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Potential of Indonesian endemic microbial consortium in degrading profenofos and chlorantraniliprole pesticide in East Java Indonesia to support agricultural ecosystems

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ABSTRACT

Synthetic pesticides on agricultural land were used by most Indonesian farmers, especially in soybean fields in East Java Indonesia especially in Jombang, Lamongan and Probolinggo regency. The soil applied with these pesticides affected the diversity of bacteria and the physical and chemical properties of the soil. This study aimed to explore, to conduct potential test and antagonistic test, and to make a consortium of microbes that could degrade Profenofos and Chlorantraniliprole pesticides in East Java Indonesia to support agricultural ecosystems. This study was exploration and experimental study. Bacterial exploration was conducted on soybean fields in Jombang, Lamongan and Probolinggo. Potential test and antagonistic test were carried out using well diffusion method and pesticide degradation test was carried out using *HPLC in house method*. Results found 39 Profenofos degrading isolates and 30 Chlorantraniliprole degrading isolates. Bacterial consortium test found 10 Profenofos degrading isolates, 3 nitrogen fixing isolates, and 2 phosphate solvent isolates which could reduce Profenofos in the control, 0, 2 and 4 days incubation each at 100%, 78.83%, 75.36% and 62.3% mg/kg and could decrease Chlorantraniliprole each by 100%, 88.46%, 74.36%, and 73.08% mg/kg. The biggest decrease occurred in the 4th day incubation.

Key words : *Chemical and Physical Characteristics of Soil, Pesticides, East Java Indonesia*

Introduction

The use of synthetic pesticides is often the main choice of farmers in Indonesia to reduce pest populations in a fast time (Laoh, *et al.* 2003). Pesticides varied depending on the type of pests that appear on various plant stages. Active ingredients of pesticides that are often applied in the areas of East Java Indonesia especially in Jombang, Lamongan and Probolinggo regency are Aceptate, Chlorantraniliprole, Dimehypo, Fenobucarb, Methomyl, Profenofos, Chlorothalonil and Chlorpyrifos (Interviews with local farmers, 2018).

The use of synthetic pesticides affected the diversity of soil microflora and soil nutrient content (Asri, *et al.*, 2018). Soil nutrients needed by plants include macro nutrients and micro nutrients. Micro nutrients are nutrients needed by plants in small

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amounts, but their function is important and irreplaceable. Macro elements are nutrients needed by plants in relatively more quantities than other nutrients (Mutmainah, 2012).

One of the most widely used pesticide group by farmers was organophosphate (Barile, 2003).These pesticides were allowed to be used in Indonesia because they could be deactivated in the environment (Barchia, 2009; Zulkarnain, 2010).They were also easily biodegradable, did not last long, and were easily disappeared naturally (Alegantina*et al*, 2005). Profenofos is a type of insecticide that belongs to the organophosphate group, has moderate toxicity and has a half-life of about 43 days (hydrolysis half-life), an average of 2 days (aerobic soil half-life) or about 3 days (anaerobic half aerobic).

Profenofos is a moderate, cancerous (carcinogenic) acute toxic chemical known as groundwater pollutants, and toxic to reproductive. Acute toxicity only occurs in the content of pure active ingredients (Tarumingkeng, 1992).

Chlorantraniliprole is a material from diamide group and the mechanism of action is attacking the nerves. It can control pests from Coleoptera, Diptera, Isoptera and Lepidoptera families such as *Spodoptera exigua* (Zhang *et al.*, 2014). Chlorantranilipole is a nerve and muscle toxin. It activates rianodin receptor and opens calcium ion channels in the sarcoplasmic reticulum of muscle cells which cause excessive release of calcium ions which disrupt the regulation of muscle contraction and result in paralysis. Insects affected by this insecticide have symptoms of stopping eating, body contraction, being inactive, and finally died.

Pesticide applications would not cause problems as long as their use is controlled and proper, but the influence on soil microbial life needs to be considered. The level of fertility in soil ecosystem depends on the role of microbial populations to be able to convert organic matter. Soil microbial population acts as an indicator of stability of the availability of nutrients when soil is contaminated with pesticides (Brookes, 1995).

Fungi and bacteria are soil microbes as bio-indicators based on the ability of life and the activities of both microflora (Cycon, and Piotrowska, 2007). This was in accordance with the statement of Newton *et al*. (2009) that the microbial response of soil to pesticides was influenced by its chemical composition and the pesticide effect was more pronounced on the community structure compared to microbial physiological activity.

Accordingly, this study would explore bacteria that could degrade Profenofos and Chlorantraniliprole in Jombang, Lamongan and Probolinggo fields, to test the potential of these microbes in degrading pesticides, to determine microbial consortiums that could degrade Profenofos and Chlorantraniliprole pesticides, and to improve soil structure.

Material and Methods

This studies were conducted in several stages, those were:

Exploration of Profenofos and Chlorantraniliprole Degrading Bacteria in Pesticides Applicated fields in East Java Indonesia

Soil samples for exploring microbes (bacteria) were taken from 2 week old soybean field in East Java Indonesia scpecialy in Jombang, Lamongan and Probolinggo regency, from 2-2.5 month old soybean field (flowering stage) in the Lamongan, and from 3 month old soybean field (harvesting time) in the Jombang. Soil samples were taken at 3 points; from the water inflow area, the middle, and the water outflow area.

Potential Test of Profenofos and Chlorantraniliprole Degrading Bacteria in East Java Indonesia (Jombang, Lamongan and Probolinggo)

The exploration of Profenofos and Chlorantraniliprole degrading bacteria was carried out using Pour Plate method. 1 g of soil samples were homogenized with 9 mL of distilled water and then diluted until 10^{-6} dilutions were obtained and then planted in nutrient agar with the addition of Profenofos and Chlorantraniliprole 0.2 mL/100 mL each. Bacterial colonies showing the presence of clear zones were isolated and morphologically characterized.

Qualitative Test of Bacterial Ability in Degrading Profenofos and Chlorantraniliprole

This test was conducted by well diffusion method with Merck agar nutrient medium added by Profenofos or Chlorantraniliprole 0.2 mL/100 mL each. The clear zone appeared around the well was measured in diameter reduced by the area of the well diameter. The diameter area expressed the abil-

ity of the bacteria in degrading Profenofos and Chlorantraniliprole pesticides.

Consortium Test of Bacterial Ability in Degrading Profenofos and Chlorantraniliprole

The microbial consortium used in this study consisted of 10 Profenofos and Chlorantraniliprole degrading bacterial isolates and 3 nitrogen-fixing bacterial isolates and 2 phosphate solvent bacterial isolates. The ability test to degrade pesticides was conducted on a bacterial consortium grown on nutrient broth medium with the addition of Profenofos and Chlorantraniliprole at a dose of 0.2 mL/100 mL medium and bacterial doses 1 mL of bacteria with a number of bacteria 10^7 cells/mL. Bacteria put into the medium were incubated at 0, 2, and 4 days and control. The incubation results were tested for pesticide residues using *HPLC in house method.*

Results

Exploration of Profenofos and Chlorantraniliprole Degrading Bacteria in Pesticides Applied fields in East Java Indonesia

Exploration of Profenofos-degrading bacteria and Chlorantraniliprole in pesticides applied in East Java Indonesia (Jombang, Lamongan and Probolinggo) (Table 1).

Table 1. The Number of Bacterial Isolates Growing in Nutrient Medium (Merck) with Addition of Profenofos and Chlorantraniliprole

No.	Origin of Microbes	The number of bacterial in medium with addition of			
		Profenofos		Chlorantraniliprole	
		-	+	-	+
1	Jombang	8	11	10	11
2	Lamongan	4	11	12	11
3	Probolinggo	0	17	14	11
	Sum	12	39	39	30
	Total	51		69	

Note:- = Clear zone absent, + = Clear zone present

Potential Test of Bacteria in Degrading Profenofos and Chlorantraniliprole in East Java Indonesia

Potential test of bacterial isolates found in soybean areas in EastJava Indonesia (Jombang, Lamongan and Probolinggo) in degrading Profenofos or Chlorantraniliprole were carried out in vitro using nutrient agar medium. The results of this test obtained data in the form of the diameter of the inhibition zone produced after 2 days of incubation (Table 2).

Qualitative Test of Bacterial Ability in Degrading Profenofos and Chlorantraniliprole

Potential test of bacterial isolates found in soybean field in Jombang, Lamongan, and Probolinggo in degrading both insecticides, Profenofos and Chlorantraniliprole, were carried out in vitro using nutrient agar medium. The results obtained data in the form of inhibition zone diameter area produced after 2 days incubation (Table 3). The results of this test obtained data with bacterial isolates to degrading Profenofos and Chlorantraniliprole could be seen in Table 3.

Consortium Test of Bacterial Ability in Degrading Profenofos and Chlorantraniliprole

From the ability test of 10 bacterial isolates that could degrade both Profenofos and Chlorantraniliprole, 7 of them are synergistic. However, in this consortium test, the 10 isolates were still used because the clear zones were still relatively small, namely 1 to 4 mm on the first day of incubation. Then the 10 bacteria isolates were consorted by adding 3 nitrogen-fixing bacteria and 2 phosphate solvent bacteria. Addition to these two types of bacteria was caused by the results of preliminary tests that the lands contaminated with pesticides had low nitrogen and phosphate content (Asri, *et al.*, 2018). Table 5 showed the results of the test of the consortium ability to degrade Profenofos and Chlorantraniliprole insecticides in control, 0 2 and 4 days incubation, as seen from the amount of pesticide residue in liquid nutrient medium added by Profenofos and Chlorantraniliprole *in vitro*.

Discussion

Exploration of Profenofos and Chlorantraniliprole Degrading Bacteria in Pesticides Applied fields in East Java Indonesia

Based on Table 1, it could be seen that there were total 120 bacterial isolates found growing on soils applied with pesticides. 39 bacterial isolates could degrade Profenofos and 30 isolates could degrade Chlorantraniliprole. According to (Rao and Subba, 1994), in each gram of soil the bacterial density

Table 2. Average and Range of Inhibition Zone Diameter of Profenofos and Chlorantraniliprole Degrading Bacteria in Soybean Fields

No.	Location	Inhibition Zone Diameter (cm)					
		Profenofos			Chlorantraniliprole		
		Number of isolate	Average	Range	Number of isolate	Average	Range
1	Jombang	11	2.32	0.50 - 3.60	11	2.46	0.50 - 4.50
2	Lamongan	11	2.20	0.10 - 4.00	11	2.15	0.40 - 4.60
3	Probolinggo	17	2.42	0.10 - 4.35	11	1.64	0.45 - 3.20

Table 3. Clear Zone Diameter of Profenofos and Chlorantraniliprole Degrading Bacteria in Jombang, Lamongan, and Probolinggo Soybean Fields (cm)

No.	Pesticide	Sample Code									
		A	B	C	D	E	F	G	H	I	J
1	Profenofos	3.9	4.0	2.6	3.1	3.15	2.5	2.75	3.85	3.5	3.55
2	Chlorantraniliprole	2.7	4.6	4.2	4.55	3.2	3.15	2.85	2.25	2.55	1.25

reached about 10^8-10^9 cells/gr. Generally, bacteria were abundant on the surface of the soil, stuck to clay-like soil with of 50-75% water content, neutral pH and temperature 25°-35°C. In this study bacterial desired conditions were fulfilled so that the number of bacterial isolates found was quite large (120 isolates). According to (Karpouzas and Walker, 2000) *Pseudomonas putida* could be isolated on a medium containing organophosphate insecticides (including Profenofos and Chlorantraniliprole). Furthermore, in the soil applied by Profenofos insecticides, *Bacillus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Citrobacter* sp., *Azotobacter* sp., and *Azospirillum* sp (Sulaeman, 2016) were also found.

The diversity of bacteria in the soil was influenced by the content of soil organic materials such as carbon and nitrogen. Taiyeb (2017) stated that if the C/N ratio was less than 25, then the soil environment conditions support for the development of microorganisms in the process of mineralization of organic matter and humus formation. In this study the C/N content ratio ranged from 6-9 (classified as low). Referring to (Taiyeb, 2017; Lugo-Perez, and Lloyd, 2009), this low C/N ratio indicated that the organic matter degradation process took place quickly and completely.

Potential Test of Bacteria in Degrading Profenofos and Chlorantraniliprole in Jombang, Lamongan and Probolinggo

Based on Table 2, it could be seen that 11 bacteria isolated from Jombang could degrade Profenofos pesticides with an incubation time of 2 days with average diameter of 2.32 cm. Bacteria isolated from Lamongan had 2.20 cm, and bacteria isolated from Probolinggo had 2.42 cm average diameter of clear zone. This was consistent with statement of (Tarumingkeng, 199[illegible] that Profenofos has a half-life, an average of 2 days (aerobic soil half-life) or about 3 days (anaerobic soil half-life). Half-life in the soil is in the range of 1 -18 days under laboratory conditions (Howard, 1989). Meanwhile, the average diameter of the clear zone produced by bacterial isolates in Jombang area in degrading the Chlorantraniliprole pesticide was 2.46 cm, Lamongan 2.15 cm, and Probolinggo 1.64 cm. The breakdown of Chlorantraniliprole could be carried out by photo degradation or biologically using soil microbes (Anonymous, 2008).

Qualitative Test of Bacterial Ability in Degrading Profenofos and Chlorantraniliprole

Based on Table 3, it could be seen that there were 10 selected isolates which could degra[illegible] both Profenofos and Chlorantraniliprole which was seen by the formation of clear zones around the colonies. The diameter of the clear zone produced by bacteria in degrading Profenofos ranged from 2.6 to 3.9 cm, while the clear zone produced in degrading Chlorantraniliprole ranged from 1.25 to 4.55 cm.

Consortium Test of Bacterial Ability in Degrading Profenofos and Chlorantraniliprole

Based on Table 4, it could be seen that microbial

Table 4. Analysis Test Result of Residual Pesticide, Nitrogen Fixing, and Phosphate Solvent in Selected Microbial Consortium

No.	Incubation time	Pesticide Residual Test Result	
		Profenofos (%)	Chlorantraniliprole (%)
1	Control	100	100
2	0 hour	78.83	88.46
3	2 days	75.36	74.36
4	4 days	62.3	73.08

consortiums were tested to break down pesticides with different incubations, those were control, 0, 2, and 4 days incubation, which showed decrease in pesticide content compared to control. In the medium added with Profenofos, the decrease in the amounts of residue were consecutively 100%, to 78.83%, 75.36% and 62.3%. Chlorantraniliprole pesticide residue also decreased consecutively from 100, 88.46, 74.36, and 73.08%. The biggest decrease occurred on day 4 incubation. Indonesia did not have residual maximum limit regulation for soil yet. So that from the results obtained, it could only be interpreted that the impact of the use of pesticides in agriculture left residue on the soil and had an impact on the soil which still need to be studied further (Brookes, 1995). This study showed decrease in soil chemical quality, specifically low C/N ratio ranged between 6-9 (Asri *et al*., 2018) where ideal C/N ratio was between 11-15 (Hardjowigeno, 2003).

Conclusion

From the results of this study, it could be concluded that 10 bacterial isolates that could degrade Profenofos and Chlorantraniliprole pesticides were found in the areas of East Java Indonesia, especially Jombang, Lamongan and Probolinggo. These bacteria could degrade pesticides with average clear zone diameter between 0.5-4.0 cm. Consortium test of 10 isolates of Profenofos and Chlorantraniliprole degrading bacteria added by 3 nitrogen fixing bacteria and 2 phosphate solvent bacteria could reduce the highest pesticide residue in the 4th day incubation.

Profenofos degradation bacteria could reduce residues in control, 0, 2 and 4 days of incubation consecutively 100%, 78.83%, 75.36% and 62.3% and could decrease Chlorantraniliprole each by 100%, 88.46%, 74.36%, and 73.08%.

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