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IDENTIFICATION OF POTENTIAL SOIL DEGRADING MICROBIALS CONTAMINATED WITH INSECTICIDES

Zhusna Nisha Maulida, Nafrida Noor Azkiya, Lailatuz Zahro, Alfini Siska Dewi, Tara Puri Ducha Rahmani, & Arnia Sari Mukaromah*

Biology Study Program, Faculty of Science and Technology, Universitas Islam Negeri Walisongo Semarang, Indonesia

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**Corresponding author*: E-mail : arnia_sm@walisongo.ac.id

Abstract

The high use of insecticides can cause soil contamination in the rice field environment, so a solution is needed to reduce the contamination and the negative impact on human health. One of the efforts that can be done to overcome this problem was by bioremediation. The bioremediation technique was chosen due to it is eco- friendly, efficient, and cost-effective in its application. However, bioremediation relies on the capacity of living organisms to absorb, accumulate, translocate and detoxify pollutants in a polluted environment. The objective of this study is to explore microbes that can be used as bioremediation agents in soil exposed to various types of insecticide contamination. The results of this study was as many as ±56 species of microbes can be used as bioremediation agents for various types of insecticides so that bioremediation needs to be carried out in order to avoid pesticide residues on soil and agricultural products.

Keywords*: Agricultural Soil; Bioremediation; Insecticides; Pesticides*

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INTRODUCTION

The agricultural sector is one of the important sectors for the economy in Indonesia, because Indonesia is dubbed as an agricultural country with a wide range of products from agriculture and plantations (Nurdiansyah & Kartika, 2020). In the development of the agricultural sector, many problems are found, such as pests and diseases that attack plants at any time. In 2021, it was predicted that when the first quarter of agricultural commodities such as cocoa would decline by 60-84% due to the cocoa pod borer, while the caterpillar pests on oil palm plants could reduce agricultural yields by 12-40% (Chieloka *et al.,* 2020). Therefore, it is necessary to make efforts to overcome the problem of pests and diseases that attack plants. General efforts made by farmers in overcoming pest attacks through spraying pesticides (Buyang & Pasaribu, 2014). Meanwhile, the use of pesticides can leave residues on the soil that can cause contamination of paddy fields, and have a negative impact on human health. Bioremediation is one of the best solutions to reduce the impact of pesticide pollution on the soil.

Bioremediation is a natural process to remove, reduce, reduce, or disable environmental pollutant in soil and water by relying on living organisms such as bacteria, fungi, and plants. The existence of bioremediation can restore a polluted environment into a clean and non-toxic environment. The implementation of the bioremediation technique was chosen because it iwas environmentally friendly, efficient, and cost-effective. However, the efficiency of bioremediation was determined by the capacity of organisms to absorb, accumulate, translocate, and detoxify pollutants in a polluted environment. Other factors that influence the efficiency of bioremediation include the physicochemical properties of the soil or water in the environment to be remedied (Irawati, 2020; Wang *et al.,* 2021).

Microbes are organisms that are able to live and adapt in various types of environments. Microbes in the soil have the ability to degrade chemical compounds in the form of organic and inorganic materials. Microbes are able to convert chemicals in the soil into water or harmless gases such as CO_2. The types of bacteria that have this ability are *Pseudomonas* and *Agrobacterium*. Meanwhile, the types of fungi that have the same ability are *Trametes hirsutus* and *Trichoderma viride*. Microbes also have the potential to produce hydrocarbon-degrading enzymes so they are widely used as bioremediation materials. Bioremediation using microbes is an alternative strategy that is quite effective and efficient because it does not cause side effects on the surrounding environment. However, microbes can degrade soil if these microbes have adapted to the environment (Lumbanraja, 2014).

Based on Dadrasnia & Agamuthu, (2013) microbes are naturally capable of degrading hydrocarbon compounds that contaminate the soil, but there are several things that need to be considered, including the specification of the type of microbes for degradation activity, and the length of time for degradation which is determined by the length of the hydrocarbon chain.

Chemical pesticides are very popular among Indonesian farmers. Chemical pesticides are synthetic chemicals used by farmers to control plant pest organisms (OPT). Pesticides are the mainstay of farmers' shields in pest control. Besides being applied to agricultural land, pesticides can also be used at home such as poison for rats, fleas, mosquitoes, cockroaches etc. Besides being effective in controlling pests, pesticides can also be toxic to other organisms, including humans (Khoiriyah, 2020). Pesticides have been widely used in the agricultural sector as a whole, especially in the plantation, horticulture and food crops sub-sectors (Ivanda & Zuhro, 2019).

Pesticides used to eradicate insect pests are insecticides. The use of insecticides is very beneficial because it can eradicate pests easily and quickly, reduce pest populations, can reduce crop failure, in food crops will increase 20 times every 25 years (Buyang & Pasaribu, 2014; Azwana *et al*., 2019).

Based on the source, pesticides are divided into three groups, namely synthetic pesticides, organic pesticides and natural pesticides. The use of pesticides is very rapid in several developing countries, especially in Indonesia. It is since pesticides have many uses and advantages in agriculture. However, the use of pesticides on crops can also cause some unwanted negative impacts. The impact of excessive use of pesticides is as results in environmental pollution, killing of natural enemies, occurrence of resistance and resurgence of pests and the emergence of residues on agricultural commodities that are harmful to humans (Singkoh & Katili, 2019).

The high use of insecticides can cause soil contamination in the rice field environment and have a negative impact on human health. The solution that can be done is through bioremediation.

The objective of this review is to identify microbes that can be used as bioremediation agents in soil exposed to insecticide contamination.

MATERIALS AND METHODS

This research used literature study method by reviewing research articles, seminar proceedings and websites related to pesticide bioremediation, especially insecticides. The research articles used have been published in national and international journals.

RESULTS AND DISCUSSION

Soil damage can occur due to certain factors such as deforestation, fires, monoculture planting and excessive use of chemicals such as pesticides. Pesticides are commonly used by farmers to repel pests, especially insecticides that can kill insect pests. However, excessive use of pesticides triggers residues that cause a decrease in soil function and even have an impact on the crops to be harvested (Puspitasari & Khaeruddin, 2016). The presence of degrading microbes can be used as an effort to clean the soil from pesticide residues, especially insecticides. The microbes used to degrade organophosphates can be seen in Table 1.

Table 1. Organophosphate Degrading Microbes

Degrading microbes	Types of Insecticides	Article Source
Aspergillus fumigatus.	Chlorpyrifos	(Anggreini *et al.,* 2019)
*Pseudomonas monteilii, Bacillus cereus,*and *Pseudomonas* sp.	Chlorpyrifos	(Sulaeman *et al.,* 2016)
Lactobacillus casei. *Saccharomyces cerevisiae, Rhodopseudomonas palustris*	Chlorpyrifos	(Rizkiyanti *et al.,* 2020)
*Rhodococcus phenolicus strain MCP1*and *Rhodococcus ruber strain MCP-2*	Monocrotophos	(Srinivasulu *et al.,* 2017)
Pseudomonas sp.	Profenophos	(Akhdiya *et al.,* 2018)
Porites sp.and *Galaxea* sp.	Triazophos	(Rafsanjani *et al.,* 2020)
Paenibacillus (Bacillus) *polymixa*, *Azospirillum lipoferum* (Beijerinck)	Chlorpyrifos, Chlorpyrifosmethyl	(Romeh & Hendawi, 2014)
Pseudomonas putidia	Chlorpyrifos	(Al-Janabi & Hashim, 2021)
Staphylococcus aureus	Chlorpyrifos	(Suman *et al.,* 2020)
Pseudomonas stutzeri	Chlorpyrifos	(Phumkhachorn & Rattanachaikunsopon, 2020)
Achromobacter xylosoxidans and *Ochrobactrum* sp.	Chlorpyrifos	(Akbar & Sultan, 2016)
Cupriavidus nantongensis X1T	Chlorpyrifos	(Shi *et al.,* 2019)
Enterobacter	Chlorpyrifos	(Niti *et al.,* 2013)
Acinetobacter sp.*, Pseudomonas* sp.*, Enterobacter* sp. and *Photobacterium* sp.	Chlorpyrifos and methyl parathion	(Kumar *et al.,* 2015)
Aeromonas hydrophila	Chlorpyrifos	(Shartooh *et al.,* 2021)
Pseudomonas aeruginosa	Chlorpyrifos	(Princess & Cintamulya, 2020)
Stenotrophomonas species and *Sphingomonas*	Chlorpyrifos	(Puspitasari & Khaeruddin, 2016)
Gigaspora spp. *(Arbuscular mycorrhizal fungi)*	Curacron and Detacron	(Setiyo *et al.,* 2014)

Organophosphate insecticides are widely used to eradicate insect pests in vegetables. This type of insecticide is used as a substitute for the organochlorine group which is widely prohibited from using, the resistance of organophosphates is moderate (Nining *et al.,* 2019), is easily degraded into less toxic compounds and works by irreversibly inhibiting the cholinestrase enzyme (Rahmawati *et al.,* 2014). The decrease in residues due to insecticides can be due to the presence of degrading bacteria. It was found that there were 28 species of microbes including 26 bacteria and 2 species of fungi. Based on the data in Table 1, it can be seen that chlorpyrifos is a type of insecticide that is widely used. Insecticide residue-degrading bacteria have specific effectiveness on agricultural soils. The combination of *Bacillus polymiksa* and *Azospirillum lipoferum* showed the best effectiveness in 100% chlorpyrifos degradation (Romeh & Hendawi, 2014) and the effectiveness of *Staphylococcus aureus* bacteria that can degrade chlorpyrifos by 99% (Dasopang & Simutuah, 2016; Suman *et al.,* 2020).

Bacteria *Porites sp.* and *Galaxea sp.* able to degrade Triazofos concentration of 50 ppm to 0.0102 ppm with a percentage of 99.76% reduction in Triazophos residue.

Besides organophosphate insecticides, many organochlorine-degrading bacteria were also found which can be seen in Table 2. Organochlorine pesticides are usually used to control pests and insects, but this type of insecticide is very persistent in the environment because of its toxicity and bioaccumulation. Based on Maulidiniawati & Oginawati (2016) pesticides containing organochlorines have many adverse effects not only on the environment but also on public health in agricultural areas. Organochlorines are subchronic and chronic so that if individuals are exposed to organochlorine too often, it causes interference with the formation of thyroid hormone, TSH levels in the blood will increase which triggers hypothyroidism.

A total of 22 microbial species that can degrade organochlorine insecticides can be seen in Table 2. Among the bacteria and organochlorine insecticides that have been found, the bacteria that have high effectiveness for degrading certain insecticides are *Pseudomonas sp.*, *Phenylobacterium sp.* and *Alcaligenes sp.* and can degrade Dicofol by 84.45%. Dicofol exhibits high toxicity and can negatively affect animals, and humans (Lu *et al.,* 2019).

Table 2. Organochlorine degrading microbes

Degrading microbes	Types of Insecticides	Article Source
Bacillus subtilis, Fusarium moniliforme, Micromonospora, Nocardia, Aspergillus, Rhizopus, Streptococcus	Heptachlor	(Ye *et al.,* 2018)
Bacillus aryabathai	Heptachlor	(Wahyuni *et al.,* 2018)
Anabaena and E. Coli	Linden	(Chaurasia *et al.,* 2013)
Pseudomonas sp., *Phenylobacterium* sp. *and Alcaligenes* sp.	Dicofool	(Pratiwi *et al.,* 2012)
Pseudomonas malleiand the fungus Trichoderma sp.	Organochlorine	(Hindersah *et al.,* 2015)
Citrobacter, Enterobacter, and *Azotobacter*	Linden	(Ardiwinata & Harsanti, 2015)
Pseudomonas fluorescens and *Bacillus polymyxa*	Aldrin	(Doolotkeldieva *et al.,* 2018)
Bacillus, Staphylococcus	Endosulfan	(Mohamed *et al.,* 2011)

Table 3. Carbamate degrading microbes

Degrading microbes	Types of Insecticides	Article Source
Bacteria Ochrobactrum thiophenivorans, Sphingomonas melonis	Methomyl	(Tatar *et al.,* 2020)
White rot fungi (*Phanerochaete chrysosporium*)	Carbofuran	(Nugraheni *et al.,* 2014)

Carbamates are pesticides that are commonly used to eradicate insect pests on fruit and vegetables. Carbamates have less persistence so they can decompose in nature in a short time and can pose a lower risk of poisoning (Wispriyono *et al.,* 2013). Carbofuran can also leave residues in the soil, contaminating and toxic to the environment so that this type of pesticide will reduce the population of various animals that are useful for agriculture (Setiawati *et al.,* 2015). Bacteria that can degrade carbamate insecticides can be seen in Table 3. Based on Nugraheni *et al.,* (2014) white rot fungi can degrade carbofuran. In this study, the potential for degradation of carbofuran was carried out by measuring the growth of white rot fungus (JPP) and the degradation of the insecticide content of carbofuran. The results showed that two white rot fungus isolates (JPP1 and JPP2) showed carbofuran degradation activity. In this study, the JPP2 isolate was identified as Phanerochaete chrysosporium, while the JPP1 isolate was not identified. The two isolates were able to degrade carbofuran with an effectiveness of 6-22% at a concentration of 100-400 g/L.

Based on Tatar *et al.,* (2020) bacteria *Ochrobacterum thiophenivorans* and *Sphingomonas melonis* showed remediation activity against the pesticide metomil by 83%. The effectiveness was strengthened by the activity of Cytochrome P4501A1 (CYP1A1), Catalase (CAT), and Glutathione S-transferase (GST) in *Gammarus pulex*. CYP1A1 is a biomarker that is sensitive to various concentrations of environmental pollutants and is associated with oxidative damage (Delescluse *et al.,* 2001). In Tatar *et al*., study (2020)P4501A1 (CYP1A1) activity in *G. pulex* associated with the investigated methomyl solution at 24 h and 96 h increased after all exposure periods. Induction of CYP1A1 and inhibition of GST activity by methomyl may contribute to the toxic effect of this insecticide in *G. pulex*. CAT was widely used as a biomarker of oxidative stress and also converts hydrogen peroxide to water and oxygen. GST also has an important role in biotransformation with various organic compounds (Łaszczyca *et al.,* 2004). In research Tatar *et al*., (2020), CAT activity decreased for 24 h and 96 h of methomyl exposure. Decreased CAT activity could be mainly related to oxidative stress induced by methomyl exposure. GST plays a major role in the biotransformation of too many components and with a wide variety of organic compounds, catalyzing the conjugation of GSH. After 8 days of bioremediation with *Ochrobacterum thiophenivorans* and *Sphingomonas melonis*, GST activity increased for 24 hours but decreased for 96 hours (P<0.05). These results were generally expected after GST plays an important role in the detoxification of electrophilic compounds (Tatar *et al.,* 2020).

Table 4. Pyreroid degrading microbes

Degrading microbes	Types of Insecticides	Article Source
Bosea eneae	Cypermethrin	(Prawitasari *et al.,* 2018)
Micrococcus sp. CPN1 Strains	Cypermethrin	(Tallur *et al.,* 2015)

Pyrethroid is an insecticide that is also widely used by farmers in Indonesia. Cypermentrin is a pyrethroid insecticide that generally has a fairly broad and effective control against many insects, but can also leave residues that can be absorbed into the soil or into food products, especially plants harvested for tubers (Buyang & Pasaribu, 2014). Bacteria that have the ability to degrade pyrethroid can be seen in Table 4. Based on Prawitasari *et al*., (2018) isolates of *Boseaeneae* can degrade pyrethroids by 87.38% through testing using liquid NMS medium with a pyrethroid concentration of 100ppm. Similar research was also conducted by Tallur *et al.,* (2015) but in this study using bacteria *Micrococcus* sp*.* CPN1 strains. Bacteria *Micrococcus* sp*.* The CPN1 strain can also degrade cypermethrin. Higher concentrations of cypermethrin were tolerated better and more rapidly by immobile cells than by freely suspended cells. In addition to pyrethroids, imidacloprid-degrading bacteria were also found which can be seen in Table 5.

Table 5. Chloronicotinyl (neonicotinoid) degrading microbes

Degrading microbes	**Types of Insecticides**	**Article Source**
Paracoccus sp. and *Achromobacter sp.*	Imidacloprid	(Gao *et al.,* 2021)
Pseudomonas mosselii strain NG1	Imidacloprid	(Bhattacherjee *et al.,* 2020)

Imidacloprid is a synthetic insecticide belonging to the neonicotinoid group which is still used to control planthoppers (Ratna & Firmansyah, 2016), like other synthetic insecticides, imidacloprid also leaves a residue that can last in the soil for 150 days (Bhattacherjee *et al.,* 2020). Research conducted (Gao *et al.,* 2021) showed that the bacteria *Paracoccus* sp. and *Achromobacter* sp. can degrade the insecticide Imidacloprite. The combination of these two bacterial strains reduced imidacloprid in the soil by 99.85% on day 15 and reached 100% on day 20. In further testing, *Paracoccus* sp. could not reduce levels of Imidaplorid, while the bacteria *Achromobacter* sp. which in the self-test can reduce imidacloprid by 53.08% on the 15th day and reach 100% on the 20th day.

Research conducted by (Bhattacherjee *et al.,* 2020) also showed that there were other bacteria that could degrade imidacloprid, namely *Pseudomonas mosselii* Strain NG1. Bacteria *Pseudomonas mosselii* Strain NG1 removed the insecticide residue by 91.42% after 67 days with a decrease in residue from 0.606 -gg-1 on day 0 to 0.052 -gg.

CONCLUSION

Bioremediation needs to be done in order to avoid leaving pesticide residues on the soil and agricultural products. From the results of this review, it was known that there were various kinds of microbes that can be used as bioremediation agents for various types of insecticides as many as ±56 species consisting of bacteria and fungi. The microbial genera that were commonly used and easy to find for bioremediation agents include the genus *Aspergillus* sp., *Pseudomonas* sp., and *Bacillus* sp. The genus *Aspergillus* is a fungus that is able to live in a medium with a high degree of acidity and sugar content. The microbial genus *Pseudomonas* is an obligate aerobic bacterium that is easy to grow on a variety of culture media, sometimes producing a sweet aroma and odor. Furthermore, there is the genus

Bacillus which is a bacterium that is obligate aerobes or facultative aerobes and positive for the catalase enzyme test. Most bacteria can degrade organophosphate pesticides.

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