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STUDY OF FUNCTIONAL MICROBES POPULATION IN PEAT SOIL TREATED WITH TWO PESTICIDES

Syaiful Anwar, Maipa Dia Pati*, Rahayu Widyastuti and Dadang

Faculty of Agriculture, Bogor Agricultural University, West Java, Indonesia.

** Corresponding author: maipadiapati.misdjan@gmail.com*

SUMMARY

Necessity in agricultural land expansion for food security in Indonesia has been faced by the fact in utilizing marginal land such as shallow peat soil in Central Kalimantan. Among problems encountered in such marginal land is incidence of pests and diseases. Common practice of farmers in dealing with this problem is the application of chemical pesticides in order to ensure production. Unintended chemical pesticides that fall on/into the soil might have an adverse effect to soil microbes activity. This research was aimed to assess the effect of pesticides on population of functional soil microbes in peat soil. The research was in the form of incubation of peat soil in pots in completely randomized factorial design with two factors. First factor was 7 combinations of pesticide types (paraquat, and buthylphenylmethyl carbamate = BPMC) and doses (50, 100, and 200% of recommended dosage). Second factor was 4 incubation times (1, 7, 14, and 28 days). After the each of incubation times, the soil samples were treated and analyzed for population of phosphate solubilizing microbes (bacteria and fungi), cellulolytic microbes (bacteria and fungi), and N_2-fixing bacteria (Azotobacter and Azospirillum). The results in general show fluctuation of microbes population with doses of pesticides and incubation. Of the six microbes studied, pesticides only significantly affecting population of Azospirillum, Azotobacter and phosphate solubilizing fungi, whilst incubation times significantly affecting all microbes population. Adverse effects to microbes population occurred on phosphate solubilizing bacteria by both pesticides, on Azotobacter and Azospirillum by paraquat, and on phosphate solubilizing fungi by BPMC, although all microbes population recovered after 7 days of incubation times. Cellulolytic microbes are the most un-affected by the two pesticides. Although there is adverse effect at the beginning of incubation time by paraquat, significantly increased population, however, found on Azospirillum by the two pesticides.

Keywords: *peat soil, pesticides, paraquate, BPMC, functional microbes*

INTRODUCTION

Agricultural activities in peatland is faced with several problems, such as the low soil fertility and the high of pests incidents (Noor, 2001). Incidences of pests to agricltural plants, among others, are caused by the change of land function from peat forest with high varieties of plants to agricultural land with low varieties of crops. One common efforts to control pests and almost always practiced by farmers in intensive agriculture is the application of agrochemical pesticides Handayanto and Hairiah, 2007) in order to ensure production. Controlling pests chemically as also practiced by farmers on peat soil in Central Kalimantan is considered as the easiest and the most practical in their applications, and faster responses of pests. On the other hand, however, chemical pesticides that fall on/ into the soil is feared would have adverse effects towards population of soil functional microbes. Only about 20% of the pesticides will arrive at targets, while the rest will fall on/ into soils then move spatially and leach to surface or ground waters (Sa'id, 1994). The present of soil functional microbes in agricultural soil is very important in supporting plant growth and production. These soil microbes have different roles in the agricultural ecosystem which related energy flow and nutrients cycles caused by main activities of the microbes (growth and development).

Adverse effects of pesticides on functional microbes population in mineral soil has been widely reported. For example, Busse et al. (2001) suggested that the herbicides glyphosate toxic to bacteria and fungi isolated from soil Ponderosa Pine Plantations, and the total population of heterotrophic bacteria decreased after application glyphosate. On peat soils that information is still lacking. Zimdhal (1993) stated that the soil contains clay and organic matter adsorbs pesticides greater than sandy soils. Since peat soil is almost entirely organic matter, the adverse effect of pesticides on the functional microbes activity in peat soil would not as drastic as it did in the mineral soil. This research was aimed to assess the effect of pesticides application on the functional microbes population in peat soil.

METHODS

This research was conducted from August 2014 to May 2015, at the Laboratory of the Department of Soil Science and Land Resource, Faculty of Agriculture, Bogor Agricultural University. This research used completely randomized factorial design with two factors. First factor was 7 combinations of types (paraquat and buthylphenylmethyl carbamate = BPMC) and doses (50, 100, and 200% of recommended dosage) of pesticides; second factor was 4 incubation times (1, 7, 14, and 28 days) with three replications. The 100% doses of pesticides were equal to 80% that will fall on/ into soils, that equal to active compound of 2208 $\mu g\ kg^{-1}$ for paraquat, and 970 $\mu g\ kg^{-1}$ for BPMC.

The treated soils were stored and incubated in open chamber in dark room at room temperature. After the each of incubation times, aliquot of the soil samples were treated and analyzed for population of phosphate solubilizing microbes (bacteria and fungi), cellulolytic microbes (bacteria and fungi), and N_2 fixing bacteria. Analysis of microbial populations were using two methods; they were total plate count (TPC) method for phosphate solubilizing microbes, cellulolytic microbes, and Azotobacter, and most probable number (MPN) method for Azospirillum.

RESULT

In general, the results showed that functional microbes population fluctuated at various doses of pesticides and incubation times. Results of ANOVA showed that the pesticide treatment does not affect the population of phosphate solubilizing bacteria (PSB), cellulolytic bacteria, and cellulolytic fungi (Table 1). On the contrary, the pesticide treatment affects the population of Azotobacter, Azospirillum and phosphate solubilizing fungi (PSF). 100% paraquat, 200% paraquat, 100% BPMC, and 200% BPMC treatments have Azospirillum population significantly higher than that of Control. Different response shown by PSF, in which 50% and 100% BPMC treatments indicates that the population PSF significantly lower than that of Control and all treatments of paraquat. Significant different is also demonstrated by Azotobacter but with a fluctuating population. Paraquat treatment tends to reduce the population of Azotobacter. Treatment of 50% BPMC is significantly higher in Azotobacter than that of the treatments of 50% and 100% paraquat, though not significantly different with that of the Control.

Table 1: Effects of pesticide treatment on average population of soil functional microbes.

Pesticide treatment	Population of functional microbes								
	PSB	Cellulolytic bacteria	Azotobacter		Azospirillum		PSF		Cellulolytic fungi
	............................(x10^5 CFU g^{-1} soil)............................						....(x10^4 CFU g^{-1} soil)...		
Control	8.80	1.22	27.10	abc	2.84	c	4.57	a	19.26
50% Paraquat	8.48	1.67	19.56	bc	5.40	bc	4.81	a	14.45
100% Paraquat	5.80	1.18	14.93	c	6.45	ab	3.16	a	12.64
200% Paraquat	5.57	1.06	20.21	abc	7.24	ab	5.39	a	19.53
50% BPMC	11.51	1.52	36.06	a	6.10	bc	1.03	b	12.39
100% BPMC	6.62	0.95	34.88	ab	8.89	a	1.65	b	11.98
200% BPMC	12.11	1.02	21.06	abc	7.49	a	5.56	a	22.38

Note: CFU = colony forming unit; PSB = phosphate solubilizing bacteria; PSF = phosphate solubilizing fungi; Different letter in the same column indicates significant different according to Duncan test at 5% level; Doses of 50, 100 dan 200% mean a half, equal to, twice of recommended dose, respectively.

Table 2 show that the incubation time real effect on the population of PSB, PSF, cellulolytic bacteria, fungi cellulolytic, Azotobacter and Azospirillum, although fluctuating but an increase in population with increasing incubation time, except PSB 7th day, Azospirillum day 14 , Azotobacter day 7, and day-to-7 PSF. At the end of the incubation period of functional microbial population groups studied significantly higher than the day-to-1. Except, the population of cellulolytic bacteria and Azospirillum which shows the effect didn't differ significantly when compared with the day 1.

Tabel 2: Effects of incubation time's on average population of soil functional microbes.

Incubation time's (Day)	Population of functional microbes											
	PSB		Cellulolytic bacteria		Azotobacter		Azospirillum		PSF		Cellulolytic fungi	
	..........................($x10^5$ CFU g^{-1} soil)...........................								..........($x10^4$ CFU g^{-1} soil)......			
1	3.37	c	0.89	b	5.71	ab	22.85	b	1.23	b	9.72	b
7	2.42	d	1.44	a	6.17	ab	16.11	b	1.05	b	12.41	b
14	21.01	a	1.62	a	4.55	b	27.27	b	7.18	a	18.57	a
28	6.85	b	0.97	ab	8.30	a	33.08	a	5.49	a	23.67	a

Note: CFU = colony forming unit; PSB = phosphate solubilizing bacteria; PSF = phosphate solubilizing fungi; Different letter in the same column indicates significant different according to Duncan test at 5% level.

The study also showed the presence of interactions between pesticide treatment with incubation time against the group Azospirillum, Azotobacter and PSB. However, after a further test Azospirillum population (Table 3) show the influences that are not real. Instead, the group PSF (Table 4) and Azotobacter (Table 5) shows the real effect is different. Table 4 shows that the negative effects due to the application of pesticides most influential groups of the PSB. The population of PSB decreased in all treatment pesticides, except for treatment of paraquat 200% on day 1. Day 7 also shows a decrease in the population of PSB on pesticide treatment, unless the treatment Paraquat BPMC 200% and 50%. on the 14th day of the downward trend seen in the treatment population Paraquat PSB 100%, 200% Paraquat, and BPMC 100%. Meanwhile, on the 28th day is not a decline in the population of PSB. this shows that the negative effects of pesticide application is shown mainly on day 1 and day 7, although it later occurred subsequent recovery in the incubation time.

Tabel 3: Effects interaction of pesticide treatment and incubation time's on average population of Azospirillum.

Pesticide treatment	Population of Azospirillum bacteria ($x10^5$ CFU g^{-1} soil)			
	Day-1	Day-7	Day-14	Day-28
Control	0.17	0.17	1.51	4.08
Paraquat 50%	0.00	0.00	5.17	7.05
Paraquat 100%	0.00	0.00	7.87	6.90
Paraquat 200%	6.91	6.91	2.84	8.20
BPMC 50%	8.43	8.43	3.97	11.83
BPMC 100%	12.24	12.24	6.43	12.23
BPMC 200%	12.24	12.24	4.08	12.23

Note: CFU = colony forming unit; PSB = phosphate solubilizing bacteria; PSF = phosphate solubilizing fungi; Different letter in the same column indicates significant different according to Duncan test at 5% level; Doses of 50, 100 dan 200% mean a half, equal to, twice of recommended dose, respectively.

Tabel 4: Effects interaction of pesticide treatment and incubation time's on average population of phosphate solubilizing bacteria.

Pesticide treatment	Population of phosphate solubilizing bacteria ($x10^5$ CFU g^{-1} soil)							
	Day-1		Day-7		Day-14		Day-28	
Control	7.46	ab	2.54	abc	22.95	a	2.25	abc
Paraquat 50%	4.50	ab	0.29	de	24.66	a	4.46	ab
Paraquat 100%	4.10	ab	0.42	cd	14.65	ab	4.06	ab
Paraquat 200%	7.56	ab	6.80	ab	4.84	ab	3.07	ab
BPMC 50%	0.00	e	2.94	ab	27.46	a	15.66	ab
BPMC 100%	0.00	e	2.10	abc	13.90	ab	10.48	ab
BPMC 200%	0.00	e	1.84	bc	38.63	a	7.96	ab

Note: CFU = colony forming unit; PSB = phosphate solubilizing bacteria; PSF = phosphate solubilizing fungi; Different letter in the same column indicates significant different according to Duncan test at 5% level; Doses of 50, 100 dan 200% mean a half, equal to, twice of recommended dose, respectively.

Table 5 below shows that there is a trend of population decline Azotobacter treatment with Paraquat 50% on day 1, Paraquat treatment 200% and BPMC 200% at day 7, all treatments of pesticides on day 14 and day 28 except BPMC treatment on day 28.

Tabel 5: Effects interaction of pesticide treatment and incubation time's on average population of Azotobacter.

Pesticide treatment	Population of Azotobacter bacteria (x10^5 CFU g^{-1} soil)							
	Day-1		Day-7		Day-14		Day-28	
Control	4.72	ab	13.49	ab	49.39	a	40.80	a
Paraquat 50%	5.50	ab	26.18	ab	20.15	ab	26.40	ab
Paraquat 100%	1.41	b	16.64	ab	23.09	ab	18.58	ab
Paraquat 200%	8.03	ab	9.52	ab	33.87	a	29.41	a
BPMC 50%	41.13	a	16.59	ab	45.78	a	40.75	a
BPMC 100%	65.10	a	19.60	ab	5.71	ab	49.11	a
BPMC 200%	34.07	a	10.78	ab	12.88	ab	26.52	ab

Note: CFU = colony forming unit; PSB = phosphate solubilizing bacteria; PSF = phosphate solubilizing fungi; Different letter in the same column indicates significant different according to Duncan test at 5% level; Doses of 50, 100 dan 200% mean a half, equal to, twice of recommended dose, respectively.

DISCUSSION

The results showed that microbial functional have different responses to the use of agrochemical in this case there are two types of pesticides (active ingredient herbicide paraquat and the insecticide active ingredient BPMC). Pesticides or pesticide dose levels can decrease or increase the population of certain microbial groups. The tendency population of Azospirillum higher pesticide treatment (Table 1), possibly due to the pesticides applied an organic pesticide. BPMC insecticide containing carbon, while the herbicide paraquat group containing carbon and nitrogen. Azospirillum bacterium groups allegedly capable of using carbon or nitrogen (nutrients) contained in pesticides to support cell metabolism. The different results expressed by Cycon and Seget, (2007) reported that the application of herbicide and insecticide diazinon linuron at high doses, reduce the population of bacteria-free N2 fastening by 40% compared to controls. Nevertheless, in the study also stated that the use of three types of selective pesticides (herbicides, insecticides and fungicides) significantly stimulates an increase in population of heterotrophic bacteria. In addition, some studies suggest that pesticides can be used as a carbon source, an energy source and a source of nutrients by some groups of soil microbes, but toxic for other microbial groups (Bhuyan *et al.*, 1993; Johansen, 2001). Instead, PSF group showed lower populations with insecticide treatments BPMC. This is presumably because the insecticide BPMC not compatible with PSF group, insecticides BPMC act as inhibitors and inhibit mycelial growth and spore germination by inhibiting the synthesis of amino acids through the path "shikimic acid". Cycon and Seget, (2007) also noted that the application of insecticides diazinon lower the total population of fungi day after application. Application of pesticides may inhibit / kill a particular microbial groups but increases the number of other microbial groups by exempting them from the competition (Hussain et al., 2009). More Akbar et al. (2012) states that all insecticides used in these studies, significantly inhibits mycelial growth and germination of conidia of entomopathogenic fungi. The incubation period (Table 2) showed that the application of pesticides generally degrade the functional microbial population at the beginning of treatment. During the incubation period of functional bacterial groups studied showed a fluctuating population, except cellulolytic fungi. However, the overall functional microbial population at the end of the incubation period is higher than the beginning of the incubation period. This, presumably because of pesticides applied a foreign substance and is toxic to some soil microbial nutrients or there is competition. Application of pesticides would hamper soil microbial populations that are not sensitive to pesticides for a while, so that the microbes will adapt first. Over time incubation, the microbial groups utilize the nutrients released from microbes that have died for growth, and or a process of degradation of the compounds of pesticides in soil within 30 days of incubation. At the beginning of the application of pesticides, some microbial populations will be affected so that the population declined, but after a period of adaptation of microbial populations is slowly returning to normal even higher (Fliessbach and Mader, 2004; Niewiadomska, 2004). Cellulolytic fungus thought to potentially solve complex carbon chain of pesticides (for their enzyme cellulase), so that the cellulolytic fungus capable of utilizing pesticides as a carbon source which then led to population increases continuously. Cellulolytic fungus is also often used as an agent for biodegradation of pesticides. Subowo (2013) states that the Aspergillus niger besides having cellulase activity can also degrade pesticides deltamethrin at 500 ppm. Maloney (2001) also states that the Aspergillus, Penicillium, Fusarium and Paecillomyces Spp. able to degrade pentachlorophenol (PCP), which is a follow-up material and are toxic pesticides. The high content of organic matter in peat, is thought to be one factor that plays an important role to support the process of functional microbial adaptation. In addition to the availability of organic material needed for the functional microbial growth and metabolism are met. Organic material also adsorbs pesticides applied, so that the negative effects of the application of pesticides on microbial functional groups can be minimized. In addition, the high content of organic material on peat soil will also increase the rate of degradation of pesticides biotic or abiotic (Roger *et al.,* 1994). Rache and Coats, (1998) in his study suggests that increased soil bacterial populations associated with biodegradation time of pesticide used. This indicates a change in microbial catabolism capabilities that allow microbes have the ability to degrade pesticides and or changes in the microbial groups (Hussain *et al.*, 2009) on peat soil after application of pesticides. The interaction between the pesticide treatment with incubation time indicates that the negative effects of pesticides seen in bacteria Azospirillum and PSB at the beginning of the incubation period (day 1 and day 7), whereas the negative effects of pesticides Azotobacter bacteria seen at the end of the incubation period (day ke- 14 and day 28). The absence of colonies PSB on day 1 after the application of insecticides BPMC, allegedly due to dilution rate used is still high, (10-4 and 10-3), there is a possibility if used lower dilutions (10-1 or 10-2) will be found the population of PSB. In addition, it is also believed to be related to the adaptability of the PSB is lower than other functional microbe against pesticide application. BPMC insecticide applications, encouraging PSB to dormancy early in the application of pesticides as a form of adaptation to environmental conditions that are less supportive of growth, and then on the 7th day when environmental conditions are more favorable, phosphate solvent bacterial colonies grow back. This could be due, pesticides applied have undergone a process of degradation on the 7th day.

Pesticides applied to soil may be used as a substrate by a specific group of microbial soil and then to degradation (Rao, 1994), in this case a group of bacteria-free N2 fastening allegedly able to utilize the pesticide as a source of nutrition for the population is not disturbed at the beginning of the application of pesticides. Rahayuningsih (2009) states that microbes play a role in the decomposition of pesticides in the soil, and pesticides used or can be used by microbes as a source of carbon to support activities / cell metabolism. Several groups of soil microbial functional besides being able to provide nutrients, it is also able to produce plant growth regulators, and act as agents of biodegradation of chemical compounds.

In this study, the negative effects of pesticides are used more demonstrated by a group of bacteria phosphate solvent (PSB), the opposite of pesticides stimulates an increase in population groups of bacteria Azotobacter and Azospirillum. In general, higher functional microbial populations present in BPMC insecticide application of the herbicide paraquat.

CONCLUSIONS

Based on the results of research that has been done, it is concluded that there are fluctuations in functional microbial populations at different doses of pesticides and incubation time. Of the six groups studied microbes, pesticides significantly affect populations of Azospirillum, Azotobacter and fungi significantly phosphate solvent, but does not affect the population of PSB, cellulolytic bacteria and fungi cellulolitic. The incubation time significantly affect all functional microbial populations studied. Both pesticides are used only negatively affects bacterial populations phosphate solvent, paraquat negative effect on the population of Azotobacter and Azospirillum, and BPMC only negatively affect the fungi populations phosphate solvent. However, the negative effect is especially true on day 1 and day 7, then some degree of recovery in the incubation period is longer. Cellulolytic microbe is a microbe groups most affected by the pesticides used. Although there are negative effects of paraquat early in the incubation period, the two pesticides significantly increase Azospirillum population.

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