TOXICITY STUDY OF ATRAZINE AND METHYL PARATHION IN POTENTIAL FUNGI SPECIES

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ABSTRACT

Pesticides are abundantly used for prevention of pests harming and affecting the yield of crops, such as atrazine and methyl parathion. However, overly used of pesticides led to accumulation of pesticides in soil and drinking water which result in severe impacts on human's health. Hence, human need to find out better remediation strategies to reduce the pollutants. In this research, mycoremediation is introduced to remediate the pollutants because it breakdown the pollutants using fungi which is an environmental friendly and cost-effective way. Objective of this research is to find out the potential fungi species that can remediate atrazine and methyl parathion based on their tolerance level against toxicity of atrazine and methyl parathion. First, 11 types of random fungi were used for screening test in PDA. After screening test, 8 selected fungi species were carried on the experiment with toxicity testing of potential fungi species in different concentration of the pesticides respectively. In toxicity test, fungi species were selected for potential fungi species because it can grow well and consistent in different tested concentrations. As result, *Trichoderma erinaceum* (I-2), *Aspergillus nidulans* (I-8) and *P. simplicissimum* (I-19) were determined to have potential to remediate atrazine with the highest mean dry mass in highest tested concentration of 20 ppm which is 0.116g, 0.073g, and 0.053g respectively. In another hand, *Penicillium chrysogenum* (I-1), *Trichoderma erinaceum* (I-2), *Aspergillus nidulans* (I-8) and *Aspergillus niger* (I-26) were determined as potential fungi species to remediate methyl parathion with the highest mean dry mass in highest tested concentration of 40 ppm which is 0.140g, 0.076g, 0.143g, and 0.163g respectively.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
°C	Degree Celsius
ppm	Parts per million
g	gram
LAF	Laminar Air Flow
LSD	Least significant different
mL	Milliliter
L	Liter
%	Percentage
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
SPSS	Statistical Package of the Social Sciences

CHAPTER 1

INTRODUCTION

In this modern era, pesticides are widely used in most of the countries in agriculture to prevent control unwanted pests or weeds that may affects the yield of crops. Pesticides are made of mixture of chemical substances that are used to alter or suppress the lifecycle or metabolism of pests and weeds (Environment Protection Authority (EPA), 2016). Most of the pesticides are aimed to kill target pests, only some of the pesticides act as attractants or repellents, defoliants (bring down only the leaves of the plant without killing it), and sterilizing agents (disrupt reproductive system of pests) (EPA, 2016). Therefore, pesticides are subjected to highly stringent quality control standards by relevant regulatory agencies like Environmental Protection Agency (EPA) in US. Pesticides also affects the pests' natural predators and other organisms nearby (Secrest, n. d.). Some residual pesticides may still be effective in the soil or plants for days or months after their application. After a period of the same pesticides used, pests and weeds will become more resistant towards the pesticides, therefore higher dose or stronger pesticides need to be used to control the populations. This situation thereby exacerbating the problem of resistant pests and superweed (Secrest, n. d.).

There is number of possible ways to clean up or reduce the pesticides in the soil, such as volatilization, incineration and chemical treatment. For volatilization and chemical treatment, large volumes of alkali or acids is used to clean up, but the wastage of it need to be disposed, which may cause another contamination. Incineration uses high temperature to destruct these compounds in the soil, but the destruction process brings up the public opposition because of the potential toxic emissions to the air, and also expensive of the more energy input (Sousa Fragoeiro, 2005). Overall for most of the physically or chemically cleaning methods are expensive and inefficient, it also may cause another severe pollution to the environment. The most efficient and environmental friendly way to reduce the pesticides contamination level in the soil or water is through bioremediation which is use of fungi or bacteria to remove the pollutants. Biodegradation by using fungi is named as mycoremediation, which use fungi to bioabsorb the pollutants or secrete extracellular enzymes to breakdown the

pollutants into non-toxic substances. Uses of mycoremdiation is less expensive than other methods, because it requires less energy input and preserves the structure of soil (Sousa Fragoeiro, 2005). Applications of mycoremediation consists of soil remediation, mycofiltration of water and hybridized multidimensional systems (using bacterial and fungi to remediate) (Singh, n. d.).

Nowadays, modern pesticides in the environment do not persist for long, act quicker and easily degraded to simple and non-toxic substances (EPA, 2016), but some of the toxic chemical substance still retained in the soil or water causing pollution to nearby organism. Pesticides that is chemically synthesis usually not easily degraded by the environment, so human or animals may get contaminated from drinking water, breathing vaporized pesticides or skin exposure after spraying the pesticides in the crops. In this experiment, two major pesticides used around the world will be discussed, which is Atrazine and Methyl Parathion, are herbicides and organophosphorus insecticides respectively. Atrazine is mostly used in crops like corns, soybeans and sugarcane, to control growth of leaf weeds. It is banned in few European countries because atrazine does not degrade easily in nature, and it widely contaminate the drinking water source of the country, people consuming the water have low levels developmental abnormalities and assuming it had possible risks as carcinogen (Tran, 2014). Moreover, Methyl Parathion had moderate toxic effect on human, mostly have neurotoxic effect on brain function after exposure to it (Agency for Toxic Substances & Disease Registry (ATSDR), 2001).

Hence, the main objective of this study is to investigate the potential of mycoremediation in remediate atrazine and methyl parathion based on their tolerance level against toxicity of both pesticides. Fungi species that have high tolerance level against toxicity of the pesticides indicating the species might have potential in remediating the pesticides.

CHAPTER 2

LITERATURE REVIEW

In the early time, disposal of wastes product into the soil has been a convenient route for wastes, but in the recent research, contaminants in the soil are highly mobile and able to find ways to other areas. Escape of chemical contaminants is a serious issue, since other environmental niches more fragile to be contaminated than the soil. Elevation of concentrations of contaminants will have severe adverse effect on organisms in the area. Normally elevation of concentrations of chemical resulted as human activity (Ashman and Puri, 2002).

Most of the soil contaminated with harmful or toxic elements in term of contamination level, including organic chemicals, oils and tars, metallic elements, pesticides residues, and radioactive materials (Bridges, 1997). These substances usually appear in the soil by intentional disposal of wastes, such as from atmospheric fallout and spillages. Among the contaminants above, pesticides are reveal as primary importance due to the continuous entry of it into the soil (Sannino and Gianfreda, 2001). Routes for pesticides contaminants including absorption by plants and eaten by animals, contaminated water source and consumed by animals and plants, contaminants enter into ground water and rivers, and volatile contaminants from soil to atmosphere (Ashman and Puri, 2002).

Pesticides contamination in groundwater has been subjected into national importance because groundwater is used as drinking water for about 50% of country population. Pesticides are able to filter down to reach the water-aquifers and affects the ground water, but it takes longer time period to become apparent in the groundwater. To protect the quality of groundwater, consideration of time lag between the application of fertilizers and pesticides to the crops is important (Perlman, 2016).

2.1 PESTICIDES

Pesticides are made of mixture of chemical substances that are used to alter or suppress the lifecycle or metabolism of pests and weeds (Environment Protection Authority (EPA), 2016). In agriculture, pests including insecticides (insects), nemotocides (nematodes), fungicides (fungi), herbicides (weeds), and rodenticides (vertebrate poisons). Most of the pesticides are aimed to kill target pests, only some of the pesticides act as attractants or repellents, defoliants (Bring down only the leaves of the plant without killing it), and sterilizing agents (disrupt reproductive system of pests) (EPA, 2016).

2.1.1 Atrazine

Atrazine is one of the most common used herbicides across the United States and Australia agricultural field to reduce the growth of broadleaf and grassy weeds. It kills the weeds by targeting the chloroplast and stopping photosynthesis. Atrazine still commonly used as weed-killer pesticides is because it reduces crop losses effectively due to unwanted weed interference and also an economical choice for low income farmers. Besides, atrazine most frequently used in corn, sugarcane and soybean (Oram, 2014). Due to the high frequent application of atrazine to the soil, it will lead to runoff and ended up in lakes and streams. Consequently, drinking water from the contaminated water source affects health of local residents. Based on an environmental health news on November 2011, woman who had consume the contaminated water with low level of atrazine have low estrogen levels and irregular menstrual cycles (Konkel, 2011). Besides that, atrazine affects children and elderly after consuming the contaminated water over long time, such as cardiovascular system problems and reproductive problems (Oregon Health Authority, 2015).

Furthermore, atrazine had been classified in Type III chemical which have possible carcinogenic to human. Some studies show that breast and prostate cancer, ovarian, and birth defects have possible association with atrazine (Vuchnich & Shochat, 2014). Atrazine in the water are measured in parts per billion (ppb), federal government had established the maximum contaminant level in the water for atrazine as 3 ppb in