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Potential Impact of Cypermethrin on Selected Groups of Soil Microorganisms

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ABSTRACT

Application of cypermethrin as insecticide during agricultural practices is a global concern due to its toxicity and potential accumulation of its metabolites in the aquatic environment. A mesocosm plot of agricultural land (500 m^2) was demarcated and deliberately contaminated with cypermethrin to study its impact on the autochthonous microorganisms. Potential effects of 3-phenoxybenzoic acid (3-PBA), a known suicide metabolite of cypermethrin, on hydrolytic activities were also investigated by using fluorescein diacetate assay (FDA). A significant increase in the abundance of total heterotrophic bacteria from 3.55 x 10^6cfu $g^{-1}$to 1.30 x 10^8cfu g^{-1}in 12 weeks as against the control was induced by the insecticide in the mesocosm. The number of starch hydrolysing bacteria increased after the second insecticide treatment from 6.25 x 10^6cfu $g^{-1}$to 2.70 x 10^7 cfu g^{-1}in three weeks. However, there was no significant increase in the microbial count of fungi, presumptive actinomycetes and nitrogen fixing bacteria during the twelve weeks of experiment. The hydrolytic activity of soil microorganisms was apparently inhibited insoil treated with different concentration of 3-PBAthan in untreated soil. These results suggest that the application of a commercial cypermethrin formulation increased the abundance of selected soil microorganisms while3-PBA reduced the soil microbial hydrolytic activity.

INTRODUCTION

Synthetic pyrethroid insecticides such as permethrin or cypermethrinare structural analogs derived from the natural pyrethrins originally isolated from the genus Chrysanthemun (Casida, 1980). Cypermethrin (α-cyano-3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethylcyclo-propanecarboxylate) is a racemic mixture consisting of eight (conventionally grouped into four*cis* and four*trans*) isomers with the *cis*-isomers showing better insecticidal activity (Miyamoto, 1981; Laskowski, 2002). Cypermethrinis typically used to control cotton ball worm *(Heliocoverpa armigera*) and to eradicate the larvae of mosquitoes and milk fishes during pond preparation in urban and agricultural environments (Collins and Cappello, 2006; Suvetha *et al*., 2010). It is also used to specifically control insect pests which damage cucumber such as *Aphis gossypi, Plutella xylostella*, *Spodoptera litura*, *Pierisrapae*, *Chrysodeoxis chalcites* and *Myzus persicae* (Lenteren, 2000; Zhang *et al*., 2007) as well asinsect ectoparasites and insect vectors carrying diseases (Spielberger*et al*., 1979; Anadón *et al*., 2009).

The insecticidal activity of pyrethroids is mainly due to their action on the sodium channels of the target insects by interfering with the nerve impulse transmission (Fortin *et al*., 2008). These pesticides, when discharged by agricultural run-offs or spray drift and in aquaculture operations, are washed into nearby water bodies and can thereby affect susceptible non-target organisms such as fish and prawn (Adhikari *et al.,* 2004; Suvetha *et al*., 2010).In addition, the pyrethroid insecticides are highly toxic to non-target insects such as honey bees (Oudou and Hansen,2002). Consequently, the pyrethroid cypermethrin has recently been identified as priority water pollutant by the European Union (Vorkamp *et al*., 2014). As cypermethrin inhibits ATPase governed active transport processes which are required to maintain the ion balance of the cell, it isinherently more toxic to aquatic organisms (Suvetha *et al.,* 2010). Studies have shown that cypermethrin possesses carcinogenic and co-carcinogenic potential (Shukla *et al*., 2002) as well as estrogenic activity (Al-Hamdani and Narasinhachary, 2011) and can upon transformation give rise to compounds apparently exhibiting endocrine activities such as3-phenoxybenzoic acid (3-PBA) (Tyler *et al*., 2000) albeit this has recently been disputed (Laffin *et al*., 2010).

The hydrophobic properties of pyrethroids generally cause strong sorption of these compounds to soil particles, which may cause formation of bound residues (Ostiz and Khan, 1994) and renders cypermethrin rather immobile in soil given its elevated log K_{OC} value (Fenoll *et al*., 2011).Formation of bound residues reduces the bioavailability of cypermethrin and has potential long-term effects on soil quality. The environmental fate of pyrethroid pesticides such as cypermethrin under ambient conditions is controlled by abiotic factors such as temperature, redox potential, pH, humidity, organic matter content and light intensity and - probably most importantly - by microbial catabolism (Chapman *et al*., 1981; Miyamoto, 1981; Laskowski, 2002; Meyer *et al*, 2013). Although cypermethrin can be photodegraded upon exposure to light (Takahashi *et al*., 1985), microbes play an essential role in transforming pyrethroid residues in the environment and a large number of cypermethrin-catabolizing isolates have been reported (Rangawasamy and Venkateswarlu, 1992; Sakata *et al*., 1992; Grant *et al*., 2002). The microbial catabolism of cypermethrin is generally initiated by hydrolysis of the ester linkage with 3-phenoxybenzoic acid being considered as a key metabolite (Miyamoto, 1981; Sakata *et al*., 1992) and can be mineralized aerobically by soil bacteria (Wittich *et al*., 1990; Schmidt, 1998) or form residues in soil (Ostiz and Khan, 1994). However, it is apparently toxic for fungi (Stratton and Cork, 1982).Similarly, exposure of human beings to pyrethroid insecticides such as cypermethrin gives rise to the biomarker 3-PBA in plasma and urine (Thiphom *et al*., 2014). However, currently there is a lack of data concerning the impact of cypermethrin-which is widely used in Nigeria (Spielberger *et al*., 1979; Smies *et al*., 1980) - upon microbial abundance and activity in Nigerian soils.

Therefore, the present study was undertaken to investigate whether application of a commercial formulation of cypermethrinat recommended dosage in the field adversely affects the abundance and activities of selected groups of soilmicro organisms. In addition, the potential effect of 3-phenoxybenzoic acid as key metabolite of cypermethrin degradation in soil upon microbial hydrolytic activity was evaluated.

MATERIALS AND METHODS

Chemicals:

A commercial formulation of cypermethrin (EC, cypermethrin 10%, m/m, 90% agricultural emulsifier and solvent) was purchased from an agricultural chemical dealer in Lagos, Nigeria and used in the experimental studies. The insecticide is typically used for crop protection in Nigeria

under different trade names (Table 1). The 3-Phenoxybenzoic acid (3-PBA), fluorescein diacetate (FDA) and fluorescein were obtained from Sigma Aldrich Chemical Co., South Africa. All other chemicals were of the highest quality commercially available.

Table 1: Commercial formulation of cypermethrin used in Nigeria agricultural soils.

Common trade name	Cyperforce, muthin, cypercot and defthin
Crops used	Maize, watermelon, cucumber and green vegetables.
Market sold	Jankara market Lagos, Tejuosho market Lagos and Isolo market Lagos.
Quantity applied (mL ha^{-1}a.i)	400

Mesocosm:

An agricultural garden (06°31′ 06.7″N and 003°21′ 03.9″E)was set up ona 10 x 50 m plot of land in the Botanical garden of the University of Lagos, located in South-West Nigeria. Cucumber crops (*Cucumis sativus*) were planted on both the experimental and control field. The crops were watered and fertilized in accordance to local grower practices. Cypermethrin (i.e. commercial formulation) was applied at the recommended dosage of 400 mL ha^{-1} of cypermethrin (EC, cypermethrin 10%, m/m, 90% agricultural emulsifier and solvent)in 500 L ha^{-1} of tap water for the cucumber crops in the experimental field (EF). The first application was done 14 days after sowing when the crops were in their vegetative stage and subsequent application was repeated fortnightly until the crops were harvested. At the time of the third and fourth insecticide application, crops were in their flowering and reproductive stages respectively. Application of cypermethrin was stopped at week eight prior to harvesting the crops. The second control field (CF) was separated from the experimental field by a distance of 50 m and no insecticide was applied. Tap water was applied to the control field throughout the experimental period. Cow dung slurry (approximately 500 g ha^{-1}) was applied at the time of thinning in both the experimental and control fields to increase crop yield.

Soil sampling:

Soil samples (0-15 cm depth) were taken at random using soil auger (2.5 cm diam.) from 7 to 10 locations in each field for analysis and mixed thoroughly to prepare a composite sample. Three composite samples were prepared likewise from each field for analysis. For estimating microbial counts, samples were collected every week from the fields before subsequent insecticide treatments. Samples were brought to the laboratory and analysed immediately to determine the abundance of different target microorganisms present in the soil.

Soil characteristics:

Soil characteristics were determined using standard methods from the Non Affiliated Soil Analysis Work Committee (1990). Soil moisture content was determined using gravimetric method. The pH of the soil samples was estimated using saturated soil samples (10:25; soil: water). Soil samples were mixed, air dried and sieved through a 2-mm mesh prior to use. The soil dry weight was established by drying of soil samples at 105°C for 48 hours.

Microbiological analysis:

The number of viable soil microorganisms was estimated by the spread plate technique. Solid media were sterilized in an autoclave (120°C for 30 min) and cooled to pouring temperature of about42°C.For each soil sample, total heterotrophic bacteria (THB), nitrogen fixing bacteria (NFB), starch hydrolysing bacteria (SHB), total heterotrophic fungi (THF) and presumptive actinomycetes were determined by plating out aliquots (0.1 ml) after decimal serial dilutions in sterile distilled water on nutrient agar (Merck, Germany), Ashby's mannitol phosphate agar (Oxoid, United Kingdom), starch agar (Oxoid, United Kingdom), potato dextrose agar (Oxoid, United Kingdom) and starch casein agar (Oxoid, United Kingdom) respectively in

triplicate. Starch hydrolysis of individual colonies was verified by flooding plates with Lugol's iodine solution and only colonies showing zones of starch hydrolysis were considered for SHB counts. All plates were incubated at 28^{o}C for 24-48h for THB, NFB, SHB, and 3-5 days for THF and presumptive actinomycetes. Results were calculated as colony-forming units (cfu) per gram soil (dry weight).

Fluorescein diacetate (FDA) hydrolysis:

Soil samples from commercial farmland which received regular cypermethrin treatment for the past ten years and Botanical garden soils which received cypermethrin treatment for three months were used to evaluate the potential effect of 3-phenoxybenzoic acid (3-PBA), on the hydrolytic activity of soil microorganisms.

The FDA assay was carried out as described by Green *et al*., (2008) with some modification. Briefly, different final concentrations of 3-PBA (0, 0.1, 0.5, 1 and 2.5 mM) were established by adding appropriate volumes from a 100 mM 3-PBA stock in 0.1 N NaOH to 250 mL Erlenmeyer flasks containing 1 g of soil sample (dry weight), and fifty millilitres of phosphate buffer (60 mM, pH 7.6). Control flasks contained autoclaved soil samples (121^{o}C for 15 min) to determine abiotic FDA hydrolysis. All flasks including controls were pre-incubated at 30^{o}C on a bench top orbital shaker at 180 rev min^{-1} in the dark. The incubation time ranged from 0 to 36 h. At the end of the pre-incubation, 0.50 ml of FDA substrate stock solution (final concentration of 2 g FDA per l ml of acetone) was added to each Erlenmeyer flask followed by 3 h incubation under the conditions specified above. All treatments were replicated four times. The reactions were stopped by addition of 2 ml of acetone to each flask with swirling. The content of each Erlenmeyer flask was centrifuged at 12000x*g* for 10 min at 4^{o}C. The supernatant was then filtered through a what man No. 2 filter paper. The hydrolytic activity (i.e. amount of fluorescein released per hour per g [dry weight] soil) was determined via UV-VIS spectrophotometry in glass cuvettes at a wavelength of 490 nm. The concentration of fluorescein released was calculated by reference to a standard curve established using authentic fluorescein.

Statistical analyses:

Statistical analyses were performed using SAS statistical software version 9.2 (Statistical Analysis Software Inc. 1990) and Prism version 5.03 (Graph pad Software, USA).Analysis of variance (ANOVA) and Fisher's least significant difference (LSD) were used to test for significant differences at 5% ($p<0.05$).

RESULTS

Soil characteristics:

The general characteristics of the soils studied is shown in Table 2. The soils were typical sandy loam and the pH values is 6.75 and 7.00 for Botanical garden and Ikorodu soils respectively.

Total Heterotrophic Bacteria (THB):

There was a notable increase in viable counts at weekly intervals after cypermethrin application in the experimental field (EF) than non-treated control field (CF) (Fig. 1). The abundance of aerobic heterotrophic bacteria increased in the cypermethrin treated field from initially 3.55 x 10^{6}cfu g^{-1}to 1.30 x 10^{8}cfu g^{-1}(week12).The observed counts for aerobic bacteria in the non-treated control field increased slightly from week 0 (3.60 x 10^{6}cfu g^{-1}) to week 12 (3.25 x 10^{7}cfu g^{-1}). However, the mean THB counts in the EF and the CF differed significantly at $p<0.05$ in weeks 7-12 the reby indicating that this might be due to the utilization of the commercial formulation of cypermethrin as an additional source for carbon and possibly nitrogen by soil heterotrophic bacteria.

Table 2: General physico-chemical properties of the soil used in the experiment

Properties	Ikorodu soil	Botanical garden soil
Sand (%)	75.90	76.75
Silt (%)	21.08	19.85
Clay (%)	1.59	1.28
Texture	Sandy loam	Sandy loam
Coarse sand	22.07	19.94
Medium sand	35.32	25.17
Fine sand	42.61	54.89
Sand grade	Coarse sand	Coarse sand
Moisture content (%)	11.92	6.30
pH (H_2O)	7.00	6.75
pH(KCl)	6.29	5.88
Total organic matter (%)	1.09	2.09
PO_4^{3-}(mg/kg)	20.00	14.40
K^+ (Cmolc/kg)	0.0054	0.0170
Temperature (^oC)	25.56	26.22
Water holding capacity (%)	80	70

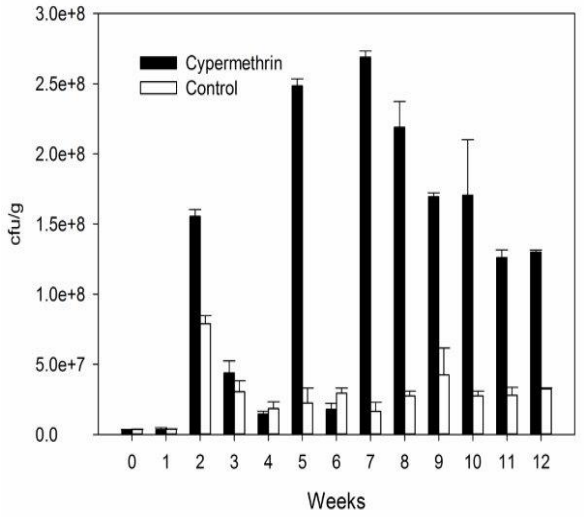

Fig. 1: Abundance of total heterotrophic bacteria (THB) in cypermethrin treated experimental (EF) and control field (CF) soil. Data points represent the mean of three replicates. Error bars represent the standard deviation.

Total heterotrophic fungi (THF):

The abundance of fungi increased somewhat in the EF ($2.30 \times 10^6$to 3.20×10^7cfu g^{-1}) between weeks 0 and 2 after cypermethrin application while the viable counts for the CF declined (Fig. 2). By the end of the experiment, the viable count for fungi decreased to 3.50×10^4cfu $g^{-1}$and 6.50×10^4 cfu g^{-1}in both the EF and CF respectively. The differences observed in the fungal count at week 12 in both the EF and CF did not differ significant at $p<0.05$.

Starch Hydrolysing Bacteria (SHB):

The number of these hydrolytically active bacteria increased after the second insecticide treatment in the EF from 6.25×10^6cfu g^{-1}(week 0)to 2.70×10^7 cfu g^{-1} (week 3)while the microbial count for the CF changed from 6.40×10^6 cfu g^{-1} (week 0) to 8.00×10^6 cfu g^{-1} (week 3) (Fig. 3). The difference in the SHB count between the two fields at the end of the experimental period differed significantly suggesting that cypermethrin application impacted on the growth and survival of SHB.

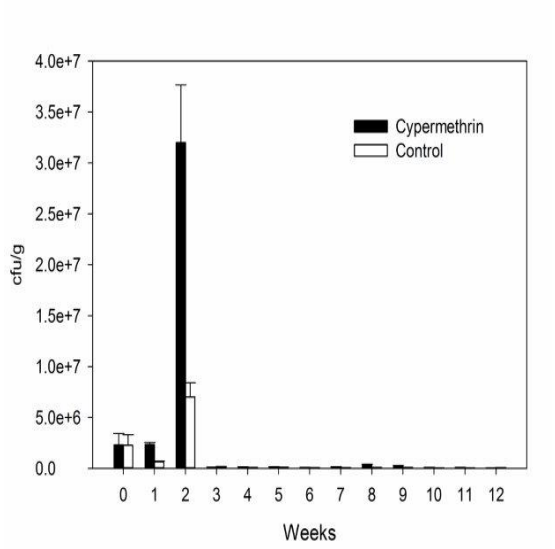

Fig. 2: Abundance of total heterotrophic fungi (THF) in cypermethrin treated experimental (EF) and control field (CF) soil. Data points represent the mean of three replicates. Error bars represent the standard deviation.

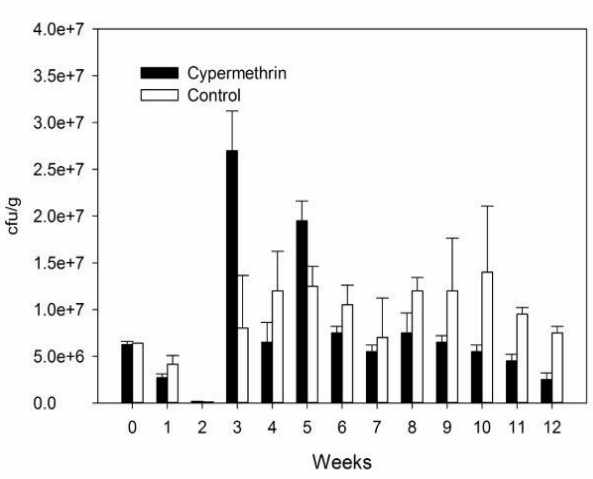

Fig. 3: Abundance of starch hydrolysing bacteria (SHB) in cypermethrin treated experimental (EF) and control field (CF) soil. Data points represent the mean of three replicates. Error bars represent the standard deviation.

Presumptive Actinomycetes:

The microbial population of presumptive actinomycetes decreased between week 0 (7.10 x 10^6cfu g^{-1}) and week 12 (2.75 x 10^5 cfu g^{-1}) in the EF (Fig. 4). The non-treated control field followed a similar trend with an initial viable count of 6.95 x 10^6cfu g^{-1}(week 0) to final count of 4.60 x 10^5cfu g^{-1}(week 12). There was no significant difference recorded in both fields between weeks 6 to 12 of the experiment indicating that the cypermethrin treatment did not exert a negative effect on the proliferation and growth of actinomycetes in the soil.

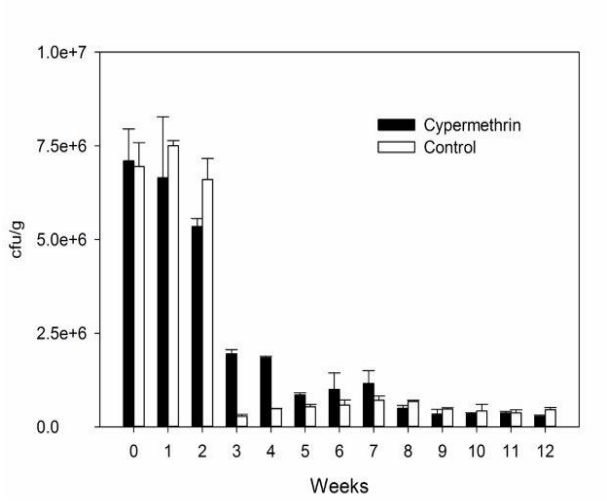

Fig. 4: Abundance of presumptive actinomycetes in cypermethrin treated experimental (EF) and control field (CF) soil. Data points represent the mean of three replicates. Error bars represent the standard deviation.

Nitrogen fixing bacteria (NFB):

The microbial counts for nitrogen fixing bacteria decreased in the experimental field over 12 weeks from 4.65 x 10^6 cfu g^{-1}(week 0) via 6.55 x 10^5 cfu g^{-1}(week 7) to finally 4.40 x 10^5 cfu g^{-1}(week 12). The non-treated control field followed a similar pattern with population count of 4.45 x 10^6 cfu g^{-1} (week 0) decreasing via 6.30 x 10^5 cfu g^{-1}(week 7) to 3.15 x 10^5 cfu g^{-1}at week 12 of the

experiment (Fig. 5). At the end of the experiment, the counts for N_2-fixing bacteria did not differ statistically in both fields thereby indicating that the insecticide treatment used in the present investigation did not cause any adverse effect on the NFB at the recommended field application rates.

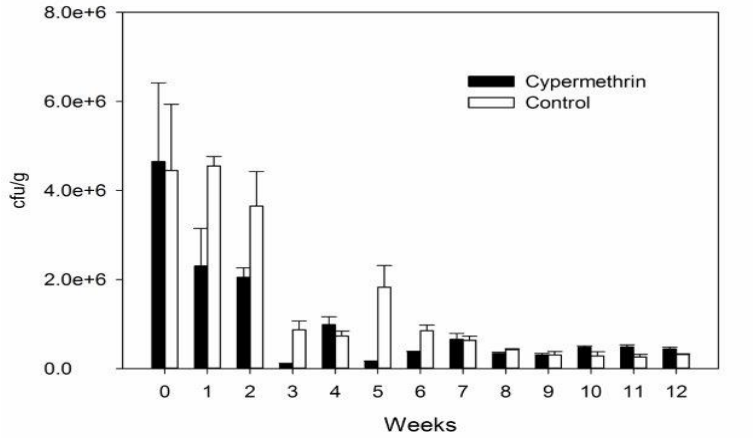

Fig. 5: Abundance of nitrogen fixing bacteria (NFB) in cypermethrin treated experimental (EF) and control field (CF) soil. Data points represent the mean of three replicates. Error bars represent the standard deviation.

Effects of 3-PBA on microbial FDA hydrolysing activity:

The FDA hydrolysis by soil microorganisms was inhibited at all tested concentrations of 3-PBA in both Ikorodu (Fig. 6) and Botanical garden soil (Fig. 7) in comparison to controls incubated under laboratory conditions throughout the exposure time of 36 h. The mean fluorescein release rates at 0 h and 0 mM 3-PBA in Ikorodu and Botanical garden soils were 1.12 mg g^{-1} soil $h^{-1}$and 1.26 mg g^{-1} soil h^{-1}while at 0 h the mean hydrolytic activity in autoclaved soil controls did not exceed 0.05mg g^{-1} soil h^{-1} Ikorodu and Botanical garden soils. At the highest concentration of 3-PBA tested (2.5 mM), the fluorescein release ratesat 36 h was limited to0.25 mg g^{-1} soil h^{-1} and 0.16 mg g^{-1} soil h^{-1}in Ikorodu and in Botanical garden soils. ANOVA showed that the difference in hydrolytic microbial activity determined with 0 mM 3-PBA and 0.1, 0.5, 1.0 and 2.5 mM 3-PBA was significant at $p<0.05$.

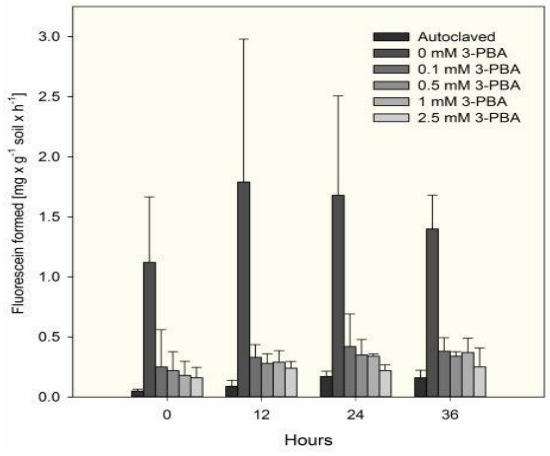

Fig. 6: Inhibitory effect of 3-phenoxybenzoic acid on FDA hydrolysis in Ikorodu soil. Data shown represent the mean of four replicates. Error bars represent the standard deviation.

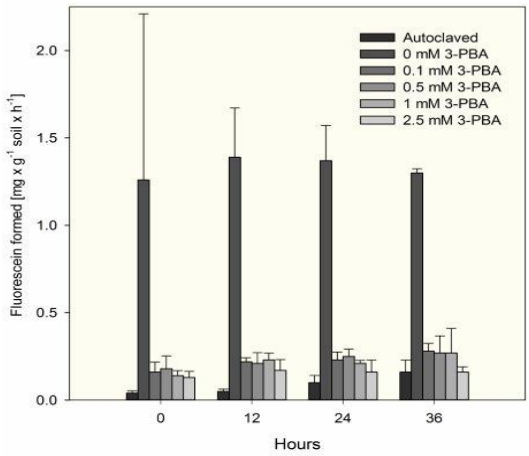

Fig. 7: Inhibitory effect of 3-phenoxybenzoic acid on FDA hydrolysis in Botanical garden soil. Data shown represent the mean of four replicates. Error bars represent the standard deviation.

DISCUSSION

In the present investigation, a commercial formulation of cypermethrin was applied fortnightly to cucumbers and viable counts for selected groups of soil microorganisms were determined over 12 weeks. The insecticide in the experimental field was not directly received by the soil and its microbial community as a result of foliar insecticide treatment and washing of the pesticides to the soil from the plant surface. The microbial count for THB was higher in the EF than the CF after the experimental period. The apparent increase in the microbial counts of THB could be due to the utilization of insecticide residues, solvent or emulsifiers present in the commercial formulation of cypermethrin. Although we have no direct evidence for cypermethrin utilization, these results might nevertheless indicate that to some degree insecticide residue and even more so emulsifiers and solvents as well as their metabolites can be used by certain aerobic heterotrophs present in the soil as additional carbon (or even nitrogen in case of the active ingredient) source. The population count for THF, presumptive actinomycetes and NFB were not statistically different between EF and CF at week 12. The microbial counts in field soil samples and the microbial hydrolytic activity of soil samples in the presence and absence of the major metabolite of cypermethrin, 3-PBA, were analysed to evaluate the potential non-target effects of cypermethrin addition to soil microorganisms. Noteworthy is the fact that Zhang *et al*., (2008) reported an increase in bacterial biomass and a decrease in fungal biomass due to cypermethrin application while Tu (1980) reported that soil microbial activities such as oxygen uptake or urease and dehydrogenasea ctivity were stimulated in soil treated with cypermethrin. Similarly, Zhang *et al*. (2009) observed a typical higher abundance of heterotrophic soil bacteria in soil treated with cypermethrin than that established for fungi. However, one week after cypermethrin treatment Tu (1980) found decreased colony counts for heterotrophic bacteria and fungi in soil samples in comparison to untreated controls. When assessing the potential impact of cypermethrin on bacterial, fungal and actinomycete counts in cotton field soils, no statistically significant changes were observed between controls and treated fields (Vig*et al*., 2008). The counts of starch hydrolysing bacteria shows a statistically significant decrease during the experimental study. Possibly neither cypermethrin nor the solvent and emulsifier present in the commercial formulation were utilized as additional carbon source for this particular group of microorganisms and at the same time might not have negatively affected their growth in the soil environment due to the low toxicity of cypermethrin towards typical amylase positive bacterial species such as *Bacillus cereus* (Ghosh *et al.,* 1997). The viable count for nitrogen fixing bacteria was similar in both the experimental and the control field thereby indicating that cypermethrin had no adverse effect on nitrogen fixing bacteria present in the treated soil which confirms earlier findings reported by Tu (1982) whereby the $EC_{50}$for a species of the N_2-fixing genus *Rhizobium* exceeded 10 g cypermethrin per L.In fact cypermethrin can stimulate the N_2-fixing activity of cyanobacteria at low concentrations (Megharaj*et al*., 1988). Overall, these results are in agreement with the assumption that the risk of soil microorganisms due to cypermethrin exposure is low (EFSA, 2014).

Although many studies have shown that pesticide application can result in a decrease in microbial biomass present in soil, other studies reported no detectable change or even an increase thereby illustrating the difficulty to quantify the net impact of pesticides or pesticide formulations on microbial populations present in different soil samples (Imfeld and Vuilleumier, 2012).It is thus not surprising that factors such as soil type and pH, temperature and organic matter content influences the impact

and persistence of pyrethroid insecticides in soil (Hussain *et al*., 2009). However, Meyer *et al.* (2013) demonstrated in a study using cypermethrin and other pyrethroids as well as three different sediment types that the elimination of the pyrethroid pesticides tested was always faster under aerobic than under anaerobic conditions for the same sediment type. They observed that in a sediment type with high organic matter content the aerobic degradation rate was lower under aerobic conditions even if the initial counts for bacteria, fungi and presumptive actinomycetes in this sediment type were higher than in a sediment type lower in organic matter content.

The hydrolysis of fluorescein diacetate into fluorescein can be used as an indicator to measure the total microbial activity present in soil (Adam and Duncan, 2001). FDA is hydrolysed by a number of different enzymes such as proteases, lipases, and esterases (Lundgren, 1981; Schnürer and Rosswall, 1982; Green *et al*., 2006). Bacterial catabolism of cypermethrin - as for other pyrethroids such as permethrin or deltamethrin is typically initiated by the hydrolysis of ester linkages present in the pyrethroids yielding the corresponding cleavage products (Miyamoto, 1981; Sakata *et al*., 1992; Schmidt, 1998; Yu and Fan, 2003; Wang *et al*., 2009). Hence, the FDA assay was used to evaluate the potential effect of 3-PBA on the hydrolytic activity of soil microbes. The significant difference ($P<0.01$) in the hydrolytic activity of soil samples in the absence and presence of 3-PBA over a range of different concentrations showed the potential of this metabolite to inhibit microbial hydrolytic activity which in turn is required to initiatethe catabolism of cypermethrin and similar pyrethroids sporting an ester bond. The absence of 3-PBA catabolising bacteria in the environment could therefore inhibit the quantitative hydrolysis of cypermethrin via a feedback inhibition due to 3-PBA build up to inhibitory concentrations. Under aerobic conditions this appears to be a somewhat improbable scenario as it is well established that 3-PBA is utilized as carbon and energy source under aerobic conditions by bacterial isolates obtained from soil (Wittich *et al*., 1990). In addition, the detection of 3-PBA residues in soil reported by Ostiz and Khan (1994) indicates that the bioavailability of this metabolite might be limited. However, anoxic conditions - for example caused by water logging of soil - are known to cause a 3-PBA build up from cypermethrin (Miyamoto, 1981).Thus it is important that even in the presence of 0.1 mM 3-PBA - the lowest concentration tested in this study - the hydrolytic activity of soil microorganisms was significantly reduced.

CONCLUSION

The viable counts established for selected groups of soil microorganisms indicates that the application of a commercial formulation of cypermethrin at the recommended field dose to agricultural soil is not likely to be detrimental to soil microbes and their activity for the majority of soil microorganisms tested in this study. The apparent tolerance of soil microorganisms to cypermethrin at the recommended field dose could be due to limited bioavailability of the active compound in soil and the microbial catabolism of the insecticide. At the concentrations of 3-PBA tested in this study, soil microbial hydrolytic activity was clearly inhibited. However, studies under field conditions would be required to verify the concentration of 3-PBA in cypermethrin treated soils under aerobic and anaerobic conditions and its effect on microbial activity under field conditions.

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