilomyces* sp., *Penicillium variabile*, *Pialophora* sp., and *Verticillium* sp. In hemic peat 10 pure isolates were found: *A. niger*, *Fusarium* sp., *Mortierella* sp., *Paecilomyces* sp., *Paecilomyces* sp., *Penicillium* sp., *Penicillium* sp., *Penicillium* sp., *Rhizopus* sp., and *T. harzianum*. Twelve pure isolates were discovered in sapric peat: *Fusarium* sp., *Paecilomyces* sp., *Paecilomyces* sp., *Penicillium* sp., *Penicillium* sp., *Penicillium* sp., *Penicillium* sp., *Penicillium* sp., *Penicillium* sp., *Penicillium* sp., *Pythium* sp., and *Trichoderma* sp..

The correlation between jengkol peel powderand different types of microorganisms, as well as the decline and growth in microorganism populations in shallot plantations on peatlands

The application of jengkol peel powderhad various effects on the population of microorganisms in the soil (Table 2).

Table 2. Total microorganisms before and after application of jengkol peel powderon shallot plantations in peatlands, South Kalimantan

Treatments	before application ($\times 10^{10}$)	after application ($\times 10^{10}$)	increase (+) or decrease (-)of microorganisms application effect (%)
t0	360	128	-47,54
t1	575	664	7,18
t2	154	455	49,43
t3	450	41	-83,30
t4	565	220	-43,95

Description: t = treatment code, t0 = Negative Control (*F. oxysporum* inoculation), t1 = Positive control (*F. oxysporum* + Benomyl fungicide), t2 = Jengkol Skin Powder 0, 125 kg/ha + *F. oxysporum* inoculation, t3 = Jengkol peel powder 0.25 kg/ha + *F. oxysporum* inoculation, t4 = Jengkol peel powder 0.375 kg/ha + *F. oxysporum* inoculation

There was a decrease in microbes' population by 47.54% in shallot plantations without botanical pesticides. While the application of chemical pesticides increased the microbial population by 7.18%, this increase was not as high as the administration of botanical pesticides with a dose of 0.125 kg/ha. When the dose of botanical pesticides was increased to 0.25 kg/ha and 0.375 kg/ha, the microbial population again experienced a considerable decline by 83.30% and 43.95%, respectively.

This research has succeeded in collecting two groups of microbes, namely bacteria and fungi. Five types of bacteria were found: *Sphinggomonas* sp., *Enterobacteria* sp., *Corynebacterium* sp., *Bacillus* sp., and *Aeromonas* sp. (Table 3); five types of fungi, namely *Trichoderma* sp., *Fusarium* spp., Mucor spp., *Aspergillus* sp., and *Penicillium* sp. from all research plots.

Table 3. Population and types of bacteria isolated in shallot plantations applied with jengkol peel powder

Treatments	Total population	species	Gram test
t0	127×10^{10} *cfu*	*Sphingomonas* sp.	Positive
t1	584×10^{10} *cfu*	*Bacillus* sp. *Aeromonas* sp.	Positive
t2	438×10^{10} *cfu*	*Bacillus* sp. *Aeromonas* sp.	Positive
t3	37×10^{10} *cfu*	*Corynebacterium* sp. *Enterobacteria* sp.	Positive

t4		216×10^{10} *cfu*	*Bacillus* sp.	Positive

Description: t = treatment code, t0 = Negative Control (*F. oxysporum* inoculation), t1 = Positive control (*F. oxysporum* + Benomyl fungicide), t2 = Jengkol Skin Powder 0, 125 kg/ha + *F. oxysporum* inoculation, t3 = Jengkol peel powder 0.25 kg/ha + *F. oxysporum* inoculation, t4 = Jengkol peel powder 0.375 kg/ha + *F. oxysporum* inoculation

Table 4 below summarizes the characteristics of each discovered bacterial genus.

Table 4. Characteristics of five types of bacteria in each research plot.

No.	Isolat code	Colony shape	optical properties	colony color	texture	Genus
1	T1A	spherical	opaque	white	rough	*Aeromonas* sp.
3	T3A	spherical	opaque	white	rough	*Aeromonas* sp.
4	T40A	spherical	slightly transparent	slightly yellowish	smooth	*Corynebacterium* sp.
5	T35A	spherical	slightly transparent	milky white	smooth	*Enterobacter* sp.
6	T421	spherical	translucent	milky white	smooth	*Enterobacter* sp.
7	T010A	spherical	slightly transparent	slightly yellowish	smooth	*Sphingomonas* sp.
8	T411	spherical	opaque	orange pink	smooth	*Bacillus* sp.
9	T231	spherical	opaque	white	dry surface wrinkle	*Bacillus* sp.
10	BT25A	spherical	opaque	white	rough	*Bacillus* sp.
11	BT210 A	spherical	opaque	white	rough	*Bacillus* sp.
12	BT410 F	spherical	transparent	yellow	smooth	*Bacillus* sp.
13	T32	spherical	opaque	cream	smooth	*Bacillus* sp.
14	T0B	spherical	opaque	cream	smooth	*Bacillus* sp.
15	T1D	spherical	opaque	white	rough	*Bacillus* sp.
16	T2B	spherical	opaque	white	rough	*Bacillus* sp.

As shown in Table 1, Table 3, and Table 4, this research has succeeded in collecting five types of bacteria, namely *Sphinggomonas* sp., *Enterobacteria*, *Corynebacterium* sp., *Bacillus* sp., and *Aeromonas* sp. with the following properties.

***Bacillus* sp**. *Bacillus* sp. isolated from shallot plantations in this study showed spherical colonies, opaque optical properties, and slight variations in colony color; some were cream, and some were white. The cream-colored colonies had a smooth texture, while the white colonies had a rough texture. *Bacillus* sp. is a PGPR (Plant Growth Promoting Rhizobacteria) that has the ability to stimulate plant growth and production. *Bacillus* can to fix N_2, dissolve phosphate, and synthesize phytohormones IAA (Indole 3- Acetic Acid). *Bacillus* sp. as PGPR can increase the availability of low nitrogen and phosphate nutrients in paddy fields. Loss of nitrogen nutrients generally occurs due to leaching and runoff in flooded soils and low availability of phosphate due to complex binding to Al^{2+} and Fe^{2+} elements. Nitrogen and phosphate availability in paddy fields can be improved by applying *Bacillus* sp. biofertilizers.

The nitrogenase test using the Acetylene Reduction Assay (ARA) method found that *Bacillus* had a nitrogenase activity of 0.05685 m ml^{-1} $hour^{-1}$ and dissolving phosphate from a $Ca_3(PO_4)^2$ with a Dissolution Index (IP) of phosphate 2.6. Phosphate solubility is caused by acetic acid, oxalic acid, lactic acid, and malic acid produced by *Bacillus* sp.. Biological fertilizers *Bacillus* sp. can reduce 25% usage of NPK fertilizer in lowland rice production (Husna *et al*., 2019).

Bacillus sp. was one of the microorganisms discovered in the peatlands where the research was conducted. Based on the relationship between the types of microbes found and the intensity of disease attacks on shallot plantations (Table 1), it can be concluded that the presence of *Bacillus* sp does not play its function as a PGPR, namely as a bioprotectant, because the intensity of molar attack remains relatively high. The role of bacteria as PGPR will increase if it is in a consortium with several other antagonistic bacteria. (Husen *et al*., 2020) discovered four bacterial consortia, each containing three bacterial strains that had complete or complementary phenotypic properties. Not only can PGPR promote plant growth, but it also possesses the ability to biologically manage plant-disturbing organisms (Kanjanasopa *et al*., 2021).

***Sphingomonas* sp.** Characteristics of *Sphingomonas* sp. isolated from shallot plantations showed round colonies, slightly transparent optical properties, and a slightly yellowish color with a smooth texture.

Sphingomonas have been isolated from various anthropogenic contaminated environments—including terrestrial (subsurface) soil (Mueller *et al*., 1990; Adkins, 1999; Momma *et al*., 1999; Bastiaens *et al*., 2000; Pinyakong *et al*., 2000; Sørensen *et al*., 2001; Cassidy *et al*., 1999; Feng *et al*., 1997; Lloyd_Jones and Lau, 1997; Meyer *et al*., 1999) and were found to have a unique ability to degrade various pollutants, including azo dyes (Stolz, 1999), chlorinated phenols (Cassidy *et al*., 1999; Crawford and Ederer, 1999), dibenzofurans (Keim *et al*., 1999; Wittich *et al*., 1999), insecticides (Nagata *et al*., 1999), and herbicides (Adkins, 1999; Kohler, 1999). In addition, *Sphingomonas* strains are frequently isolated as decomposers of polycyclic aromatic hydrocarbons (PAHs) from contaminated soil (Mueller *et al*., 1990; Khan *et al*., 1996; Bastiaens *et al*., 2000;

Pinyakong *et al*., 2000). PAHs are highly hydrophobic toxic chemicals with low solubility in water, making them less available for natural bacterial degradation. Due to their ubiquitous distribution and diverse catabolic ability against persistent organic pollutants, *Sphingomonas* strains can be considered important biocatalysts for soil bioremediation.

***Enterobacteria* sp**. *Enterobacteriaceae* sp. isolated from shallot plantations showed spherical colonies, slightly transparent and translucent optical properties, milky white colony color, and smooth texture.

Enterobacteriaceae are facultative aerobic microbes (Kersters *et al*., 2006) that anaerobically degrade sugars into acetate, CO_2, H_2, formate, lactate, succinate, and ethanol (White, 2007), supplemented by various carbon sources (Ku¨sel *et al*., 2008). These findings suggest that (Daniela *et al*., 2009) facultative aerobes compete for dissolved organic carbon in the anoxic microzone of aerated soils (Daniela *et al*., 2009)under anoxic conditions similar to those formed in anoxic slurries. Both facultative aerobes and obligate anaerobes from methanogenic soils mainly assimilate [13C-U]-glucose under anoxic conditions .(Hamberger *et al*. 2008; Wu¨st *et al*., 2009)

***Corynebacterium* sp.** *Corynebacterium* sp. isolated from shallot plantations showed round colonies, slightly transparent optical properties, slightly yellowish colony color, and smooth texture.

Corynebacterium is a genus of Gram-positive and mostly aerobic rod-shaped bacteria. One of the *Corynebacterium* species is *C. glutamicum* which is gram-positive, a non-motile bacterium belonging to the phylum Actinobacteria. *C. glutamicum* is a glutamate-producing bacterium (Kinoshita *et al*., 2005). Due to its great glutamate and lysine production capacity, it has become a widely used organism in biotechnology (Kelle *et al*.; Kumagai *et al*., 2000; Kimura *et al*., 2013). Apart from glucose, it can utilize various other carbon sources such as fructose, sucrose, gluconate, acetate, propionate, pyruvate, L-lactate, ethanol, glutamic amino acids, and serine (Kalinowski *et al*.; Netzer *et al*.; Cocaign *et al*., 1993; Claes *et al*., 2002). *C. glutamicum* has a potential gene that allows it to catabolize Neu5Ac as the sole carbon source (Holder *et al*. 2011)

***Aeromonas* sp.** *Aeromonas* sp. isolated from shallot plantations showed spherical colonies, opaque optical properties, white colony color, and rough texture.

Today, the genus *Aeromonas* contains 24 legally published species. Bergey's Manual of Systematic Bacteriology, Second Edition (Bergey's) permits far less (Martin-Carnahan and Joseph, 2005). *Aeromonas sp*. is halophilic, does not form spores, gram-negative, and is widely distributed in soil, food, and aquatic environments. The three main pathogenic species of this genus are *Aeromonas hydrophila*, *A. caviae*, and *A. sobria* (Tomas). These biochemically distinct species have now been further subdivided into DNA hybridization groups (Igbinosa *et al*. 2012). The characteristics of each genus of fungi found in this study are presented in Table 5.

Table 5. The results of macroscopic and microscopic identification of fungi on shallot plantations applied with botanical pesticides

No	Isolat code	Macroscopis		Microscopis			Genus
		Colony color	Colony shape and texture	Conidiophores / hyphae	Conidia/spore	Fialid	
1.	CT431	green	sperical	branched	round	Erect,short	*Trichoderma* spp.
2.	CT410B CT45	dark green	rounded, circular smooth and embossed surface	cylindrical	round	erect	*Aspergillus* spp.
3.	CT231 CT311	white	Round, smooth and slippery surface, cotton-like surface	Branched, insulated	Ovoid, crescent	single	*Fusarium* spp.
4.	CT0	pale white to gray	Grew upward, thinly fibrous	unbranched, not insulated, transparent	round, transparent	single	*Mucor* spp.
5.	CT35A	greenish-white then turned bottle green	Irregular	Branched, hyaline	round	Erect	*Penicillium* sp.

The presence of microbes is influenced by environmental factors in which they are found. Environmental elements affecting peat soil decomposition include soil acidity (pH), soil temperature, soil moisture, vegetation, and the level of decomposition of peat soil. (Sutedjo *et al*., 1991) stated that soil acidity is a factor controlling the type of microbial diversity. The acidity of the environment (pH of the substrate) is critical for microbial development since certain enzymes are only active at a specific pH. The measurement showed that the soil pH was in the range of 3.55-4.11. Generally, microbes can grow at pH below 7 (Gandjar *et al*., 2006). Similar results were also reported by (Waluyo), that microbes are aerobic organisms and have a wide pH range, ranging from 2.0 to 8.5.

As shown in Table 1 and Table 5, this research succeeded in collecting five types of fungi, namely *Trichoderma* sp., *Fusarium* sp., *Aspergillus* sp., *Scopulariopsis* sp. and *Penicillium* sp. with the following properties:

Trichoderma **sp.** has green colonies, spherical shape, branched conidiophores, spherical conidia with erect phialides position and short size, belonging to the ascomycetes class, antagonistic to plant diseases because they have antifungal activity. *Trichoderma* occurs naturally in forest soils, agricultural fields, and on woody substrates.

Several studies have found that *Trichoderma* is one of the fungi that can act as a biocontrol agent due to its antagonistic nature toward other fungi, particularly pathogenic fungi. The antagonistic activity may include competition, parasitism, predation, or the formation of toxins such as antibiotics. This biocontrol agent can be extracted from *Trichoderma* and utilized to treat crop damage caused by pathogens for biotechnological applications.

As a biological agent, *Trichoderma* has the potential to maintain plant resistance systems, for example, from attack by pathogens such as pathogenic fungi. Because the intensity of the moler disease attack is still high in the shallot plantation under study, the presence of *Trichoderma* has not yet functioned as an antagonistic agent. This is most likely because the ability and mechanism by which *Trichoderma* inhibits pathogen growth vary between species. This ability gap is influenced by ecological factors that affect metabolite production. *Trichoderma* produces volatile and non-volatile metabolites.

The nutritional composition of the media affects the outcomes of these metabolites. *Trichoderma* produces chitinolytic protein and chitinase enzymes when in chitin-rich conditions. This enzyme contributes to the improvement of biocontrol actions against chitin-containing diseases.

***Aspergillus* sp.** *Aspergillus* sp. isolates isolated from shallot plantations showed characteristic dark green colony color, rounded colony shape, circular colony texture, smooth and embossed surface, cylindrical conidiophores, spherical conidia, and erect phialides. This genus, *Aspergillus*, has septate hyphae and hyaline. It is supported by (Noerfitryani and Hamzah, 2017), who reported that the macroscopic characteristics of the *Aspergillus* fungus on PDA media are that its surface is light green to dark green and black, had a flour-like texture, with microscopic characteristics, namely conidia spherical in shape, with septate hyphae and hyaline. According to (Isroi, 2008), *Aspergillus* sp. is a phosphate solubilizing fungus that has been proven to dissolve phosphate from poorly soluble sources. *Aspergillus* sp. also can dissolve insoluble inorganic phosphates by secreting organic acids (Saraswati *et al*., 2007). In addition, according to (Saraswati *et al*., 2007), *Aspergillus* sp. is capable of producing proteases that function in the transformation of organic nitrogen (in the form of protein) in the soil and other organic waste materials into inorganic N (NH_4^+), which the Kintamani Siamese citrus plant can utilize.

One of the *Aspergillus* species is *A. niger*. *A. niger* produces steroids (Lima *et al*., 2019). This species is a major source of citric acid and accounts for more than 99% of global citric acid production, or more than 1.4 million tonnes per year. *A. niger* is also commonly used to produce enzymes, including glucose oxidase, lysozyme, and lactase (Thom and Church, 1926).

Another *Aspergillus* species is *A. nidulans* (*Emericella nidulans*). *A. nidulans* is a pioneering organism to have its genome sequenced by researchers at the Broad Institute. In 2008, seven other *Aspergillus* species had their genomes sequenced: *A. niger* (two strains), industrially applicable *A. oryzae*, and *A. terreus*, and the pathogens *A. clavatus*, *A. fischerianus* (*Neosartorya fischeri*), *A. flavus*, and *A. fumigatus* (two strains). *A. fischerianus* is rarely pathogenic but is closely related to the common pathogen *A. fumigatus*. One of the reasons for sequencing the *A. fischerianus* genome was to have a better understanding of *A. fumigatus*' pathogenicity.

***Fusarium* sp.** *Fusarium* sp. isolates isolated from shallot plantations showed the characteristics of white colony color, round colony shape, smooth and slippery colony texture, branched, insulated cotton-like surface, ovoid-shaped conidiophores, and crescent-shaped conidia.

Fusarium is a soil saprophyte but can be pathogenic to plants. These molds can induce root rot and contribute to decomposition. t is in accordance with (Saragih, 2009), who found five dominant decomposers, namely *Fusarium* sp., *A. ochraceus*, *A. niger*, *Monascus rube*, and *Trichoderma* sp..

Most of the *Fusarium* species are economically important plant pathogens. *Fusarium* can be endophytic or saprophyte. As a pathogen, *Fusarium* causes various diseases in agricultural, horticultural, and forestry crops (Moore *et al*. 2001; Ploetz, 2001; Summerell *et al*. 2001; Burgess, 1981, Burgess *et al*., 1994). Over 81 commercially significant crops have been afflicted by at least one disease caused by the *Fusarium* fungus ((Leslie and Surnrnerell, 2006) *Fusarium* causes pre- and post-emergence damping-off (Palmero *et al*., 2009). *Fusarium* fungi are often found as endophytes in various plants in agricultural ecosystems (Kuldau and Yates, 2000; Leslie *et al*. 1990; Burgess, 1981). While the *Fusarium* fungus can infect interior plant tissues without causing symptoms, it can develop disease symptoms when plants are subjected to drought or other environmental stresses (Burgess, 1981).

***Mucor* spp.** The colonies were pale white to gray. The colonies grew upward. The surface of the colonies was thinly fibrous, conidiophores unbranched, conidia not insulated, round in shape, and transparent in color.

Mucor sp. is one type of mold found in the microbial isolation carried out in this study. Mold can grow on peat soil because of the substrate produced by wood containing lignin and cellulose. According to (Sennang *et al*., 2012), organic compounds such as lignin and cellulose provide energy and food for soil mould. Peat soil is a rich source of energy and food for soil molds. The deeper the peat, the lower the oxygen condition (Barchia, 2012). This is due to the low intensity of light that can penetrate the soil and the peat soil environment, which is generally always waterlogged. It is a factor that influences the presence of molds and how molds decompose peat organic materials. The more mature the peat, the more decomposing molds are discovered (Buckman and Brady, 1982). (Saragih, 2009) found two types of mold at the fibric peat maturity level (*Aspergillus* sp. and *Mucor* sp.), five types of mold at the hemic maturity level (*P. chrysogenum*, *Mucor* sp., *P. digitatum*, *Culvularia* sp., and *Penicillium* sp.), and four types of mold at sapric maturity level (*Aspergillus* sp. 1, *Aspergillus* sp. 2, *Fusarium* sp., and *P. chrysogenum*).

***Penicillium* sp.** Isolates of *Penicillium* sp. isolated in this study had the characteristics: at first, the colony's surface was greenish-white then turned bottle green, there were white cotton fibers, irregular colony shape and texture, branched conidiophores, hyaline conidia, and erect phialides. On the sixth day following incubation, colonies filled the Petri dish. Conidia walls are smooth. Conidiophores have smooth walls, branched conidiophores, and have metulae and phialides.

The genus *Penicillium* has septate hyphae and hyaline. It is consistent with the findings of (Anggraeni and Usman, 2015), who discovered that colonies of *Penicillium* sp. begin as white and then change turquoise, greenish-grey, olive-grey, and occasionally yellow or reddish. Meanwhile, the microscopic form of the fungus *Penicillium* sp. has hyaline hyphae, spherical unicellular conidia, and a set of phialides. According to reports, *Penicillium* sp. is capable of protecting plants against pathogen attack while simultaneously promoting plant development (Rozali, 2015). Additionally, *Penicillium* serves as a decomposer, contributing to soil fertility (Purwati and Hamidah, 2018). According to (Yuleli, 2009), *Penicillium* sp. is a soil microorganism whose job is to deliver nutrients by converting insoluble inorganic phosphate compounds into soluble forms (H_2PO_4 and HPO_4) that plants can absorb. Microbes with a high capacity for phosphorus (P) dissolution usually have a high capacity for potassium (K) dissolution too (Wulandari *et al*., 2013).

Application of synthetic pesticides and botanical pesticides to microbial biodiversity, species richness, dominance index, and microbial balance index in shallot plantations

The application of botanical pesticides has various effects on the diversity index, species richness index, dominance index, and microbial balance index in shallot plants (Table 6 and Table 7).

Table 6. The average diversity, species richness, dominance, and microbial balance index in shallot plants given the peel powder of jengkol fruit peel vegetable pesticide on peatlands.

Treatmens	Species Diversity Index (H′)	Species Richness Index (R)	Domination Index (D)	Microbial Balance Index (E)
t0	1.18	0.83	0.34	0.73
t1	1.23	0.94	0.43	-0.08
t2	1.65	1.32	0.23	-0.12
t3	1.22	1.67	0.45	-0.12
t4	1.67	2.23	0.27	0.91

Description: t = treatment code, t0 = Negative Control (*F. oxysporum* inoculation), t1 = Positive control (*F. oxysporum* + Benomyl fungicide), t2 = Jengkol Skin Powder 0, 125 kg/ha + *F. oxysporum* inoculation, t3 = Jengkol peel powder 0.25 kg/ha + *F. oxysporum* inoculation, t4 = Jengkol peel powder 0.375 kg/ha + *F. oxysporum* inoculation

Biodiversity refers to the variety of species and their distribution patterns within an organism or living natural resources and ecosystems. Biodiversity consists of two components: the total number of species per unit area and evenness (abundance, dominance, and spatial distribution of individual species). A biodiversity index is a metric that integrates these two concepts into a single number. The variables were then combined into a single value regarding the number of species, relative species abundance, homogeneity, and sample plot size. For this reason, the biodiversity index depends on the richness index, the diversity index, and evenness index (Barnes *et al*., 1997).

Table 7. Status of diversity, richness, dominance and balance of microbial populations index in shallot plantations applied with jengkol peel botanical pesticides

Treatmens	Standard of Species Diversity Index (H')	Species Diversity Index (H')	Standard of Species Richness Index (R)	Species Richness Index (R)	Standard of Domination Index (D	Domination Index (D)	Standard of Microbial Balance Index (E)	Microbial Balance Index (E)
t0	1,0 < H'≤ 3,0 : medium	1.18	R ≤ 3,5 : low	0.83	D < 0.5: low	0.34	E >0.5: high	0.73
t1	1,0 < H'≤ 3,0 : medium	1.23	R ≤ 3,5 : low	0.94	D < 0.5: low	0.43	E < 0.5: low	- 0.08
t2	1,0 < H'≤ 3,0 : medium	1.65	R ≤ 3,5 : low	1.32	D < 0.5: low	0.23	E < 0.5: low	- 0.12
t3	1,0 < H'≤ 3,0 : medium	1.22	R ≤ 3,5 : low	1.67	D < 0.5: low	0.45	E < 0.5: low	- 0.12
t4	1,0 < H'≤ 3,0 : medium	1.67	R ≤ 3,5 : low	2.23	D < 0.5: low	0.27	E > 0.5: high	0.91

Description: t = treatment code, t0 = Negative Control (*F. oxysporum* inoculation), t1 = Positive control (*F. oxysporum* + Benomyl fungicide), t2 = Jengkol Skin Powder 0, 125 kg/ha + *F. oxysporum* inoculation, t3 = Jengkol peel powder 0.25 kg/ha + *F. oxysporum* inoculation, t4 = Jengkol peel powder 0.375 kg/ha + *F. oxysporum* inoculation

Table 7 shows that the Shannon Wiener (H') diversity index value in shallot plantations area ranged from 1.18 to 1.67. According to (Krebs, 1985), this value is in the moderate diversity category because the H' value is in the range of 1-3. Diversity in the medium category indicates that the community's species are relatively diversified, and the ecosystem is stable (Odum and Barrett, 2017).

The Margalef species richness index (R') in the research location ranges from 0.83 - 2.23. According to (Magurran, 1988), a richness index of R 3 is considered low. This situation indicates that the shallot plantation contains a limited number of microbial species. The development and distribution of soil microbes are generally influenced by biotic and abiotic factors in their living environment, including soil oxygen and vegetation (Jha *et al*., 1992; Rao, 1994). Aerobic microbes require oxygen in their respiration to develop properly, which means they require a well-aerated environment (Suharni, 2008). Based on the type of land, the research location was swampland with inadequate aeration. This condition can be a limiting factor for development. Only certain microbes can adapt, resulting in a low number of microbial species found in the research location.

Vegetation is another environmental factor that affects the number of microbial species. Vegetation on the soil surface can modify microbial communities (Bezemer *et al*., 2006; Carney

and Matson, 2006). Variations in the number and types of these plants affect the composition of the soil microbial community (Han *et al*., 2007; Zul *et al*., 2007; Liu *et al*., 2020). (Bernadip *et al*., 2015) found that the shallot rhizosphere in several observation areas has a soil pH that tends to be acidic. This may also be a limitation of the low microbial species present in the study site.

The dominance index (D) was between 0.23 and 0.45, considered the low category. This suggests that microbial species are diversified in the shallot plantation, with no dominant species. This is in line with (Odum and Barrett, 2017) statement that the range of dominance index values starts from 0-1. If the value obtained is near zero, it indicates no species that completely dominate other species in the observed community structure.

Evenness Index (E) describes the distribution of species in a community, and this value is related to the Dominance index (D). The evenness index in shallot plantations ranged from -0.08 to 0.91. This value is included in the low to the high category, but it is included in the low category when viewed from the average value of 0.26. It depicts that the distribution of species in the community is not evenly distributed. A species dominates, but its dominance is not extreme if it is associated with a low dominance index value.

CONCLUSIONS

1. The application of jengkol peel powder could suppress the attack of moler disease on shallots. The lowest attack intensity was achieved using botanical pesticides at 0.375 kg/ha, or 60%.
2. Biodiversity in shallot plantations was quite good due to a moderate diversity index and the absence of dominant species.
3. Application of botanical pesticides at a dose of 0.125 kg/ha could increase the microbial population in the soil, but the intensity of moler disease on shallots was still high (86.3%).
4. The type of microbe found was *Trichoderma* sp., *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Mucor* sp., *Aeromonas* sp., *Corynebacterium* sp., *Enterobacter* sp., *Sphingomonas* sp., and *Bacillus* sp..
5. Microbial population is affected by pesticide application. Plants that were not treated with botanical pesticides or chemical pesticides had a drop in the number of microbes, whereas chemical pesticides increased the number of microbes in the rhizosphere.
6. The application of botanical pesticides had a varied impact as the dose increased, and then it affected the decrease in microbes in the shallot rhizosphere.

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