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Effect of Biopesticides Neem Extract (Azadirachta Indica) treatments on Soil Biochemical Properties and Plant Growth Promoting Rhizobacteria Viabilities

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Abstract

Recently the use of biopesticide is becoming popular. The neem seed contains Azadirachtin which is the most important component as a biopesticide. However, it is not known how the effect of this compound on PGPR and soil biochemical properties. This study aims to determine the impact of neem extract biopesticides (Azadirachta indica) on the viability and activity of plant growth-promoting rhizobacteria (PGPR). The method used is in vitro assay, by implanting disc blank which has been treated with the variation of biopesticide concentration of neem extract by 0 % (control), 2.5 %, 5 % and 10 % on 11 PGPR isolates. In Vivo assay was conducted by applying biopesticide neem extract directly into soil with different concentrations and soil biochemical properties monitored include soil respiration, Phosphomonoesterase (PME-ase), and urease. The results showed that the neem extract biopesticide (Azadirachta indica) was able to inhibit 8 of 11 PGPR isolates. The eight isolates were *Pseudomonas* sp 4a, *Bacillus* sp 4e, *Bulkholderia* sp III b, *Brevundimonas* sp PIKO, *Pseudochrobactrum* sp L7TO3, *Bacillus* sp 140B, *Microbacterium* sp. AA2, and *Bacillus* sp. AA1. Meanwhile, *Pseudomonas* sp PP2, *Bulkholderia* sp AD71, and *Pseudomonas* sp PS isolates were not inhibited. The value of the soil respiration test was proportional to the PGPR population number. In the PME-ase activity, measurement decreased as biopesticide extract treatments at higher concentrations. Similar results were observed in the Urease activity. From this work, it must be considered on the application of biopesticide neem extract in the plantations, because it could produce a negative effect on PGPR and soil biochemical properties.

Keywords: *Azadirachta indica*, PGPR, PME-ase, soil respiration, Urease
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Introduction

Recently, the impact of excessive the use of pesticides is not only making crops pests and diseases more resistant, but also polluting nature and poisoning agricultural products. For this reason it is very urgent to reduce the use of pesticides and switch to organic pesticides such as the use of neem extract to control pests and diseases.

Singh et al. (2000) reported the use of Neemazal product of Neem combinated with PGPR to control powdery mildew (*Erysiphe pisi*) of pea (*Pisum sativum*). Neem extract contains a complex mixture of molecules, including normal hydrocarbons, phenolics compounds, terpenoids, alkaloids and glycosides (Hossain et al., 2013). Morgan (2009) reported that azadirachtin is the main active ingredient of Neem extract and has an antifeedant and toxic effect on insects. Azadirachtin can be extracted from neem oil which is not only cheap but also

available on the market. Neem 4.5% mixture is effective for repelling insect pests but does not cause the death of *T. cinnabarinus* (Mansour et al., 1997). However, information about the effects of neem extract on soil microorganisms and plant growth promoting rhizobacteria (PGPR) are still rare.

These various microorganisms present on the rhizosphere and usually can be used as indicator soil quality through their enzymatic activities such as; N fixation, P solubilization and respiration. In this study, the impact of neem extract as organic pesticides on soil microorganisms and their enzyme activity are therefore studied.

Material and Methods

Bacterial isolates & Neem extract

Ten isolates of PGPR of liquid organic biofertilizer inoculants were used in the in vitro test. These bacterial isolates showed multi enzyme activity such as P-solubilization, IAA production, protein degradation, and N-fixation. The isolates were grown under appropriate selective media prior the use in the experiments. The neem extract was gift from Chemical Research Center, Indonesian Institute of Sciences and it was prepared according Savitri and Meliana (2017).

Agar disk-diffusion method of PGPR isolate

Agar plates are inoculated with a Standardized inoculum of the test microorganism. Then, filter paper discs (about 6mm in diameter), containing neem biopesticide at a desired concentration (2,5%, 5%, 10%, and 0% as control) are placed on the agar surface. The Petri dishes are incubated under room temperature conditions (about 28-30^{0}C).

Soil treatments

500 gr soil sample were collected and put in the pot. Series of treatments were arranged as following: Control Soil only (K); Soil+ PGPR isolates (TP); Soil+ PGPR isolates + neem extract 2,5 % (TP2,5); Soil+ PGPR isolates + neem extract 5% (TP5); Soil+ PGPR isolates + neem extract 10% (TP10)

Total of general soil bacteria

The colony forming unit (CFU) of bacteria determined in one gram of soil sample by using a ten fold serial sterilized aquadest dilution technique, and followed by inoculation of drop of each dilution onto the surface of the appropriate agar plate in petridish after a 48-hour-incubation at 28ºC. Microbial growth observed under magnified plate counter.

Soil enzyme analysis

Phosphomonoesterase measurement pursues to Margesin (1996) method. Urease measurement adapted from Kandeler (1996) method. Soil Induce Respiration (SIR) measured to follow Beck et al. (1996) method.

Results and Discussion

The results of this study indicated that each PGPR isolate tested gives different responses. As shown in the table 2 that *Pseudomonas* sp PP2, *Bulkholderia* sp AD71 and *Pseudomonas* sp PS did not show any inhibition zones even though the addition of 10% biopesticide (Table 1.). This means that all three isolates were very resistant to the presence of neem extract biopesticides. while *Bacillus* sp 4e, *Pseudochrobactrum* sp L7TO3 and *Bacillus* sp. AA1 were relatively susceptible, by treating disks containing of 2.5% neem extract with high inhibition zone (+++). The other, *Pseudomonas* sp 4a, *Bulkholderia* sp III b, *Bacillus* sp 140B and *Microbacterium* sp. AA2, belonged to medium resistance to neem extract biopesticides at a concentration of 5%. The emergence of different resistance of PGPR to neem extract biopesticides is very possible due to differences in the metabolic system, however further study are needed.

Table 1. Inhibition zone of PGPR isolate under agar disk-diffusion with different neem extract concentration.

No	Isolate	Neem extract concentration (%)							
		0		2.5		5		10	
1	*Pseudomonas* sp 4a	-	-	-	-	++	+++	+++	+++
2	*Bulkholderia* sp III b	-	-	-	-	++	+++	+++	+++
3	*Bacillus* sp 4e,	-	-	+++	+++	+++	+++	++++	++++
4	*Brevundimonas* sp PIKO	-	-	-	-	-	+++	+++	+++
5	*Pseudomonas* sp PP2	-	-	-	-	-	-	-	-
6	*Bulkholderia* sp AD71	-	-	-	-	-	-	-	-
7	*Pseudomonas* sp PS	-	-	-	-	-	-	-	-
8	*Pseudochrobactrum* sp L7TO3	-	-	+++	+++	++++	++++	++++	++++
9	*Bacillus* sp 140B	-	-	-	-	+++	+++	+++	+++
10	*Microbacterium* sp. AA2	-	-	-	-	++++	++++	++++	++++
11	*Bacillus* sp. AA1	-	-	+++	++	+++	+++	+++	+++

Note :

(-) = No inhibition zone
(+) = inhibition zone 0,5 – 5 mm
(++) = inhibition zone 5 – 10 mm
(+++) = inhibition zone 10 – 15 mm
(++++) = inhibition zone ≥ 15 mm

The negative effect of chemical pesticide synthesis on soil biochemistry is well known. According to Antonius et all (2005) the number of colony forming unit of general bacteria of conventional farming is lower than organic farming. Further more it was reported that the number of specific group of microorganisme including fungi, phosphate solubilizing bacteria, N-fixing bacteria and proteolitic bacteria were also relatively lower in the conventional farming than in organic farming. Therefore we analyzed here the effect of biopesticides on general bacteria grown on Nutrient broth media. It was interesting instead of decreasing, the number colony forming unit of soil treated neem extract biopesticides were parallel with the increasing percentage biopesticide treatments (Fig1.).

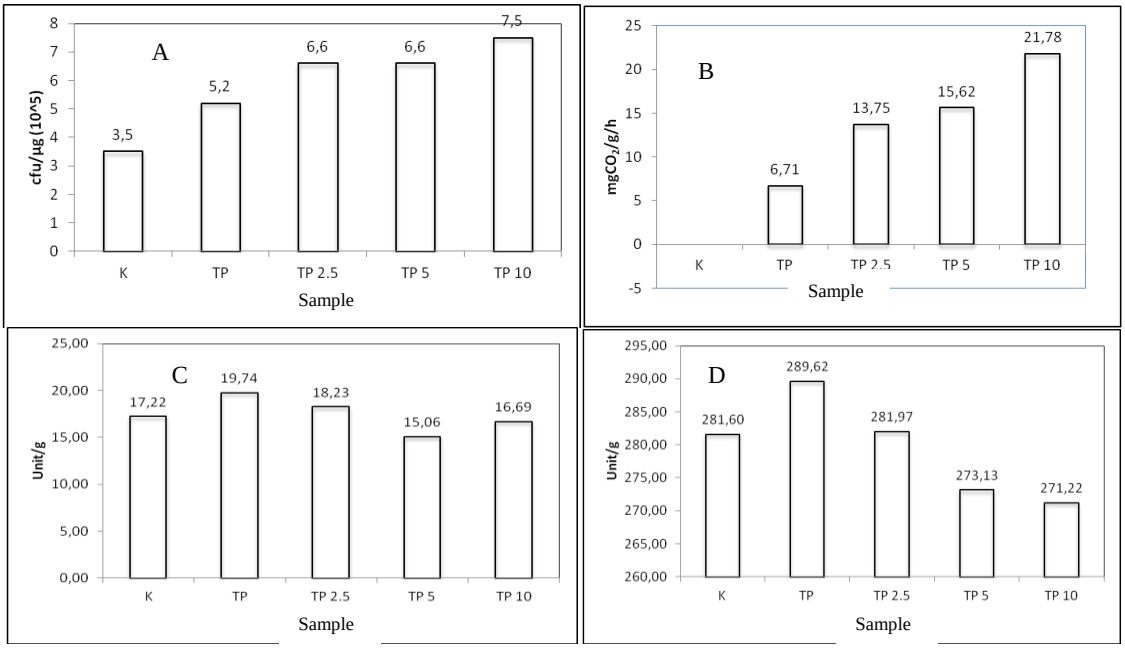

Figure 1. Effect of neem extract on population general soil bacteria (A), Respiration rate (B), Phosphomonoesterase enzyme activity (C) and Urease activity (D)

These data was different with in vitro assay, where 3 isolates were completely not inhibited by the presence of neem extract biopesticides and 7 other isolates were inhibited at leas at concentration of 2,5 % cotaining biopesticide. It might be under natural condition in the soil, automatically the concentraion of organic pesticides more deluted or in other possibility that toxic effect will be absorbed organic material containing in the soil, then will be less toxic or even can be consumed as nutrition, since the number of CFU were increasing as the concentration of biopesticides increased. However, Sarawaneeyaruk et al. (2015) reported that application of azadirachtin and neem extract could reduce the number of root nodules on mung bean plants, as well as the *T. Asperellum* population in the rhizosphere. The ammonia production during the decomposition of neem oil and seed cakes, increased soil alkalinity, resulting in an increase in antimicrobial activity (Dubey et al. 2009).

The activity of phosphomonoesterase enzyme in the soil was not effected by the treatment of biopesticide. It can be seen the Fig.1c, the activity of phospho monoesterase were all almost simmilar, including control, even when the soil sampel were treated with different concentration of neem extract biopesticide. Incontrast with urease soil activity, was rather sensitive to neem extract biopesticides (Fig. 1d. At higher treatment of neem extract biopesticides the activity of urease more inhibited. From this data showed that biopesticide was inhibitor for urease but not for Phodphomonoesterase. However this inhibition seemed to be non specific, because only least then 10 %.

Conclusions and Outlook

Neem extract are widely used as an alternative biopesticide in agriculture to control insect populations. This study found that neem extract usage inhibited the growth of same PGPR during *in vitro* assay and reduced the number general soil microbes. Neem extract in particular inhibited the activity of soil urease enzyme. Therefore, the use of neem extract for high concentration and for long period should be conducted with care, i.e. by combination of resistant PGPR isolates and applying soil ameliorants to support soil biodiversity.

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