MYCODEGRADATION OF DIMETHOATE IN ARABLE SOIL AND OF ITS RESIDUE IN THE LEAVES OF *Telfairia occidentalis* Hook. F.

ISSN: 2141 – 3290
www.wojast.com

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ABSTRACT

Pesticide residue in food and the environment has remained a major cause for concern to man. This study investigated the fungal degradation of dimethoate in the soils by an indigenous strain of *Aspergillus aculeatus*, by evaluating dimethoate residue in the soil and *Telfairia occidentalis* leaves. The experimental field was laid out in a 5x5 Randomized Complete Block Design. Fungi were isolated from the soil of the experimental site using the spread plate technique. *T. occidentalis* seed was thereafter planted at the rate of 3 seeds per hole, 60 cm apart and with 1metre inter row spacing. One of fungal species, *Aspergillus aculeatus*, isolated from test soil was mass cultured and aseptically returned into the soil of the experimental site after the application of 0.07% of dimethoate to control insect pests on *T. occidentalis*. Gas Chromatography with Pulsed Flame Photometric Detector was used in determining dimethoate residue in both soil and the leaves of *T. occidentalis* planted on soil. Data on dimethoate residue detected in both the leaf and soil samples were analyzed using the one-way ANOVA, while means were separated using the Least Significant Difference (LSD) at 95% confidence interval. The application of fungal spores to the experimental soil caused a significant ($p<0.05$) reduction of 11.22% and 32.66% in the dimethoate residue in the soil and *T. occidentalis* leave samples respectively. These results indicate the ability of *A. aculeatus* to reduce dimethoate residues in soil and edible plant samples. The presence of dimethoate in the leaves of *T. occidentalis* and non-target areas calls for strict regulation in the application of pesticides.

INTRODUCTION

In Africa, the consumption of indigenous vegetables is usually encouraged because they are essential for a healthy and balanced diet, as well as for adding variety and flavour to the menu (Botwe *et al.,* 2011). Additionally, it is likely that plants will continue to be a valuable source of new and improved drugs after their possible chemical manipulation (Sharma *et al.,* 2006). Hence, the contamination of these vegetables with pesticide used in their protection poses a threat to their quality and safety, as the pesticides usually leave often toxic residues in the tissues of the plants to which they are intended as protection agents.

Pesticide residues constitute the elements of a pesticide which are deposited on or in food, or any component of the environment after their application to food crops, spillage or dumping (Dasika *et al.,* 2012). Hence, pesticide residue analysis is an important process in the determination of the safety of certain pesticides (Dasika *et al.,* 2012).

Usually, physical and chemical cleanup techniques are expensive and sometimes not very efficient (Rhodes, 2014). However, biological techniques such as bioremediation via microorganisms have proven very effective (Schoefs *et al.,* 2004). An example of these biological methods is *mycoremediation,* which is based on the use of fungi for the elimination of toxic wastes from the environment. The major aim of this research was to evaluate the potential application of this remediation strategy to remove dimethoate from the environment and reduce its uptake by edible vegetable.

MATERIALS AND METHODS

Experimental Design. This experiment was laid out in a Randomized Complete Block Design (RCBD). The field dimension was 20m × 20m and this was divided into five equal blocks of 3 × 20m spaced one metre apart from each other, with five equal plots per block (3 × 3m).

Fungal Isolation

Soil samples were collected from each block and bulked together. Under an aseptic condition, serial dilutions were carried out on this bulked soil sample for fungal isolation. The isolation was done according to the spread plate method of Okungbowa and Oviasogie (2010) and, Sanyaolu and Kolawole-Joseph (2015) using Potato Dextrose Agar (PDA) as the culture medium. The desired fungus was then inoculated into another freshly prepared PDA and repeatedly subcultured to obtain pure cultures of the fungus.

Identification of Fungus

Identification of the fungus was done using the morphological technique of spore and mycelium characteristics as documented by Deacon (1980) as well as molecular methods where fungal DNA were extracted with Zymo Research (ZR) and its quality and quantity were verified by Nanodrop spectrophotometer. Fungal universal Primers were used both for Polymerase Chain Reaction (PCR) and sequencing of the desired genes.

Application of Remediating Agent

Three seeds of *T. occidentalis* were sown per hole. After sprouting, dimethoate was diluted at the recommended application rate of 7 ml dimethoate to 10 litres of water and evenly applied to the applicable experimental spots using a Knapsack sprayer. Extreme care was taken to avoid dimethoate getting to the slots that were not to receive the dimethoate treatment. The fungus *A. aculeatus* was mass cultured in 100 plates, and under aseptic conditions, the spores were harvested from the plates using a presterilized spatula into sterile distilled water. The spores so harvested were then inoculated into the field at the rate of 250,000 spores/ml 48 hours after the application of dimethoate.

The following were the 5 Treatments used in this work: T1 = *A. aculeatus* + dimethoate + *T. occidentalis*; T2 = Dimethoate + *T. occidentalis* – *A. aculeatus*; T3 = *A. aculeatus* + dimethoate – *T. occidentalis*; T4 = *A. aculeatus* – dimethoate + *T. occidentalis*; T5 = Control (i.e. Nothing added).

Analysis of Dimethoate Residue. The soil samples and plant materials were analyzed for the presence of dimethoate residues, using Gas Chromatography (HP 5890/6890) with Pulsed Flame Photometric Detector.

The extraction and the analysis of the dimethoate residue was carried out using a modified approach of Luke and Doose (1984) for multiple residue extraction as reported by Sanyaolu (2018), while its determination was followed using the European Commission Regulation (EC) No 396/2005 (2005).

The pulverized samples were weighed and preserved below 4^0C until analysis. Ten grams of the soil and plant sample were each extracted and later transferred to the extracting bottle that was cocked with TFE-fluorocarbon. The phosphate buffer (50 ml) was added, followed by the pH measurement with the addition of sulphuric acid (1+1) and sodium hydroxide solution (400 g/l) for pH adjustment to 7. One gramme of sodium chloride was added to the sample, sealed and shaken so as to dissolve the salt. The redistilled analytical grade methylene chloride (20 ml) was measured and poured into the sample. The sample was extracted through sonication for 30 minutes. The extract was filtered into the Erlenmeyer flask. The extraction was repeated twice with fresh solvent, and the filtrate was combined. The combined extract was dried by pouring through a drying column containing a 10 cm column of anhydrous sodium sulphate (previously rinsed with methylene chloride), and the filtrate was concentrated in the concentrator flask with a stream of nitrogen. The clean-up of the concentrated extract was

followed by packing the column with the florisil. The wall of the concentrator flask was rinsed with methyl tert-butyl ether (MTBE) so as to bring the final volume of the extract to 5.0 ml.

Statistical Analysis

Data obtained from the dimethoate residue analysis from the soil and *T. occidentalis* were analyzed using the one-way Analysis of Variance (ANOVA), using the Statistical Package for Social Sciences (SPSS, Version 20.1) and means were separated using Least Significant Difference (LSD) at the 95% confidence level. The relationships among the residues of dimethoate in the soil samples and its residues in the plant samples were established by bivariate correlation method. Correlation method was also used to determine the relationship between the soil and plant variables, and to identify the plant variables that were influenced by the soil variables.

RESULTS

Dimethoate Residue in Soils and its Uptake in *T. occidentalis* leaves.

The test fungus, *A. aculeatus*, produces dark brown to black conidia and is very related to *A. niger.* It is distinguished from *A. niger* by *Aspergillus* heads bearing only phialides. The mean residue of dimethoate detected in the soil and the leaves of *T. occidentalis* are shown in Table 1. The concentration of dimethoate in the soil was highest in Treatment 2 (13.90 µg/kg), and next to this value was that of Treatment 3 (12.34 µg/kg). Dimethoate residues in Treatments 1 (10.32 µg/kg) and 4 (10.31 µg/kg) were not significantly ($p \geq 0.05$) different from each other, although they were significantly ($p < 0.05$) lower than Treatments 2 and 3 (Table 1). Also, the control Treatment (T5) contained a significantly ($p < 0.05$) lower concentration of dimethoate (7.08 µg/kg) than the other 4 Treatments (Table 1).

Dimethoate residue uptake in *T. occidentalis* leaves varied remarkably.. There was a significantly ($p < 0.05$) higher rate of dimethoate uptake in T2 plants (6.98 µg/kg) than in T1 plants (4.70 µg/kg). The least value of 2.68 µg/k) were also detected in the leaves of *T. occidentalis* in T4 (Table 1). The results of this study also showed a corresponding high level of dimethoate residues in *T. occidentalis* where there was a high concentration of dimethoate in the soil, and vice versa (Table 2).

Table 1: Mean dimethoate residues in soil and leaves of *Telfairia occidentalis* (µg/kg)

Treatments	Soil residue (µg/kg)	Residue in leaves of *T. occidentalis* (µg/kg)
T1 (*A. aculeatus* + Dimethoate + *T. occidentalis*)	10.32 ± 2.17^{b}	4.70 ± 0.24^{b}
T2 (Dimethoate + *T. occidentalis*)	13.90 ± 0.05^{d}	6.98 ± 0.03^{c}
T3 (*A. aculeatus* + Dimethoate)	12.34 ± 0.10^{c}	NA
T4 (*A. aculeatus* + *T. occidentalis*)	10.31 ± 0.36^{b}	2.68 ± 0.05^{a}
T5 (Control)	7.08 ± 0.20^{a}	NA

Means in the same column followed by the same superscript are not significantly different ($p \leq 0.05$) using LSD; NA = not applicable.

Table 2: Comparisons between the concentrations of dimethoate residue in the soil and *T. occidentalis* (µg/kg)

Treatments	Soil residue (µg/kg)	Residue in leaves of *T. occidentalis* (µg/kg)	Bioaccumulation Factor
T1	10.32 ± 2.17^{a}	4.70 ± 0.24^{b}	0.46
T2	13.90 ± 0.05^{a}	6.98 ± 0.03^{b}	0.50
T4	10.31 ± 0.36^{a}	2.68 ± 0.05^{a}	0.26

Residue of Dimethoate indicated for the same Treatments with different superscripts are significantly different at $p < 0.05$.

DISCUSSION

Although a number of studies exist that deal with degradation of pesticides by fungi, not many of them focus on the uptake of these pesticides by plants. Dimethoate residues were deposited in the soil and were taken up by the *T. occidentalis* cultivated there. Treatment of select plots with *A. aculeatus* led to a reduction in the mean level of dimethoate residue deposited in the soil. The high residue level in Treatment 2 was probably as a result of the absence of *A. aculeatus*. The levels of dimethoate residue in Treatments 1 and 4 were not significantly different ($p<0.05$), probably because of the action of the *A. aculeatus* in the degradation of the dimethoate in Treatment 1, a position which agrees with the findings of Karanth (1992). The presence of dimethoate residues in Treatment 4 which was not subjected to the dimethoate treatment may probably be due to natural occurrences like action of runoff, volatilization and probably rain splash, a position which agrees with the findings of Keikotlhaile and Spanoghe (2011). These events could also be responsible for the presence of dimethoate residues in the control Treatment (T5) and leaves of *T. occidentalis* in Treatment 4, as well as other non-target areas.

The inoculation of *A. aculeatus* into Treatments 1, 3 and 4 was probably responsible for the lower level of the mean dimethoate residues than in Treatment 2, where *A. aculeatus* was not applied. Although Treatments 1 and 3 were similarly treated with both dimethoate and *A. aculeatus*, degradation was more effective in Treatment 1. This was probably due to the presence of the *T. occidentalis* in T1, as there is a synergistic relationship between the plant roots and other fungi in the rhizosphere. The presence of the *T. occidentalis* could have aided the degradation process better by supplying the *A. aculeatus* with more nutrients for its metabolic processes (Harrison, 2005). However, degradation was still effective in Treatment 3, as the dimethoate residue in this Treatment was significantly lower than that of Treatment 2. A probable reason for the degradation of dimethoate in Treatments 1 and 3 could be that within the toxic environment, *A. aculeatus* could have responded with a greater reproductive effort through increased conidia production (Pelizza *et al.,* 2015). *A. aculeatus* was probably able to degrade the dimethoate because of its ability to utilize the dimethoate or its derivatives for its metabolic processes (Christian *et al.,* 2005; Hasan, 1999). It is obvious from the results of the GC analysis that *A. aculeatus* played a major role in the degradation of the dimethoate in the soil. This is in consonance with the works done by Liu *et al.* (2001) where *A. niger* was successfully used to degrade dimethoate, and Sanyaolu (2018) where the pesticide lambda-cyhalothrin was degraded in the soil by *A. niger.*

The maximum residue limit (MRL), which is the highest legally tolerable pesticide residue in food or feed when pesticides are applied in accordance with good agricultural practices, is set for different food items to monitor residue levels in these food items (FAO/WHO, 2004; FAO, 2005). The mean concentrations of dimethoate in this study, however, fell below the FAO/WHO 2007 benchmark of 0.02 mg/kg (20.0 µg/kg) for dimethoate in vegetables. Similar results were obtained by Sanyaolu (2018) where *A. niger* was able to reduce lambda-cyhalothrin residue to acceptable levels in soil and *Lactuca sativa* plants.

CONCLUSION AND RECOMMENDATION

It is evident from the study that dimethoate can be deposited in the soil and *T. occidentalis* tends to absorb and accumulate this pesticide in its tissues, although at levels below the MRL. Results also revealed that *A. aculeatus* showed some promise at degrading dimethoate residue in the soil as well as a concomitant reduction of its translocation to the leaves of *T. occidentalis*. This fungal strain could be beneficial for efficient biodegradation of other organophosphate pesticides.

REFERENCES

Botwe, B. O., Ntow, W. J., Kelderman, P. , Drechsel, P., Carboo, D., Nartey, V. K. and Gijzen, H. J. (2011). Pesticide residue contamination of vegetables and their public health

implications in Ghana. *Journal of Environmental Issues and Agriculture in Developing Countries*, 3(2): 1 – 18.

Christian, V., Shrivastava, R., Shukla, D., Modi, H. A. and Vyas, B. R. (2005). Degradation of xenobiotic compounds by lignin-degrading white-rot fungi: Enzymology and mechanisms involved. *Indian Journal of Experimental Biology,* 43(4): 301 – 312.

Dasika, R., Tangirala, S. and Naishadham, P. (2012). Pesticide residue analysis of fruits and vegetables. *Journal of Environmental Chemistry and Ecotoxicology,* 4(2): 19 – 28.

Deacon, J.W. (1980). Introduction to Modern Mycology. Blackwell Scientific Publications, London. 197pp.

European Commission Regulation (EC) No 396/2005 of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. Off J Eur Union L 70/1, 16.3.2005, with later amendments

FAO (2005). *Specifications and Evaluations for Agricultural Pesticides – Dimethoate.*http://www.fao.org/ag/agpp/Pesticid/Default.htm. (Retrieved on 8th August 2018).

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization) (2004). *Pesticide Residues in Food*. Joint FAO/WHO Meeting on Pesticide Residues, Evaluations Part I – Residues. *FAO Plant Production and Protection Paper,* 177: 364 – 367.

Harrison, M. J. (2005). Signaling in the arbuscular mycorrhizal symbiosis. *Annual Review of Microbiology*, 59: 19 – 42

Hasan, H. A. H. (1999). Fungal utilization of organophosphate pesticides and their degradation by *Aspergillus flavus* and *A. sydowii* in soil. *Folia Microbiologica,* 44: 77 – 84.

Karanth, N. G. K. (1992). Abatement of pesticide residues through biodegradation. *In:* V. P. Agarwal and S. V. S. Rana (Eds.) *Environment and Biodegradation*. Society of Biosciences, India, 352p.

Keikotlhaile, B. M. and Spanoghe, P. (2011). *Pesticide Residues in Fruits and Vegetables.* 22p. http://cdn.intechopen.com/pdfs-wm/13013.pdf (Retrieved on 9th April 2017)

Liu, Y. H., Chung, Y. C. and Xiong, Y. (2001). Purification and characterization of a dimethoate-degrading enzyme of *Aspergillusniger* ZHY256, isolated from sewage. *Applied and Environmental Microbiology,* 67: 3746 – 3749.

Luke, M.A. and Doose, G.M. (1984). A rapid analysis for pesticides in milk and oilseeds. *Bull. Environ. Contam. Toxicol*. 32: 651-656.

Okungbowa, F. I. and Oviasogie, E. F. (2010). Antimicrobial effect of grapefruit crude extracts on selected bacterial isolates. *International Journal of Biomedical Pharmaceutical Sciences,*5(1): 68 – 70.

Pelizza, S. A., Scorsetti, A. C., Fogel, M. N., Pacheco-Marino, S. G., Stenglein, S. A., Cabello, M. N. and Lange, C. E. (2015). Compatibility between entomopathogenic fungi and biorational insecticides in toxicity against *Ronderosia bergi* under laboratory conditions. *BioControl,* 60: 81 – 91.

Rhodes, C. J. (2014). Mycoremediation (bioremediation with fungi) – growing mushrooms to clean the earth. *Chemical Speciation and Bioavailability,* 26(3): 196 – 198.*Sanyaolu, A. A.A. and Kolawole-Joseph, O. (2015). Mycodeterioration of some nutritional components of palm oil and processed Capsicum frutescens (L.) Kuntze. Nigerian Journal of Mycology, 7: 55 – 74.*

Sanyaolu, A. A.A. and Kolawole-Joseph, O. (2015). Mycodeterioration of some nutritional components of palm oil and processed *Capsicum frutescens* (L.) Kuntze. *Nigerian Journal of Mycology,* 7: 55 – 74.

Sanyaolu, A. A. A. (2018). Verification of Aspergillus niger as a mycoremediation agent of lambda-cyhalothrin and associated heavy metals in Lactuca sativa (L.) leaf. *Journal of Applied Sciences and Environmental Management,* 22(5): 621 – 624.

Schoefs, O., Perrier, M. and Samson, R. (2004). Estimation of contaminant depletion in unsaturated soils using a reduced-order biodegradation model and carbon dioxide measurement. *Applied Microbiology and Biotechnology,* 64: 256 – 261.

Schuster, E. and Schröder, D. (1990). Side-effects of sequentially applied pesticides on non-target soil microorganisms: Field experiments. *Soil Biology and Biochemistry,*22: 367 – 373.

Sharma, R. K., Agrawal, M. and Marshall, F. M. (2006). Heavy metals contamination in vegetables grown in water irrigated areas of Varanasi, *India Bulletin of Environmental Contamination and Toxicology,* 77: 312 – 318.