Isolation and Morphological Characterization of Culturable Soil Microorganisms from Local Shallot Fields in Palu, Central Sulawesi, Exposed to Different Insecticide Intensities

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ABSTRACT

Soil microbial communities can be influenced by environmental factors such as insecticide exposure and the resulting chemical residues. The purpose of this study was to isolate and characterize rhizosphere microorganisms of local shallots from Palu from fields with different levels of insecticide exposure. The approach used was an isolation method using selective media for bacteria, actinomycetes, and fungi, followed by colony morphology characterization and Gram staining for bacteria and actinomycetes. A total of 26 isolates were obtained from this study, including 9 bacterial isolates, 4 actinomycete isolates, and 13 representative fungal isolates. Morphological characterization showed that actinomycete isolates had dry colonies, powdery/rough texture, and formed aerial mycelium, while fungal isolates were dominated by *Aspergillus niger*, *Aspergillus flavus*, and *Trichoderma*. Soil pH values between locations were relatively uniform, while soil moisture showed variation and tended to be lower at high-intensity locations. The formation of rhizosphere microbial community structures from land with different insecticide exposures, so that these findings can be used as a basis for developing soil health bioindicators and local biological agent candidates to support sustainable shallot farming.

1. Introduction

Agricultural activities involving the indiscriminate input of agrochemicals, such as fertilizers, pesticides, and/or herbicides, directly impact various fundamental areas, including the environment, public health, and the global economy [1, [2], [3]. These agricultural practices aim to increase crop yields and meet increasing global production demands [4]. However, they have significant impacts, particularly on soil conditions and food quality. The continued use of agrochemicals results in chemical residues remaining in agricultural land for long periods [5], [6]. Furthermore, the application of pesticides/insecticides to control pests and diseases triggers the emergence of resistance and the emergence of new opportunistic pests due to the loss of competitors. This has the additional effect of altering the highly diverse soil microbial community, characterized by complex interactions between microbial species and functional groups. It is known that soil microorganisms, such as bacteria, fungi, and actinomycetes, play a crucial role in the global C and N cycles and are responsible for approximately 90% of all organic matter decomposition [7], [8].

Soil microbes comprise a far more diverse community than plant and animal communities, with less than 5% of all microbes identified and described [9], [10]. It is widely recognized that soil microbes play a crucial role in soil nutrient cycling and plant health, contributing to various

ecological and physiological functions. The presence and diversity of soil microorganisms are strongly influenced by environmental conditions, including cultivation practices and pesticide use [11]. The local shallot cultivation system in Palu, Central Sulawesi, employs an intensive farming system that uses insecticides to suppress major pest populations, such as *Liriomyza* sp. (leafminer flies) and *Spodoptera* sp. (*army caterpillars*) [12], [13], which can cause leaf damage, reduce photosynthesis, and ultimately reduce yields [14]. Insecticide applications at varying doses and frequencies have the potential to impact soil microbial communities, either by reducing specific microbial populations or by altering their composition and diversity [15], [16].

Insecticide use can affect soil biological activity and suppress sensitive microorganisms, while other microbes may be able to adapt or even utilize pesticide residues as a carbon source. Therefore, it is important to understand how varying insecticide application rates in shallot fields relate to the diversity of soil microorganisms. This study aimed to identify soil microorganisms in shallot fields receiving varying levels of insecticide application through a culture-based isolation and identification approach. The media culture method was used to isolate microorganisms that can grow on both selective and general media, allowing for initial identification based on colony morphology and microscopic characteristics. The results of this study are expected to provide an overview of the diversity of soil microbes that can be cultured in shallot fields and provide a basis for understanding the potential impacts of insecticide use on soil microbial communities.

2. Method

2.1. Soil Sample Collection

Soil samples for microbial isolation were collected from three locations in local shallot cultivation centers in Palu, Central Sulawesi, with different levels of insecticide use: Bulupountu Jaya 1 = 1.007387°S, 119.95046°E (Location 1); Bulupountu Jaya 2 = 1.010701°S, 119.9526°E (Location 2); Maku = 1.044374°S, 119.890816°E (Location 3). As a control, soil samples were also taken from Guntarano = 0.771237°S. 119.884310E (Location 4) (without onion cultivation and no insecticide use) in March 2025. The insecticide intensity indicators and sample codes are presented as follows: SHI1 = Guntarano Village (low intensity: 1 active ingredient, applied as needed, with some use of organic pesticides); SHI2 = Bulupountu Village (medium intensity: up to 3 active ingredients, applied twice weekly at the recommended dosage); SHI3 = Maku Village (high intensity: ≥4 active ingredients, applied more than three times weekly until harvest, often exceeding the recommended dosage). The topsoil was excavated to a depth of 0–20 cm to sample the root zone (rhizosphere) of local Palu shallot plants. At each insecticide application level, 100 grams of samples were taken from five random locations using a sterile spatula and then composited into one sample per treatment [17]. The soil samples were placed in sterile polyethylene plastic bags, labeled, and stored in a cool box prior to laboratory analysis.

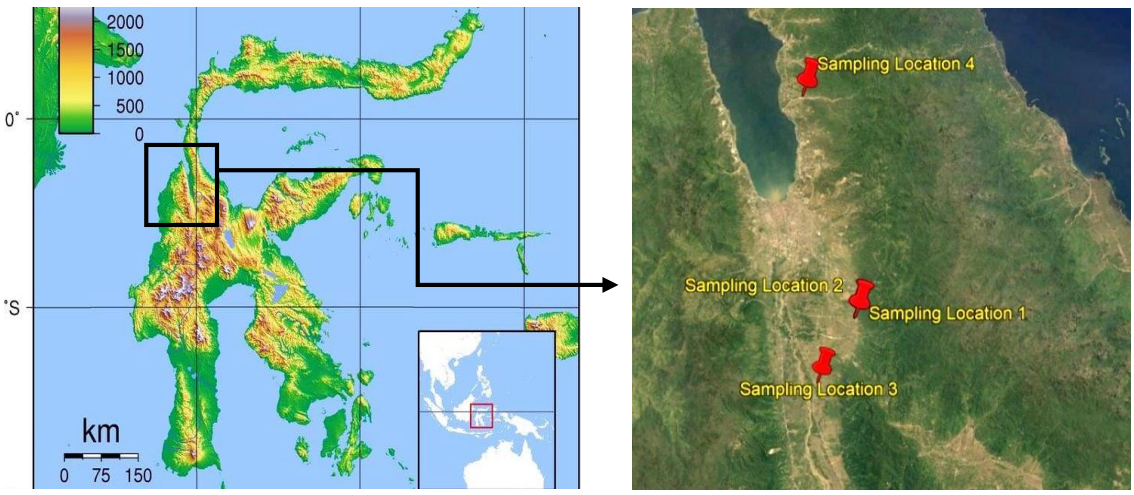


Fig. 1. Sampling location at the local shallot planting center in Palu, Central Sulawesi Province

2.2. Soil Microbial Isolation Using Culture and Isolate Purification Methods

The serial dilution method was used to isolate soil microbial samples. A one gram sample of soil was taken using a sterile spatula, placed in 9 mL of sterile physiological NaCl solution (0.85%) and then homogenized [18], [19]. The soil suspension was serially diluted to 10^{-4} or 10^{-6}, followed by inoculation using the spread plate method. The resulting dilutions were inoculated into selective culture media: nutrient agar (NA) + 50 mg/L Nystatin for bacteria, Potato Dextrose Agar (PDA) + 100 mg/L Chloramphenicol for fungi, and Starch Casein Agar (SCA) + 50 mg/L Nystatin for actinomycetes [20], [21]. All types of antifungal and antibacterial agents were added after the media had been sterilized in an autoclave at 121°C for 15 minutes, at a temperature of approximately 45–50°C. The plates were then incubated at 28–30°C for 2–7 days (depending on the microbial group). After the incubation period, the bacterial and actinomycete isolates were purified using the streak plate method, and the fungi were isolated by hyphal tip removal until a single, homogeneous colony was obtained.

2.3. Morphological Characterization of Soil Microbes

Morphological characterization of soil microbial colonies for bacteria includes colony shape, color, size, elevation, edge, and texture, while fungi are characterized based on the color of the surface and back of the colony, texture (cottony/starchy), growth pattern, and microscopic structures such as hyphae (septate/aseptate), conidiophores, sporangia, and spore shape [22], [23], [24]. Meanwhile, in actinomycetes, morphological characterization is determined from colonies that are generally dry or starchy, filamentous, form aerial mycelium, variations in colony color and pigment.

2.4. Gram test, pH and Soil Moisture Measurement

The Gram staining method, used to differentiate between Gram-positive and Gram-negative bacteria and their cell shapes, refers to the method proposed by [25]. The process begins by preparing a preparation of pure bacterial colonies and actinomycetes on a glass slide, fixing them, then treating them with crystal violet, iodine, decolorizing them with alcohol, and counterstaining them with safranin, then observing them under a microscope [26], [27]. pH observation is carried out by referring to the [28] method by determining a soil-water suspension at a ratio of 1:2.5 (w/v), homogenizing it, allowing it to stand for ±30 minutes, and then measuring it using a calibrated pH meter. Meanwhile, soil moisture is determined using the gravimetric method, which involves weighing the wet weight of the sample, drying it in an oven at 105°C for 24 hours until a constant weight is reached, and then calculating the moisture percentage based on the difference between the wet and dry weights.

3. Results and Discussion

3.1. Identification and Morphological Characterization of Soil Microbes

It is clear from the results that 21 bacterial isolates were successfully isolated from fields with varying levels of insecticide exposure. However, only nine isolates were successfully characterized after colony purification (Table 1). Eight of the nine isolates exhibited circular colonies, while only one isolate, BSHI0_3, exhibited irregular colonies. The colony color was predominantly creamy white, with one isolate yellowish-white. The colony margin type was predominantly entire, while others exhibited undulate and lobate margins.

Tabel 1. Morphological characteristics of bacterial isolates on NA medium.

No	Isolate Code	Colony shape	Margin	Elevation	Surface	Color	Opacity	Texture	Note
1	**BSHI0_1**	Circular	Entire	Raised	Smooth	Creamy white	Opaque	Mucoid	Uniform colonies
2	**BSHI0_3**	Irregular	Lobate	Flat	Rough	Yellowish-white	Opaque	Dry	Uneven distribution
3	**BSHI1_1**	Circular	Entire	Convex	Smooth	Creamy	Opaque	Mucoid	Dense growth
4	**BSHI1_2**	Circular	Undulate	Raised	Smooth	White	Opaque	Butyrous	Colonies clustered
5	**BSHI1_3**	Circular	Entire	Flat	Smooth	Off-white	Opaque	Dry	Thin layer growth
6	**BSHI2_1**	Circular	Entire	Raised	Smooth	Cream	Opaque	Mucoid	Glossy colonies
7	**BSHI2_3**	Circular	Undulate	Flat	Smooth	Pale white	Opaque	Dry	Spread growth
8	**BSHI3_2**	Circular	Entire	Convex	Smooth	White	Opaque	Butyrous	Dense colonies
9	**BSHI3_3**	Circular	Entire	Flat	Smooth	Cream	Opaque	Dry	Uniform colonies

Note: The prefix B indicates bacterial isolates. BSHI0 = Comparison site without shallot cultivation (control); BSHI1 = Guntarano Village (low intensity: 1 active ingredient, applied as needed, with some use of organic pesticides); BSHI2 = Bulupountu Village (medium intensity: up to 3 active ingredients, applied twice per week according to recommended dosage); BSHI3 = Maku Village (high intensity: ≥4 active ingredients, applied more than three times per week until harvest, often exceeding the recommended dosage).

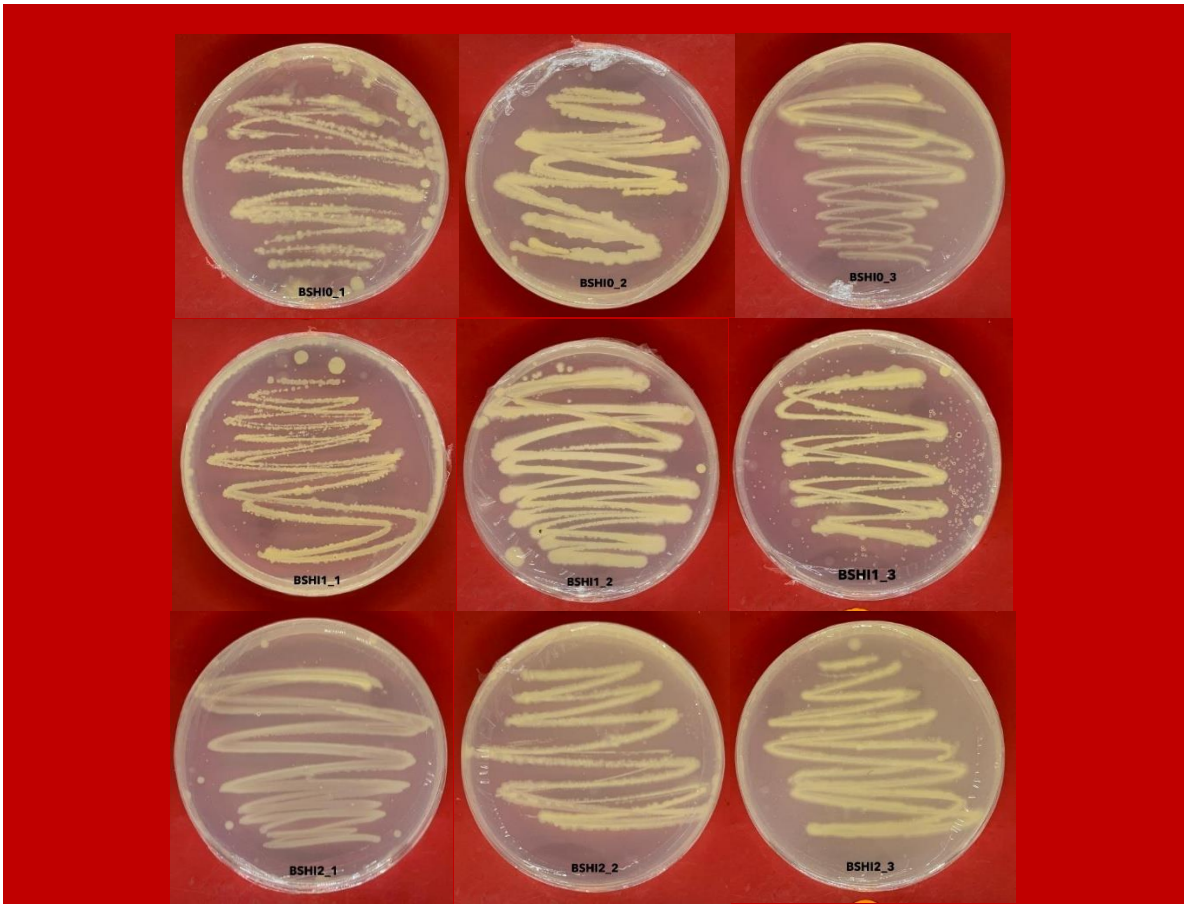


Fig. 2. Morphological analysis of bacterial colonies growing on NA selective media from local red onion fields in Palu exposed to insecticides

Observations revealed varied colony growth patterns, confirming the bacterial diversity present at the study site. Isolates BSHI0_1, BSHI1_1, and BSHI2_1 are likely capable of producing exopolysaccharides (EPS), which play a role in rhizosphere colonization. This is indicated by their mucoid texture, while isolates with a dry texture indicate the potential for bacteria to be more resistant to environmental stress. [29] reported a similar finding, stating that the mucoid texture on the surface of bacterial colonies is influenced by an extracellular matrix factor. This contrasts with [30] who suggested that high stress tolerance in bacteria is due to their ability to produce osmoprotectants and exopolysaccharides.

Table 2. Morphological characteristics of actinomycete isolates on SCA media

No.	Isolate Code	Colony Shape	Surface/ Texture	Aerial Mycelium Color (upper)	Substrate Color (reverse)	Note
1	**ASHI1_1**	Irregular	Compact, granular	Grayish white	Yellow	Spreading colony
2	**ASHI1_2**	Irregular, spreading	Dry, powdery	Grayish white	Cream-yellowish	Resembling *Streptomyces*
3	**ASHI2_1**	Irregular, flat	Dry, rough	Grayish white	Pale white	Dense growth
4	**ASHI3_1**	Irregular	Dry, filamentous	Yellowish white	Pale yellow	Spreading colony

Note: The prefix A indicates Actynomycetes isolates. ASHI0 = Comparison site without shallot cultivation (control); ASHI1 = Guntarano Village (low intensity: 1 active ingredient, applied as needed, with some use of organic pesticides); ASHI2 = Bulupountu Village (medium intensity: up to 3 active ingredients, applied twice per week according to recommended dosage); ASHI3 = Maku Village (high intensity: ≥4 active ingredients, applied more than three times per week until harvest, often exceeding the recommended dosage).

The results, as seen in Table 2 and Figure 3, show that 11 actinomycete isolates were successfully isolated from rhizosphere samples of local shallots from Palu in fields exposed to different insecticides. However, based on the morphological characteristics of the colonies on specific Starch Casein Agar (SCA) media, only 4 representative isolates were obtained, namely ASHI1_1, ASHI1_2, ASHI2_1, and ASHI3_1. Colonies with irregular shapes were predominantly found, accompanied by various surface texture variations. In general, the color of the aerial mycelium ranged from grayish white to yellowish white, while the color of the substrate (reverse) produced varied.

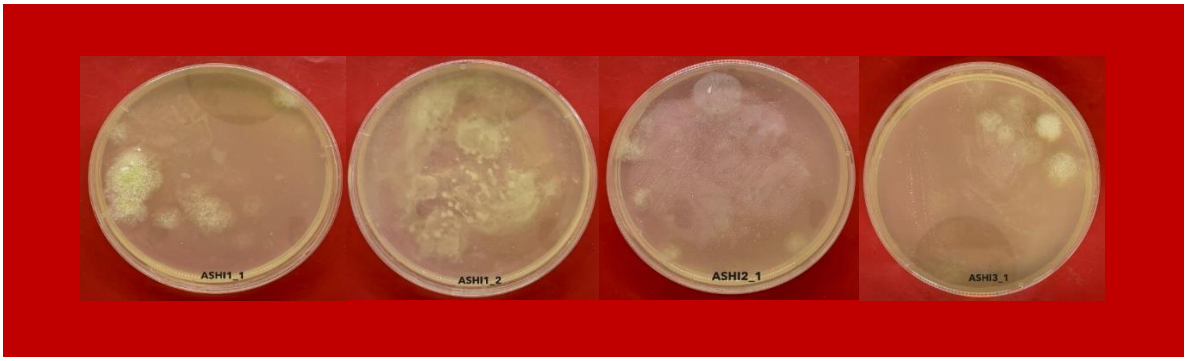


Fig. 3. Morphological analysis of Actynomicetes colonies growing on SCA selective media from local red onion fields in Palu exposed to insecticides

Based on morphological characterization, the isolates obtained exhibited typical actinomycete characteristics, characterized by the presence of aerial mycelium, dry colonies with a rough/dusty texture, and distinct reverse pigmentation. The actinomycete findings in the insecticide-exposed field indicate that these isolates are known to have the ability to withstand environmental stress. This is evident from the spores they produce, although the number of isolates found was limited due to selection from insecticide exposure. It is known that direct application of insecticides will reduce the abundance of soil microbes and inhibit the growth of sensitive microbes through the toxic effects of

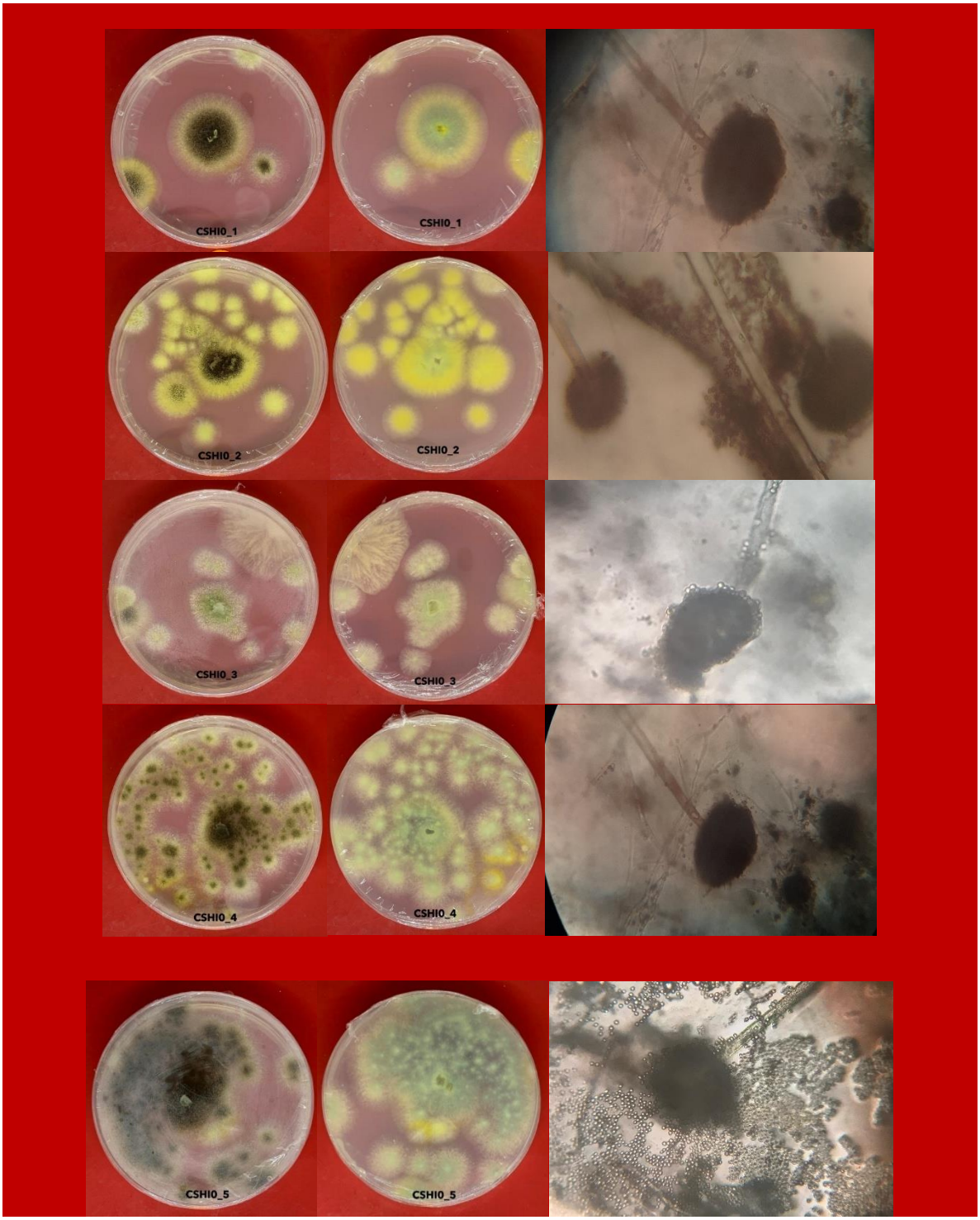
the chemicals [31], [32]. These research results confirm that only tolerant isolates can survive and thrive, while sensitive isolates are easily eliminated.

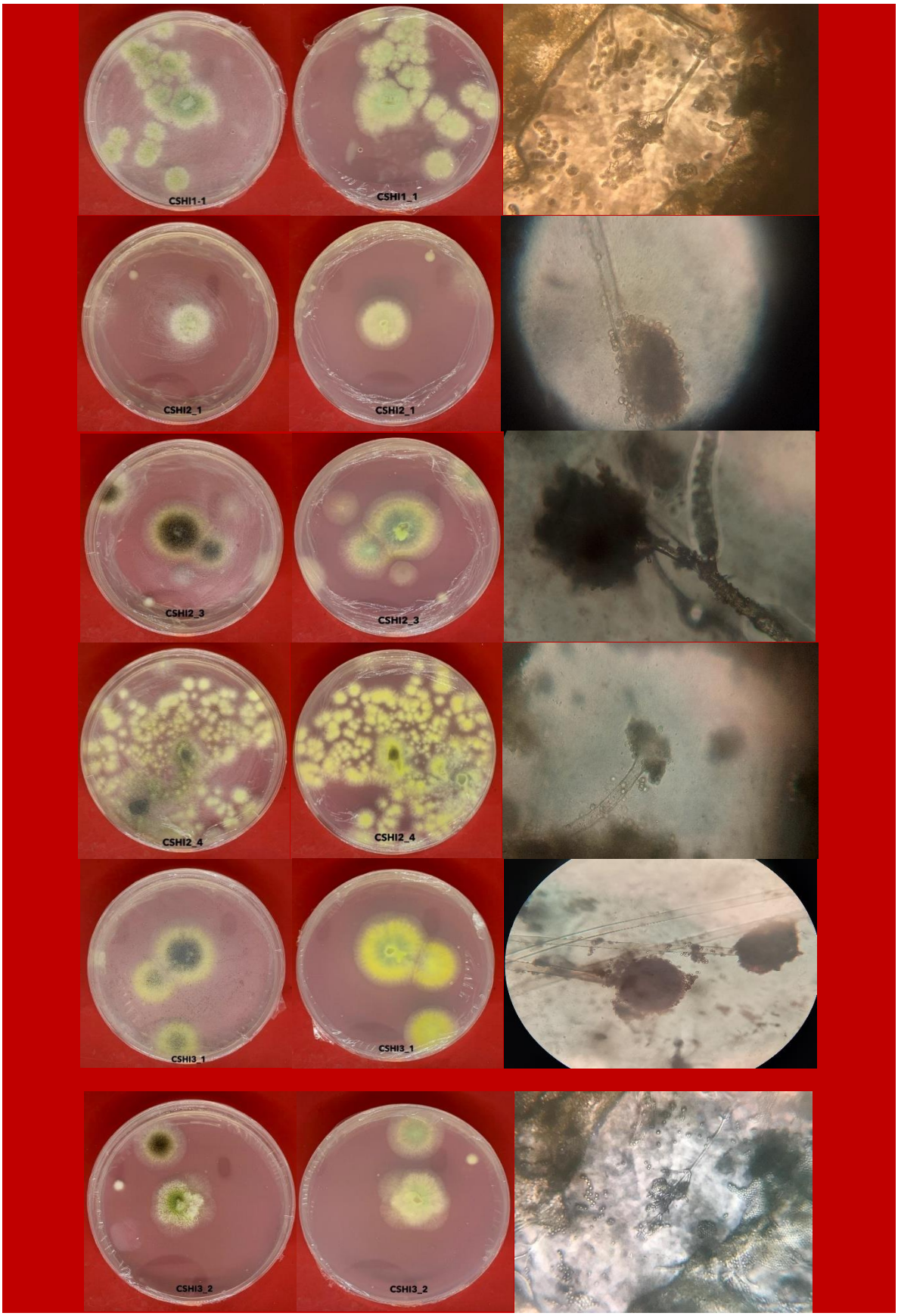
Table 3. Macroscopic Characteristics of Fungal Isolates on PDA Medium

No	Isolate Code	Colony Shape	Colony Margin	Topography	Surface Texture	Color	
						Obverse	Reverse
1	**CSHI0_1**	Circular, large colony	Entire	Raised at center	Velvety	Dark green to bluish-green	Pale yellow
2	**CSHI0_2**	Irregular, multiple colonies	Undulate	Flat	Powdery	Bright yellow with dark green center	Pale yellow
3	**CSHI0_3**	Circular, spreading colony	Entire to irregular	Flat with radial folds	Velvety to floccose	Light green to whitish	Pale yellow
4	**CSHI0_4**	Irregular, dense colony	Undulate	Flat	Powdery	Dark green mixed with yellow	Yellowish brown
5	**CSHI0_5**	Irregular, widely spreading, zonate colonies	Undulate / irregular	Center raised with clustered patches	Velvety to powdery, localized granular patches	Dark center (green-black) with light-green areas and yellow spots	Yellowish brown, diffuse pigment
6	**CSHI1_1**	Circular, spreading	Entire to slightly undulate	Flat to slightly raised, center elevated	Powdery to velvety	Light green to whitish green	Cream to pale yellow
7	**CSHI2_1**	Circular, small, slow growth	Entire	Flat	Smooth to slightly powdery	Creamy white	Pale cream
8	**CSHI2_3**	Circular, multiple colonies	Entire	Raised at center	Velvety to powdery	Greenish-black with white–yellow zones	Yellowish brown
9	**CSHI2_4**	Irregular, widely spreading	Undulate	Flat	Powdery	Yellowish-green with black spots	Pale yellow
10	**CSHI3_1**	Circular, 2–3 adjacent colonies	Entire	Center elevated, contrasting peripheral zone	Velvety powdery at margin	Dark greenish-black center with bright-yellow margin	Pale yellow
11	**CSHI3_2**	Circular, small–medium colonies	Entire	Slightly raised center	Velvety–smooth, relatively compact	Light-green/white center with faint green periphery	Whitish–pale yellow / cream
12	**CSHI3_3**	Circular, compact with radial halo	ntire, well-defined	Strongly raised center with radiating floccose halo	Floccose (hairy/radiating) periphery, dense center	Dark center surrounded by white floccose ring and yellow-green zone	Pale yellow to yellowish brown
13	**CSHI3_4**	Circular, single large colony	Entire, distinct	Center raised, margin relatively flat	Velvety, compact center	Dark green–black center with yellowish-green halo	Pale yellow

Note: The prefix C indicates fungi isolates. CSHI0 = Comparison site without shallot cultivation (control); CSHI1 = Guntarano Village (low intensity: 1 active ingredient, applied as needed, with some use of organic pesticides); CSHI2 = Bulupountu Village (medium intensity: up to 3 active ingredients, applied twice per week according to recommended dosage); CSHI3 = Maku Village (high intensity: ≥4 active ingredients, applied more than three times per week until harvest, often exceeding the recommended dosage).

Strong evidence of the presence of soil microbes (fungi) was found in the land exposed to insecticides with an initial number of 34 isolates during isolation, after selective morphological characterization, 13 isolates were successfully subcultured (Tabel 3). Variations in morphology, ranging from colony shape consisting of 2 (circular and irregular), margin (entire to undulate), topography (flat to raised/center elevated), surface texture (velvety, powdery, floccose), as well as variations in colony color (green, white, to blackish green) and reverse color (pale yellow to yellowish brown).




CSHI1-1
CSHI1_1
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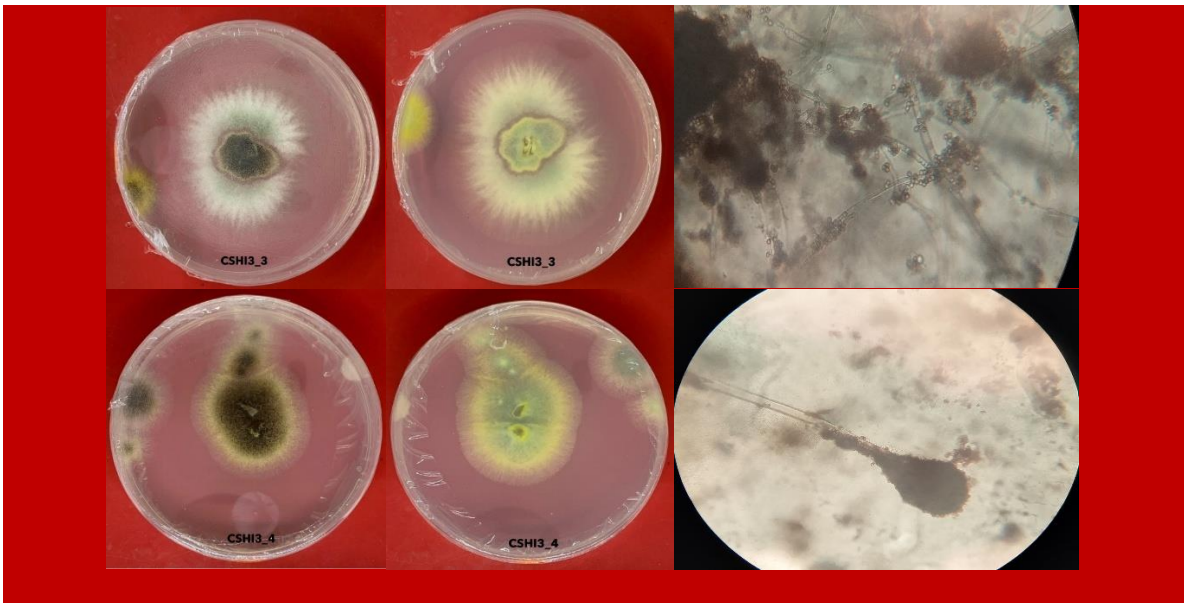

Fig. 4. Macroscopic (top and bottom views) and microscopic morphological characteristics of fungi on PDA

Based on Figure 4, macroscopically, several isolates showed contrasting color zones (e.g., the center of the colony was darker than the edges). Meanwhile, from the bottom (reverse) view, the isolates showed variations in pigmentation, with some isolates producing diffuse pigment that spread across the medium. Microscopically, the dominant fungal isolates found were from the Aspergillus niger, Aspergillus flavus, and Trichoderma groups. The distinctive characteristics of the colonies growing on PDA media were their varying green to blackish-green color, powdery/velvety texture, and differences in the resulting reverse pigmentation. Control plots (without shallot cultivation and without insecticide exposure) tended to produce a higher number of fungal isolates than plots with high and medium management intensity. This indicates the presence of selection pressure from the more tolerant and adaptive fungal microbes that survived. [33] reported that *Aspergillus spp*. and *Trichoderma* are known to be highly adaptable microbial groups, enabling them to survive environmental conditions resulting from insecticide inputs in the cultivation system.

3.2. Gram Test and Environmental Observation (pH and Soil Moisture)

Two isolates, ASHI1_1 and ASHI3_1, were found to be predominantly Gram-positive, with actinomycete-like bacteria, while the other, BSHI3_3, was bacterial (Table 7). The actinomycete group in this study was more adaptable to environmental conditions exposed to insecticides, especially since the two bacterial and actinomycete isolates found came from fields with high insecticide application intensity. [34] suggests that Gram-positive bacteria have thicker cell walls, making them more resistant to environmental stress, including chemical stress. Therefore, microbes with thicker cell walls are more tolerant.

Table 7. Gram Test on Bacteria and Actinomycetes in Local Palu Shallot Fields Exposed to Insecticides with Different Intensities

No	Isolate Code	Gram	
		Positive (+)	Negativ (-)
1	BSHI0_1		(-)
2	BSHI0_3		(-)
3	BSHI1_1		(-)
4	BSHI1_2		(-)
5	BSHI1_3		(-)
6	BSHI2_1		(-)
7	BSHI2_3		(-)
8	BSHI3_2		(-)
9	BSHI3_3	(+)	
17	ASHI1_1	(+)	
18	ASHI1_2		(-)
19	ASHI2_1		(-)
20	ASHI3_1	(+)	

Note: The prefix B indicates bacterial isolates, prefix A indicates Actynomycetes isolates. SHI0 = Comparison site without shallot cultivation (control); SHI1 = Guntarano Village (low intensity: 1 active ingredient, applied as needed, with some use of organic pesticides); SHI2 = Bulupountu Village (medium intensity: up to 3 active ingredients, applied twice per week according to recommended dosage); SHI3 = Maku Village (high intensity: ≥4 active ingredients, applied more than three times per week until harvest, often exceeding the recommended dosage).

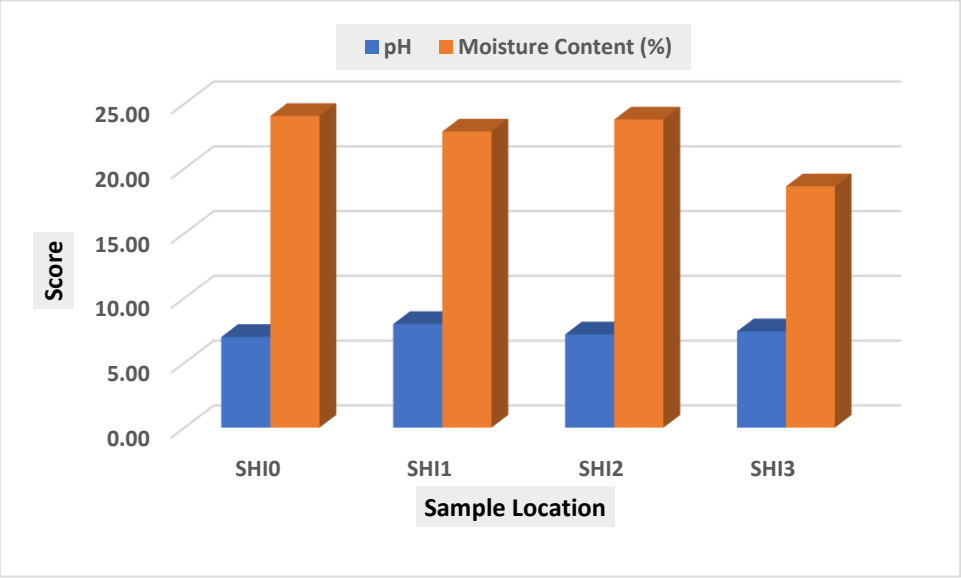

Fig. 5. Soil pH and moisture data at the research location

The soil pH values shown in Figure 5 show uniformity between locations, ranging from neutral to slightly acidic. Conversely, soil moisture levels showed more pronounced variation, with higher values at SHI0–SHI2 and decreasing at SHI3. The uniformity of pH between locations indicates that pH is not a primary factor differentiating microbial communities. The results of this study indicate that soil moisture has the potential to influence microbial activity and abundance. Drier soil conditions at the high-intensity site (SHI3) may be an additional selection factor that strengthens the dominance of microbes that are more tolerant to environmental stress.

A clear selection pattern is evident in the results of this study, where the isolates obtained generally have diverse morphological characteristics. This is in line with [35] which reported that insecticides can affect the structure of soil microbial communities and reduce the diversity of sensitive groups. In addition, there are environmental factors that play a role in supporting this statement, where the pH indicated from each location is relatively uniform so that this cannot be used as a major differentiating factor. A significant effect is seen from the resulting humidity values. Land with high exposure intensity produces relatively drier humidity than other locations, so this has the potential to be an additional selection pressure for microbes that are more tolerant to environmental stress. In addition, the combination of differences in humidity and the intensity of insecticide exposure is thought to play a role in shaping the structure of the rhizosphere microbial community at each location, before further testing is carried out to assess the functional potential of the selected isolates.

4. Conclusion

There was a change in the composition of culturable soil microorganisms based on the results of isolation and overall morphological characterization obtained 26 isolates, consisting of 9 bacterial isolates, 13 fungal isolates, and 4 actinomycete isolates. Bacterial and actinomycete isolates were dominated by groups that are adaptive to agricultural soil conditions, while fungal isolates were dominated by Aspergillus niger, Aspergillus flavus, and Trichoderma, with a higher number of isolates in the control field compared to the field with high insecticide intensity. Soil pH conditions between locations were relatively uniform, while moisture variations were more pronounced and are thought to play a role as an additional selection factor. The local microbial isolates found have the

potential to be used as biological indicators of soil health as well as initial candidates for the development of biological agents to support a more sustainable shallot farming system.

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