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Effect of the chlorinated hydrocarbon herbicide, paraquat dichloride, on the growth properties and diversity of soil fungi

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Abstract

This study explored acute herbicide-induced changes in soil fungal biomass, relative abundance, diversity and hyphal extension in response to five different application concentrations (1.625mg/kg – 25.00mg/kg) of paraquat dichloride using the media dilution technique. There was a general decline in all the parameters investigated with increasing concentration and contact time. Of all the isolates obtained, *Penicillium* and *Fusarium* were the most frequently occurring amongst the filamentous fungi while *Saccharomyces* was the more frequently occurring yeast. Investigation of soil mycelial biomass revealed that the lowest dry weight was attained at the highest application rate of 25mg/kg representing a 61.71% reduction in mycelial biomass compared to the control. Based on the tolerance indices, the three isolates studied could be ranked in increasing order of tolerance as *Mucor* < *Fusarium* < *Penicillium*. *Mucor* showed no linear extension at concentrations of 3.25mg/kg and above while *Penicillium* exhibited linear extension even at 25mg/kg. At paraquat dichloride concentrations of 6.50mg/kg and over, growth inhibition levels for total soil fungi were over 50%; for *Mucor*, it was 100% at concentrations over 3.25mg/kg. *Fusarium* only exhibited 100% growth inhibition at the highest application concentration of 25mg/kg. *Penicillium* and total soil fungi were inhibited by 94% and 75% respectively at 25mg/kg. Statistically, soil fungal abundance and linear extension at herbicide concentrations of 3.25mg/kg and over were significantly different (at 95% confidence interval) from those obtained in the control experiments. It was concluded that the soil fungal community structure was radically altered by acute exposure to the herbicide, paraquat dichloride.

Keywords: Herbicide; Fungi; *Fusarium*; *Mucor*; Paraquat dichloride; *Penicillium*

Introduction

Pesticide residues in the environment are considered a hazard and public safety challenge. Environmental pollution, in the case of pesticides, is often precipitated by excessive and sustained use of chemical pesticides, frequently resulting in ecological and human health impacts that demand intervention measures (Mnif *et al.* 2011; Degrendele *et al.* 2022). It has been asserted that the impact of pesticide pollution transcends all environmental media impinging on the quality of ground water, inland and coastal waters, soils and the atmosphere (Balmer *et al.* 2019; Silva *et al.* 2019). Some pesticide groups are known to accumulate in produce leading to pesticide poisoning (Tayade *et al.* 2013) while others have been associated with a decline in biodiversity in soil and water ecosystems and the interruption of ecosystem services (Brühl & Zaller, 2019). Pesticides are known to impact on the function and abundance of bacteria, fungi and actinomycetes in soil ecosystems. Findings from a study by Doolotkeldieva *et al.* (2018) revealed that at elevated concentrations, pesticides impede vital cellular function in microbes and precipitate a decline in microbial biodiversity. Similar conclusions were reached in another study that

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reported a drop in the abundance and diversity of soil fungi in the presence of commonly used pesticides (El Ghany *et al*. 2015).

Paraquat dichloride (N,N-dimethyl-4,4-bipyridinium dichloride) is an organochlorine herbicide used regularly by farmers in developing countries although it is currently banned in countries across Europe and the Americas (Nesheim *et al*. 2005; Bang *et al*. 2015). It has the chemical formula $C_{12}H_{14}Cl_2N_2$ and is also known as methyl viologen. Like most synthetic organochlorine compounds, it is strongly toxic to organisms across trophic levels. It is a potent inhibitor of photosynthesis in green plants. It functions by blocking oxidised nicotinamide adenine dinucleotide phosphate ($NADP^+$) reduction within the chloroplast which leads to cell death (Reczek *et al*. 2017). The herbicide has been associated with both bioaccumulation and biomagnification and its residues and degradation by-products will often remain in the ecosystem for several years (Huang *et al*. 2019). Consumption of pesticide-tainted food crops by higher animals and man may precipitate cancers, immune system suppression, organ failure and reproductory deficiencies (Kuo *et al*. 2012; Frimpong *et al*. 2018; Shadnia *et al*. 2018). While some researchers consider paraquat dichloride relatively biodegradable, several other studies deem it less so; having established its half-life in soil as 3 – 6.6 years and its minimum lethal dose as 35 mg/kg in humans (Pateiro-Moure *et al*. 2009; Tsai, 2013; Huang *et al*. 2019; Tang and Maggi, 2021).

The activities of soil fungi are pivotal to soil quality, structure, fertility and nutrient levels. Additionally, fungi are important drivers of the decomposition process in soil and aid nutrient uptake by plants (Fraç *et al*. 2018). With current annual global pesticide use placed at around 4.1 million tonnes and further increases in annual usage projected (FAO 2021), the need to fully understand the extent of the problem including a clear understanding of possible acute toxicity effects on ecosystem players has arisen. This study explored the modifications to soil fungal community structure by investigating changes in fungal biomass, relative abundance and diversity in response to the chlorinated hydrocarbon herbicide, paraquat dichloride. It further investigated the impact of the pesticide on linear extension of fungal hyphae in selected soil fungal isolates.

Materials and methods

Sample collection

the soil used in the laboratory microcosms were collected from the University of Port Harcourt agricultural farm from plots that had not previously been subjected to pesticide treatment. Soil cores were extracted at different points within the selected plots from the surface to a depth of about 15 cm in different parts of each plot and then mixed thoroughly to form a large composite sample.

Experimental design for soil-based study

Before use, debris and plant material were removed from the soil then it was sieved using a 4 mm mesh sieve. The microcosms each contained 1000 g of sample soil. Soil moisture content was maintained at 60 % water holding capacity.

Paraquat dichloride was applied to the soil in replicated microcosms at five different application concentrations of 25.0 mg/kg, 12.5 mg/kg, 6.5 mg/ kg, 3.25 mg/kg and 1.625 mg/kg. Sampling was carried out at 24h intervals for 5 days. The control study consisted of the set up without any herbicide applied. The parameters monitored were fungal diversity and fungal abundance as total fungal counts.

Isolation and characterisation of soil fungi

About 1 g of soil sample was suspended in 9 ml of sterile normal saline and shaken thoroughly prior to serial dilution. Approximately 0.1 ml aliquots from suitable dilutions were then plated out on potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) amended with lactic acid to inhibit bacterial growth. The spread plate technique was employed. The inoculated petri plates were incubated at room temperature for 72 h. Discrete colonies were sub-cultured unto fresh medium in order to obtain pure isolates. The plates were used to determine fungal abundance.

The isolates obtained were first characterised via their macroscopic characteristics and then observed under a microscope using lactophenol blue stain. Identification of the fungal isolates was done as described by Dobranik & Zac (1999) and Cheesborough (2006). Sugar fermentation and oxidation–fermentation tests were further used to differentiate between the yeast isolates.

Determination of fungal abundance

The total fungal counts in the herbicide-treated soil samples were determined from the incubated media Petri plates (as described above). Only plates with colony counts between 30 and 300 were considered. Counts were expressed as colony forming units per gram of soil.

Effect of paraquat dichloride on mycelial biomass

Media containing paraquat dichloride at the five different test concentrations were prepared in three-fold replicates in Erlenmeyer flasks. About 10 g of

soil sample was introduced into 100 ml of herbicide-amended acidified potato dextrose broth (PDB). The flasks were plugged with non-absorbent cotton wool and incubated at room temperature for 5 days with agitation at 200 rpm. The fungal mycelia were harvested by filtration 5 days after seeding using Whatmann No. 1 filter paper of known weight. The harvested mycelia on the filter paper was washed with distilled water then dried in an oven at 50 °C – 60 °C. The mycelial dry weight was determined after drying. Broth with no herbicide amendment served as the control.

Effect of paraquat dichloride on hyphal linear extension

The agar dilution method was employed in the determination of the effect of the herbicide on hyphal extension in the isolates identified as *Penicillium, Mucor* and *Fusarium* spp. These were selected because they were able to grow at the higher concentrations of the herbicide. Approximately 6 mm diameter discs from 72 h old pure culture of the test fungal strains were individually introduced into a 6 mm well at the centre of individual 100 mm Petri plates containing acidified PDA with paraquat dichloride incorporated at the five different concentrations of 1.65 mg/kg – 25 mg/kg. The set-ups were replicated and the control study consisted of unamended PDA with the test isolate disc inoculated at the centre. The plates were incubated at room temperature and the hyphal extension was measured at 24 h intervals using callipers and a ruler. Multiple measurements were taken at right angles to each other across the Petri plates.

Determination of day five tolerance indices

two factors were considered for estimation of tolerance indices – the total fungal counts and the hyphal linear extension. With regards to fungal abundance, the tolerance indices at the different concentrations were determined using Equation (1). For the 3 selected fungal isolates, the tolerance indices were ascertained based on the hyphal extension of the selected fungal isolates on day 5 using Equation (2) outlined below.

$$TI_5 = \frac{\text{Total fungal count in herbicide-incorporated medium}}{\text{Total fungal count in Control}} \quad (1)$$

$$TI_5 = \frac{\text{Linear extension of test fungus in herbicide-incorporated medium}}{\text{Linear extension of test fungus in Control}} \quad (2)$$

Where: TI_5 – Tolerance Index on Day 5

Determination of growth inhibition levels

the level of herbicide-induced growth inhibition of the soil fungi at the end of the 5-day study was determined firstly based on fungal abundance and secondly, based on the impact of paraquat dichloride on hyphal extension on *Penicillium*, *Mucor* and *Fusarium* spp. using Equations (3) and (4) respectively.

$$\text{Inhibition (\%)} = \frac{C(\text{control}) - C(\text{sample})}{C(\text{control})} \times 100 \quad (3)$$

Where: C(control) – Total fungal count in Control on Day 5; C(sample) – Total fungal count in test soil sample on Day 5

$$\text{Inhibition (\%)} = \frac{LEc - LEi}{LEc} \times 100 \quad (4)$$

Where: LEc – Hyphal linear extension in Control on Day5; LEi – Hyphal linear extension in test isolate on Day5

Data analyses

testing was done in replicates and the data expressed as mean values relative to standard deviation. One and two factor analysis of variance was used to define the relationships between fungal growth properties and paraquat dichloride concentration at 95 % confidence interval within groups and between groups. The data were analysed using Microsoft Excel® 2016 and SPSS® 23.0.

Results

Generally, the untreated soil had higher fungal diversity (Figure 1) and abundance (Figure 2) than the treated soils and both diversity and abundance were seen to decline with rising herbicide concentration and, for abundance, increasing contact time after the acclimatisation period. There were marked variations in soil fungal diversity and abundance in the herbicide-treated soils compared to the untreated controls (Figures 1 and 2). Of all the isolates obtained, *Penicillium* and *Fusarium* were the most frequently occurring amongst the filamentous fungi while *Saccharomyces* was the more frequently occurring yeast. The only other yeast isolate obtained was *Candida*. *Aspergillus niger*, *Aspergillus flavus*, *Saccharomyces* and *Candida* spp. did not occur at herbicide concentrations of 3.25 mg/kg and above. Only *Penicillium* sp. was able to growth at the herbicide concentration of 25 mg/kg while both *Penicillium* and *Fusarium* were the sole isolates at 12.5 mg/kg.

At higher herbicide concentrations of 12.5 mg/kg and 25 mg/kg, a lag in total fungal counts (TFC) was observed till around days 2 – 3. For the lowest applied herbicide concentration, TFC matched control counts by day 3 of the study and even exceeded it slightly by the end of the study (Day 5). At 3.25 mg/kg, there was an initial steady decline in TFC followed by a gradual increase in abundance. This initial decline was not seen at other applied herbicide concentrations. As presented in Figure 3, soil mycelial biomass did not vary distinctly from the control at 1.625 mg/kg herbicide concentration. Mycelial biomass diminished with

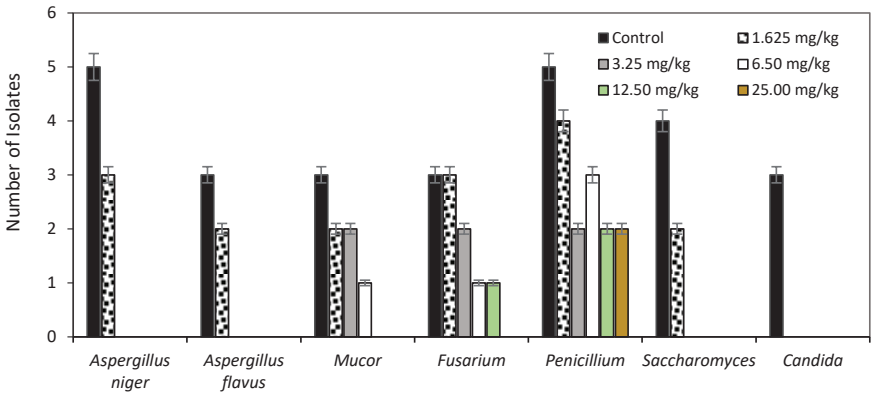

Figure 1. Diversity of soil fungi at different herbicide concentrations. Bars represent the standard error.

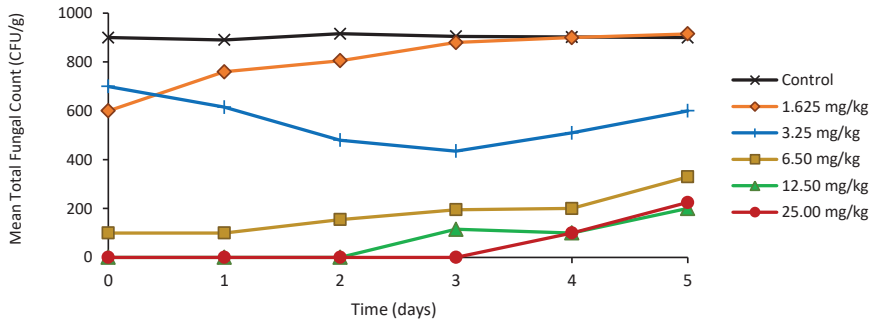

Figure 2. Variation in the abundance of soil fungi at different herbicide concentrations during the study

increasing concentrations. The lowest biomass weight was attained at the highest application rate of 25 mg/kg. This represented a 61.71 % reduction in mycelial biomass on Day 5 of the study compared to the Control. Mycelial biomass dropped by 0.90 %, 18.0 %, 29.2 % and 54.8 % at 1.625 mg/kg, 3.25 mg/kg, 6.50 mg/kg and 12.50 mg/kg herbicide concentration respectively by the end of the study.

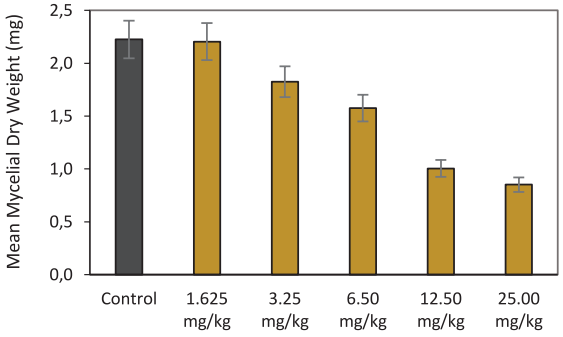

Figure 3. Soil mycelial biomass at varying concentrations of paraquat dichloride by the end of the study. Bars represent the standard deviation from the mean.

The effect of the different concentrations of paraquat dichloride on hyphal linear extension of *Fusarium*, *Mucor* and *Penicillium* spp. and the herbicide tolerance indices of the soil fungi are outlined in Tables 1 and 2 respectively while the observed growth inhibition levels at the end of the study are depicted in Figure 4. Of the three isolates, studied, *Mucor* showed no linear extension at concentrations of 3.25 mg/kg and above while *Penicillium* exhibited linear extension even at 25 mg/kg, albeit limited. The growth properties of *Penicillium* sp. was relatively unaffected at herbicide concentration of 1.625 mg/kg but showed a lull in linear extension on Day 1. Even where the linear extension of the isolates was not fully inhibited based on Day 5 results, the extension rates appeared reduced with all three isolates showing no visible linear extension on Day 1 in contrast to observations in the control studies.

Based on the tolerance indices (Table 2), the isolates could be ranked in decreasing order of tolerance as *Penicillium* > *Fusarium* > *Mucor*. This trend in response to different concentrations of the herbicide buttresses the results obtained for growth and diversity (Figure 1) and linear extension (Table 1).

Tolerance indices with regards to the total fungal counts revealed very high tolerance (≥ 1.00) at 1.625 mg/kg, moderate tolerance (0.60 – 0.79) at 3.25 mg/kg and low (0.40 – 0.59) to very low tolerance (0.00 – 0.39) at subsequent higher herbicide concentrations. *Mucor* sp. was only able to tolerate the herbicide at the minimum con-

Table 1. Effect of varying concentrations of paraquat dichloride on fungal hyphal linear extension

	Herbicide Concentration (mg/kg)	Mean Hyphal Linear Extension (cm)				
***Fusarium* sp.**		**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
	0.00 (Control)	0.3	0.9	2.5	3.2	4.5
	1.625	–	0.6	1.6	1.9	2.6
	3.25	–	0.5	1.5	1.8	2.3
	6.50	–	0.4	0.9	1.6	1.6
	12.50	–	–	–	0.4	0.9
	25.00	–	–	–	–	–
***Mucor* sp.**		**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
	0.00 (Control)	–	3.7	4.3	4.82	**
	1.625	–	0.3	0.5	0.9	1.3
	3.25	–	–	–	–	–
	6.50	–	–	–	–	–
	12.50	–	–	–	–	–
	25.00	–	–	–	–	–
***Penicillium* sp.**		**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
	0.00 (Control)	0.8	2.8	4.0	5.0	**
	1.625	–	2.5	3.8	4.3	**
	3.25	–	1.3	1.5	1.7	2.0
	6.50	–	1.1	1.3	1.5	1.8
	12.50	–	0.9	1.2	1.4	1.5
	25.00	–	–	–	–	0.3

**: 5 cm Limit of Petri plate radius reached
– : No linear extension observed

centration of 1.625 mg/kg. The isolate exhibited very low tolerance (≤ 0.39) at all concentrations of paraquat dichloride. Even though *Penicillium* sp. was the most tolerant of the test isolates (based on its ability to grow at higher herbicide concentrations) and demonstrated very high tolerance (≥ 1.00) at 1.625 mg/kg herbicide concentration, the isolate displayed low to very low tolerance to higher concentrations of paraquat dichloride. For *Fusarium* sp., the tolerance indices obtained implied low tolerance (0.40 – 0.59) at 1.625 mg/kg and 3.25 mg/kg and very low tolerance (≤0.39) at higher concentrations. *Mucor* sp. showed the lowest tolerance overall compared to other fungal isolates and total soil fungi.

The growth of total soil fungi and *Penicillium* sp. were not impeded at the minimum herbicide concentration of 1.625 mg/kg. At concentrations of 6.5 mg/kg and over, growth inhibition levels for total soil fungi were over 50 % while *Mucor* demonstrated growth inhibition levels of 100 % at 3.25 mg/kg and above. At the highest concentration of 25 mg/kg, growth of *Fusarium* and *Mucor* were inhibited by 100 % while *Penicillium* and total soil fungi were inhibited by 94 % and 75 % respectively.

Statistically, clear trends were exhibited across the groups. Soil fungal counts at concentrations of 3.25 mg/kg and over were significantly different from the counts obtained in the control and from counts obtained at 1.625 mg/kg but were not significantly different from each other. The same trend was seen with regards to mean mycelial dry weight. Likewise hyphal linear extension for the three test isolates differed significantly from each other (*Penicillium* differed significantly from *Fusarium* and *Mucor* and so on) and from their individual control studies. Analyses within groups, however, showed that for *Penicillium* and *Fusarium*, the observed linear extension did not

Table 2. Herbicide tolerance indices of soil fungi

Herbicide Concentration (mg/kg)	*Fusarium* sp.	*Mucor* sp.	*Penicillium* sp.	Soil Fungi*
1.625	0.58	0.27	1.00	1.02
3.25	0.51	0.00	0.40	0.67
6.50	0.33	0.00	0.36	0.37
12.50	0.20	0.00	0.30	0.22
25.00	0.00	0.00	0.06	0.25

* Based on mean total fungal counts

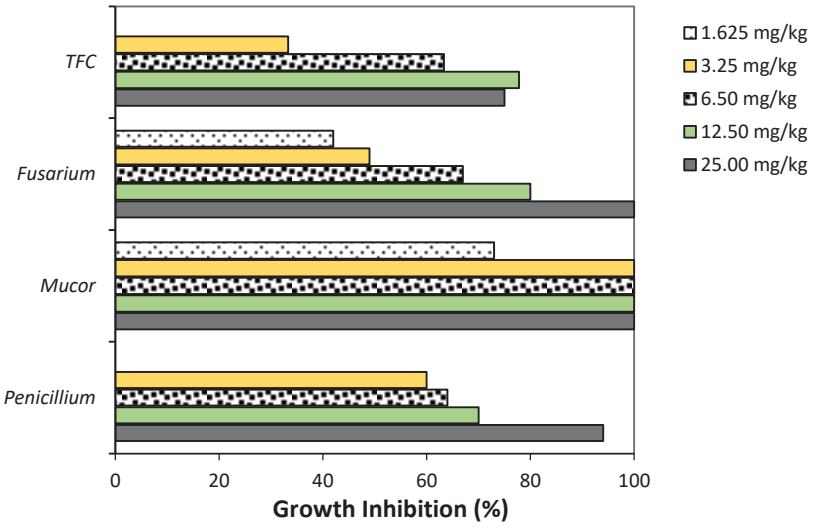

Fig. 4. Growth inhibition levels of paraquat dichloride on soil fungi at the end of the study. TFC – Total fungal count

differ significantly from one concentration level to the other at 95 % confidence interval.

Discussion

The concentration of paraquat dichloride and exposure time were shown to impact on all the parameters investigated – soil fungal diversity and abundance, hyphal linear extension and mycelial biomass. These findings are in tandem with similar studies. Organochlorine pesticides are well-known to bioaccumulate across the trophic levels and diminish fungal populations in soil systems (El-Ghany & Masmali, 2016; Jayaraj *et al*. 2016). A previous study by Vazquez *et al*. (2021) highlighted an abrupt decline in the diversity and abundance of cultivable fungi during a 5-day study with the pesticide, glyphosate. Their study further confirmed the drop in fungal biomass with increasing pesticide concentration. These alterations in soil fungal diversity and counts with the application of pesticides is further confirmed by Streletskii *et al*. (2022). A concentration of 1 µl/100 ml paraquat dichloride produced reduced abundance in *Aspergillus niger* with a 34 mm zone of inhibition in agar diffusion studies (Geetha *et al*. 2016) while the insecticide, endosulfan at 100 g/L hampered the growth of *Aspergillus terrus* and *Clasdosporium oxysporum* (Mukherjee & Mittal, 2005).

Bending *et al*. (2002) attributed the loss of soil diversity and abundance, following exposure to pesticides, to cellular lysis. They maintain that acute exposure to herbicides may often cause disruptions to cell membrane transport and other similar systems leading to cell death. Surviving species will often be those with the genetic and enzymatic predisposition to resist the effects of the herbicide. These species will typically proliferate rapidly increasing their overall biomass in order to limit any competition and to enhance the likelihood of survival (Rongchapo *et al*. 2016). For several fungi, the initial decline in biomass and population counts eventually gives way to degradation of the herbicide due to enhanced enzymatic activity (Mougin *et al*. 2002). The results from the present study revealed an overall decline in all the parameters as herbicide concentrations increased. The significance of herbicide concentration was underscored in a study by Hata *et al*. (1986) where the yeast, *Lipomyces* sp. was able to totally eliminate (100 % degradation) paraquat dichloride from tainted broth at 27 ppm herbicide concentration, however, when the concentration was doubled to 54 ppm, degradation levels dropped below 10 %. Reports from Fang *et al*. (2008), for *Verticillium* sp. on the organophosphate insecticide, chlorpyrifos at varying concentrations also made similar deductions.

Fungi are typically reputed for their adaptation proficiency skills in polluted and extreme environments. The delayed growth seen with both the total fungal counts and the hyphal linear extension in the fungal isolates in the presence of the herbicide is likely indicative of a period of adaptation during which the fungus acclimatises to the environmental change. This period allows the organism to harness its genetic capacity leading to modified gene expression and protein activity which, in turn, allows the organism to continue normal metabolic functions at cellular level. In some cases, metabolic functions are exacerbated (Gao *et al*. 2011; Selbmann *et al*. 2013). In congruence with the effect of herbicides and other pesticides on the soil fungal abundance, mycelial biomass has also been found to decline on acute exposure to these chemicals. Mycelial dry weight in *Trichoderma harzianum* reduced by 91.09% – 92.25 % in response to the insecticide,

profenofos whereas acute exposure to the herbicide, Fluazifop-P-Butyl led to reductions in mycelial dry weight of between 5.81 % and 43.02% (Ali & Ramadan, 2019).

From the tolerance indices and inhibition levels obtained, it can be concluded that soil fungi in the present study had a somewhat low tolerance threshold for acute exposure to paraquat dichloride. The soil fungi further demonstrated decreasing tolerance with increasing herbicide concentrations. This variation in tolerance levels may be a function of dissimilarities in fungal cell membrane characteristics and adhesion properties. The levels of fungal growth inhibition obtained in the current study corresponds with those reported in a 7-week study by Al-Ani *et al*. (2019) that confirmed a reduction in the activity and counts of soil fungi by up to 65 % (for counts) following the application of varying concentrations of the pesticides, glyphosate, malathion and alphacypermethrin. Comparable to the current study, the observed decline was seen to be greater with increasing pesticide concentration and contact time. These findings are, however, somewhat in contrast to those of Sahid *et al*. (1992) who reported that at 250 mg/kg, paraquat dichloride elicited relatively low inhibition of 29 % in soil fungal counts. Toxicant exposure time alongside concentration has been affirmed as a key driver of toxicity (Connell *et al*. 2016).

The low tolerance of *Mucor*, *Fusarium* and *Penicillium* spp. is unexpected as these fungal genera have been associated with the biodegradation of different pesticide groups. Andy *et al*. (2015) reported that *Penicillium* and *Mucor* alongside *Aspergillus niger* and *Rhizopus*, effectively degraded paraquat dichloride *in vitro*. Akin to the current study, however, cell mass yield gradually dwindled as paraquat concentrations climbed. As with the results obtained in current study as well, *Penicillium* sp. proved more resilient than other fungal groups at higher paraquat concentrations. Several studies also confirm the degradation of commonly used hydrocarbon pesticides by *Fusarium* spp. (Guillen-Jimenez *et al*. 2012; Shi *et al*. 2018; Bhatt *et al*. 2020). Noting the biodegradation reports from other studies, it would have be expected that the fungal isolates would express some level of resistance to the herbicide. This was not the case in the present study. This would suggest that toxicity may not necessarily denote the absence of biodegradation capacity in affected microorganisms.

The inhibitory effects of paraquat dichloride in the current study were seen at concentrations slightly lower than the range obtainable in the field. The recommended field application rate (RFAR) for paraquat dichloride is 2 – 5 kg/ha which corresponds to about 20 – 50 mg/kg. The results from the current study are therefore worrisome considering that the paraquat dichloride concentrations used in this study lie at the lower limits of these RFAR values and that soil fungal counts dropped by about 75 % at herbicide levels of 25 mg/kg. It should, nevertheless, be noted that the conclusions of the current study may not necessarily be indicative of the effect that paraquat dichloride would produce in the field. While it has been established that pesticides inhibit fungal populations both *in vitro* and in field studies, growth inhibition tends to be more significant in laboratory investigations than in field applications where factors like weather conditions come into play (Rakesh Sharma *et al*. 2011). Furthermore, some studies indicate that pesticide exposure will normally alter soil fungal community drastically in the short term (acute) but not the long term (Singh *et al*. 2020; Vazquez *et al*. 2021). This does not warrant or validate the indiscriminate use of pesticides in the environment as while recovery in the long term has been asserted, the findings from the current study infer that continued and repeated pesticide application even at recommended field application rates could lead to the emergence of an altered equilibrium state with the loss of crucial biological components of the soil ecosystem and the impairment of fungal community structure.

Conclusion

This study revealed that acute exposure to paraquat dichloride had an inhibitory impact on soil fungal diversity and growth properties. The reduced diversity and inhibition of growth in soil fungi by paraquat dichloride was evidenced by diminished numbers of fungal species detected, and reduced fungal abundance, mycelial biomass and hyphal linear extension when comparisons were made to the control studies. The findings of this study raise concerns for the inordinate and unregulated use of paraquat dichloride in soil. It is, therefore, advocated that the herbicide should only be employed, as required, at the lowest recommended concentrations over limited timeframes.

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Conflicts of Interest

There are no known conflicts of interest associated with this study.

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