Identification of fungi originated from soil polluted by Dichloro Diphenyl Trichloroethane (DDT)

Lita Nasution[1,2], Darma Bakti[3*]

[1] Student of Doctoral Program at Management of Natural Resources and Environment Department, Universitas Sumatera Utara, Indonesia
[2]Lecturer of Agrotechnology Department, Faculty of Agriculture, Al Azhar University, Medan, Indonesia
[3]Lecturer of Agrotechnology Department, Faculty of Agriculture, Universitas Sumatera Utara, Indonesia

[*]Email: darma@usu.ac.id

Abstract. Since the last twenty years Merdeka Village in Merdeka Subdistrict, Tanah Karo District, Indonesia had been the centre of horticultural plant production for the North Sumatra Province. Insecticide commonly used by farmers in this area was of organochloric active ingredient, namely Dichloro Diphenyl Trichloroethane (DDT).The DDT was an insecticide that was degraded very difficultly by the soil, was toxic and persistent in the soil for decades. This insecticide caused changes in soil microbiology, including fungi. Soil sampling using a soil drill at a depth of 0-20 cm with a diagonal sampling method of 45 sample points. Subsequently the soil samples were taken to the Pesticide Laboratory at the North Sumatra Plantation Seed Germination Center for extraction using the Quechers Tube Method using three material variables, namely sodium acetate (Na-acetate), primary secondary amine (PSA) and octadecylsilane (C18) with Gas Chromatography-Mass Spectrometry (GC-MS). The results showed that DDT derivatives were found in B o'p'DDD soil at the amount of 1.6 µgKg-1, and at C o'p'DDD soil at 2.2 µgKg-1. The DDT residues in both locations B and C were classified as dangerous because they exceeded the DDT standard of 0.7 µgKg-1 dry soil which refered to the Canadian Environmental Quality Guidelines (CCME). Soil samples containing five grams of DDT residue were then isolated and fungi growing on the soil were identified. Identification of fungi showed that *Mucor* sp, *Fusarium* sp and *Trichophyton* sp were found.

Keywords: DDT residue polluted soil, mushroom identification, Merdeka Village, *Mucor* sp, *Fusarium* sp, *Trichophyton* sp.

1. Introduction

Since twenty years ago, leaf onion vegetable farmers in Merdeka Village, Merdeka Subdistrict, Tanah Karo District. Merdeka District, Karo Regency generally used insecticides to control plant pests. Intensive use of insecticides left residues in the soil and plants and caused death of various types of beneficial microorganisms, and even enter the body of fishes, animals and aquatic biota [1].

Some important effects caused by insecticides were the changes in the ecological balance of soil microflora [2]. To reduce the negative effects of chemicals it was necessary to conduct research on microorganisms that was potential to be used as degradator of chemicals contained in insecticides. Indigenously microorganisms was able to be developed from agricultural land contaminated with DDT [3].

In Rachel Carson's book entitled "Silent Spring" it was able to be seen a very clear picture of the negative effects caused by the use of prolonged synthetic chemicals [4]. Insecticides had a negative impact on the environment that it was able to accumulate in the bodies of insects, plants, fish and birds [5], and cause disturbances in the ecological balance of soil microflora and changes in soil microflora. One type of dangerous chemical insecticide was DDT [6]. The DDT insecticide belong to the organochlor group. Its metabolites are dichlorodiphenyl dichlorethylene-DDE, dichlorodiphenyldi-chloroethane - DDD, and methoxychlor, hexachlorobenzene (HCB), hexachlorocyclohexane group [α-HCH, ß-HCH, Y-HCH], the cyclodiene group [aldrin, dieldrin, endrin, chlordane, nonachlor, heptachlor andheptachlor-epoxide], and chlorinated hydrocarbons [dodecachlorine, Toxaphene, and chlordecone] [7]. All DDT-forming isomers have a relative molecular weight of about 354.5. The pp 'DDT temperature is around 108.5 to 109ºC and the pressure is around 2.53 x 10-5 Pa (1.9 X 10^{-7} mmHg) at a temperature of 20ºC. The DDT is a mixture of organic solvents (100 $ml^{-1}g$) benzene, 110; cyclohexanone, 100; chloroform, 96; solvents, 4-10; ethanol, 1.5. The DDT is highly insoluble in water (solubility of about 1µg $liter^{-1}$) but is highly soluble in animal fat [8]. In Pakistan, DDT residues were found at 5 cm from the surface of sandy loam soil [9] DDT is able to survive for 4-35 years in the soil and is immobile [10], resulting in slow environmental damage. The DDT found in 1973 was very dangerous to life and the environment, because it left very long persistent residues and was able to accumulate in tissues through the food chain, was very stable both in water, in the soil, in plant and animal tissues [11]. Some fungi commonly found on the soil were *Fusarium* sp, *Mucor* sp, *Trichoderma* sp, and others. In India, *Mucor* sp fungi isolated from Bhitarkania mangrove forest was also found [12].

In this study, soil contaminated with DDT residues was extracted using Quechers Tube method and applying three material variables namely sodium acetate (Na-acetate), primary secondary amine (PSA) and octadecylsilane (C18) with Gas Chromatography-Mass Spectrometry (GC-MS) instrument. The soil sample preparation method was fast, easy, cheap, effective, sturdy and safe or QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) [13], and has been used in multi-pesticide residue analysis on various sample matrices. QuEChERS method had several advantages such as simple, had only a few stages of analysis and effective in the cleanup process. This method was also environmentally friendly because it required only a small amount of organic solvents, which was in line with the current new trend of "green analytical chemistry" [14,15]. This method was proved to be effective in analyzing pesticide residues in sample matrices of fruits and vegetables matrix.

2. Methods

2.1. Taking Soil Samples Containing DDT Residues

The first soil samples were taken at point B {$3^0$12 '10, $42^{2'}$ North, $98^0$29 '55, $44^{2'}$ East 3012 '10, 342' North. $98^0$29 '56, $22^{2'}$ East $3^0$12 '07, $66^{2'}$ North, $98^0$29 '54, $90^{2'}$ East $3^0$12 '07,$76^{2'}$ North, $98^0$29 '55, $00^{2'}$ East} and Point C {$3^0$12 '07, $73^{2'}$ North $98^0$29 '56 , 192 '. $3^0$12 '07, $60^{2'}$North $98^0$29 '56. $3^0$12' 06, $93^{2'}$ North $98^0$29' 56. $3^0$12 '07, $46^{2'}$ North $98^0$29 '55} by placing an anchoring in a diagonal direction, then tie a plastic rope over the anchoring wood to form a diagonal line. After the rope has been installed, the next step was to drill the soil using a soil drill with a depth of 0-20 cm at twenty-five holes at point B. After the soil was drilled at every single point, the soil was put directly into a two kilogram plastic bag, then a sticker label was put on each plastic bag, where the location, the point name and the coordinate of the point using the GPS Test Plus of the soil sample being taken was written down. This was done to get twenty points of drilled holes in a diagonal line. Next, after finishing taking the soil samples on a diagonal line, the step was repeated to take soil samples on the other diagonal line in a different direction until it formed a letter of X, with the number of fifteen drilled holes, so totally the holes taken at point B was 45 drilled points. The next step was to collect all the soil samples that had been packaged by mixing it into one composite soil sample and brought it to the Pesticide Laboratory at the North Sumatra Plantation Plant Seed Center. This soil sample was extracted using Quechers Tube Method with a Gas Chromatography-Mass Spectrometry (GC-MS) instrument to detect its level of insecticide residues contained in the soil.

2.2. Soil Sample Preparation

The soil samples were taken to the Pesticide Laboratory at the North Sumatra Plantation Seed Germination Center for extraction using the Quechers tube method using three material variables namely sodium acetate (Na-acetate), primary secondary amine (PSA) and octadecylsilane (C18) [16]) with Gas Chromatography-Mass Spectrometry (GC-MS). A 2.5 g of homogenized soil was weighed using Mettler Toledo. Each soil was taken from soil samples B and C, then be sifted so that it became smooth and evenly mixed. Then, the soil was sprinkled to the media by sifting it, so that it was evenly distributed throughout the Sabaraud Dektrosa Agar media. The 2.5gr of soil sample was divided into 10 Agar Extracted Sabaraud mushroom media. Each 2.5 grams soil sample was taken and put into Dextrose Sabaraud mushroom growing media which contained Clorampenicol and Cycloheksamide as antibiotics so that the fungi growth would be better and be protected from bacteria caused by contaminants. Early identification of fungi was done macroscopically and microscopically on growing fungal colonies. Then purification of each fungus was carried out [17].

2.3. Mushroom Isolation

Composite soil samples were taken by uniting all soil samples and mixing them evenly in the biosafety cabinet. Composite soil samples of B and C of 0.25 grams were cultured into Sabaraud Dektrosa Agar media. Then it was cultured at room temperature 27^0C by placing it in a dry place and being given a lamp waiting for three to six days to be placed in the biosafety cabinet just to see its fertility whether the growth of the fungus was good or not and then identified. Furthermore, the fungal isolates from the sample B and C were observed; if there was a contamination in the media, then the fungus was transferred to the new media and the isolation process was repeated so that the pure isolates of the fungus were obtained.

2.4. Mushroom Identification

Mushroom culture that grew on soil samples B and C was identified by observing the kind of fungus. Next, it was transferred to the Potato Dextrosa medium in order to get the pure species of the fungus grown in the soil. When observed microscopically at 40x100 magnification, it was seen that *Mucor* sp had sporangia as its reproduction tools [17].

2.5. Mushroom Staining

The structure of the growing pure fungus was seen using a wet preparation of Lactophenol cotton blue. Filamentous fungus colonies were taken with cellotape and then placed into the media where the fungus was slightly pressed and then placed on a glass object containing lactophenol cotton blue [1] and was seen under a microscope with 10x and 40x magnification. Then, microscopic images were taken. Then the images were seen and observed microscopically; if fungus colonies were yeast gram staining procedures were carried out like what has been done in bacterial staining.

3. Result and Discussion

Recently farmers use synthetic insecticides in their agriculture practices. Synthetic insecticides contain inorganic compound that when accumulated in the soil can cause negative effects, so that some important microbes in soil destroyed [1], and the ecological balance of the soil is disturbed [2]. Synthetic insecticide used by farmers called DDT is one of the compounds that can interfere with the ecological balance of the soil [6], therefore DDT is very dangerous for the soil so an effort is needed to degrade DDT. Identification of microorganisms that can adapt to DDT contaminated soil can be used as an alternative to DDT degradation agents.

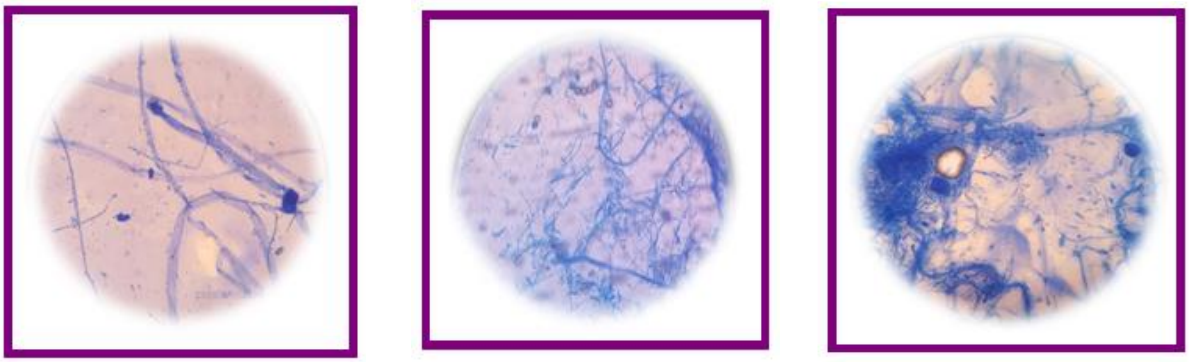
The results showed that soil samples at locations B and C were contaminated by DDT. The DDT residue testing results can be seen in Table 1.

Table 1. DDT Residue Testing Results

No.	Locus	DDT Consentration
1.	B	1.6 µg Kg^{-1}
2.	C	2.2 µg Kg^{-1}
3.	Standard DDT *	0.7 µg Kg^{-1}

Standard DDT * based on CCME [15]

Results in Table 1. showed that the DDT concentration at the test site had exceeded the normal limits, so that it was concluded to be positively contaminated with DDT contamination. The DDT residues in both locations B and C were classified as dangerous because they had exceeded the DDT standard of 0.7 µgKg-1 dry soil which refered to the Canadian Environmental Quality Guidelines (CCME) [18]. Microscopic identification results can be seen ini.



a **b** **c**

Figure 1. Fungus **a.** Mushroom *Mucor sp* **b.** Mushroom *Thrichophyton* sp. **c.** Mushroom *Fusarium* sp

The results showed that fungi adapted to DDT contaminated conditions were identified. The visualization of Fusarium in Figure 1 was matched with fungus identification standards. Figure 1a showed hyphal colony morphology form like white thread, at certain parts there were sporangium and sporangiophore in the form of black dots like pin needles and microscopic features of hyphae without septum; there were sporangium and sporangiospores so it was identified as *Mucor* sp. The classification of *Mucor* sp is shown in Table 2.

On the other hand, Figure 1b was more likely to have a denser arrangement and had a special characteristic that microscopically it had insulated hyphae, spiral hyphae, mace macroconidia was found and it had a thin cell wall consisting of 6-12 cells, macroscopically coarse-powdered colonies were found and the protruding middle part was visible, so Figure 1b was identified as mushroom Trichophyton sp. The *Trichophyton* sp classification is shown in Table 2.

In Figure 1c it was seen that the tissue forms were elongated and tight and characterized by having a microconidia and macroconidia structure, the colony surface was purple and the edges were jagged and had a rough, fibrous and wavy surface. In the soil, this fungus formed branched conidiophores and macroconidiium which was crescent shaped or was like a small stalk banana and were in pairs so that it was classified as *Fusarium sp*. The classification of the fungi *Fusarium sp* is shown in Table 2.

Table 2. Classification of *Mucor* sp, *Trichophyton* sp, *Fusarium* sp Fungi

No	Classification of Fungi	*Mucor* sp	*Trichophyton* sp	*Fusarium* sp
1	Super Kingdom	Eukaryota	Eukaryota	Eukaryota
2	Kingdom	Fungy	Fungy	Fungy
3	Divisio	Zygomycota	Ascomycota	Ascomycota
4	Class	Zygomecetes	Euascomycetes	Sordariomycetes
5	Ordo	Mucorals	Onygenales	Hypocreales
6	Family	Mucoraceae	Arthrodermataceae	Netriaceae
7	Genus	Mucor	Trichophyton	Fusarium
8	Species	*Mucor* sp	*Trichophyton* sp	*Fusarium* sp

Based on the discussion, *Mucor* sp*, Trichophyton* sp and *Fusarium* sp were identified in DDT contaminated soil. The presence of these three fungi was identified by the study [12] which found them under mangrove forests in Bhitarkania, India. The fungus was able to survive in the soil contaminated with DDT and the three fungi were parasites on plants. This showed that the mushroom microflora in the soil was very few in number and also affected the exuberancy of vegetable plants that grew in these locations.

The results showed that the fungus microflora in the soil were very small in number. This indicated that the soil was relatively infertile for horticultural crops to grow. It caused plants that grow in soil containing DDT residues to have residual levels in plants, as well. Under normal conditions where the soil was free from DDT insecticide residues, such as in forest soil, tens of fungus species can be isolated.

4. Conclusion

The examination of two soil samples B and C showed that the soils contained DDT insecticide residues with a concentration of 1.6 µg Kg^{-1} and 2.2 µg Kg^{-1} at location B and C, respectively. In both locations, only three types of fungi were able to live in the top soil: they were identified as *Fusarium* sp, *Mucor* sp and *Thricophyton* sp.

5. References

[1] Bell, W.R. Dalton, J.B. Mac Call, C.M. Karram, S. Pearce, D.T Memon, W. Lee, R. Carroll, K.C. Lyons, J.L. Gireesh, E.D. Trivedi, J.B. Cettomai, D. Smith, B.R. Chang, T. Tochen, L. Ratchford, J.N. Harrison, D.M. Ostrow, L.W. Stevens, R.D. Chen Li and Zhang, S.X. 2013. Iatgrogenic Exserohilum Infection of The Central Nervous System: Mycological Identification and Histopathological Findings. Modern Pathology. 26. Pp. 166-170. Doi: 10.1038/modpathol.2012.208 Source: PubMed.

[2] Tang, J. Zhang, M. Cheng, G and Lu, Y. 2009. Preparation and Evaluation of Sol-Gel Glass Based Immunoaffinity Column and Their Potential Use In Determination of Diazinon In Water and Soil Samples With High Perfomance Liquid Chromatography. Analytical Letters Journal. 42. 243-254. Doi: 10.1080/00032710802585709.

[3] Hindersah, R., Rachman, W., Fitriatin, B.N and Nursyamsi, D. 2013. The Population of Microbes in the Rhizosphere and Growth Caisim (Brassica juncea) in Soil Contaminated with Insecticides Organochlorines After Konsoria Microbial Applications and Compost. Journal of Indonesian Nature 15(2): 115-120.

[4] Cutright, T.J and Erdem, Z. 2012. Overview of The Bioremediation and The Degradation Pathways of DDT. Journal of Adnan Menderes University Agricultural Faculty. 9(2). 39-45.

[5] Carson, R. 2002. Silent Spring 40th Anniversay Edition. Houghton Mifflin Company. Boston. New York.

[6] Vanita, B. and Veena, S. 2015. Impact of Insecticides Use in Agriculture. Internasional Journal

of Development Research and Engineering. 2(2):1-21.

[7] Abd El-Ghany, T.M., Masmali, I.A and Negin,M. 2015. Insecticide-fungyInteraction and Their Impact on Fungal Population in Agricultural Soil. International Journal of Microbiology and Allied Sciences (IJOMAS). 1(4). 18-22. ISSN 2382-5537.

[8] Mwangi, K. Boga, H. I. Muigai, A. W. Kiiyukia, C and Tsanuo, M. 2010. Degradation of Dichlorodiphenyl Trichloroethane (DDT) by Bacterial Isolates from Cultivated and Uncultivated Soil. African Journal of Microbiolog Research. 4(3). 185-196.

[9] Anwar, T., Ahmad, I and Tahir, S. 2012. Determination of Insecticide Residues in Soil of Nawabshah District, Sindh. Pakistan. Pakistan. J. Zool. 44(1). 87-93.

[10] Corona-Cruz, A., G., Gold-Bouchot, M., Dutierez-Rojas, O., Monroy-Hermosillo and E.Favela.1999. Anaerobic-aerobic Biodegradation of DDT (1,1,1-trichloro-2, 2-bis (p-chlorophenyl) ethane) in Soil. Bull. Environ. Contam. Toxicol., 63 :219-225.

[11] Thierfelder, C and Wall, P.C. 2012. Effect of Conservation Agriculture on Soil Quality and Productivity in Contrasting Agro-Ecological Environments of Zimbabwe. Soil Use and Management Journal. 28. 209-220. Doi:10.1111/j.1475-2743.2012.00406.

[12] Kannan, K., Tanabe, S., Williams, R.J and Tatsukawa, R. 1994. Persistant Organochlorine Residues in Foodstuffs from Australia, Papua New Guinea and The Solomon Islands: Contamination Levels and Dietary Exposure. The Science of the Total Environment 153: 29-49.

[13] Jeong In-Seek, Kwak Byung-Man, Ahn Jang-Hyuk and Jeong Seung-Hwan, 2012. Analitical Methods Determination of Pesticide Residues in Milk Using a Quechers Based Method Developed By Response Surface Methodology. Food Chemistry Journal (133). Pp. 473-481. Doi:10.1016/j.foodchem.2012.01.004.

[14] Ziaee, A. Zia, M. Bayat, M and Hashemi, J. 2016. Molecular Identification of Mucor and Lichtheimia Species in Pure Cultures of Zygomycetes. Jundishapur Microbiol. 9 (4) e35237. Doi : 10.5812/jjm.35237.

[15] Canadian Environmental Quality Guidelines, Canadian Council of Ministers of The Environment, CCME 1999, Including updates of 2001 and 2002.

[16] Anastassiades, M., Lehotay, S.J., Stajnbaher, D. and Schenck, F.J. 2003. Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for The Determination of Pesticide Residues in Produce. Journal of AOAC International, 86: 412-431

[17] Zhang, Y., Hu, D., Zeng, S., Lu, P., Zhang, K., Chen, L., and Song, B. 2015. Multiresidue Determination of Pyrethroid Pesticide Residues in Pepper Through a Modified QuEChERS Method and Gas Chromatography with Electron Capture Detection. Biomed. Chromatog

[18] Biziuk, M. and Stocka, J. 2015. Multiresidue Methods for Determination of Currently Used Pesticides in Fruits and Vegetables using QuEChERS Technique. International Journal of Environmental Science and Development, 6(1)

[19] Thakur, M. Lyncoln, L.Niyonzima, F.N and More, S.S. 2014.Isolation, Purification and Characterization of Fungal Extracelullar L-Asparaginase From Mucor hiemalis. J. Biocatal Biotrasformation.2-2. http://dx.doi.org/10.4172/2324-9099.1000108.