Preliminary study of the ability of indigenous fungi from river as an alternative to degrade pesticides and their effect on insect larvae

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Abstract. Agriculture relies on pesticides for crop protection, but their negative impacts on the environment, human health, and ecosystems are concerning. Pesticide residues in river water, especially in agricultural areas, threaten organism survival. This study aims to explore fungi's role in mitigating these negative effects and their potential to degrade pesticide residues. Fungi were isolated from river water in Banyumas regency's Mengaji and Prukut rivers, where pesticides are used in surrounding rice fields. The fungi's potential was tested by inoculating them on PDA-chlorpyrifos medium, measuring mycelium diameter, and conducting a clear zone test to assess pesticide degradation capability. Indigenous fungal isolates from Mengaji and Prukut showed diameters ranging from 1.0-7.1 cm. Eleven isolates demonstrated pesticide degradation abilities, producing clear zones, including I12, 121, I24, and I25. Isolate I24, identified as *Trichoderma* sp., was the most effective. Environmental parameters (current speed, temperature, pH, O2) were measured at nine collection sites, influencing fungal diversity. Additionally, *Trichoderma* sp. is effective at rapidly degrading pesticides and are safe for non-target insects. This preliminary study highlights fungi's potential as future agricultural technology for managing pesticide residues.

1 Introduction

Rivers are vital freshwater sources that flow from high to low elevations, sustaining diverse ecosystems. In tropical Indonesia, rivers host numerous microorganisms, including fungi, which thrive on plant residues, parasitize aquatic organisms, or attach to fish. These fungi play a crucial role as decomposers in aquatic habitats, primarily comprising Ascomycota, Basidiomycota, and Chytridiomycota groups due to their cellulose-degrading abilities [1][2].

Fungi are instrumental in detoxifying and degrading toxic compounds in contaminated environments, including pesticides [3]. Some fungi can degrade insecticides like chlorpyrifos through enzymatic actions, converting these complex compounds into less

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harmful substances [4][5][6][7]. This degradation process involves various biochemical pathways, making fungi a potential tool for bioremediation.

Chlorpyrifos, an organophosphate insecticide, accounts for 36% of the global insecticide market[8]. It poses severe environmental and health threats, affecting non-target organisms and causing various health issues in humans, including neurological damage and increased cancer risk [9][10][11][12]. Traditional remediation methods like sorption, phytoremediation, and chemical treatments have limitations, often leaving toxic byproducts behind [13][14].

Bioremediation using fungi offers a promising alternative. Various fungi, such as Fusarium oxysporum and *Trichoderma* species, have shown potential in degrading pesticides like chlorpyrifos [15]. Previous studies have isolated fungi from Mengaji and Prukut Rivers in Banyumas Regency, but broader sampling is needed to explore their pesticide-degrading capabilities and interactions with common rice field organisms like insect larvae [16]. This study aims to investigate fungi's role in mitigating pesticide impacts and their potential as sustainable agricultural technology.

2 Materials and Methods

2.1 Preparation of research tools and materials

2.1.1 Sampling

Water sampling was carried out in Mengaji and Prukut Rivers in Banyumas Regency (Fig.1 and Fig. 2). The Mengaji River consists and the Prukut River consists of nine sites. Parameters measured from river water samples are current velocity, temperature, pH, and O2. River water is taken with a glass bottle that has been sterilized by autoclaving for 15 minutes at a temperature of 121ºC and a pressure of 2 atm. The glass bottle is placed opposite the flow of water until it is full and then closed tightly [17].

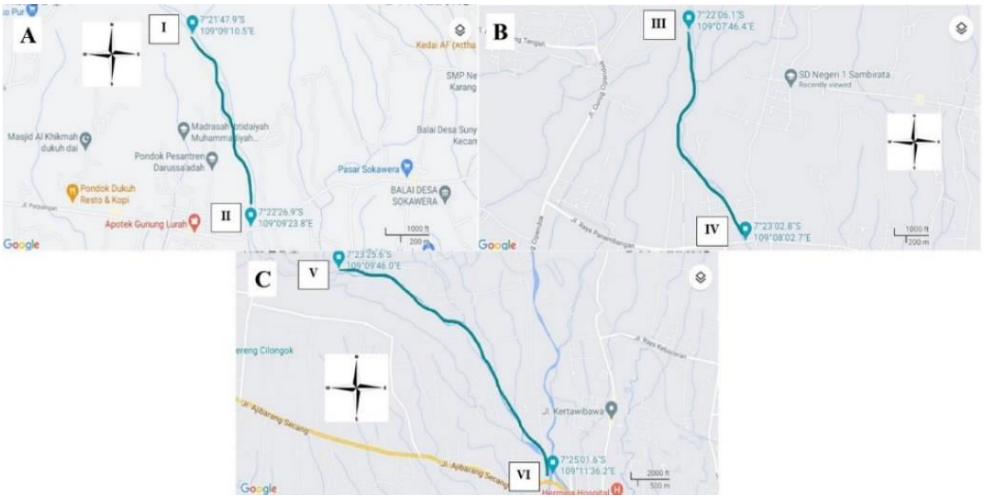

Fig 1. Map of sampling Site. A. Site I and II Mengaji River, Banyumas Regency, B. Site III and IV of the Prukut River, Banyumas Regency,C. Site V and VI of the Mengaji River, Banyumas Regency.

Fig 2. A. Site I, B. Site II, C. Site III, D. Site IV, E. Site V, F. Site VI.

2.1.2 Tool sterilization

Tools such as test tubes, Erlenmeyer, and Petri dishes were sterilized using an autoclave at 121 ºC with a pressure of 2 ATM for 15 minutes. The ose needle was chemically sterilized by spraying 70% alcohol, then burned directly over a bunsen flame before use.

2.1.3 Preparation of PDA media

Potatoes are cut into small pieces and then weighed with an analytical balance as much as 200g. Potatoes are boiled with 500 mL of distilled water until soft. Potato cooking water was filtered and then added 20 g dextrose and 15 g agar and homogenized. The media was added with distilled water up to 1 L and homogenized. When it was clear, the media was transferred to an Erlenmeyer flask and added with 250 mg chloramphenicol and then homogenized. The media was sterilized using an autoclave for 15 minutes at 121 ºC and 2 atm pressure.

2.2 Obtaining the fungi

2.2.1 Isolation of river water fungi

Test tubes containing nine mL of distilled water and sterilized using an autoclave for 15 minutes at 121 ºC and 2 atm pressure were prepared. Multilevel dilutions were carried out using micropipettes and tubes in a ratio of 1:9 (one mL of water sample and nine mL of distilled water) up to a dilution of 10-6. Planting or platting was done by pour plate (pouring the sample first followed by PDA media) at the last two dilutions of 10-5 and 10-6 with duplo cups. The cup is closed tightly using a wrapper. The results of planting were incubated for 3 -7 x 24 hours. Observations were made every day [18].

2.2.2 Purification of isolation results

Fungi that have grown are inoculated in new PDA media as much as one ose until it becomes a pure culture and avoids contamination. Incubation was carried out 3 - 7 x 24 hours. Observations were made every day to ensure the fungi remained pure and uncontaminated.

2.3 Potency assay

2.3.1 Fungal activity test in degrading pesticides

Medium for pesticide treatment was made by mixing 250 mL of PDA medium and 50 mg/L chlorpyrifos in an Erlenmeyer flask, then put into a shaker at 115 rpm for 30 minutes. Purified fungal isolates were inoculated on PDA medium that had been given chlorpyrifos. Incubation was carried out for 7 and 14 days at room temperature. The results observed were in the form of colony diameter and were carried out three times. Colony diameter was measured and it was seen which fungi had great potential to degrade [19]. Colony diameter measurement is done with the following formula [20].

$$D = \frac{d1+d2+d3+d4}{4} \quad (1)$$

Description:
d1 = colony diameter on the vertical line (cm)
d2 = colony diameter on diagonal line 1 (cm)
d3 = colony diameter on diagonal line 2 (cm)
d4 = diameter of growth on the horizontal line (cm)

2.3.2 Identification of superior fungi

Fungal identification is carried out up to the genus level [21]. The identification of fungi was carried out up to the genus level. Identification methods used based on morphology are colony morphology and microscopy. The method of identification of fungi based on colony morphology (macroscopic) involves observing the physical characteristics of fungal colonies that can be seen with the naked eye. Parameters observed include color, texture, shape, size, and edge growth of colonies [22]. Meanwhile, microscopic identification involves observing the cellular structure of fungi with the help of a microscope. This observation includes the identification of spores, hyphae, and other reproductive structures. Spores can be observed in terms of their shape, color, and grouping pattern. Hyphae are also examined for whether they are septate (encysted) or aseptate (unencysted), and how they branch [23].

2.4 Affect insect larvae

A fungal suspension that has been calculated for spore density is prepared. Then, 12 glass jars were prepared and filled with 180 ml of distilled water. The fungal suspension from the culture suspension (dilution) was transferred as much as 20 ml using a micropipette into three glass jars (three replicates). After that, stir using a stirring rod to make it homogeneous. The prepared insect larvae were placed into 12 glass jars. Afterward, larval mortality and changes that occurred within 9 days were observed.

3 Results

Sampling of river water as a source of fungal isolates was carried out at nine sites of the Mengaji and Prukut rivers. After isolation on PDA media, several fungal isolates were found. Each site produces a different number of fungi (Table 1). The isolates obtained were tested for their ability to degrade pesticides containing the active ingredient chlorpyrifos through a series of tests to determine the effectiveness of the isolates in breaking down chlorpyrifos compounds into simpler and less harmful components. The results of these tests are shown by measuring the average colony diameter (Table 2), as well as the presence or absence of a clear zone around the colony (Table 3). The diameter of the fungal colonies formed on PDA media enriched with chlorpyrifos is presented in detail in Table 2, Figure 2, and Figure 3..

Table 1. Fungi isolation results of each site

No	Isolat	Site River
1	I1	Site I (Mengaji River)
2	I2	
3	I3	
4	I4	Site II (Mengaji River)
5	I5	
6	I6	
7	I7	
8	I8	
9	I9	Site III (Prukut River)
10	I10	
11	I11	
12	I12	
13	I13	Site IV (Prukut River)
14	I14	
15	I15	
16	I16	Site V (Mengaji River)
17	I17	
18	I18	
19	I19	
20	I20	
21	I21	Site VI (Mengaji River)
22	I22	
23	I23	
24	I24	
25	I25	
26	I26	Site VII (Mengaji River)
27	I27	
28	I28	
29	I29	Site VIII (Mengaji River)
30	I30	
31	I31	
32	I32	Site IX (Mengaji River)
33	I33	
34	I34	

Table 2. Mean Colony Diameter at 7 and 14 days

Isolates	Colony diameter (cm)	
	7 days	**14 days**
Control	0.00 ± 0.00a	0.00 ± 0.00ij
I1	0.758 ± 0.057bcd	2.2 ± 0.5efgh
I2	1.158 ±0.23fgh	2.0 ± 0.25cde
I3	1.13 ± 0.326defg	1.9 ± 0.321cdefg
I4	1.20 ± 0.139fgh	1.1 ± 0.1bc
I5	0.98 ± 0.052cdefgh	1.5 ± 0.16cde
I6	1.18 ± 0.115fgh	1 ± 0,057b
I7	0.858 ± 0.087bcdef	3,2 ± 0,057jk
I8	1.30 ± 0.189gh	1,7 ± 1,21cdef
I9	1.158 ± 0.339fgh	1,2 ± 0,152bc
I10	1.108 ± 0.212defgh	1,4 ± 0,217bcde
I11	1.225 ± 0.198fgh	5.6 ± 0.714m
I12	1.15 ± 0.46efgh	1.60 ± 0.1cdef
I13	0.90 ± 0.195bcdef	1.6 ± 0 cdef
I14	1.10 ± 0.025defgh	3.00 ± 0.00ij
I15	0.958 ± 0.014bcdefg	2.8 ± 0.251hij
I16	1.13 ± 0.123defgh	1.32 ± 0.246bcd
I17	0.76 ± 0.115bcde	1.1 ± 0.057b
I18	0.60 ± 0.00b	1.2 ± 0.1527bc
I19	0.63 ± 0.057bc	1.7 ± 0.322bcdef
I20	1.475 ± 0.26h	4.00 ± 0l
I21	1.30 ± 0.90gh	2.2 ± 1.442efgh
I22	1.416 ± 0.40h	1.6 ± 0.153bcdef
I23	0.66 ± 0.14bc	3.9 ± 0.153kl
I24	1.39 ± 0.20h	7.1 ± 0.61n
I25	1.80 ± 0.146i	1.56 ± 0.057bcdef
I26	0.78± 0.057bcd	2.10 ± 0.10defgh
I27	1.05 ± 0.025defgh	1.75 ± 0.05bcdef
I28	0.875 ± 0.087bcdef	2.00 ± 0.00defg
I29	1 ± 0.123defgh	2.53 ±0.57ghi
I30	1.265 ± 0.198fgh	2.03 ± 0.57defg
I31	1.015 ± 0.025defgh	1.16 ± 0.38bc
I32	0.58 ± 0.00b	2.60 ± 0.10ghij
I33	1.3 ± 0.189gh	2.19 ± 1.36defg
I34	1.095 ± 0.025defgh	2.19 ± 1.36defg

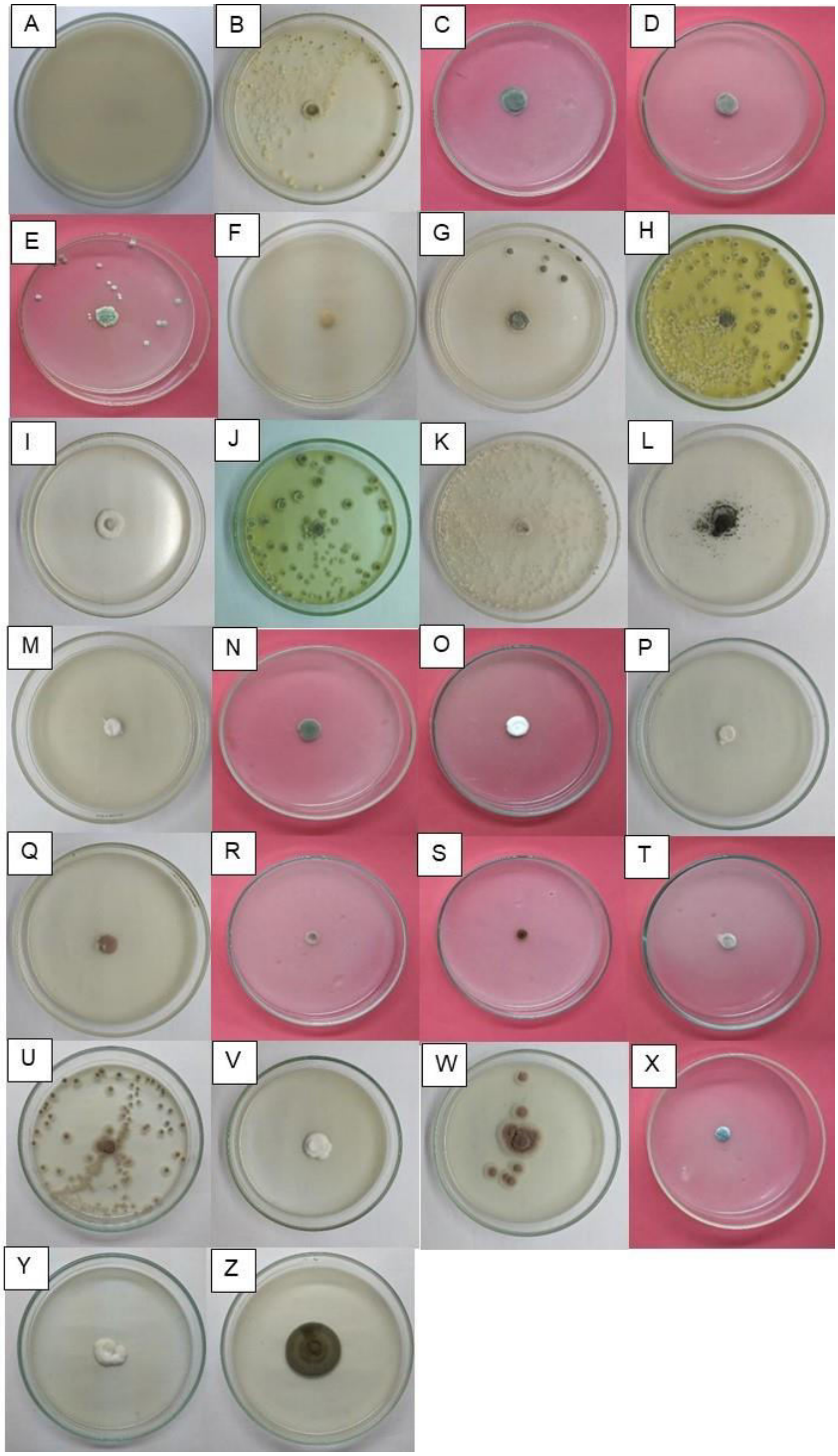

Fig. 3. Diameter of fungi (Observed 7x24 hours). (A). Kontrol; (B). I1; (C). I2; (D). I3; (E). I4; (F). I5; (G). I6; (H). I7; (I). I8; (J). I9; (K). I10; (L). I11; (M). I12; (N). I13; (O). I14; (P). I15; (Q). I16; (R). I17; (S). I18; (T). I19; (U). I20; (V). I21; (W). I22; (X). I23; (Y). I24; (Z). I25.

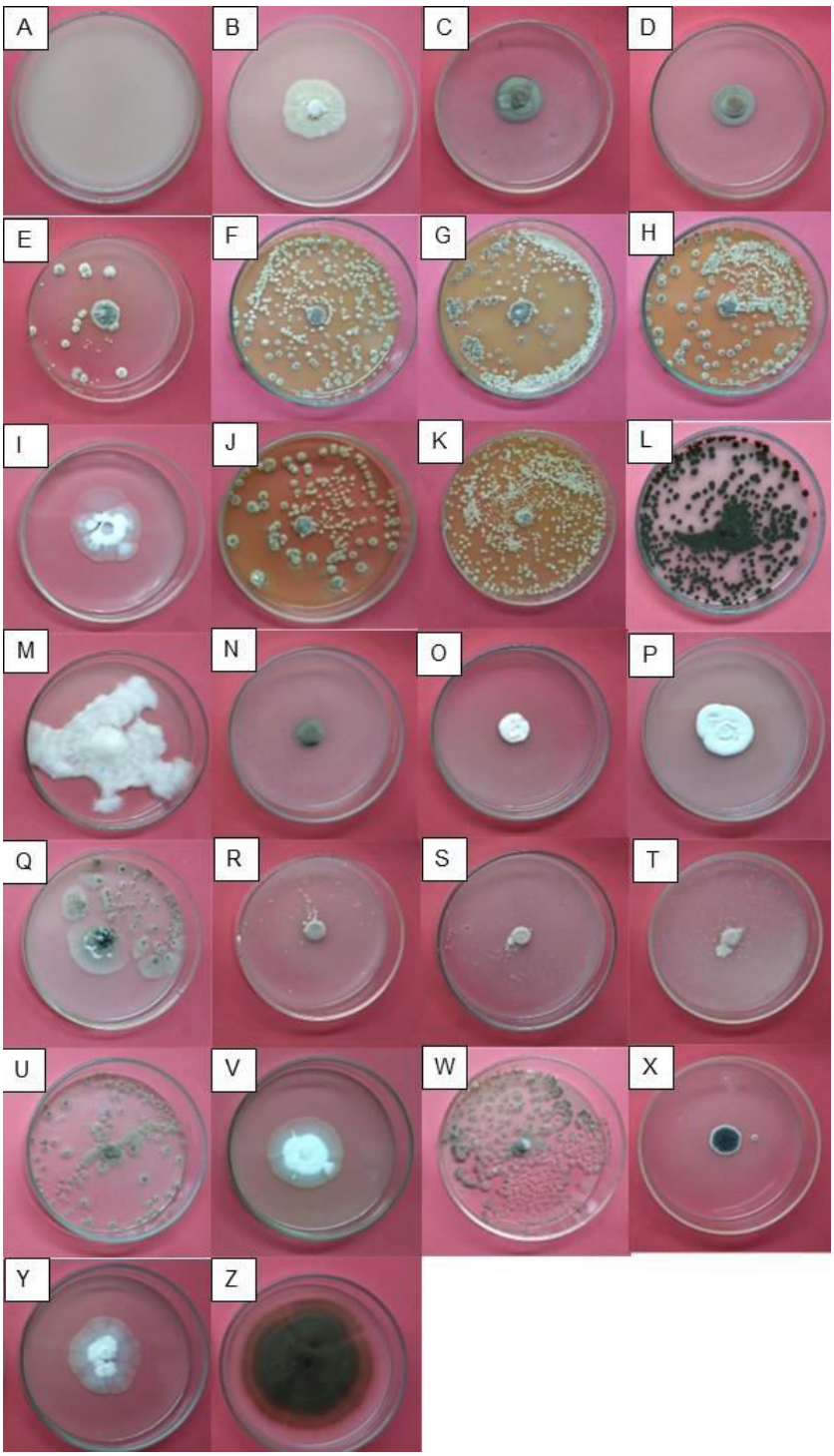

Fig. 4. Diameter of fungi (Observed 14x24 hours). (A). Control; (B). I1; (C). I2; (D). I3; (E). I4; (F). I5; (G). I6; (H). I7; (I). I8; (J). I9; (K). I10; (L). I11; (M). I12; (N). I13; (O). I14; (P). I15; (Q). I16; (R). I17; (S). I18; (T). I19; (U). I20; (V). I21; (W). I22; (X). I23; (Y). I24; (Z). I25.

Table 3. Observation results of clear zone and yellow zone

No	Isolate	Repetition			Color Change
		I	II	III	
	Control	-	-	-	No color change
1	I1	-	-	-	No color change
2	I2	-	-	-	No color change
3	I3	+	+	+	Clear
4	I4	-	-	-	Yellow
5	I5	-	-	-	Yellow
6	I6	-	-	-	Yellow
7	I7	-	-	-	Yellow
8	I8	+	+	+	Clear
9	I9	-	-	-	Yellow
10	I10	-	-	-	Yellow
11	I11	-	-	-	No color change
12	I12	+	+	+	Clear
13	I13	+	+	+	Clear
14	I14	+	+	+	Clear
15	I15	+	+	+	Clear
16	I16	-	-	-	No color change
17	I17	+	+	+	Clear
18	I18	+	+	+	Clear
19	I19	-	-	-	No color change
20	I20	+	+	+	Clear
21	I21	+	+	+	Clear
22	I22	+	+	+	Clear
23	I23	-	-	-	No color change
24	I24	+	+	+	Clear
25	I25	++	++	++	Very Clear
26	I26	-	-	-	No color change
27	I27	-	-	-	No color change
28	I28	-	-	-	No color change
29	I29	-	-	-	No color change
30	I30	-	-	-	No color change
31	I31	-	-	-	No color change
32	I32	-	-	-	No color change
34	I34	-	-	-	No color change

Figure 3 displays the changes in the diameter of the fungal colonies measured at time intervals of 7 and 14 days of observation, providing an overview of the development of colony growth within the period. The presence of clear zones on solid media containing pesticides is an important indicator in the study of pesticide degradation by fungi. A clear zone is an area around a fungal colony on solid media that does not show the growth of other microorganisms or that is brightened due to the degradation of a particular substance (I3, I8, I12, I13, I14, I15, I17, I18, I20, I21, I22, I24, I25), in this case pesticides. The observation made is that the formation of a clear zone indicates that the fungus has the ability to metabolize or degrade pesticides present in the media. The clear zone indicates the area where the pesticide has been effectively degraded by the fungus, allowing the fungus to grow well around the area. Clear zones are used as a screening method to identify potential fungi in bioremediation. The clear zone formed around a fungal colony on solid media containing pesticides indicates the degradation activity of the pesticide by the fungus. Fungi that are effective in degrading pesticides will produce enzymes that break down pesticide molecules into simpler and less toxic compounds, which are visible as clear zones.

Table 4. Best fungal isolate characters

Parameters observed	Isolate character	Reference
Colony Color	Greenish black	Greenish black with a smooth texture [13]
Colony Shape	Smooth	
Conidiophores	Smooth and long, branched	Branched [13]
Phialid	Shaped like a pyramid	The phialids are shaped like pyramids.
Conidia	Oval-shaped and interlocking, smooth-walled	Conidia are smooth-walled, semi-spherical to short oval and clustered together [13] Oval conidia attached to each other, long conidiophores, and pyramid-like phialids [30]

Characterization was carried out on fungi that have a high ability to degrade pesticides, identification up to the genus level. Observations of isolate I24 macroscopically are shown in Fig. 5A. Microscopic observation is shown in Fig. 5B.

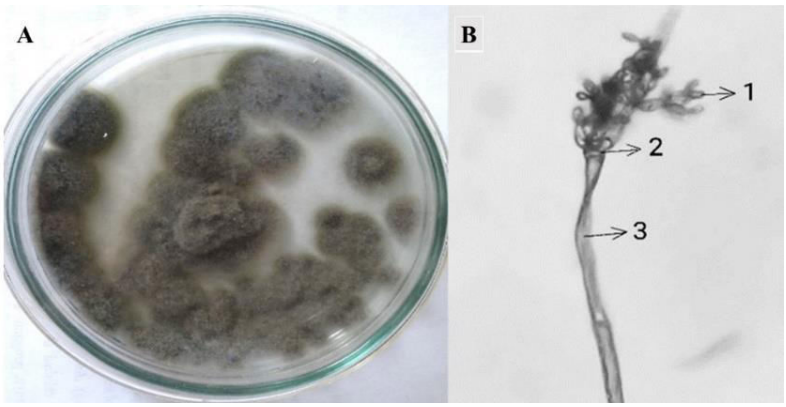

Fig. 5. A. Fungal colonies 14 days on PDA media. B. Morphology of the fungus at 400x magnification (1) Conidia, (2) Fialid, and (3) Conidiophores.

Data on water quality in the Mengaji and Prukut Rivers, Banyumas Regency are presented in Table 5. The effectiveness of superior pesticide-degrading fungal isolates was evaluated for its impact on non-target insects. The results are shown in Fig.6. When the observation was conducted for five days, no insect larvae mortality was detected.

Table 5. Water quality data of Mengaji and Prukut Rivers

No	Variable	Site								
		I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**
1	Current speed (m/s)	2.3	2	1.2	0.96	1.2	0.7	2.4	2.1	1.44
2	Temperature (^{o}C)	20	21	20	21	22	27	21	21.6	21.6
3	pH	7	7	7	7	6.5	6.5	7	7	7
4	O2	8.4	8.2	8.1	7.9	7.5	7.2	8.6	8.5	8.4

Fig. 6. Larvae caused by the *Trichoderma* sp.

4 Discussion

A total of 34 fungal isolates were found in 9 sites of the Prukut River and Mengaji River, with Sites II, V, and VI having the five most isolates (Table 1), which is thought to be related to pesticide spraying activities in the area, affecting environmental quality and river organisms [17][19][24].

The isolates obtained by the fungus were tested for their ability to degrade chlorpyrifos-active pesticides, including observations of colony diameter, clear zone, and water quality. The diameter of fungal colonies carried out on 7 and 14 hours observation (Table 2, Fig. 2, and Fig. 3) shows that isolates I25 had a largest average diameter value compared to other isolates (7.03 ± 0.60 cm). Therefore, I25 isolated from Mengaji River Banyumas Regency are isolates that have the best ability to degrade pesticides. Fungi that grow well in media containing chlorpyrifos produce a large mycelium growth diameter of more than 3 cm and can be interpreted that the fungus is able to metabolize optimally [25]. Conversely, the smaller the average value of colony diameter, the smaller the ability to degrade pesticides made from chlorpyrifos.

To evaluate the degradation kinetics by isolate I25 over time, observations were made on days 7 and 14. The colony diameter of I25 on day 7 increased until day 14, with a

significant clear zone around the colony. This indicates that the fungus is not only able to grow well, but also shows strong enzymatic activity in degrading chlorpyrifos.

The kinetics of pesticide degradation by fungal isolates can be explained through several biochemical mechanisms. Enzymes such as chlorpyrifos-degrading enzymes (e.g. phosphotriesterases) produced by fungi play a key role in this process. The degradation mechanism starts with the hydrolysis of chlorpyrifos to 3,5,6-trichloro-2-pyridinol (TCP), which is then further broken down by the fungi [26].

Fungi from the genus Aspergillus and Penicillium have the ability to degrade chlorpyrifos through hydrolytic and oxidative pathways. These enzymes catalyzed the ester bond breaking reaction and significantly increased enzymatic activity in chlorpyrifos-containing media [27].

Organophosphate pesticides are a fairly large class of pesticides. Organophosphate pesticides contain the chemical group H3PO4 (phosphoric acid) which is toxic but the active ingredient of the pesticide. Organophosphates are chemically classified into phosphoric acid esters or thiophosphoric acid which are insecticides as the most acutely toxic and carcinogenic insect repellent. Its effects include blocking the transmission of nerve impulses by binding to the enzyme acetylcholinesterase. One type of pesticide that is part of the organophosphate group is chlorpyrifos. Chlorpyrifos has a broad spectrum and is an organophosphate insecticide that can suppress insect growth and activity. Chlorpyrifos has the chemical name 0,0-diethyl 0-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate. Its Chemical Abstracts Service (CAS) number is 2921-88-2.

The fungus was able to degrade chlorpyrifos and 3,5,6-trichloro-2-pyridinol (TCP) and was able to utilize it as a sole carbon source. The degradation mechanism shows that chlorpyrifos is hydrolyzed into TCP, dechlorinated 2-Pyridinol, and then pyridine ring catabolism occurs. Fungi living in chlorpyrifos-contaminated environments produced 100% and 94.3% degradation percentages. Chlorpyrifos is hydrolyzed into TCP and DETP. TCP breakdown results in ring breakage. DETP breakdown produces H2O and CO2.

Chemical compounds from pesticides are utilized by fungi for their growth. Chemical compounds are used by microorganisms for growth and reproduction through the oxidation process [28]. The degradation mechanism of chlorpyrifos-active pesticides chlorpyrifos is hydrolyzed to TCP (trichlorophenylmethylodosalicylate), dechlorinated 2- Pyridinol, and then catabolism of the pyridine ring occurs [29]. The mechanism of chlorpyrifos biodegradation is that chlorpyrifos is broken down into TCP (trichlorophenylmethylenodosalicylate) and dechlorinated 2- Pyridinol. The breakdown of TCP results in ring damage. The breakdown of dechlorinated 2- Pyridinol produces H2O and CO2 [14].

Clear zones on solid media containing pesticides indicate the ability of fungi to degrade pesticides, used as indicators in bioremediation studies to identify fungi that are effective in metabolizing pesticides. Isolate I24 was rated best in two parameters, namely colony diameter and clear zone

Fungi that have a high ability to degrade pesticides are identified up to the genus stage. Observations on isolate I 24 macroscopically show that the colony is greenish black with a smooth texture (Fig. 5A). Microscopic observations showed that the walls of the conidiophores were smooth and long, had pyramid-shaped phialids, oval-shaped conidia and clustered together (Fig. 5B). *Trichoderma* sp. colonies are greenish black with a smooth texture [23]. *Trichoderma* sp. has conidia that are smooth-walled, semi-round to short oval and clustered together. The conidiophores are branched and the phialids are shaped like pyramids. *Trichoderma* sp. has oval-shaped conidia that stick together, long conidiophores, and a pyramid-like phialid shape [30].

Trichoderma sp. can dissolve phosphate into organic phosphate compounds and produce the hormone IAA. This fungus can also spur plant growth and decompose lignin.

Trichoderma sp. can be packaged into biofertilizer to help plant growth and degrade pesticide residues on agricultural land. Biofertilizer is made by preparing *Trichoderma viride* [25] which has the ability to dissolve phosphate compounds, produce IAA, and decompose cellulose and lignin. The fungal mycelium was mixed with compost, 10% NPK, and 10% guano fertilizer, then applied to rice plants as much as 100 g/pot, with fertilization carried out twice, namely when the rice was 2 weeks old and at flowering. The addition of Trichoderma viride biofertilizer was able to increase the growth of Ciherang rice varieties with plant height reaching 100.45 cm and the number of tillers up to 31.20, superior to chemical fertilizers with plant height of 87.42 cm and the number of tillers 31.75, and compost with plant height of 68.47 cm and the number of tillers 7.00.

The ability of fungi to degrade pesticides is influenced by the availability of pesticides or metabolites, physiological conditions, proliferation, and sustainability of microorganism populations [21]. The reaction of microorganisms to chemicals such as pesticides varies, and the processes carried out include degradation, transformation, and absorption of toxic substances [31]. Nutrients such as carbon, nitrogen, and phosphorus are required for the growth and metabolism of microorganisms.

Based on water quality data Table 5, *Trichoderma* sp. came from Site VI of Mengaji River, Banyumas Regency with water quality data including current speed of 0.7 m/s, temperature of 27ºC, pH 6.5, and oxygen content of 7.2. Each site has pesticide spraying activities. Pesticide spraying activities cause the surrounding environment to become polluted so that organisms must have good adaptation. In addition, Site VI has the lowest current speed, the highest temperature, acidic pH, and the lowest oxygen content. Trichoderma sp. has a life span at pH 3-7 and temperature 7-41ºC [32]. This is in accordance with water quality data where Site VI has an acidic pH and high temperature.

Evaluation of the effectiveness of superior isolates of fungi in pesticide degradation and their impact on non-target insects, found very encouraging results (Fig.6). Based on observations over a five-day period, no insect larval deaths were detected. This indicates that the identified fungal isolates are not only effective in degrading pesticides, but are also safe for non-target insects. The effectiveness of pesticide degradation by this fungal isolate was good, meaning that the pesticide degraded quickly before it could reach dangerous concentrations for non-target insects. The rapid degradation process reduces pesticide residues in the environment, so insect larvae remain safe during the observation period.

Fungi as pesticide degradation agents function to break down pesticides into less toxic or non-toxic compounds, reducing negative impacts on non-target organisms such as beneficial insects. The use of effective pesticide degradation agents can reduce pesticide residues, maintain ecosystem balance, and support sustainable agricultural productivity. Within the framework of Integrated Pest Management (IPM), pesticide degradation agents can reduce reliance on chemical pesticides. Although fungal species have been selected, the use of fungi in in-situ bioremediation in agricultural environments faces challenges such as adjustment of techniques, interaction with other microorganisms, and the time required for degradation. To overcome these challenges, optimization strategies such as biostimulation, use of nanotechnology, and advanced culture techniques can be applied [33, 34]. Future improved isolates could be used for pesticide degradation in the field, overcoming stunted growth and low viability in killing insects, in line with studies showing the effectiveness of isolates such as *Pseudomonas* sp. LBKURCC419 in degrading naphthalene [35] and *Penicillium rolfsii* Y-1 in overcoming radicle growth inhibition [36], without harming non-target insect species [37].

5 Conclusions

Based on the results and discussion, it can be concluded that:

1. Fungal isolates obtained from nine sites of the Mengaji and Prukut Rivers in Banyumas Regency consisted of 34 isolates.
2. Fungal isolates from Mengaji River and Prukut Banyumas Regency that have the best ability to degrade pesticides are *Trichoderma* sp.

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