IMPACT OF DIMETHOATE ON THE MYCELIA GROWTH OF SOME SOIL INHABITING FUNGI

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ABSTRACT

Application of pesticides, even under Good Agricultural Practice (GAP) has severally been reported to have had adverse effect on the ecology (including a disruption and a shift in the microbiota) of the place where they were applied. The effect of the organophosphate pesticide, dimethoate, on the growth of some soil-inhabiting fungi was evaluated under laboratory condition. Fungi were isolated from the soil of the experimental site using the spread plate method. The identified fungal isolates were cultured on Potato Dextrose Agar and exposed to dimethoate using the fume growth test. The same set of fungi were also cultured without dimethoate fume as a control. The radial growth rate of individual fungus was measured every two days for eight days using a ruler. Results showed that some of the fungi such as *Aspergillus. aculeatus* strain MR10, *A. aculeatinus* isolate LrBF25, *A. aculeatus* strain SC7e, *A. niger* strain ac, *A. terreus* strain KJ1121 and *A. terreus* isolate AMK973-II demonstrated good growth under the dimethoate fume, while mycelial growth of others such as *Peniccilium daleae* isolate pdy1, *A. foetidus* strain af and *Fomitopsis meliae* voucher JV 1109/40-J were remarkably inhibited by dimethoate fume. These results indicate the ability of dimethoate to inhibit the growth of some fungi while some other fungi could utilize dimethoate for their metabolism and growth, thus are promising agents for dimethoate degradation and remediation of contaminated environment.

INTRODUCTION

Microorganisms are ubiquitous in the environment, fungi and bacteria being the most abundant (Yassin and Almouqatea, 2010), a sizeable proportion living either as parasites or saprophytes in the soil. These soil microbial communities are affected by many factors, including pesticide use (Haleem *et al.*, 2013). Pesticides are chemicals used in agriculture to protect crops and livestock against insects, fungi, weeds and other pests which could reduce farm productivity. It is also used to protect public health by controlling the vectors of tropical diseases like mosquitoes. Pesticides can prevent, destroy, repel or mitigate any pest (Cassida, 2009). Although pesticides have numerous beneficial effects, they are toxic by design, even to beneficial soil biota.

Organophosphorus (OPs) compounds constitute a heterogeneous category of chemicals that are used as pesticides, herbicides, and have been used in the past for the production of chemical gases during the World War (Bowls *et al.*, 2003). Individual organophosphate pesticides vary widely in acute toxicity but collectively, they are among the most acutely toxic chemicals used in agriculture. Dimethoate is a non-systemic, broad-spectrum organophosphate insecticide which affects soil microbial diversity (Haleem *et al.*, 2013). The aim of this research was to identify fungi that can potentially be used as a cleanup agent against dimethoate persistence and or pollution, while the specific objective was to evaluate the effect of dimethoate on the growth of certain fungi isolated from the soil.

MATERIALS AND METHODS

Collection of Soil Samples for Fungal Isolation and Culture

Soil samples were obtained from different parts of the $5m^2$ experimental site located behind the Screen House of the Department of Botany and Ecological Studies ($5^{o}02'26.4"N$, $7^{o}58'45.5"E$), University of Uyo Main Campus under aseptic conditions. Serial dilutions were carried out for

fungal isolation. The spread plate technique (ref) was employed using Potato Dextrose Agar (PDA) as the analytical medium. The PDA plates were inoculated with the desired diluents and incubated at room temperature until the sporulation of fungal colonies occurred (Okungbowa and Oviasogie, 2010; Sanyaolu and Kolawole-Joseph, 2015). Using freshly prepared PDA, the test fungi were repeatedly subculture to obtain pure or axenic cultures of each of the different fungi used for the assay. All fungi were incubated at room temperature (29^0C – 31^0C) for 8 days before the assay.

Fume Test of Isolated Fungi and Determination of Radial Growth Rate

Adopting a modified method of Amund *et al*. (1987), as described by Sanyaolu *et al.* (2018), isolated strains were tested on the basis of their tolerance todimethoate. A fume test was carried out to determine which strains had a favourable growth in the presence of dimethoate. Filter papers immersed in dimethoate were allowed to drain for approximately 5 mins and were thereafter placed inside the cover of the petri-dishes during the incubation of the fungi for each of the dilution strenght. As a control, the same fungal cultures were grown on the PDA medium without the dimethoate.

The effect of the dimethoate on the radial growth rate of the isolates tested was estimated by measuring the radius of the mycelia extension (in centimeters) using a ruler. Observations of colony growth were made every two days until the eighth day, and compared with the control. The experiment was performed in three replications.

Identification of Test Fungi

Identification of the fungi was done using both morphological techniques as described by Deacon (1980) and molecular techniques 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.

RESULTS

The fungal strains recovered from the field at the initial stage of isolation included *Aspergillus aculeatus* strain MR10, *Aspergillus aculeatinus* isolate LrBF25, *Aspergillus aculeatus* strain SC7e, *Penicillium daleae* isolate pdy1, *Aspergillus foetidus* strain af, *Aspergillus niger* strain ac, *Fomitopsis meliae* voucher JV 1109/40-J, *Aspergillus terreus* strain KJ1121, and *Aspergillus terreus* isolate AMK973-II.

There were considerable variations among these fungi in their response to the fume test. The underlisted strains and species from the Aspergilli genera namely *Aspergillus aculeatus* strain MR10, *A. aculeatinus* isolate LrBF25, *A. aculeatus* strain SC7e, *A. niger* strain ac, *A. terreus* strain KJ1121 and *A. terreus* isolate AMK973-II) showed more aggressive mycelial growth in the presence of dimethoate, while the other species and strains such as *Penicillium daleae* isolate pdy1, *A. foetidus* strain af and *Fomitopsis meliae* voucher JV 1109/40-J showed moderate or inhibited growth (Figure 1). *A. aculeatus* strain MR10 (3.40 cm), *A. aculeatinus* isolate LrBF25 (3.67 cm), *A. aculeatus* strain SC7e (4.23 cm), *A. niger* strain ac (4.00 cm), *A. terreus* strain KJ1121 (3.60 cm) and *A. terreus* isolate AMK973-II (3.83 cm) exhibited remarkable growth growth after eight days of culturing, *A. aculeatus* strain SC7e (4.23 cm) demonstrated the best growth. *P. daleae* isolate pdy1 and *A. foetidus* strain af showed moderate growth after eight days (2.93 and 3.07 cm respectively). *F. meliae* recorded the poorest growth after day eight (0.43 cm), significantly lower than the value of the control (1.37 cm).

Apart from *A. aculeatus* strain SC7e, a general trend was observed in the growth of the fungi under fume. At eight days, the fungal colonies reached only 50 – 60% of the diameter of the control. However, the dimethoate seemed to stimulate the growth of *A. aculeatus* strain SC7e, as it was observed to show even more vigorous growth under fume than in the control (Figures.1 and 2). It was also observed that sporulation seemed to be less vigorous in the fungal

strains exposed to dimethoate, and the exposed isolates exhibited slow growth and reduced vigour during subsequent subculturing.

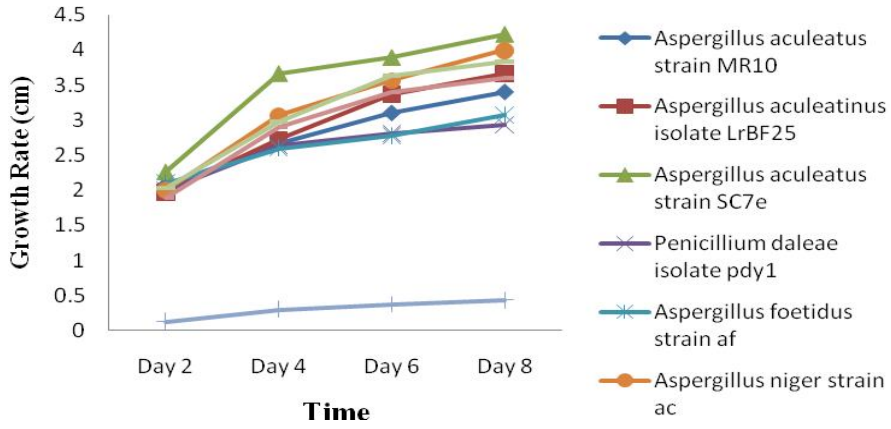

Figure 1: Radial growth of fungi under dimethoate fume after eight days of incubation.

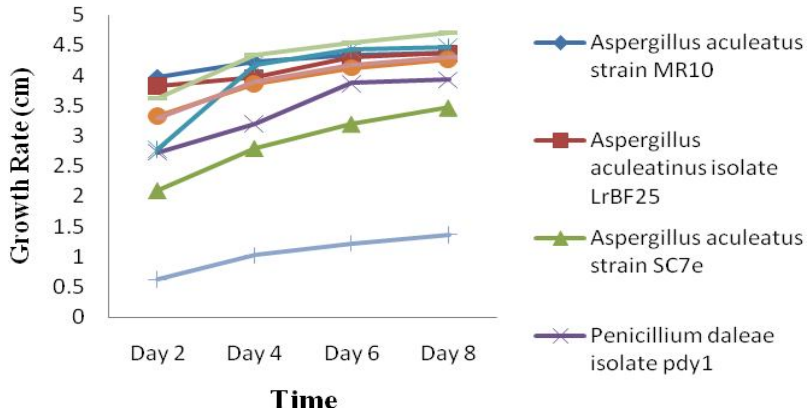

Figure 2: Radial growth of fungi not exposed to dimethoate (control) after eight days of incubation

DISCUSSION

Various studies have indicated that different fungal species, or different strains of the same species react differently to the same pesticide (Tkaczuk *et al.*, 2012). The results of this study have shown differences among the fungi in their ability to tolerate the pesticide dimethoate. For instance, *A. aculeatus* demonstrated favourable growth when exposed to dimethoate fume , while the growth of *A. foetidus* was slightly inhibited. Similar results about the impact of a pesticide on four species of *Hirsutella* were reported by Tkaczuk and Mietkiewski (2005). The ability of some of the fungal strains to effectively grow on exposure to dimethoate fume indicates their ability to metabolize dimethoate and utilize it as secondary nutrients for their growth (Christian *et al.*, 2005; Hassan, 1999). This could also explain why *A. aculeatus* thrived even better under dimethoate fume than in the control.. For a few of the fungi, such as *F. meliae*, *P. daleae* and *A. foetidus*, the dimethoate had a negative effect on their growth. The mycelial growth of *Penicillium daleae* isolate pdy1, *A. foetidus* strain af and *Fomitopsis meliae* voucher JV 1109/40-J were markedly inhibited in the presence of dimethoate (and possibly other organophosphates - OPs)

Also, the reduced and delayed sporulation of some of the fungal strains during subsequent subculturing indicates a negative impact of dimethoate on the physiology of the affected fungal

strains. This implies that some pesticides can reduce growth and sporulation in certain fungi and is in accordance with the work done by Tkaczuk *et al.* (2015). Tkaczuk *et al.* (2015) reported that the pesticides chizalofop-P-ethyl, glyphosate and lambda-cyhalothrin inhibited the growth of the fungi *Hirsutella nodulosa* and *Beauveria bassiana.* The results posits *Aspergillus aculeatus* strain MR10, *A. aculeatinus* isolate LrBF25, *A. aculeatus* strain SC7e, *A. niger* strain ac, *A. terreus* strain KJ1121 and *A. terreus* isolate AMK973-II) as good candidates for dimethoate (and possibly other OPs) degradation and cleanup in the soil.

CONCLUSION

The number of fungal strains isolated from the study site indicates the vast diversity of the fungal community that exist in any given environment. The fume test has revealed that some of the fungal strains encountered in this study could be excellent candidates for dimethoate degradation/cleanup. The active ingredients in OP pesticides might be capable of precipitating an extensive alteration in the molecular and physiological characteristics of fungi.

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