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Evaluating biotoxicity of cypermethrin towards non-targeted organisms by using phytotoxic, cytotoxic and antimicrobial bioassays

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Abstract

Pesticides are inevitably used in the horticulture, aquaculture, and agriculture to prevent pests' infestation and support systematic sustainability. A minute concentration of pesticides reaches the target community while rest of the volume either bulks-up in soil or reaches groundwater and aquatic systems through different channeling processes. Presence of pesticides is undesirable in these systems and may pose long term environmental hazards by interacting with the non-targeted communities in the vicinity. The current study was conducted to project the biotoxicity profile of an abundantly used pesticide 'Cypermethrin' by combining various toxicology parameters including Phytotoxicity, Cytotoxicity, Antibacterial and Antifungal activity assays. Evaluation of phytotoxicity of Cypermethrin showed a 50% reduction in radish seeds' viability on exposure to 100ppm pesticide concentration. A significant decrease in the root and shoot length was recorded on 50 - 1000 ppm pesticide concentration. Cytotoxic profiling of Cypermethrin revealed detrimental effects of pesticide on the *Artemia salina* larvae, killing 50% of the population at a concentration of 11.161µg/L. Cypermethrin significantly inhibited growth of non-targeted bacterial species at a concentration of 10ppm and more, while showing drastic effect on the growth of *Escherichia coli*. Antifungal assay affirmed biotoxicity of pesticide towards fungal domain by reducing the growth of fungal species at a pesticide concentration of 10ppm and more with highest impact on the growth of *Physarum polycephalum*. By using a combination of novel and conventional model organisms, the current study reports biotoxicity of Cypermethrin which is an abundantly used pesticide in agricultural systems.

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Introduction

Pesticides are continuously under usage in agriculture and aquatic systems to control the pests (Grosch, 1967; Liu *et al.,* 2009; Matthews, 2015). Pesticides comprise a large group of substances with diverse chemical and physical properties. Based upon chemical composition pesticides are categorized in four different groups: Pyrethroids, Carbamates, Organochlorides, and Organophosphates. Pyrethroids are abundantly used in Aquaculture, Horticulture, Agriculture, Veterinary and Domestic setups to control insects (Bragança *et al.,* 2018). Cypermethrin (CYP), *[cyano-(3-phenoxyphenyl) methyl] 3-(2,2-dichloroethenyl)-2,2-dimethylcyclop ropane-1-carboxylate* is an abundantly used synthetic type II pyrethroid. Cypermethrin is plentifully used in domestic and agricultural environments in place of Carbamates, Organochlorines, and Organophosp hates due to shorter half-life, broad target spectrum and lower toxicity to the environment (Atamanalp *et al.,* 2002; González-Doncel *et al.,* 2004; Knisel, 1993).

Pesticides are designed specifically for the pests targeting selective bioprocesses to minimize the impact on non-targeted organisms (Zhang *et al.,* 2011). A very small concentration of pesticide reaches the target organisms while the rest of the pesticide bulks up in the environment (Carriger *et al.,* 2006). Despite of specific design, pesticides interact with non-target organisms in the environment as well, leaving long-lasting toxic effects on the ecosystem (Hua *et al.,* 2009; Omar and Abdel-Sater, 2001). Presence of excessive pesticide residues can pioneer excessive severe eco-toxicological problems while affecting all biological domains. Analysis of the bio-toxicity of pesticides is an on-going process. The model toxicity studies analyzing impact of pesticides on non-target organisms help in the prediction of pesticides' role in their micro-niches (Bajet *et al.,* 2012). Importance of toxicity studies for the abundantly used pesticides cannot be stressed enough due to expected insight into the role of pesticides in their surrounding environment.

Several studies have been conducted so far, reporting the interaction of Cypermethrin with surrounding communities (González-Doncel *et al.,* 2004; Li *et al.,* 2005; Sheng *et al.,* 2004; Willis and Ling, 2004). But, there is a scarcity of comprehensive research studies reporting the impact of Cypermethrin on non-targeted biological domains. Increased usage of Pyrethroid pesticides and studies reporting a broad range of positive and negative effects, demand further investigation of impact of Cypermethrin on organismic toxicity. The present study was aimed to explore the detailed biotoxicity profile of Cypermethrin towards various biological domains for a comprehensive insight into its role in the environment. To achieve the aim of conducted study different toxicity assays were performed including Cytotoxicity (Brine Shrimp-*Artemia salina* larval mortality Bioassay), Phytotoxicty (Radish-*Raphanus sativus* seeds Bioassay) and Antimicrobial Bioassays such as Antibacterial and Antifungal Assays.

Materials and methods

This research work was conducted at Microbiology Research Laboratory (MRL), Quaid-i-Azam University, Islamabad, Pakistan. Cypermethrin was purchased from Sigma Aldrich. Growth media from Oxoid were used to culture microorganisms.

Phytotoxicity Testing

Phytotoxicty of Cypermethrin was tested by using pre-established Radish Seed Bioassay with slight modifications (Turker and Camper, 2002). For Radish (*Raphanus sativus*) seed germination, three parameters were analyzed for the establishment of results namely Root length, Shoot length and Number of germinated/viable seeds. Five different concentrations of Cypermethrin were prepared in deionized distilled water corresponding to 10, 20, 50, 100, 1000ppm pesticide concentration.

Radish seeds were surface sterilized by sequential rinsing with autoclaved distilled water, 10% Sodium Hypochlorite solution and autoclaved distilled water. Autoclaved sterilized Petri plates were lined with sterilized Whatman filter paper. A 5-ml aliquot of pesticide solution was added on the filter paper. After that, five radish seeds were transferred on the filter paper with a sterile pair of forceps maintaining an equal distance between all the seeds.

The experiment was repeated with all different concentrations of pesticides. Control was prepared by adding 5ml of distilled water instead of the pesticide solution. Petri plates were covered and incubated in the dark at 25°C. After every 24 hours, plates were checked for moisture and growth. After incubation of 5 days, three parameters of germination were observed: Number of Viable Seeds, Root Length, and Shoot Length. The experiment was repeated to confirm the reproducibility of results. Seeds were counted as germinated if the plumule was at least 2mm long. Root and shoot length was measured in 'millimeters' by using a measuring tape and percentage inhibition was calculated by the following equation:

Percentage Inhibition:

$$\frac{\text{Mean growth in Cypermethrin exposed seed}}{\text{Mean growth in control seed}} \times 100 \quad \text{Eq. 1}$$

Cytotoxicity Testing

To affirm cytotoxicity of the Cypermethrin, Brine Shrimp Bioassay also as *Artemia salina* Larval Mortality Assay was used with slight modifications. Artificial seawater was prepared in the laboratory by dissolving 34.0 g of commercially available seawater in 1 liter of deionized distilled water and pH was maintained at 8.0. After an artificial aeration of the sea water by magnetic stirring for 2 hours, seawater was poured into a bi-partitioned tank (9 x 6 in). The bigger compartment of the tank was covered with aluminum foil after adding Brine Shrimp (*A. salina*) eggs and the other compartment was left exposed to the light under a lamp. The setup was maintained at 28°C for 24 hours. After 24 hours, one may observe actively swimming *A. salina* larvae (nauplii) in the illuminated compartment of the tank.

Cypermethrin was dissolved in deionized distilled water to achieve pesticide dilutions between the ranges of 0 to 30 ppb (µg/L) concentrations. Pesticide dilutions were added to the labeled test tubes and after that 20 larvae were transferred to each tube by using Pasteur pipette and magnifying glass. Control was established by adding 20 larvae into artificial seawater with no pesticide residues and the experiment was performed in triplicates. All the test vials were incubated at 28°C for 24 hours under illumination. After the incubation, numbers of dead and alive larvae were counted under a magnifying glass and percentage mortality was calculated by the given equation.

Percentage mortality of *A. salina* larvae

$$\frac{\textit{Total number of A. salina larvae} - \textit{Number of alive A. salina larvae}}{\textit{Total number of A. salina larvae}} \times 100$$

Eq. 2

Antibacterial Activity Testing

Bacterial growth inhibition assays were performed by using two methods: (i) Spectrophotometric Method (ii) Well diffusion Method. Three bacterial candidates were used to test the bio-inhibition activity of Cypermethrin: *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 15442) and *Staphylococcus aureus* (ATCC 6538). Bacterial stock cultures were maintained on nutrient agar medium slants.

Spectrophotometric Method

Bacterial growth inhibition activity of Cypermethrin was analyzed by slightly modifying the previously reported method (Slabbert, 1986). Inhibition assay was conducted in the Mineral Salt Medium (MSM) with the following composition per liter of deionized distilled autoclaved water: K_2HPO_4 2.0g, KH_2PO_4 7.0g, NH_4NO_3 1.0g,mg$SO_4.7H_2O$ 0.1g, $FeSO_4.7H_2O$ 0.01g, $MnSO_4.6H_2O$ 0.002g, $ZnSO_4.7H_2O$ 0.001g and $CuSO_4.7H2O$ 0.0001g. Seven different concentrations of Cypermethrin were prepared in sterile MSM corresponding to 0 - 1000ppm (mg/L) pesticide concentration. For performing the antibacterial assay, bacterial specimens were sub-cultured in nutrient broth medium. After inoculating 50ml broth medium with the pure bacterial colony, medium was incubated at 37°C for 20 to 24 hours. After incubation, the optical density of the growth medium was analyzed by spectrophotometer. Freshly prepared sterilized medium was added in the growth suspension to achieve an optical density of 0.6 at 600 nm. After that each tube containing 9ml of a particular concentration of the pesticide was inoculated with 1ml of bacterial growth suspension. Control was prepared by adding 1ml of growth suspension in

sterile MSM with no pesticide residues. Tubes were incubated at 37°C for 24 hours in a shaking incubator (120 rpm). After incubation, samples were withdrawn out of test tubes, vortexed and analyzed by measuring optical density at 600 nm by using a UV-Vis spectrophotometer. Experiment was performed in triplicates. Percentage growth inhibition (%) was calculated by given equation:

$$\text{Percentage Growth Inhibition (\%)} = \frac{(\text{Growth OD in control sample} - \text{Growth OD in pesticide exposed sample})}{\text{Growth OD in control sample}} \times 100$$

Eq. 3

Well Diffusion Method

Bacterial growth inhibition activity of Cypermethrin was analyzed by using Well Diffusion Method as well (NCCLS, 1993). Briefly, bacterial colonies were inoculated on Luria-Bertani (LB) agar plates and incubated at 37°C for 24 hours. Bacterial suspension was prepared by picking up one colony from incubated LB agar plate and suspending it in the sterile 1N saline solution. Following vortex, bacterial growth suspension was spread over Mueller-Hinton (MH) agar plate supplemented with 0.1% Fluconazole w/v by using a sterile swab. Following inoculation of plates with bacterial cultures, eight equidistant wells with a diameter of 8mm were punched into LB agar plates for adding different concentrations of pesticide. Eight different concentrations of Cypermethrin were prepared in acetone corresponding to 1, 5, 10, 25, 50, 100, 500 and 1000ppm pesticide concentration. Plates were left at room temperature in sterile environment after filling up labeled wells with 100µl of corresponding pesticide concentrations to allow diffusion of pesticide; control contained acetone with no pesticide residues. After 30 minutes, plates in upright position were shifted to incubator at 37°C for 24 hours. Growth inhibition zone was measured after incubation by taking four perpendicular measurements in mm and later averaging them. Experiment was conducted in triplicates for each bacterial isolate.

Antifungal Activity Testing

In vitro antifungal activity was carried out by Disc Diffusion method (Bhalodia and Shukla, 2011). Growth of three fungal isolates was screened against inhibition capability of different levels of Cypermethrin. Fungal candidates used for antifungal assay are: *Aspergillus flavus, Cladosporium sphaerospermum* and *Physarum polycephalum*. Fungal cultures were maintained on Potato Dextrose Agar (PDA) slants.

Briefly, 5 mm disc of actively growing fungal mycelium was excised from a 72 hours old culture and was transferred to the center of Mueller-Hinton (MH) agar plate with 0.1% w/v Ciprofloxacin. Cypermethrin was diluted over a range of 1 to 1000ppm concentration: 1, 5, 10, 25, 50, 100, 500 and 1000ppm pesticide concentration. Sterile 6 mm sized discs were saturated with corresponding pesticide dilutions and were transferred to MH agar plate already seeded with fungal isolate. Procedure was repeated with all fungal isolates in triplicates. Plates were incubated at 28°C for 48 -96 hours. After incubation period, bio-inhibition zones were measured by taking four perpendicular measurements in mm and later averaging them. Fungicidal activity of Cypermethrin was depicted as the average of diameter (mm) of zone of fungal growth inhibition.

Data Analysis

All the methods reported in this study were conducted either in duplicates (Phytotoxic Analysis) or triplicates (Cytotoxic Analysis, Antibacterial and Antifungal Assays). Microsoft Excel 2016 was used for calculation of Mean, Standard Deviation and Standard Error along with Graphical representation of data. For Cytotoxicity analysis LD20, LD50, LD80 and LD100 were calculated by Linear Regression equation in Microsoft Excel 2016. SPSS (IBM SPSS, OS X, Version 23.0., Armonk, NY, USA) was used to calculate significant differences between datasets by using MANOVA (Multivariate analysis of Variance) and Post-hoc Analysis (Tukey's Test).

Results and discussion

Exposure of natural habitats to pollutants may affect indigenous communities due to toxic and xenobiotic nature of the pollutants. Pesticides are continuously sprayed in the environment leading to environmental pollution of terrestrial and aquatic ecosystems.

This study was conducted to specifically assess impact of Cypermethrin on the non-targeted communities present in environment. Test organisms from different biological domains were used to establish toxicity profile of the pesticide.

Phytotoxicity Testing

Studies assessing the impact of pesticides on various parameters related to plants' growth hold immense importance as the pesticides are continuously directly sprayed on the crops to avoid pests' infestation. Along with targeting insects and pests, pesticides can also harm different stages of plants' growth by targeting seed germination, root and shoot length, number of leaves and branches. Despite of in-compensable importance of the agricultural sustainability, insufficient number of studies have been conducted so far to predict toxicity of pesticides towards non-targeted plants (Shakir *et al.,* 2016). A limited number of studies have been conducted so far to evaluate impact of specifically Cypermethrin on non-targeted plants' community; till date to best of our knowledge no study has been conducted to analyze toxicity of Cypermethrin towards *R. sativus* (radish) seeds' germination.

The current study investigated effect of Cypermethrin residues' exposure on three important factors related to the plants' growth i.e. seed germination, root length and shoot length. Presence of smaller concentration of Cypermethrin i.e. 10ppm did not affect *R. sativus* (radish) seeds' germination, but a dose-dependent significant decrease was witnessed on the seeds' viability in presence of higher concentrations of Cypermethrin (Fig. 1). Number of seeds germinated at 20ppm (20mg kg^{-1} pesticide concentration) or higher were significantly different from the number of seeds germinated in control setup and the setup exposed to 10ppm pesticide concentration ($p < 0.05$). Similar findings have been reported earlier showing no significant effect on the number of seeds per bud when the seeds were exposed to lower pesticide concentration; while increase in the pesticide concentration led to drastic impact on *Vigna unguiculata* (Cowpea) seeds' germination (Obidola *et al.,* 2019).

Another research has been recorded earlier where presence of up to 500µg kg^{-1} Cypermethrin didn't cause any significant difference on the seed germination of *Cucumis sativus* (Bragança *et al.,* 2018). One other study reported that out of six test weeds only two weeds were affected by Deltamethrin - a closely related Pyrethroid (Hanley and Whiting, 2005). Stable response of the seeds towards lower concentration of pesticide implies presence of a response mechanism in the seeds that might help seeds resist certain environmental perturbations.

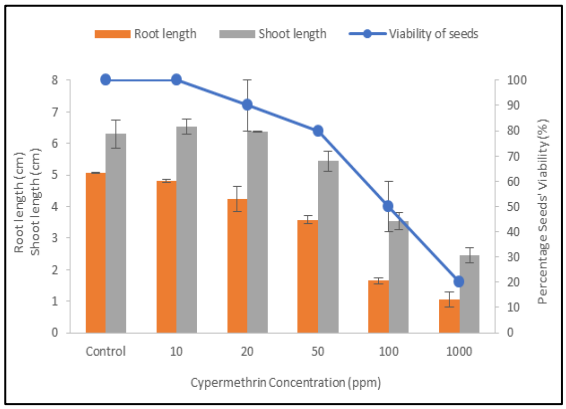

Fig. 1. Graphical representation of impact of different concentrations of Cypermethrin on *Raphanus sativus* (Radish). Parameters used: Root length, Shoot length, Number of viable seeds.

Impact of pesticide on root and shoot growth of *R. sativus* was also studied to establish phototoxic dynamics of Cypermethrin. Presence of pesticide residues during the growth of seedlings significantly affected growth of root and shoot ($p < 0.05$). A dose dependent significant decrease was observed in the root growth of *R. sativus* (radish) seedlings on high concentrations of Cypermethrin exposure (Fig. 1). Results of our study are supported by a previously conducted study reporting significant dose-dependent decrease in Chinese cabbage (*Pakchoi*) seeds' radicle growth (Liu *et al.,* 2009). Our results also coincide with one previously conducted study which reported significant decrease in radical length of *Vigna unguiculata* (Cowpea) on exposure to higher concentrations of Cypermethrin (Obidola *et al.,* 2019). The mentioned study also showed a slight stimulatory effect in the presence of lower percentage of Cypermethrin residues which supports another

previously conducted study showing slight to moderate increase in root growth of *Cucumis sativus* on exposure to Cypermethrin (Bragança *et al.,* 2018). Our study is not completely contradicting reported studies as our study also showed lower degree decline in root growth on lower pesticide exposure i.e. at 10 – 20ppm pesticide concentration in comparison to higher exposure of pesticide. The difference in the results may be attributed to many conditions e.g. Experimental design, Plant species used for toxicity studies and type of solvent/medium used for the controls.

Results of shoot growth studies showed significant increase on lower pesticide exposure i.e. 10ppm pesticide concentration while slightly higher pesticide presence did not cause any significant shoot growth stimulation or inhibition (Fig. 1). Higher pesticide exposure at 50-1000ppm pesticide concentration caused significant dose-dependent inhibition of plumule (shoot) growth showcasing up to 61% inhibition at 1000ppm pesticide concentration. Inhibition of shoot growth at higher concentration is in accordance with a previously published study that tested and reported reduction in shoot growth of 4 different pulses seeds at higher concentrations of Cypermethrin (Srivastava, 2014).

Pesticide dose-dependent reduction in shoot growth of Chinese cabbage seedlings has been reported earlier causing up to 100% inhibition of plumule growth (Liu *et al.,* 2009). Cypermethrin exposure affected root growth more severely in comparison to shoot growth. The highest tested pesticide concentration- 1000ppm inhibited 79% of root growth in comparison to 61% inhibition of shoot growth. Findings of this study are supported by previously reported studies showing more drastic impact on root growth as compared to shoot and other plant organs growth in presence of pesticides and other environmental perturbations (Bragança *et al.,* 2018; Liu *et al.,* 2009; Rede *et al.,* 2016). One of the major causes of enhanced sensitivity of roots towards pollutants is direct contact and exposure of roots to the soil medium that originally serves as reservoir for pollutants accumulation.

Cytotoxicity Testing

Pesticides may reach aquatic system by two different ways: (i) adsorption of pesticide through soil bed and access to ground water (ii) direct usage of pesticide in aquaculture to control pests (insects) infestation. Presence of pesticide residues in the watersheds and ground water wells has been reported earlier that further strengthens the environmental concerns over exposure of aquatic ecosystems to pesticides (Gilliom *et al.,* 2006; Staley *et al.,* 2015).

Evaluation of bioactivity of pollutants by employing aquatic test organisms including Insects, Crustaceans and Fish can help in prospecting eco-hazardous fate of the pollutants due to higher sensitivity of these organisms. One of the most established and economical method used to analyze impact of pollutants on the aquatic systems is Brine Shrimp Bioassay. Cypermethrin and other related Pyrethroids have not received much deserved attention in contrast to other classes of pesticides due to its higher degradability in the environment. But certain studies have reported that even 1pg/L concentration of Pyrethroids may prove to be lethal for aquatic organisms (Mian and Mulla, 1992; Werner and Moran, 2008).

Cytotoxic activity of Cypermethrin was tested towards *A. salina* which is specie of brine shrimp – aquatic crustaceans. A significant dose dependent inhibition was recorded on the viability of larvae in response to Cypermethrin exposure as shown in Fig. 2 ($p < 0.05$). Acute cytotoxicity of Cypermethrin was further evaluated by calculating LD20, LD50, LD80 and LD100 after 24 hours which is as follows: 1.897, 11.161, 20.426 and 26.602 ppb (µg/L). Pesticide concentration as low as 11.161 ppb (µg/L) may kill 50 percent population of *A. salina* larvae which are not even the prime target of Cypermethrin. A previously conducted study reported lethal effects of Cypermethrin residues towards other crustaceans- *Thamnocephalus platyurus*was and *Artemia fransiscana while reporting LC50 after 24 hours as* 0.67mg/l and 4.72mg/l respectively (Sánchez-Fortún and Barahona, 2005). Cypermethrin also affected assemblage of Shrimp *Macrobrachium lar* with an LC 50 of 1.07µg/L after 48 hours of exposure (Bajet *et al.,* 2012).

Cypermethrin has been also reported to show an inhibitory effect towards other invertebrates such as *Daphnia magna* HB with an LC50 of 4.81 ± 0.40mg/L (Sheng *et al.,* 2004). This study testifies previously conducted studies reporting acute toxicity of Cypermethrin towards non-targeted communities at low exposure levels.

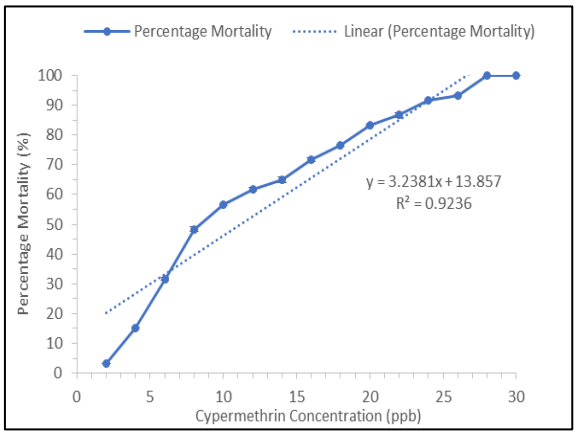

Fig. 2. Graphical representation of cytotoxic effect of different concentrations of Cypermethrin on *Artemia salina* larvae' mortality.

Antibacterial Activity Testing

Toxicity of Cypermethrin was tested towards three bacterial species*: Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus*. Cypermethrin caused significant inhibition in growth of all bacterial species ($p < 0.05$) as shown in Fig. 3. Spectrophotometric studies revealed high antibacterial activity of Cypermethrin residues Fig. 3a. Highest inhibition of 68% was observed at 1000ppm pesticide concentration against *E. coli,* while *P. aeruginosa* and *S. aureus* were found to be more tolerant against pesticide showing lower inhibition values: 53.78% and 62.61%.

Well diffusion method confirmed results obtained by spectrophotometric method Fig. 3b. Biggest inhibition zone was observed for *E. coli* with a diameter of 23.5 ± 1.5 mm at 1000ppm pesticide concentration. Wells containing 1ppm Cypermethrin concentration didn't show any inhibitory effect on any of the bacterial species that's why they are not included in the graphical presentation of antibacterial activity of pesticide. Non-significant reduction in the growth of *P. aeruginosa* was observed at lower pesticide exposure i.e. 5 and 10ppm pesticide concentration ($p > 0.05$). With increase in pesticide exposure a significant reduction in terms of inhibition zones was observed ($p < 0.05$). For E. coli, a significant dose dependent inhibition was observed on all pesticide concentrations ($p < 0.05$). Presence of 5ppm pesticide concentration caused a non-significant reduction in the growth of *S. aureus* ($p > 0.05$), while higher concentrations significantly inhibited the growth of bacteria ($p < 0.05$). The order of bacterial species' resistibility towards the presence of Cypermethrin was evaluated as: *P. aeruginosa* > *S. aureus* > *E. coli*.

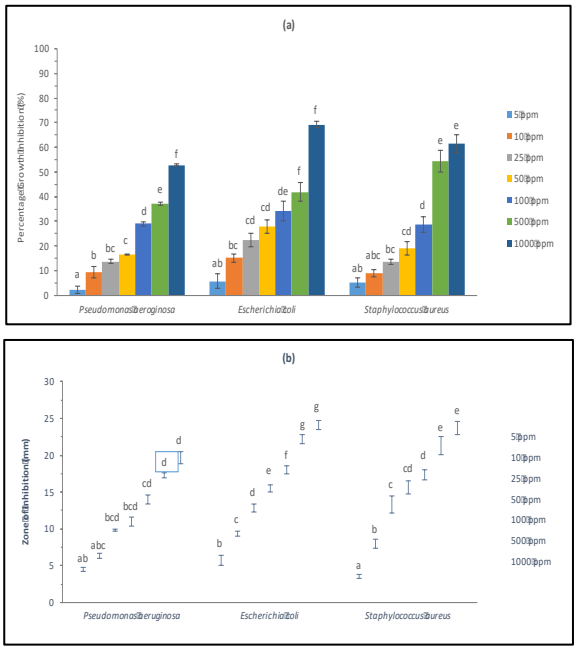

Fig. 3. Graphical representation of Bacterial Growth Inhibition in response to Cypermethrin exposure (a) Spectrophotometric Method (b) Well Diffusion Method.

Several studies have been reported earlier showing ability of these bacterial species to grow in the presence of Cypermethrin while actively degrading isomers of the pesticide. Our findings report acute (short-term) toxicity of Cypermethrin towards these test bacterial species which may correspond to the time required for acclimatization of the bacterial species towards presence of pollutants and/or pesticides. A degradation study conducted earlier showed an initial decrease in the growth of

P. aeruginosa cells followed by the enhanced growth after period of acclimatization that supports our findings (Shamsuddeen and Inuwa, 2013). Another previously reported study calculated maximum resistible concentration of Cypermethrin for *S. aureus, P. aeruginosa* and *E. coli* as 0.5ml/L,1ml/L and 2.5ml/L respectively (Nisharaj *et al.,* 2012). The present study also reports tolerance of lower pesticide concentrations by all three-bacterial species corresponding to the Cypermethrin concentration of 1ppm and 5ppm with no significant reduction in bacterial growth ($p > 0.05$). High concentrations of pesticide left acute toxic impact on the bacterial growth which can be attributed to the time required for acclimatization of bacterial species to high pitch environmental perturbations.

Antifungal Activity Testing

Fungicidal potential of Cypermethrin was tested against three fungal species: *Aspergillus flavus, Cladosporium sphaerospermum* and *Physarum polycephalum*. A pesticide concentration of 1ppm did not inhibit fungal growth while presence of 5ppm pesticide concentration slightly inhibited growth of *A. flavus* and *C. sphaerospermum* Fig. 4. A dose dependent significant inhibition was observed in response to pesticide exposure on higher concentrations that is 10 - 1000ppm ($p < 0.05$).

Ability of fungal species to resist against Cypermethrin was found to be in following order: *C. sphaerospermum* > *A. flavus* > *P. polycephalum*. There have been a research report earlier reporting inhibition of rhizosphere fungi including *A. flavus* in response to Cypermethrin exposure (Abdel-Hafez *et al.,* 2010). In contrast, another study was reported showing ability of *A. flavus* to survive in Cypermethrin contaminated soil resisting up to 800ppm pesticide concentration in soil (Sethi *et al.,* 2015). Contrast in different studies may be attributed to the different research design, environmental conditions, abundance and growth stage of fungi in the soil or environment before experimentation. To the best of our knowledge till date, no study has been conducted earlier to study binary interaction of Cypermethrin or any other pesticide with *C. sphaerospermum* and *P. polycephalum*. Interaction of *C. sphaerospermum* has been reported earlier with essential oils demonstrating capability of fungal specie to enact as an bio-indicator organism (Morandim *et al.,* 2010). Investigating the interaction of new microbial species with pesticides is an interesting research area to plot biotoxicity profile of pesticides more precisely.

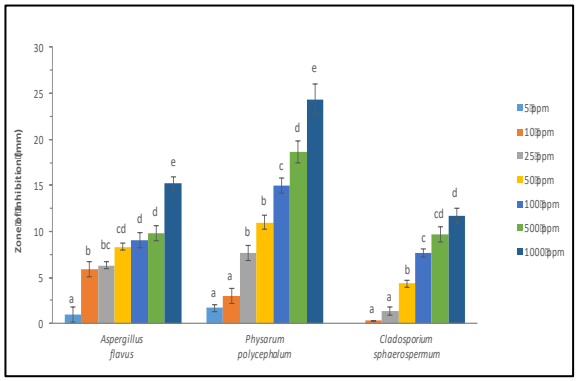

Fig. 4. Graphical representation of Antifungal activity of Cypermethrin.

Conclusions

The present study is the first comprehensive study exploring impact of Cypermethrin on four different biological domains. Despite of specific design of the pesticide, pesticide affects non-targeted community as well. Cypermethrin caused significant reduction in the *Raphanus sativus* (radish) seeds' germination as monitored by Seeds' viability, Root length and shoot length ($p < 0.05$). Cytotoxic profile of Cypermethrin concludes lethal toxicity of the pesticide; pesticide concentration as low as 11.161µg/L can kill half of the population of test organisms used i.e. *Artemia salina*. Antibacterial and Antifungal assays concluded restriction of test species' growth on exposure to higher concentrations of pesticides mimicking environmental conditions where pesticide residues keep on accumulating along the timeline.

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