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Avoidance Response: A More Important Determinant of Population Immediate Decline in Guppy Fish (*Poecilia reticulata* Peters, 1859) Exposed to Pesticides

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the ability of Paraquat (herbicide) and Dichlorvos (insecticide) to elicit avoidance response in the population of Guppy fish, *Poecilia reticulata* and predict the population immediate decline (PID) of *P. reticulata* when exposed to both agrochemicals. A 96-h forced system (FS) bioassay was each conducted in five duplicate systems, each with a control experiment. The avoidance response was examined using a non-forced multi-compartmented static system (NFS). The guppies (n = 3 guppies per concentration of 6 compartments in quadruplet) were exposed to a gradient of Paraquat (0, 2.5, 5.0, 7.5, 10.0 and 15.0 mgL^{-1}) and Dichlorvos (0, 1.6, 2.6, 3.0, 3.2 and 4.0 mgL^{-1}). Their distributions were examined at 20 min intervals for a 3-h period (n=9observation periods). The results from 96-h FS were dose-dependent with the highest percentage mortality being 85.7% for Paraquat and 78.6% for Dichlorvos in their respective highest concentrations. The 3-h NFS exposure showed statistically significant

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concentration-dependent spatial avoidance. The *P. reticulata* avoided the lowest concentration of Paraquat (2.5mgL^{-1}) and Dichlorvos (1.6 mgL^{-1}) by 68.3% and 48.3% respectively. The avoidance increased significantly ($p < 0.005$) to 75%, for the highest concentrations of both test chemicals (15.0 and 4.0 mgL^{-1}). The 3-hr AC_{50} value revealed that the guppies were more sensitive to Paraquat (0.37 mgL^{-1}) than Dichlorvos (1.73 mgL^{-1}). The PID was driven by the avoidance behavior (NFS) of the guppies rather than mortality (FS). The FS heretofore overestimate the environmental risk of contamination due to mandatory exposure of mobile organisms to contaminants. Rather than the traditional FS systems, NFS should be adopted and incorporated into typical bioassays for better predictive results.

Keywords: Population Immediate Decline (PID); Paraquat; Dichlorvos; Non-forced system; Forced system; Multi-compartmented Static System; Environmental Risk Assessment.

1. INTRODUCTION

There has been a worldwide concern about the ecological impacts of economic poisons (synthetic pesticides) on living organisms, especially non-target species. Farming is the biggest consumer of pesticides, where they are employed for pest control to enhance crop yield. Pesticides are also useful in mitigating the spread of bugs, microbes, parasites, and as biocidal agents for protecting various items of value [1]. Given the broad-spectrum nature of most pesticides, inadvertent exposures often result in harm to biota and humans [2]. Exposure to low concentrations of pesticides has been attributed to detrimental effects on various life stages of organisms [3]. Pesticides at a high concentration are known to diminish the endurance, development and multiplication of fish, and produce numerous obvious consequences for fish [4]. The hazardousness of a synthetic chemical depends on the actual concentration of the substance entering an organism i.e., its bioavailability or its concentration at the target receptor in living organisms [5]. In order to avert the most harmful effects of pesticides, there is a need to understand the species-specific responses of the various organism used and build relevant quantitative data to guide extension services [6]. To this end, this study investigated the acute toxicity and avoidance responses of an important mosquito bio-control fish, the guppy fish, *Poecilia reticulata* to pesticides, Paraquat and Dichlovors.

A major challenge in assessing responses of organisms to toxicants such as pesticides is the bioassay design. In much toxicity testing bioassays where the usual reactions, such as tolerance, growth, prevalence, and common endpoints are investigated, forced exposure system are typically used. This involves the introduction of organisms into a confined space for the purpose of exposure to pre-determined concentrations of toxicants. Despite the fact that this method can help in predicting the impact of continuous exposure to pollutant, it cannot predict the ways in which organisms respond under normal circumstances where an organism has the capacity to move away from contaminated areas [7].

In nature, animals such as fishes often have the freedom to move away from unfavorable conditions. Thus, in a non-forced environment, avoidance can be used as a plausible response to assess the potential population decline of aquatic organisms, driven by complex stress, and subsequent consequences for community structure and environmental dynamics [8,9,10]. Avoidance responses should be considered an integral tool for traditional assessments, which improves the biological importance of assessing the natural hazards of pollution [7,8].

In the present study, a non-forced contamination gradient has been adopted to understand the spatial distribution and rapid population decline of guppy fishes upon exposure to Paraquat and Dichlorvos. Hence, the objectives of this investigation were: (i) To determine the capacity of Paraquat and Dichlorvos to induce avoidance response in *P. reticulata*, (ii) To predict the Population Immediate Decline (PID) at the local scale, caused by exposure to both pesticides (iii) Determine whether mortality can be overestimated when using forced and continuous exposure to assess hazardous effects.

2. MATERIALS AND METHODS

Test Animal: Acquisition and Acclimation

Guppy fishes, *Poecilia reticulata* (Chordata, Poeciliidae, Animalia, Actinopterygii) were collected using a scoop net from storm channels at the Faculty of Science, University of Lagos and transported to the laboratory in a tank filled with water from the point of collection. The use of the fish was approved by the Ethics Committee on the Use of Animals (Lagos University Teaching Hospital). The organisms were acclimated under laboratory conditions at the Ecotoxicology Laboratory of the Department of Zoology, University of Lagos for at least one week prior to the tests, following OECD guidelines [11]. Only F1 generations were employed in the actual bioassay. Fishes were kept in 50L aquaria filled with dechlorinated municipal water. The cultures were constantly aerated and the water was renewed by 30% every week. The animals were fed daily, with commercial Coppens® fish food until 24 h prior to the experiments. The conditions in the culture media were maintained as follows: pH (6.4 - 7.2), conductivity (0.15 - 0.30 $mS{\cdot}cm^{-1}$), total dissolved solids (113-125 $mg{\cdot}L^{-1}$), photoperiod (12:12-h. light: dark) and temperature (25.8 to 26.3°C).

Test Chemicals

The Pesticides for this study were the herbicide; Paraforce, (manufacturer: Nanjing Redsum Biochemistry Co Ltd., Nanjing City, China), active ingredient 276g of Paraquat dichloride (200g Paraquat/L) and Sniper (manufacturer: Forward (Beihai) Hepu Pesticide Co. Ltd. Guangxi, China), active ingredient $1000gL^{-1}$ of 2,3-dichlorovinyldimethyl phosphate (DDVP). Both pesticides were procured from Lagos State Agricultural Input Supply Office, Oko Oba Agege, Lagos State. Prior to each toxicity and avoidance test, stock solutions of Paraquat dichloride and Dichlorvos were prepared at concentrations of 1000 mgL^{-1} each dispensing an aliquot to be measured out in distilled water. Subsequent dilutions were made with culture water in the experimental tanks. Both pesticides were stored at room temperatures (<33˚C) prior to the bioassay.

Acute Toxicity Tests Using Forced Exposure Systems (FS)

Acute toxicity tests were performed with the fish for the two pesticides in order to determine the LC_{50} values for each pesticide before the avoidance tests. The Dichlorvos and Paraquat concentrations used in the tests were 0, 1.60, 2.60, 3.00, 3.20 and 4.00 mgL^{-1} and 0, 2.50, 5.00, 7.50, 10.00 and 15.00 mgL^{-1} respectively. The tests were carried out in duplicate using 7 fishes per 3L glass aquarium per concentration, at a temperature of 26.20 ± 0.4 ^{0}C and a photoperiod of 12:12 -h (light: dark).

Design of the Non-Forced Multi-Compartment Exposure System (NFS)

Non-forced avoidance tests were performed in a free-decision, static multi-compartment system, based on the method of [12]. The system was made out of interconnected compartments developed PET bottles without any tainting material. The chambers were linked with non-lethal transparent plastic paste (Zuma PVC glue). The system had an all-out length of 186 cm and a total volume of 9000 ml, and each compartment had a volume of 1500 ml. For the experiment, the compartments were loaded up with 1 L of culture medium containing each of the test pesticides at various concentrations.

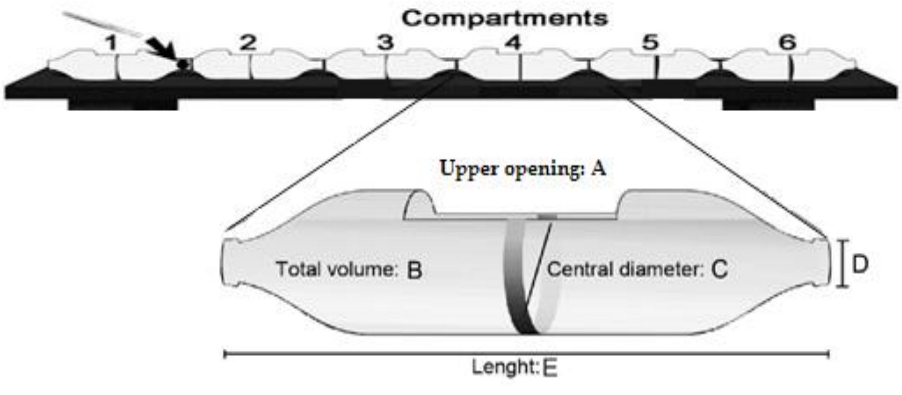

Fig. 1. Schematic diagram of the static Non-Forced multi-compartment System (NFS) utilized in the avoidance tests (A = 18 × 6 cm; B =1500 mL; C = 9 cm; D = 2 cm; E = 31 cm)
Source: [12]

Calibration of the Non-Forced Multi-Compartment System (NFS)

The linear gradient of the contaminant was first validated without fishes in the NFS experiments. For this, various concentrations (0, 0.2, 0.4, 0.6, 0.8, and 1.0 mgL^{-1}) of analytical grade sodium chloride (NaCl) in distilled water were prepared. NaCl concentrations were measured indirectly by conductivity at 0 hr (initial) and after 3 hr (final). The initial concentrations (at 0 h) within each compartment were (mean and standard deviation): 0.07 ± 0, 0.14 ± 0, 0.29 ± 0.004, 0.51 ± 0.004, 0.71 ± 0.004, and 1.125 ± 0.005 mgL^{-1}). Before dispensing the NaCl salt in the system, the compartments were isolated from each other using plasticine plugs. After the introduction of the different amounts of NaCl in the compartments, the plugs were removed, resulting in a linear gradient. The calibration was carried out in quadruplets, in the dark. Subsequently, a control distribution experiment was performed to confirm that the fish did not exhibit a preference for any particular compartment. To this end, three organisms were introduced into each compartment containing only culture water (no gradient) and the number of fishes in each compartment was recorded after 3-h.

Avoidance Tests with Paraquat and Dichlorvos

The avoidance tests were performed separately in an arrangement of NFS, mimicking a spatially more extensive and more permanent slope in accordance with the 96-h LC_{50} values of FS of the guppies, singly to both pesticides. The concentrations were as follows (in mgL^{-1}): 0 (control), 2.50, 5.00, 7.50, 10.00 and 15.00 and 0 (control), 1.60, 2.60, 3.00, 3.20 and 4.00 for Paraquat and Dichlorvos respectively. In each test, three fishes were introduced per concentration, totaling 18 individuals in each system. The procedures used to dispense the test chemicals and fishes in the system were those described in the previous calibration procedure. The tests were completed in quadruplets, in the dark to minimize external interferences.

Statistical Analysis

The proportions of organisms recorded in each compartment at various experimental times were arcsine transformed. The arbitrary dispersion of organisms in the control medium and avoidance tests with both pesticides were evaluated with a mixed model (time as a repetitive measure, within factor, and compartment as between factor) analysis of variance (ANOVA). Mauchly's test was utilized as a sphericity test. Where sphericity was infringed (the variances of the differences are not equal: $p < 0.05$), Greenhouse-Geisser correction was applied ([6]. See Tables S1a, S1b, S1c, S2a, S2b, S2c, S3a, S3b and S3c- Supplementary Material).

The outcome of the acute tests was analyzed using Probit analysis [13] to estimate the 96-h LC_{50} values for the individual test chemical and the corresponding 95% confidence interval (CI). Unidirectional ANOVA was used followed by an LSD multiple comparisons post hoc test ($p < 0.05$) to analyze the results of the calibration tests and avoidance tests. For the calculation of avoidance (%), the number of avoiders for each concentration was determined as the difference between the number of expected organisms (N_E) and the number of organisms observed (N_O): Avoiders = N_E - N_O. The N_E was determined as described by Moreira-Santos et al. [7]: for the compartment with the highest concentration of the test chemical, the N_E was equal to the number of fish introduced into the compartment at the beginning of the test; for the remaining compartments, N_E included the organisms initially introduced into the compartment, plus the organisms introduced into the adjacent compartment with greater concentration. Initially, since each compartment contained three fishes, the value of N_E was 3 for the most contaminated compartment (considered the first compartment). The N_E value for the adjacent compartment (second compartment) was 6 (3 organisms inserted in this compartment plus 3 organisms that are expected to move from the first compartment), while for the last compartment (sixth, not contaminated) it was 18. With respect to the number of organisms observed for each concentration, the organisms found in that compartment and those found in higher concentrations were considered as N_O. Finally, for the control compartment, because this compartment contained the culture medium, it was not expected to be avoided; therefore, it was 18. The avoidance percentage for each compartment was calculated as follows: (Avoiders / N_E) * 100. The avoidance percentages for each concentration were used to obtain AC_{50} values (concentration that 50% of the exposed organisms avoids) and the corresponding 95% confidence interval using Probit analysis. The avoidance and mortality percentages were subsequently integrated to calculate the PID_x (x in percentage) induced by each chemical test concentration that simultaneously caused a 'y' mortality percentage (i.e., the 96-h LC_y) and an avoidance percentage 'w' (that is, the 3-hour AC_w), as described by Rosa et al [8].

(100y+100w-yw)/100

The calculation of the immediate population decline was based on the fact that some of the fish first flee (% avoidance), and that mortality is then determined based on the remaining organisms (those that did not show evasion). The PID_{50} values (the concentration that causes an immediate population decrease of 50% of the exposed organisms) and the corresponding confidence interval were also obtained using Probit analysis. All statistical analysis was performed using SPSS Version 20 (IBM).

3. RESULTS

Acute Toxicity Test of Paraquat and Dichlorvos to *Poecilia reticulate*

The percentages responses in the 96-h FS acute toxicity test recorded at various concentrations of Paraquat and Dichlorvos are presented in Tables S2 and S3-Supplementary Material respectively.

In particular, for the FS exposure to Paraquat mortality per concentrations after 96-h were 0% (control), 0% (2.5 mgL^{-1}), 21.43% (5.0 mgL^{-1}), 35.71% (7.5 mgL^{-1}), 71.43% (10.0 mgL^{-1}), 85.71% (15.0 mgL^{-1}) while for Dichlorvos exposure, it was 0% (control), 21.43% (1.6 mgL^{-1}), 42.86% (2.6 mgL^{-1}), 50% (3.0 mgL^{-1}), 50.0% (3.2 mgL^{-1}), 78.57% (4.0 mgL^{-1}). Unconsciousness and loss of steadiness were displayed by test organisms in the first 24-h at highest concentrations of both chemicals. The measure of relative potency of toxicants (i.e., toxicity factor) ascribed Dichlorvos to be more lethal to the guppies than Paraquat by a factor of 2.9 (Table 1). Similar trends were followed at 24hr, 48hr and 72hr of forced exposure (Tables S4a, S4b and S4c - Supplementary Material).

Avoidance System Calibration and Control Tests

The results of the calibration of the avoidance system using NaCl revealed that in the absence of the guppy fishes, the gradient was maintained after 3-h ($p < 0.05$; $F_{5, 18} = 755.024$), since statistically significant differences ($p<0.05$) existed among the concentrations in the compartments. This affirmed the accuracy and appropriateness of the set-up for the avoidance experiment. However, the fitness of the NaCl gradient curve was checked by linear regression analysis of the initial and final concentrations after 3-h (Y= 0.115 + 7764; $r^2 = 0.974$ and r = 0.987). After 3-h, the mean and standard deviation values for the NaCl concentrations in the validation test without fish were 0.08 ± 0.007, 0.18 ± 0.008, 0.34 ± 0.0008, 0.52 ± 0.001, 0.7 ± 0.02, and 1.02 ± 0.048 mgL^{-1} (Table S1 - Supplementary Material). In the control fish distribution experiment without Paraquat and Dichlorvos, an arbitrary conveyance with no statistically significant differences in the percentages (with no inclination for any compartment) of the organisms throughout the system were observed after 3-h ($p > 0.05$; $F_{5, 18} = 2.16$). The mean distribution and standard deviation at the end of the control avoidance test of *P. reticulata* (Mean± standard deviation) per compartment were as follows: 3.25 ± 0.5, 2.50 ± 0.58, 3.25 ± 0.5, 2.50 ± 0.58, 3.25 ± 0.5, and 3.0 ± 0.59 respectively. The relative percentages and standard deviations of organisms observed in each compartment at the end of the 3-h observation are presented in Fig. 2.

Avoidance Response of the Guppy Fish, *Poecilia reticulata* to the Exposed Pesticides

In the NFS tests with each pesticide, the distribution of *P. reticulata* was concentration-dependent indicating avoidance behavior. In the

Table 1. Comparative assessment of relative 96-h acute toxicity of Herbicide (Paraquat) and Insecticide (Dichlorvos) mg/L acting singly against *Poecilia reticulata* in Forced System (FS)

Chemical	LC_{50} (Interval)	Slope ± SE	Probit line equation	DF	TF
Paraquat (mgL^{-1})	8.20 (6.69 – 10.09)	4.46 ± 1.0	Y= -3.75 + 4.13x	3	2.9
Dichlorvos (mgL^{-1})	2.84 (N/A)	3.66 ± 1.26	Y= -1.63 + 3.63x	3	1

KEY: CL: Confidence Limit, DF: Degree of Freedom, SE: Standard Error, T.F.: Toxicity Factor NA: Not Available

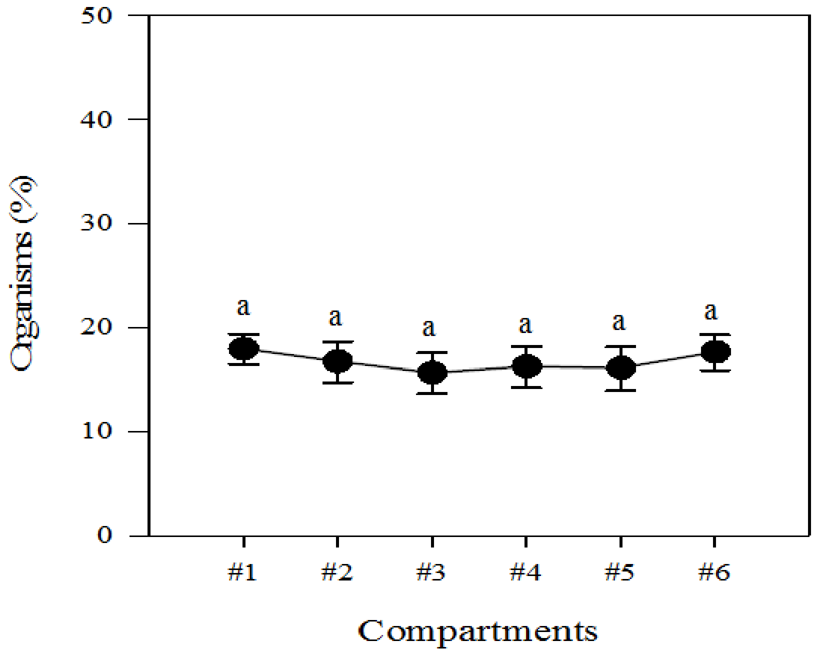

Fig. 2. Mean percentage and standard deviation (n = 9 observation periods) of the distribution of guppy fish (*Poecilia reticulata*) in the culture medium during control tests recorded in each compartment for 3-h. The letters represent statistically significant differences

highest concentrations of 15.0 mgL^{-1} and 4.0 mgL^{-1} of Paraquat and Dichlorvos exposures respectively, 54 organisms (75%) were found to avoid both considering the four replicates (a total of 72 organisms), while zero organisms (0%) avoided the control compartments (0 mgL^{-1}) as the fishes tend to migrate towards uncontaminated compartments (Tables S4 and S5 - Supplementary Material).

The statistical analysis (Unidirectional ANOVA and LSD multiple comparison post hoc test) of *P. reticulata* distribution in the gradient of Paraquat ($F_{5, 18}$ = 206.80 $p < 0.05$) and Dichlorvos ($F_{5, 18}$ = 126.96 $p < 0.05$) indicated that the organisms significantly preferred the less contaminated compartments for survival during the 3-h exposure period. In the presence of the contaminants, the organisms of lesser-contaminated compartments displayed changes in the pattern of interaction such as competition and aggressiveness among the test organisms occurred i.e., preferential behavior. Largely, *P. reticulata* migrated from more polluted compartments to uncontaminated compartments thus maintaining a strategic distance from the harmful concentration of test chemicals.

The mean distributions of fishes were determined by concentration gradient with individuals tending to migrate to less contaminated compartments. At 1-h of exposure to both pesticides, the guppy fish distribution in the contaminant gradients indicated that they preferred significantly (Paraquat $F_{5, 18}$ = 98.509 $p < 0.05$; Dichlorvos $F_{5, 18}$ = 38.12 $p < 0.05$) the uncontaminated compartments. A similar statistically significant response pattern was observed after 2-h (Paraquat $F_{5, 18}$ = 94.25 $p < 0.05$; Dichlorvos $F_{5, 18}$ = 29.25 $p < 0.05$) and 3-h of exposure (Paraquat $F_{5, 18}$ = 206.8 $p < 0.05$; Dichlorvos $F_{5, 18}$ = 126.96 $p < 0.05$) (Figs. 3 and 4).

Population Immediate Decline (PID)

Regarding avoidance and mortality, Population Immediate Decline (PID) was predicted from the number of organisms that moved towards other compartments and non-avoiders that might die. If mortality alone is considered, the hazard is underestimated, because part of the population which are mobile (i.e., avoiders) are not considered in the estimation of population decrease. In all the scenarios, Population Immediate Decline (PID) curves from Non-Forced System (NFS) followed the same trend with the avoidance curves of *P. reticulata* for both test chemicals, as no mortality was recorded in the non-forced system (NFS). The population decline curves for various possible scenarios are represented in Fig. 5.

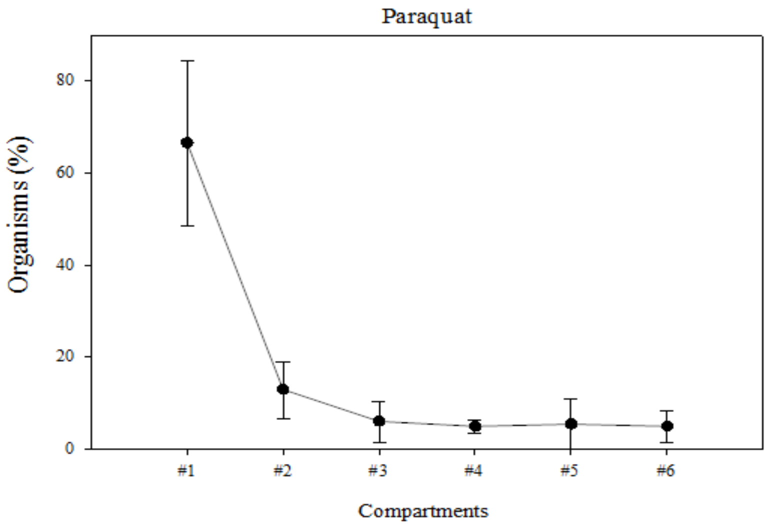

Fig. 3. Mean percentage and standard deviation (n = 9 observation periods) of guppy fish (*Poecilia reticulata*) exposed to contamination gradients of Paraquat pesticide recorded in each compartment for 3h. Different letters represent statistically significant difference

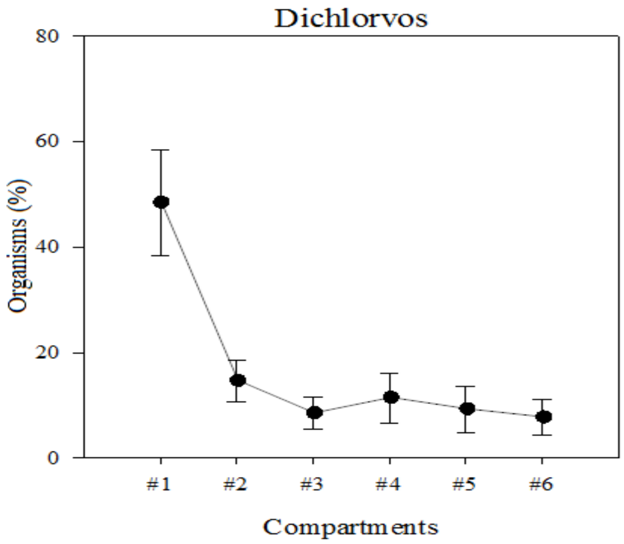

Fig. 4. Mean percentage and standard deviation (n = 9 observation periods) of guppy fish (*Poecilia reticulata*) exposed to contamination gradients of Dichlorvos pesticide recorded in each compartment for 3h. Different letters represent statistically significant difference

AC_{50}, LC_{50}, and PID_{50}

The calculated median concentrations of the pesticides causing avoidance (AC_{50}), mortality (LC_{50}) and population immediate decline (PID_{50}) of exposed guppies exposed to both pesticides considering mortality in the FS and NFS are presented in Table 2. The AC_{50} obtained specifically for Paraquat (0.37 mgL^{-1}) at 96-h of mortality and 3-h of avoidance was about 4.7 times more sensitive than Dichlorvos (1.73 mgL^{-1}). The comparison of AC_{50} and LC_{50} values showed that the avoidance response was approximately 2 (Dichlorvos) to 22 (Paraquat) times more sensitive than mortality (LC_{50} value). Similar trends were observed at 24-h, 48-h, and 72-h of mortality and 3-hr of avoidance response. In each case, Paraquat (116, 41 and 28 times) was profoundly more sensitive than Dichlorvos (12, 3 and 2 times) with respect to Avoidance-concentration to Mortality-concentration (Tables S5a, S5b and S5c - Supplementary Material). The PID_{50} and AC_{50} values were similar in non-forced exposure since there was no mortality in the non forced exposure system.

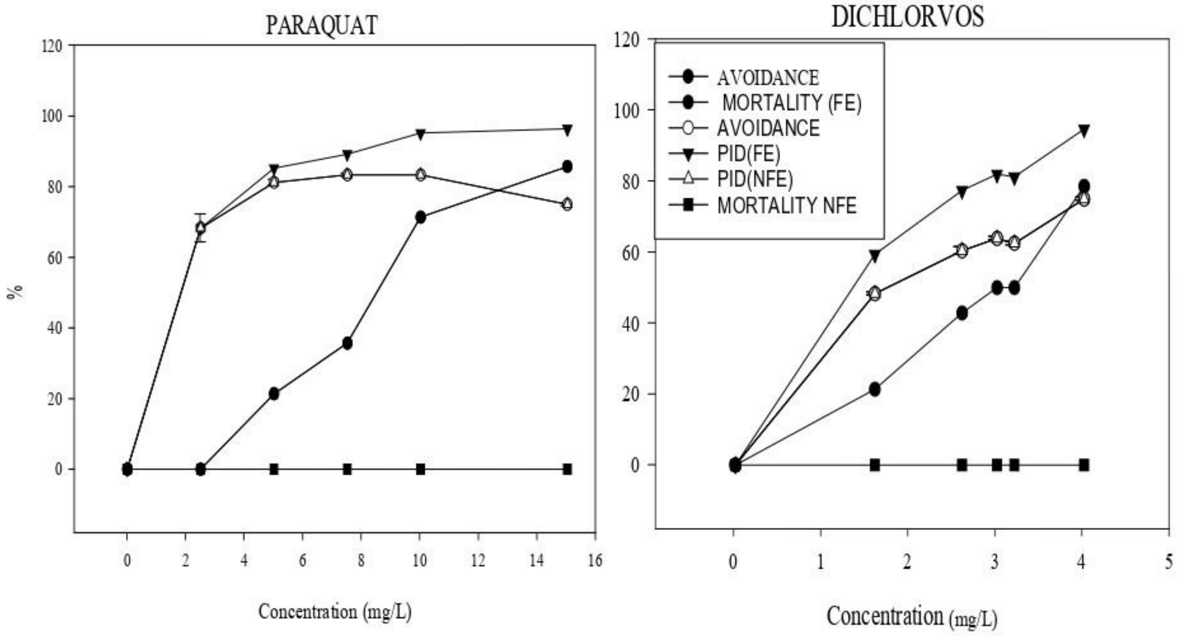

Fig. 5. Concentration-response curves for the avoidance (Non-Forced System) and mortality (Forced System) responses, and the calculated PID (Population Immediate Decline) for *Poecilia reticulata* exposed to Paraquat herbicide and Dichlorvos insecticide

Table 2. Values (in mg/L) of AC_{50}, LC_{50}, and PID_{50} (median concentrations that cause avoidance, mortality, and population immediate decline, respectively) in Guppy fish, *Poecilia reticulata* exposed to Paraquat and Dichlorvos in Forced System (FS) and Non-Forced multi-compartment exposure Systems (NFS)

Chemicals	AC_{50} (NFS)	LC_{50} (NFS)	PID_{50} (NFS)	LC_{50} (FS)	PID_{50} (FS)
Paraquat mgL-1(CL) 10.09)	0.37 (N/A)	> 15	0.37 (N/A)	8.20 (6.69-	1.34 (0.34 – 2.14)
Dichlorvos mgL^{-1}(CL)	1.73 (N/A)	> 4	1.73 (N/A)	2.85 (N/A)	1.31 (0.56 – 1.69)

KEY: CL: Confidence Limit, N/A: Not Available. NFS: Non-forced System FS: Forced System

4. DISCUSSION

The findings from this study have elucidated the responses of the important mosquito bio-control fish, *P. reticulata* to two selected agrochemicals typically used for insect pest and weed control in urban farms. The 96-h LC_{50} values for Paraquat and Dichlorvos (8.20 and 2.84mgL^{-1} respectively) obtained from this study demonstrated that both pesticides were acutely toxic to *P. reticulata* thus, threatening their survival especially in chronic exposure situations due to runoffs from farmlands. This threat is heightened by the fact that the toxicity of both pesticides increased with duration within the 96-h period. The 96hr-LC_{50}, value of Dichlorvos exposure in the present study was slightly higher than 1.84mgL^{-1}reported by Günde and Yerli, [14] for the same species. Several authors have also reported similar acute toxicity responses to Dichlorvos even with other fish species, pointing to a rather consistent pattern of response to this insecticide exposure. For example, some reported LC_{50} values following exposures to Dichlorvos include; *Anabas testudineus* - 2.35 mgL^{-1} [15, 3]; *Aphanius iberus* - 3.17mgL^{-1} [16]; 2.3mgL^{-1}[17] and *Dicentrarchus labrax* - 3.5mgL^{-1} [16].

In general, the WHO [18] noted that for freshwater and estuarine fishes, Dichlorvos is ranked as moderate to highly toxic and 96-hLC_{50} values have been found to range from 0.2 to 12mgL^{-1}, within which the values for the current study lie.

The 96-h LC_{50} of Paraquat on the exposed guppies in this study (8.20 mgL^{-1}) was also comparable to values from previous studies such as that of Kamwing and Furtado [19] who reported 96-h LC_{50} of 7ppm for *Raspora trilineats*. Oloruntuyi et al. [20] however reported 90 ppm 96-h LC_{50} value for Paraquat on *Clarias gariepinus* at a temperature range of 24.5-26.5°C which is similar to the conditions for the present study.

The findings from this study indicated that the *P. reticulata* was able to detect the gradient of both pesticides, implying that the fishes have innate survival abilities provided there is an opportunity to escape toxic conditions. At the highest concentrations of 15.0 and 4.0 mgL^{-1} for Paraquat and Dichlorvos respectively, *P. reticulata* recorded the highest levels of avoidance of 75% each demonstrating their high capacity for survival as three-quarters of their population were able to move away from toxic conditions. Despite these favorable levels of avoidance, 25% of the guppy populations remain at such high risk and they are unable to move away as in FS, then they may die. The consolation lies with the fact that the peak concentrations in the NFS exposures are less toxic than the LC_{50} values of both pesticides, thus enhancing their chances of survival in typical field conditions. Similarly, high levels of avoidance have been reported in the estuarine White leg shrimp *Litopenaeus vannamei* (80%) and the marine fish *Rachycentron canadum*(60%) upon exposure to contaminant gradients of copper for 3-h [9]. Regarding contaminants of emerging concerns, studies of avoidance using NFS have been mainly performed with fishes. For instance, around 50% of a population of zebra fish (*Danio rerio*) avoided 1.4 mgL^{-1} of the fungicide (Pyrimethanil) [21,22]. Only 22% of *P. reticulata* exposed under the same system for 4 h avoided triclosan concentrations as low as 0.2 mgL^{-1} [23]. Morse so, the AC_{50} for *P. reticulata* exposed to a bisphenol gradient was reported to be as low as 0.15 mgL^{-1} [24]. This implies that based on the chemistry of various pollutants, different responses are expected for the same species, thus indicating that pollution management for the sake of protecting a species must be toxicant specific. This also calls for studies involving exposure to combinations at environmentally relevant concentrations to effectively model avoidance responses.

Organisms' behavioral response to the presence of a contaminant by avoiding contaminated sites is a protective strategy to minimize toxic impact [25,26,23]. The use of a non-forced exposure approach gives a simple demonstration of the immediate response of organisms due to the presence of contaminants and the potential for loss of species diversity due to large-scale migration towards less unpleasant habitats [8,27]. Avoidance responses, despite their immediate value for organism survival, might result in disruptions in ecosystems, which receive migrants as well [7]. Apparently, avoidance would be easier and practical when pollutants arise from point sources and/or from unidirectional sources, moving along water current than in cases of diffused sources or in large water bodies such as seas where turbulence is high. While migration from the contaminated area is a solution for the avoiders [28], the consequences to the ecosystem could prompt the loss of abundance and biodiversity [29]. For instance, avoidance by the shrimps, that involves an intermediate trophic level [30], might constrain the measure of food accessible to organisms in the upper trophic strata and decrease the predation pressure on lower trophic levels.

Investigation of population immediate decline in the two pesticide contamination scenarios (i.e., Paraquat and Dichlorvos exposures) demonstrated that avoidance assumed an increasingly more important role in population decline for all concentrations, and was the determining factor in the Population Immediate Decline (PID). At higher concentrations of both test chemicals, the effect on the fish behavior and swimming patterns was turbulent. Oliveira et al. [31] observed that the swimming pattern of *D. rerio* got modified at triclosan concentrations of 400 and 500 mg/L. Such consequence of treatment might impair avoidance response as fishes might lose orientation, which could have been of value for their avoidance movement. The typical patterns of avoidance and mortality reported in the literature propose that an underlying avoidance reaction defines the population decrease in the very short-term, while at higher concentrations, the ability to avoid contamination becomes impaired [22]. Failures in the ability to identify contamination and consequent loss of the ability to avoid toxic concentrations have been observed in Cladocerans and Copepods [32], amphibians [33], and fishes [34]. In other studies, where PID was assessed, avoidance also tend to occur at concentrations lower than mortality [8,23], demonstrating that under conditions of gradual and heterogeneous contamination, the contaminated area might lose part of the organism population due to its escaping from contamination with conceivable longer-term results on local ecosystem structure and functioning. In cases where the contaminant was observed to cause unconsciousness in the organisms [32] and avoidance is prevented, the mortality in the short term might play a more significant role for the PID [12,34].

Regarding the non-forced exposure data, the PID caused by exposure to both test chemicals in this study was exclusively determined by the avoidance response rather than mortality. In any case, when the PID values were determined based on mortality results from the forced exposure, the outcomes were overestimated, since the concentrations which caused mortality in the FS were higher than in the NFS. The capacity to identify a pollution gradient and escape to a more favorable habitat reduces the probability of suffering lethal effects. Notwithstanding, mortality data from FS is valuable for understanding the toxicity potential of a given concentration, and is more realistic for scenarios where a contamination gradient is not expected such as in ponds, lakes and slow-flowing waters. Furthermore, there is a high level of uncertainty in mortality data derived from a NFS, since dead organisms found in a given concentration cannot be unequivocally linked to that concentration, because they could have migrated from another concentration. Thus, caution should be exercised in the use of mortality data from a NFS scenario for the calculation of the PID. Such mortality data from NFS may be employed to provide an overview of contamination effects at the ecosystem or landscape level, instead of at the individual level or at the local spatial scale.

5. CONCLUSION

The outcomes of this study revealed the acute sensitivity of *P. reticulata* to both pesticides triggering its avoidance towards less polluted compartments. The responses observed indicated a decline in the population of *P. reticulata* exposed to various concentrations of both pesticides, which was primarily driven by avoidance behavior. The use of Forced System (FS) may be misleading in evaluating realistic environmental risks of the pesticides because spatial avoidance is not considered a possible response. Lethal effects can be overestimated in FS, due to mandatory confinement of naturally mobile organisms. Thus, it is advised that both types of exposure systems should be simultaneously integrated in ecological risk assessments for the most realistic recommendations.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICS APPROVAL

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This study followed the principles in the Declaration of Helsinki on the humane treatment of animals used in research and the principles in the AVMA Guidelines for the euthanasia of animals [35].

SUPPLEMENTARY MATERIALS

Supplementary material is available in the following link:
https://journalajee.com/index.php/AJEE/libraryFiles/downloadPublic/9

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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