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Isolation and potential evaluation of organophosphate-indigenous degrading fungi from Singolangu Farmland, Magetan, Indonesia

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Abstract. *Pujiati, Hertanti, Kiswardianta RB, Fatimah, Ramadhan R, Ni'matuzahroh. 2025. Isolation and potential evaluation of organophosphate-indigenous degrading fungi from Singolangu Farmland, Magetan, Indonesia. Biodiversitas 26: 166-177.* Pesticide use is prevalent in Indonesian agriculture, especially in vegetable farming regions like Singolangu Hamlet, Sarangan Village, Plaosan, Magetan, East Java. While pesticides effectively control pests, their overuse and misuse have caused soil contamination, health disruptions, and decreased agricultural productivity. This study examines the potential of indigenous fungi from Singolangu to remediate soil polluted by organophosphate pesticides, specifically chlorpyrifos and profenofos. Twelve indigenous mold isolates were isolated and ex-situ bioaugmentation was performed with six treatments. The levels of N, P, K, pH, and organophosphate pesticide residues were analyzed. *Aspergillus flavus* was the most effective in bioremediation, achieving significant results within four weeks and displaying N, P, K, pH values of 101 ppm, 99 ppm, 306 ppm, and 6, respectively. The KPSK Formula (a mixture of all Fungi KPS1 to KPS12) which utilized a 12-isolate consortium, also showed significant results, with N, P, K, and pH values of 57 ppm, 59 ppm, 138 ppm, and 6, respectively. The control soil had 150 ppm of chlorpyrifos and 29 ppm of profenofos left over from the pesticides, but the bioremediation treatment KPS3 lowered these levels to 97 ppm of chlorpyrifos and 5.5 ppm of profenofos. This study indicates that indigenous fungi can efficiently degrade profenofos and chlorpyrifos pesticides, either individually or in consortium.

Keywords: Agricultural soil, bioremediation, chlorpyrifos, Indigenous fungi, profenofos

INTRODUCTION

Agriculture plays a crucial role in Indonesia, underpinning the nation's food security. The Central Bureau of Statistics (BPS) reported that food crop areas covered 10,657,275 ha in 2020 and 10,515,323 ha in 2021. This sector significantly impacts the economy, contributing Rp1,354 trillion to the GDP, which accounts for 12.4% of the national total, and provides essential services like food security, employment, and poverty reduction (Mukhlis and Gürçam 2022). Despite its importance, various issues with agricultural land continue to hinder productivity.

Farmers widely perceive pesticides to be an effective and easy solution to eliminate Plant Pest Organisms quickly. The use of pesticides has detrimental effects on the agricultural environment. It poses a threat to human and animal life due to the accumulation of pesticide residues in agricultural products and water sources (Jasrotia et al. 2024). Previous studies have highlighted that only 20% of pesticides are applied accurately, while the remaining 80-90% are applied incorrectly, which often results in ineffective pesticide use (Sun et al. 2018; Schreiber et al. 2018). The impact of pesticides on the environment is exacerbated by their persistence and tendency to penetrate soil layers, thereby causing pollution in undesirable areas and aquatic habitats (Jumaeva 2023). The excessive use of pesticides can lead to decreased soil fertility, which is an indication of polluted agricultural soil. The use of pesticides, disrupts soil quality by reducing the microbial contributions to soil fertility, such as soil formation and nutrient cycling (Srinivasulu et al. 2024; Fernández-Triana et al. 2024).

Pesticides significantly affect soil nutrient availability and crop productivity, impeding the nitrogen-fixing process essential for plant growth. The need for immediate action is clear, as excessive pesticide contamination in soil poses global environmental and health risks. Pesticides like glyphosate, paraquat, chlorpyrifos, and profenofos often exceed regulatory limits, impacting over 1.88 million km² of treated land, with hotspots in South America and Asia, particularly Brazil, Argentina, and China (Tang and Maggi

2021). Continuous use of pesticides damages soil biodiversity and results in the leaching of approximately 0.2 million metric tons of pesticides below the root zone annually. Many vegetables and other horticultural plants are planted in Singolangu Hamlet. Pesticides are sprayed regularly, often as a preventive measure, to protect these plants. Based on qualitative data gathered through interviews with farmers in this region, the agricultural soil exhibits significant compaction and is associated with frequent crop failures. The interviewees attributed these issues to the injudicious application of chemical fertilizers and pesticides on their agricultural lands. The detrimental effects of excessive agrochemical use in this locality are manifested through the hardened soil structure and diminished vegetable productivity.

Indigenous fungi have diverse ecological functions, including tolerance to environmental stressors and interactions with other soil organisms. These fungi present in soil exhibit a wide range of ecological roles and adaptations, which contribute to soil health and fertility. They play a crucial part in enhancing soil biodiversity through various mechanisms. Fungi exhibit unique traits that make them more effective decomposers than bacteria in various ecosystems. Their high moisture tolerance, rapid growth rate, and dense hyphal structure enhance their degradation capabilities compared to bacteria (Yu et al. 2023). These traits include bioactivity, distinct growth forms, and resilience in polluted environments. Fungi also form dormant structures like ascospores and rhizome spores to survive unfavorable conditions (Ramirez 2022). Under favorable conditions, fungi decompose pesticides metabolically through their hyphae. Examples of such fungi include *Trichoderma*, *Fusarium*, *Penicillium*, and *Aspergillus* (Bisht et al. 2019). Fungi are vital in maintaining material cycles and recycling organic contaminants in soil ecosystems. They offer an alternative to conventional bioremediation, particularly using indigenous fungi from contaminated soils. This method provides eco-friendly solutions to reduce pesticide residues, promote sustainable agriculture, and minimize environmental impacts (Raffa and Chiampo 2021).

This investigation examines the potential of indigenous fungi to enhance soil quality by remediating pesticide-polluted soil. Through the isolation, characterization, and evaluation of mold formulations, the study aims to demonstrate the significant role of indigenous fungi in improving soil quality. The identification of fungi in soil contaminated with organophosphate pesticide residues and the evaluation of their impact on soil fertility, including N, P, K, pH, and pesticide residue levels, will provide valuable insights into the promising future of sustainable agriculture.

MATERIALS AND METHODS

Study area

The study area is location at Singolangu Hamlet, Sarangan Village, Plaosan Sub-district, Magetan, East Java, Indonesia, and it is situated at an altitude of 1,338 meters above sea level (masl) (Figure 1). This area is located at the base of Mount Lawu in Magetan, which is additionally renowned as the "village of milk", within the vicinity of the Telega Sarangan Magetan tourist destination. The primary commodity in this region is vegetables. The types of vegetables grown here include cabbage, leeks, carrots, celery, potatoes, shallots, mustard greens, chicory, broccoli, cauliflower, and chickpeas (Figure 2).

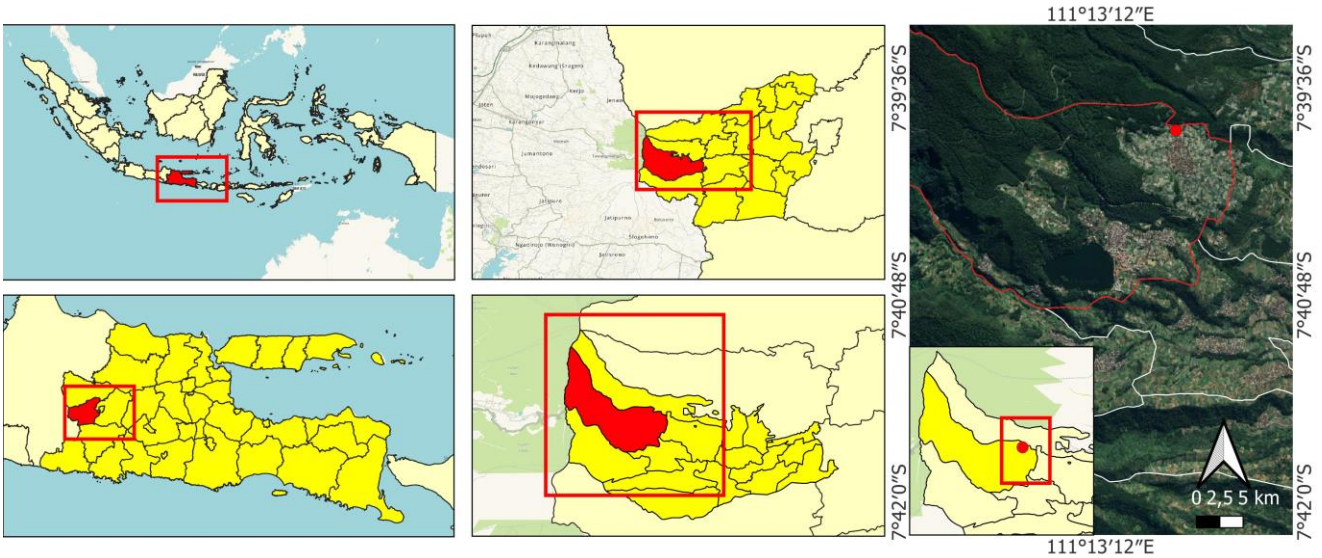


Figure 1. Location of Singolangu Hamlet, Plaosan, Magetan, 1,338 masl (7º39’51.7216" S, 111º13’28.3136" E) and the detected sites in Sarangan Village, Plaosan Sub-district, Magetan, East Java, Indonesia

Procedures

Soil sampling

Soil sampling was conducted using a simple random sampling method at a vegetable farming site in Singolangu Hamlet, Sarangan Village, Magetan, East Java, Indonesia which had been exposed to pesticides. Samples were collected at a depth of 5-30 cm below the surface (Efeoğlu et al. 2022) and stored in a dry ice box. The soil samples were transported to the PGRI Madiun University laboratory and then cleaned for debris and sieved to obtain a homogeneous sample.

Soil sample pretreatment

The soil samples were treated with organophosphate pesticides, including chlorpyrifos (brand name: Fostin 610 EC) and profenofos (brand name: Curacron 500 EC). These pesticides were each dissolved in 1 L of water at a concentration of 2 mL. The soil was soaked for 30 days, dried, pulverized, and sieved. The soil samples containing pesticide residues were divided into 24 groups, each weighing 50 g, with four control groups and 20 treatment groups. Different types of indigenous mold isolates were used for each treatment group.

Mold isolation

Czapek Dox Agar (CDA) was used as the isolation medium for the fungi. The ingredients were heated in a glass beaker with 1000 mL of distilled water and sterilized in an autoclave for 15 minutes at 121°C and 1-2 atm pressure. The soil sample was weighed and suspended in 9 mL sterile distilled water (10 g/90 mL) to perform multilevel tenfold serial dilution. Dilutions of 10^{-3}, 10^{-4}, and 10^{-5} were used to isolate the mold. The mold grown on the isolation media was then transferred to PDA media in test tubes.

Preparation of fungal stock cultures

Indigenous fungi, initially grown on CDA media from soil samples, were subsequently subcultured onto slant media for stock culture preservation and observation. Four replicates were prepared for each fungal isolate. The stock culture preparation involved suspending the rejuvenated fungi in 600 mL of physiological saline, followed by the addition of 6 g of NaCl (10 g NaCl/L) and a pesticide seditester. The cultures were then incubated at room temperature on a shaker platform operating at 150 rpm for a period of 4 days

Macroscopic and microscopic examination of fungal cultures

Direct visual inspection of fungal cultures on agar plates constituted the macroscopic examination. This process involved characterizing the mold isolates by observing the pigmentation of their conidia and mycelia. For microscopic analysis, the morphological features of spores, conidia, and hyphal structures were scrutinized

Bioremediation test

The bioremediation trial began with the preparation of stock cultures using selected mold isolates. Six treatment groups were established, each containing 50 g of sterile soil mixed with pesticide. To ensure homogeneity, the contaminated soil samples were cleaned and sifted. Observations were conducted weekly for four weeks, on days 7, 14, 21, and 28. The degradation of chlorpyrifos and profenofos was analyzed using a liquid chromatography-mass spectrometry (LC-MS) system. The LC-MS was equipped with a C18 column (1.7 to 5 µm mm, particle size 5 µm). Separation of intermediates was performed using a 60:40 (v/v) mixture of acetonitrile and water as the mobile phase, with a flow rate of 0.5 mL/min at room temperature (Naik et al. 2022; Tabasum et al. 2022).

Data collection

Data were analyzed both descriptively and quantitatively. Descriptive data focused on identifying the types of mold isolated from pesticide-contaminated soil in Singolangu Hamlet, Magetan, Indonesia. Quantitative data, N, P, K levels, pH values, and residual levels of chlorpyrifos and profenofos pesticides were determined. The measurements for N, P, K, and pH levels were obtained using a digital soil N, P, K tester, while the pesticide residue levels were measured using LC-MS.

RESULTS AND DISCUSSION

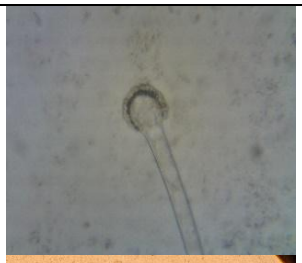
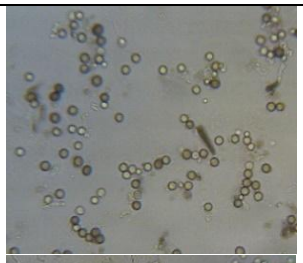
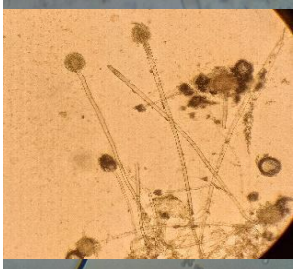
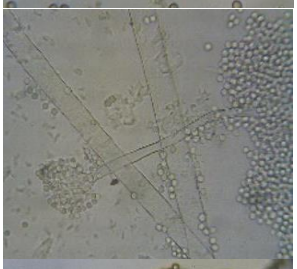
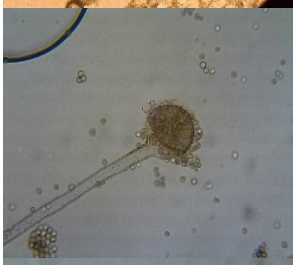
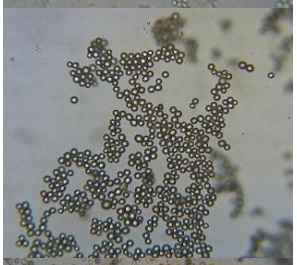
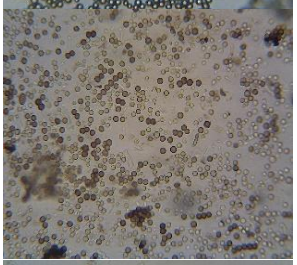
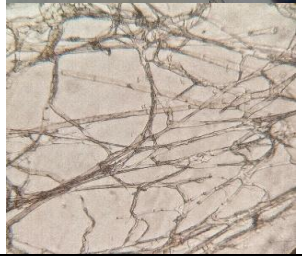
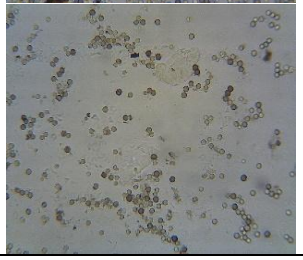
The bioremediation of soil contaminated with pesticide residues using indigenous fungi isolated via CDA media resulted in several key achievement parameters, including the chemical indicators in the treatment soil. These levels include N, P, K, pH, and organophosphate pesticide (chlorpyrifos and profenofos) reduction levels.

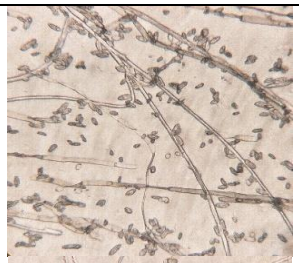
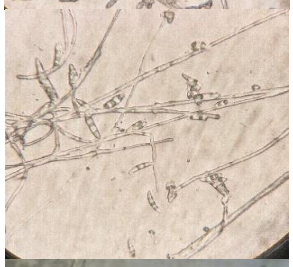
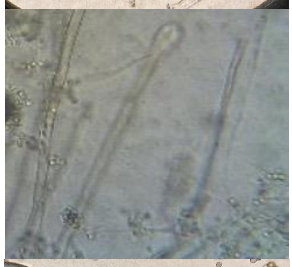
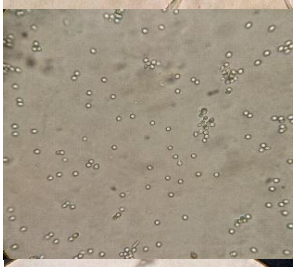
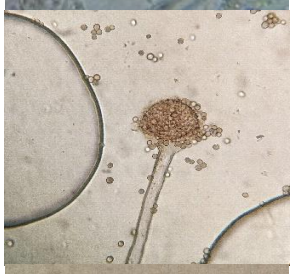
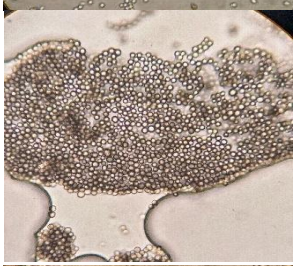
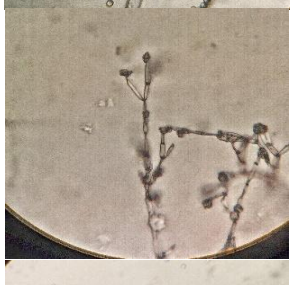
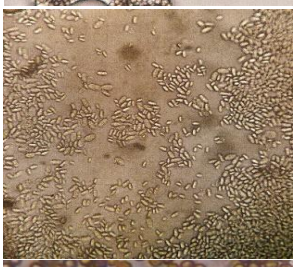
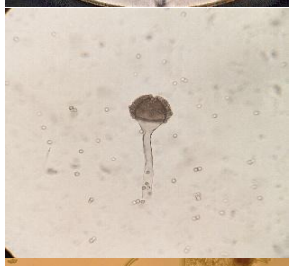
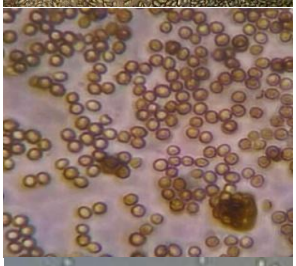
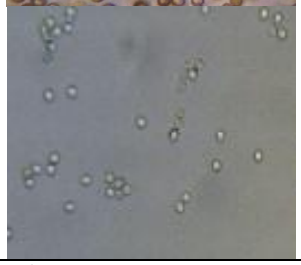
Figure 2. Sampling site condition

Indigenous fungi

This study involved the meticulous isolation and identification of 12 indigenous fungal isolates from soil samples containing organophosphate pesticides. The identification of these fungi was performed with utmost care, both macroscopically and microscopically, with results compared to supporting journals to ensure accuracy. The reference indigenous fungi that were successfully isolated were primarily spp. of *Aspergillus* (Table 1). The samples analyzed revealed mould species from four genera (i) *Aspergillus*; (ii) *Phoma*; (iii) *Cladophialophora*; and (iv) *Fusarium*, with two species remaining unclassified. *Aspergillus* was the most prevalent genus, represented by seven isolates (i) *Aspergillus fumigatus*; (ii) *Aspergillus* sp.; (iii) *Aspergillus flavus*; (iv) *Aspergillus niger*; (v) *Aspergillus tamarii*; (vi) *Aspergillus oryzae*, and (vii) *Aspergillus sydowii*. A single isolate of *Phoma* sp. was identified from the *Phoma* genus. The analysis also yielded one isolate each from the *Cladophialophora* and *Fusarium* genera, specifically *Cladophialophora* sp. and *Fusarium solani*, respectively.

Table 1. Indigenous Fungi from Singolangu Hamlet, Singolangu, Magetan, East Java, Indonesia

Isolate code	Mold pictures	Conidia images	Spores images	References
KPS_1				*Aspergillus fumigatus* (Kumar et al. 2021)
KPS_2				*Aspergillus* sp. (Glässnerová et al. 2022)
KPS_3				*Aspergillus flavus* (Bharose et al. 2017)
KPS_4				*Aspergillus niger* (Mohapatra et al. 2021)
KPS_5				*Phoma* sp. (Devi et al. 2018)

KPS$_6$				*Cladophialophora* sp. (Levin et al. 2004)
KPS$_7$				*Fusarium solani* (Wasti et al. 2020)
KPS$_8$				Unidentified
KPS$_9$				*Aspergillus tamarii* (Guchi et al. 2014)
KPS$_{10}$				Unidentified
KPS$_{11}$				*Aspergillus oryzae* (Jagat et al. 2021)
KPS$_{12}$				*Aspergillus sydowii* (Jurjevic et al. 2012; Wang et al. 2021)

Notes: KPS1: *Aspergillus fumigatus*; KPS2: *Aspergillus* sp; KPS3: *Aspergillus flavus*; KPS4: *Aspergillus niger*; KPS5: *Phoma* sp.; KPS6: *Cladophialophora* sp.; KPS7: *Fusarium solani*; KPS8: Unidentified species; KPS9: *Aspergillus tamarii*; KPS10: Unidentified species; KPS11: *Aspergillus oryzae*; KPS12: *Aspergillus sydowii*

In sum, twelve potential fungal isolates were detected in the study. Four of the twelve mold isolates were chosen for further testing in the bioremediation process, namely KPS1, KPS2, KPS3, and KPS4 based on their dominant presence in the isolation results. In the bioremediation test, these fungi were also compared with the entire consortium, which consisted of a mixture of KPS1 to KPS12 (KPSK, Kapang Singolangu Konsorsium). The bioremediation process involved assessing the quality of the soil, which included evaluating its N, P, K, and pH levels, as shown in Table 2 and Figure 3. Additionally, the levels of degraded pesticides were also quantified, as depicted in Figure 4.

Table 2. Bioremediation test results: N, P, K, and pH levels of remediated soil

Treatments	Fermentation time															
	W_1				W_2				W_3				W_4			
	N	P	K	pH	N	P	K	pH	N	P	K	pH	N	P	K	pH
KPS_0	4	5	8	5	4	3	9	5	4	4	11	5	3	2	7	5
KPS_1	22	39	112	5	33	63	209	5	54	85	254	6	65	90	284	6
KPS_2	24	34	99	6	47	69	135	6	65	82	196	6	93	103	220	6
KPS_3	37	24	148	6	48	33	181	6	68	60	207	6	101	99	306	6
KPS_4	43	23	54	5	46	41	110	5	57	52	143	6	64	65	176	6
KPS_K	33	42	92	5	44	45	109	5	50	47	123	5	57	59	138	5

Notes: KPS0: Control treatment; KPS1: *Aspergillus fumigatus*; KPS2: *Aspergillus* sp; KPS3: *Aspergillus flavus*; KPS4: *Aspergillus niger*; KPSK: A mixture of all fungi KPS1 to KPS12 ; W1: Week 1; W2: Week 2, W3: Week 3; W4: Week 4

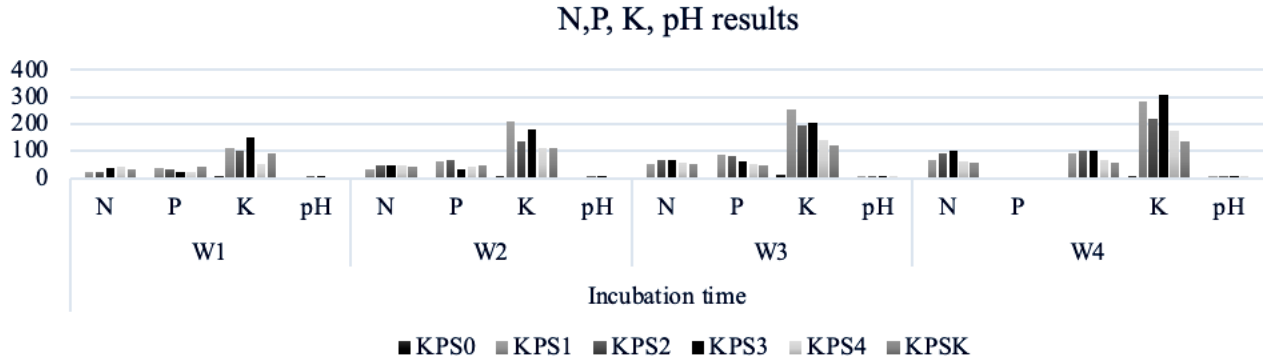


Figure 3. N, P, K, pH results. KPS0: Control treatment; KPS1: *Aspergillus fumigatus*; KPS2: *Aspergillus* sp; KPS3: *Aspergillus flavus*; KPS4: *Aspergillus niger*; KPSK: A mixture of all Fungi KPS1 to KPS12; W1: Week 1; W2: Week 2, W3: Week 3; W4: Week 4

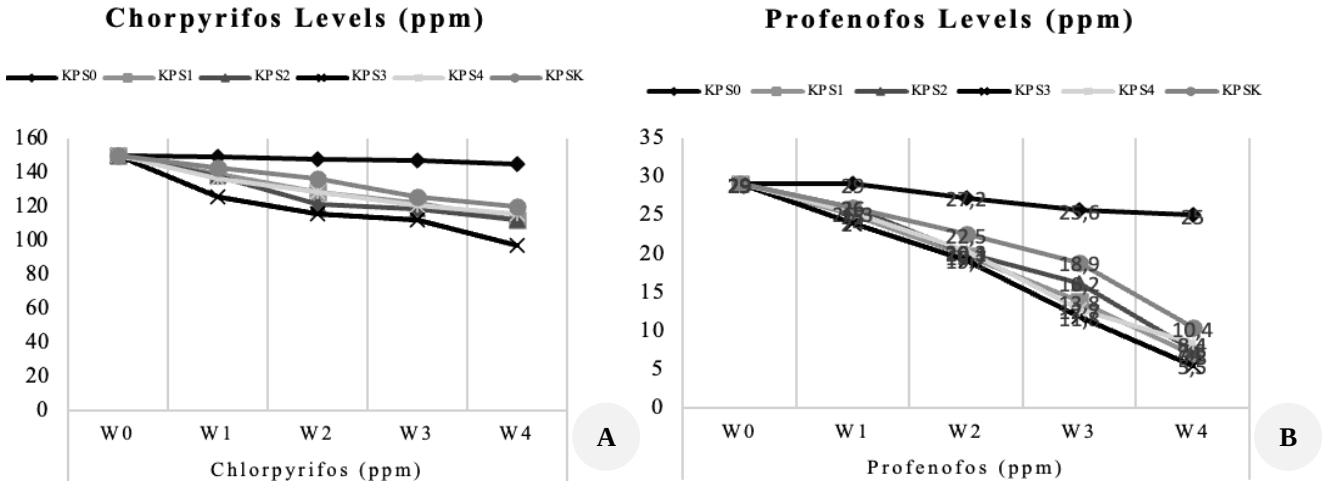


Figure 4. The levels of A. Chlorpyrifos; B. Profenofos in different treatments. KPS0: Control treatment; KPS1: First type of Singolangu potential mold; KPS2: Second type of Singolangu potential mold; KPS3: Third type of Singolangu potential mold; KPS4: Fourth type of Singolangu potential mold; and KPSK: A mixture of all Fungi KPS1 to KPS12

Based on comprehensive research and rigorous mold characterization. This diverse assemblage of fungal species encompasses multiple genera, with a predominance of *Aspergillus* species. The presence of unidentified species (KPS8 and KPS10) indicates the potential existence of novel or less common fungal taxa within the studied environment. KPS1 (first type of Singolangu potential mold), KPS2 (second type of Singolangu potential mold), KPS3 (third type of Singolangu potential mold), KPS4 (fourth type of Singolangu potential mold), and KPSK(A mixture of all Fungi KPS1 to KPS12).

The levels of nitrogen (N), Phosphate (P), Potassium (K), and pH levels in the soil were tested weekly on days 7, 14, 21, and 28. The data indicate that the control treatment, designated as KPS0, consistently had the lowest average nitrogen (N) value among all treatments. This observation was confirmed by the NPK test, which revealed an average N value of 3-4 ppm in the control soil every week. In contrast, the treated soil had a significantly higher N value compared to the control, but it was not significantly different from the other treatments. The highest average N value was observed in the KPS3 treatment, which reached 101 ppm at the end of the measurement in week 4. The N values in formulas KPS1, KPS2, KPS4, and KPSK (A mixture of all Fungi KPS1 to KPS12) also increased every week, indicating that the duration of the bioremediation process conducted by indigenous fungi is influenced by time.

The highest N value recorded in the test was in week 4. Phosphorus content in the control soil remained stable, fluctuating between 2 and 5 ppm each week. On the other hand, the values in the soil treatments with the mold formula showed a consistent increase every week, as illustrated in Table 2 and Figure 3. Soil using the KPS2 mold formula has proven to be the most advantageous, offering an average of 33 ppm, 69 ppm, 82 ppm, and 103 ppm per week. According to the analysis of P levels in the soil, the treatment soil has been found to possess elevated levels of phosphorus, with a value of >33 ppm. Conversely, the control soil exhibited exceptionally low phosphate concentrations, consistently below 10 ppm. These measurements were assessed against established criteria for available phosphate in the soil, as detailed in Table 3.

According to the data collected by measuring potassium levels in a control soil every week for a month, the average potassium level in the soil was found to be less than 10 ppm, except for 11 ppm in week 3. Although the potassium level increased in week 3, it was still within the low range. The treatments applied were KPS0 (control treatment), KPS1 (first type of Singolangu potential mold), KPS2 (second type of Singolangu potential mold), KPS3 (third type of Singolangu potential mold), KPS4 (fourth type of Singolangu potential mold), and KPSK (A mixture of all Fungi KPS1 to KPS12). The treatment with the indigenous mold formula resulted in potassium levels above 60 ppm, indicating that the potassium content in the soil was very high. The effect of time on the performance of indigenous fungi in the formula was observed, but it did not affect the control. The effect of the K value on time can be seen in Figure 3.

The K value in the control soil was found to be in the very low range, while the KPS1, KPS2, KPS3, KPS4, and KPSK treatments resulted in values within the very high range. These results were obtained by comparing the results and criteria for K content in the soil presented in Table 4.

Bioremediation test results: Pesticide levels

A comparison of pesticide residue levels between the control and KPS3 treatments showed that the control treatment soil had high levels of chlorpyrifos residue at 150 mg/kg. In contrast, soil treated with KPS3 formula had significantly lower levels at 97 mg/kg. These results suggest that the KPS3 formula can degrade organophosphate pesticides with the active ingredient chlorpyrifos. A similar trend was observed with the organophosphate pesticides and the active ingredient profenofos. The control soil had 29 mg/kg of profenofos residue, while the KPS3-treated soil had much lower levels at 5.5 mg/kg. The results of the LC-MS test results for pesticide residues are shown as illustrated in Figure 4.

Discussion

The bioremediation of soil contaminated with pesticide residues using indigenous fungi isolated via CDA media has shown several achievements, including changes in the physiochemical parameters in the treatment soil. These levels include N, P, K, pH, and pesticide reduction levels.

Indigenous fungi

Twelve isolates of indigenous fungi were obtained from soil samples containing organophosphate pesticides. These fungi were identified both macroscopically and microscopically, and the results were compared to supporting journals. The reference indigenous fungi that were successfully isolated were primarily *Aspergillus* spp. *Aspergillus* species exhibit a high prevalence in soils contaminated with pesticides due to their remarkable capacity to degrade these compounds, utilizing them as nutrient sources. This capability is attributed to their diverse enzymatic machinery, which enables them to decompose complex pesticide molecules into simpler, less toxic substances. *Aspergillus* species, such as *Aspergillus niger* and *Aspergillus flavus*, have demonstrated efficacy in degrading organophosphorus and organochlorine pesticides, resulting in their abundance in contaminated soils.

This biodegradation process not only contributes to the reduction of pesticide toxicity but also facilitates the growth and proliferation of these fungi in such environments (Matúš et al. 2023). As decomposers, they break down organic matter, which is crucial for maintaining soil health and fertility. Indigenous fungi, including various fungi, play a vital role in nutrient cycling by facilitating the decomposition of organic matter and the mineralization of essential nutrients such as nitrogen and phosphorus. Hence, indigenous fungi are integral to maintaining ecosystem functionality through their multifaceted roles in nutrient cycling. They are vital in the biodegradation of organic waste materials, acting on cellulose, lignin, gums, and other organic compounds, which contribute to nutrient cycling and soil health. For instance, *A. niger* and *A. candidus* have

been shown to effectively decompose wheat crop residues, enhancing soil organic matter and fertility (Awasthi et al. 2017; Singh et al. 2020).

Table 3. Criteria for available phosphorus in soil

Element	Units	Indicators		
		Low	Medium	High
Phosphate	mg/kg (ppm)	10-15	16-25	26-33

Note: Source: Annappa et al. (2024)

Table 4. Criteria for potassium elements in soil

Element	Unit	Indicators				
		Very Low	Low	Medium	High	Very High
Potassium	mg/kg (ppm)	<10	10-20	21-40	41-60	>60

Note: Source: Bahadur et al. (2016)

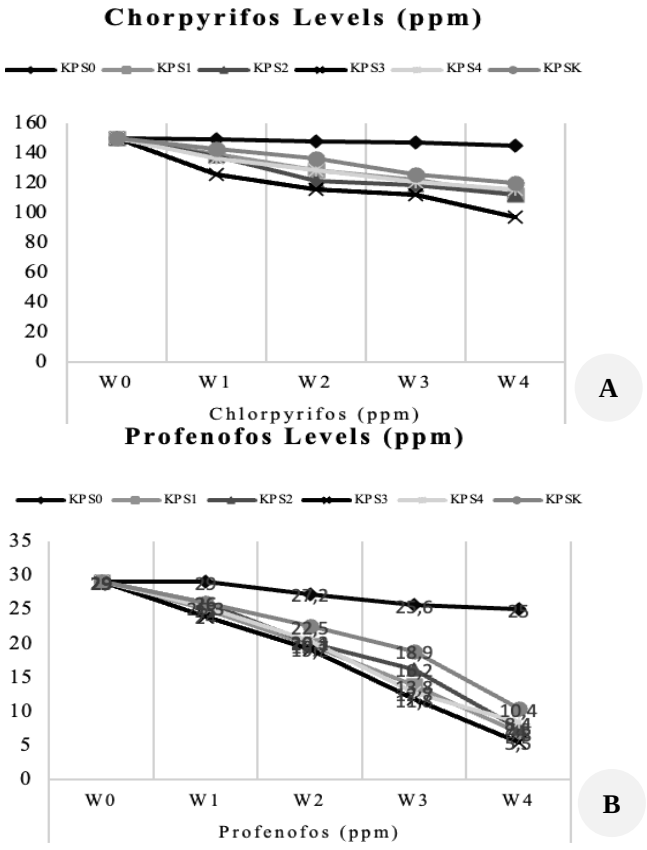


Figure 4. The levels of A. Chlorpyrifos; B. Profenofos in different treatments. KPS0: Control treatment; KPS1: First type of Singolangu potential mold; KPS2: Second type of Singolangu potential mold; KPS3: Third type of Singolangu potential mold; KPS4: Fourth type of Singolangu potential mold; KPSK: A mixture of all fungi KPS1 to KPS12

N, P, K, and pH results

The N levels in the control treatment showed minimal variation, likely due to the presence of indigenous fungi in the soil, which are in line with natural conditions without additional fungi provided. The soil contaminated with pesticide residues and treated with a mixture of all fungi KPS1 to KPS12 (KPSK) recorded the lowest N content. This is due to the presence of fungi with antagonistic properties, which disrupt the growth and development of other fungi (Ismail et al. 2019). The increase in N content in the soil treated with the indigenous mold formula is attributed to the fungi's nitrogen-fixing activity, enhancing soil nitrification. Nitrogen fixation is a crucial function of fungi. Fungi might be able to increase soil nitrification by releasing compounds called brachial acetone from the roots of plants that grow with fungi (Odokonyero et al. 2017). These compounds stop the process of nitrification from happening. A different study found that fungi can improve the movement of nitrogen through hyphae (Caravaca et al. 2005) and the activity of aquaporins, which move Meena et al. (2017a)

Phosphorus (P) is a crucial element for plants, as it facilitates the transportation of energy and carbohydrate components. Moreover, phosphorus accelerates the development of flowers, fruit and seed ripening, and root growth (Ahmed et al. 2024). The increase in phosphate levels within the research soil indicates that the application of indigenous fungi in the treatment also affects the duration of bioremediation. Nevertheless, the control treatment did not show any impact on the duration of bioremediation. Phosphate in soil is often in an insoluble form, limiting plant growth (Tian et al. 2021). Consequently, farmers continue to apply phosphate fertilizers even when the soil contains high to very high total phosphate (Etesami 2020). Phosphate-solubilizing microbes are capable of dissolving insoluble phosphate, allowing plants to acquire the necessary nutrients for growth (Timofeeva et al. 2022). To date, microbial-enriched organic fertilizer has been formulated using a consortium of several superior microbial species. However, this method could be more efficient as it requires various types of superior microbe (Ptaszek et al. 2023).

Therefore, there is a need for multifunctional microbes that can serve two or more functions. Phosphorus can be converted into a form usable by plants through the action of phosphate-solubilizing microorganisms (Yadav et al. 2019). These microorganisms are commonly used to enhance plant growth and development by increasing phosphorus uptake. Among the fungi, phosphate-solubilizing properties are significant in making soil phosphate available for plants (Meena et al. 2017b). Several fungi possess phosphate-solubilizing properties, including *Saccharomycopsis schoenii*, *Cryptococcus luteolus*, *Trichosporon beigelii*, *Rhodotorula aurantiaca*, *Kluyveromyces walti*, *Neosartorya fischeri* var. *fischeri*, *Candida montana*, *Penicillium purpurogenum* var. *rubrisclerotium*, and *Zygoascus hellenicus* (Birhanu et al. 2017). In bioremediation, a mold formula containing the genera *Aspergillus* and *Fusarium* is used, both of which are phosphate-solubilizing genera. Research indicates that

phosphate-solubilizing fungi belong to the genera *Fusarium* sp., *Aspergillus* sp., and *Penicillium* sp. (Meena et al. 2017b).

Phosphate-solubilizing fungi possess the potential to serve as biofertilizers. These fungi can convert insoluble soil phosphate into soluble phosphate due to the secretion of various organic acids. Under optimal conditions, they can mobilize or solubilize approximately 40-50 kg of phosphorus, resulting in a 20-30% increase in crop yield (Khan et al. 2013). The relationship between plants and fungi plays a significant role in plant function and the ecology of plant communities (Vimal et al. 2017). Mycorrhizae, along with plant root hairs, increase the surface area of the soil-root interface as they are crucial components at the soil-root interface through their extraradical hyphae. Therefore, fungi enhance plant nutrient uptake, particularly phosphate. Additionally, the fungi produce substantial amounts of phosphatase, which helps the plant access insoluble, condensed, and complex phosphate in the soil so that it can be utilized (Jyothi and Basaiah 2023).

There are two stages of phosphate reduction by phosphate-solubilizing fungi: chemical and biological (Kumari and Nanayakkara 2017; Yang et al. 2022). The chemical mechanism begins when mold secretes low-molecular-weight organic acids into the soil. These acids form stable complexes with P-binding cations like Al and Fe in acidic soils. Each phosphate-solubilizing mold has a genetically distinct capacity to excrete various types and quantities of organic acids, with the nature of the acids being more crucial than their quantity. The efficiency of these acids varies based on soil microenvironmental conditions. Biological phosphate reduction occurs through the production of enzymes such as phosphatase, which is secreted by roots and soil microorganisms. Phosphatase is produced when phosphate availability is low and becomes less effective when phosphate levels are high. During the mineralization of organic matter, phosphatase enzymes convert organic phosphate compounds into inorganic forms accessible to plants, breaking down bound phosphate into available forms (Verma et al. 2017; Rawat et al. 2021).

These stages involve two processes, Chemical and biological Mechanism. The chemical mechanism commences when the mold secretes several low-molecular-weight organic acids from its metabolism into the soil. These organic acids can form stable complexes with P-binding cations in the soil, such as Al and Fe, which are P binders in acidic soils. Each phosphate-solubilizing mold has a genetically distinct ability to excrete different types and amounts of organic acids. The nature of the organic acids is more critical than the amount produced. This can be seen from the differences in the capacity of each phosphate-solubilizing mold to dissolve P. The efficacy of the organic acids produced is contingent upon the microenvironmental conditions of the soil. Biological phosphate reduction occurs because these microorganisms produce enzymes, including phosphatase enzymes. Phosphatase is an enzyme that is produced when phosphate availability is low; if phosphate availability is high, the phosphatase enzyme is less useful, or microbial production of phosphate is ineffective. Phosphatase is secreted by roots and microorganisms in the soil. In the mineralization process of organic matter, organic phosphate compounds are decomposed into inorganic phosphate forms that are accessible to plants with the aid of phosphatase enzymes. Phosphatase enzymes can break bound phosphate into its available forms.

Potassium is vital for enzymatic processes related to N metabolism and the formation of plant materials (Du et al. 2017). It is the third most important nutrient after nitrogen and phosphorus and is absorbed by plants in the form of K+ ions (Zou et al. 2024). The positive charge of potassium helps neutralize the electrical charge caused by the negative charge of nitrate, phosphate, or other elements (Rizwan et al. 2021; Novair et al. 2023). The availability of potassium is interchangeable and can be absorbed by plants depending on the addition from outside, fixation by the soil itself, and the addition of potassium (Aguilar-Rosero et al. 2022). Mold formula KPS 1 is believed to be *Aspergillus fumigatus*, while KPS 4 is thought to be *A. niger*, which is known to be a potassium solvent. Potential fungi as potassium-soluble biofertilizers, including *Aspergillus* sp. (*Aspergillus terreus*, *A. fumigatus*, and *A. niger),* and ectomycorrhizal fungi (Suleman et al. 2022; Olaniyan et al. 2022).

Soil pH measurements revealed that the control treatment remained acidic, indicating a less fertile soil for plant growth. This finding is in line with research that suggests the application of potassium fertilizer, such as the potassium solubilizing mold isolates in this study, can increase soil pH. The significance of this finding is that higher doses may be more effective than lower doses (Olaniyan et al. 2022). The pH value measurement chart showed a shift in the soil's pH from acidic to neutral with the original mold formulation. By week 4, the pH value of the soil treated with all formulations had reached 6, indicating a significant change in pH and underscoring the importance of this research in soil management.

Pesticide residue levels in soil

The degradation rate of chlorpyrifos after four weeks was approximately 53 mg/kg, while profenofos showed a degradation rate of around 23.5 mg/kg. In terms of environmental remediation, indigenous fungi obtained from soils treated with organophosphate pesticides exhibit a high tolerance for polluted environments. They can break down insecticides, herbicides, polychlorinated organics, and other hydrophobic aromatic compounds. The degradation process begins with the removal of active groups through hydrolysis, dealkylation, and dehalogenation, resulting in the formation of aliphatic or aromatic compounds. The induction of several enzymes follows this to complete the degradation (Mohapatra et al. 2018). Most of the indigenous fungi successfully isolated from soil contaminated with pesticide residues belong to the genera *Aspergillus* and *Fusarium*. Some mold strains used in pesticide biodegradation/ bioremediation include *Fusarium* sp., *Aspergillus* sp., and *Microsphaeropsis* sp. (Birolli et al. 2018; Kumar et al. 2021). Mold possesses the ability to biodegrade diazinon via distinct biochemical pathways involving specific enzymes and metabolic processes. Among these organisms, *Aspergillus* sp. has shown remarkable efficiency in degrading diazinon.

This process is facilitated by enzymes such as hydrolase, acid phosphatase, laccase, cytochrome P450, and flavin monooxygenase, which play critical roles in breaking down diazinon into less harmful metabolites (Wu et al. 2021)

Researchers frequently identify *Aspergillus* species due to its distinctive macroscopic structure and ubiquitous presence in soil. According to previous studies (Barberis et al. 2019), *A. oryzae*, a mold species found in the KPS 3 formula, could reduce 76% of the initial concentration of organophosphate pesticides in synthetic media after a 30-day incubation period. Another study also indicated that *A. niger* mold is utilized as a biofertilizer or microbial fertilizer due to its capacity to break down cellulose into simple C compounds and dissolve phosphate rock into organic phosphate compounds that plants can readily absorb (David et al. 2023). *A. niger* mold is also present in the KPS 4 formula with N, P, and K values that are comparable to other mold formulas. Furthermore, *A. sydowii* mold with isolate code KPS 12 was studied, and the results demonstrated the mold's potential for biocatalytic processes in reactions that typically employ toxic compounds, as well as its primary potential as a degrader of chlorpyrifos, methyl parathion, and profenofos (Soares et al. 2021).

In conclusion, this study has demonstrated the success of the bioremediation process using indigenous mold species in pesticide-contaminated soil. We identified 12 such species, with the genus *Aspergillus* being the most prevalent, followed by other species such as *Phoma* sp., *Cladophialophora* sp., *Alternaria* sp., and *Streptomyces* sp., as well as one unidentified species. The use of indigenous fungi for the bioremediation of pesticide-contaminated soil and the duration of incubation time has a significant impact on the levels of N, P, K, and pH in the soil. The best results for N, P, and K levels were observed at week 4, while the worst results were at week 1. The highest levels of N and K were achieved with the KPS3 formula associated with *Aspergillus* sp. At the same time, the best P values were recorded with the KPS2 formula, another species of *Aspergillus* but a different species. The application of indigenous mold formulas and the duration of bioremediation significantly reduced the levels of pesticide residues in the soil. In the soil containing the KPS3 formula, 53 mg/kg of chlorpyrifos active ingredient, was successfully degraded, while 23.5 mg/kg of profenofos was successfully degraded.

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