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Research article

Pretreatment of mosquito larvae with ultraviolet-B and nitropolycyclic aromatic hydrocarbons induces increased sensitivity to permethrin toxicity

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ABSTRACT

Nitropolycyclic aromatic hydrocarbons (Nitro-PAH) are highly toxic PHA derivatives. Nitro-PHAs are emitted by carbonaceous materials and PHA post-emission transformation, which causes water and environmental pollution and also exists as carcinogenic and immunotoxic agents. UV light has been shown to cause DNA damage and improves the covalent binding of PAH to DNA significantly. Mosquito breeding grounds are pools of water that can be large open zones or encased ponds with varying levels of sunlight exposure. This research was performed to assess the combined effects of UV-B exposure and Nitro-PAH on the physiological function of *Culex quinquefasciatus* larvae. To assess the impact of UV-B irradiation and Nitro-PAH exposure on mosquito vectors, parameters were examined: (1) Nitro-PAH availability and its impact on cell fatalities; (2) the detoxifying abilities of cytochrome P450, glutathione-S-transferase, and esterase; (3) the reactions to Reactive Oxygen Species; and (4) The resistance of mosquito larvae to three synthetic pesticides (temephos, imidacloprid, and permethrin). UV-B and Nitro-PAH treatment caused cellular damage and increased major detoxification enzymes such as α & β-esterase, cytoP450, CAT, GST, and POX. The levels of oxidative stress, ROS and protein carbonyl content, nitrite, ascorbic acid and thiobarbituric acid were decreased significantly. Toxicology bioassays revealed that UV-B + Nitro-PAH exposure significantly increased larval susceptibility. The current study concludes that prior exposure to Nitro-PAHs and UV-B may make mosquito larvae more vulnerable to chemical insecticides.

1. Introduction

Rivers, lakes, and ponds are examples of aquatic habitats that provide essential services and goods to human communities. They are the most polluted ecosystems, with contaminants such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) from skin-care products and agrochemicals (Zhang et al., 2010). Because of their toxic effects to humans, nitropolycyclic aromatic hydrocarbons (Nitro--PAH) are a key problem (Bandowe and Meusel, 2017). The biotransformation of PAHs into secondary extremely toxic metabolites by cytochrome P450 monooxygenase enzyme activity is strongly linked to the toxic effects of PAH in biological entities (Reynaud and Deschaux, 2006). Nitroaromatic products are extremely harmful and genotoxic and the majority of them are known or suspected carcinogens (Lotufo et al., 2009; Padda et al., 2003).

Several NPAHs are toxic pollutants to environment, since they share the same properties as agrochemicals, making them hazardous to human health (Tiwari et al., 2019). The nitroaromatic component (monocyclic

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and heterocyclic) can interact with genetic material and cause genotoxicity were extensively studied and evaluated (Purohit and Basu, 2000; Singh et al., 2015; Gooch et al., 2017; Basu, 2018). Nitroaromatic and oxidation/reduction components can directly damage DNA or form oligomers that cause mutagenesis due to nucleotide misincorporation during DNA replication. The position of the anion on the aromatic ring as well as the presence of many other functional moieties, according to structural and spectroscopic studies, can influence the genotoxicity and carcinogenic effects of these contaminants (Purohit and Basu, 2000; Basu, 2018). PAHs absorb UV (280–400 nm) and visible (400–700 nm) light due to their facilitating photolysis and biotransformation (Haritash and Kaushik, 2009). PAH photodegradation may produce metabolites that are far more detrimental than the parent compounds (Abbas et al., 2018).

UV irradiation is known to produce reactive oxygen species (ROS), which are reactive chemical compounds (hydroxyl radical, superoxide anion, and so on) capable of severely damaging cellular components, DNA, lipids, and proteins, eventually leading to apoptosis (Fu et al., 2012). PAHs have been shown to stimulate ROS production in *Phaeodactylum tricornutum* (Liping and Binghui, 2008) when exposed to fluoranthene and copper. UV-B rays could have a considerable impact on plant-phytophagous insect interactions (Paul and Gwynn-Jones, 2003; Murugan et al., 2016). Because of UV-B radiation's negative effects, its use in pest control has been considered, particularly for insects and mites that are farmland serious pests that are the most challenging to control owing to their rapid rate of reproduction and later growth of pesticide resistance (Dermauw et al., 2013; Ramkumar et al., 2019). A most common effect of increased UV-B exposure on crops is a reduction in the density of arthropod herbivory (Rousseaux et al., 2004).

Mosquitoes transmit a wide range of pathogenic organisms, which can cause serious infections in humans. Due to the lack of cost-effective and time-consuming chemotherapy drugs for the majority of mosquito-borne illnesses, existing control measures focus on reducing vector populations through the use of synthetic pesticides (Hemingway et al., 2006; Stoops et al., 2019; Dusfour et al., 2019). Despite this, chemical use has negative consequences, such as low mosquito selectivity and toxic effects on non-target organisms (Zhang et al., 2010; Ramkumar et al., 2014; Stoops et al., 2019). The majority of a mosquito's life is spent in water, where the four larval stages and pupa can develop. These breeding sites, which are highly polluted by natural or anthropogenic activities, including farmlands as well as densely populated areas, can produce large UV gradients in addition to the effect of contaminants (Jurado et al., 2012; Zhang et al., 2010). PAHs alter mosquito metabolism, increasing their tolerance to synthetic pesticides while causing no significant toxicity (Poupardin et al., 2012). Furthermore, exposing mosquito larvae to UV-A throughout their larval development enhanced detoxifying enzymes as well as epigenetics in accordance with an earlier reported (Tetreau et al., 2013). As a result, sunlight exposure may have a significant impact on the efficacy of insecticide-based mosquito control methods. The current study aimed to see combined exposure to UV-B light and Nitro-PAHs influenced mosquito larvae physiology (cell death rates, detoxifying enzymatic activity, and free radicals accumulations) and mosquito tolerance to permethrin, imidacloprid and temephos insecticides.

2. Materials and methods

2.1. Mosquito strain

The mosquito larva (*Culex quinquefasciatus*) was used in all assays. The larvae were kept in the laboratory under controlled conditions at 27 °C with 70–80% of relative humidity and 14:10 light/ dark ratio. A 3:1 mixture of dog biscuit and yeast granules was fed to the larvae.

2.2. Nitro-PAH + UV-B irradiation

UV-B light with 280–315 nm range with the luminance of 300 W/cm^2 was employed as the origin to irradiate *Cx. quinquefasciatus* larvae. Before being used in our studies, 250 larvae were randomly selected and exposed to Nitro-PAH (Nitro-anthracene) for 24 h at different concentration (0.001–0.008 ppm), with 1-h exposure to UV-B light and the control and Nitro-PHA only (Tetreau et al., 2013; WHO, 1998). The samples were stored at −80 °C for further studies.

2.3. Localization of nitro-PAHs and detection of cell death

When exposed the Nitro-PAH to UV light, it glow brightly (Xia et al., 2013). To assess the localization of Nitro-PAH within mosquito body tissue, larvae exposed to significant concentrations (50 mg/L) of Nitro-PAH were observed using an Olympus phase contrast microscope with light source and fluorescence. To identify cell fatalities caused by Nitro-PAH + UV-B light exposure, 20 larvae from each treatment were submerged in a mixture of 40 g/L propidium iodide (PI) (Thermo Fisher Scientific, Cat. No. P1304MP) for 3 h in the absence of light cleaned and placed in purified water for 1 h. The mosquito's larvae were examined under fluorescence with the excitation and emission of 450–490 and 510–540 nm respectively, which allowed to identify, which encompasses the nuclei of dead/damaged cells.

2.4. Sample preparation

The mosquito larvae of *C. quinquefasciatus* were weighed before being homogenized. The mosquito larvae were homogenized in ice-cold phosphate buffer (pH 7.2) in a 0.1 g/bw to 1 ml of buffer ratio. The homogenates were subjected to centrifugation for 15 min at 4 °C at 10,000 g, and the supernatant was used for further analysis. Protein contents were measured using Lowry's method (1951) (Ramkumar and Shivakumar, 2015).

2.5. Detoxification enzyme activities

2.5.1. Cytochrome P450 assay

The cytochrome P450 activity was determined using a modified method developed by Brogdon et al. (1989). In brief, 20 μl of supernatant was extracted from a 200 μl of homogenate containing 6.3 mM TMBZ solution (pH 5.0). Afterward, 80 μl of 0.063M potassium phosphate buffer (pH 7.2) and 25 μl of 3% H_2O_2 were added to each aliquot. 2 controls were prepared using 20 μl of homogenizing buffer does not contain the enzymatic source for each sample. Upon 30 min at room temperature, absorbance at 630 nm was observed. The measured absorbance was translated as the formation of the end product using a Cytochrome C standard curve. Overall cytochrome P450 activity was measured in p mol equivalent cytochrome P450 mg protein^{-1} min^{-1} (Ramkumar and Shivakumar, 2015).

2.5.2. Glutathione- S-transferase (GST) assay

The GST assay was carried out using the protocol described by Habig et al. (1974), with minor modifications. In brief: To 2.78 ml of sodium phosphate buffer (pH 6.5), 50 l of 50 mM 1-chloro-2,4-dinitrobenzene and 150 l of 50 mM reduced glutathione were incorporated (pH 6.5). The enzyme stock was then added to the sample in a volume of 20 μl. The process was performed twice. The components were rotated and incubated for 3 min at 20 °C before being shifted to a cuvette and the absorbance was measured at 340 nm with a Systronics 118 UV-Vis Spectrophotometer type 2205. The absorbance at 340 nm was measured for 10–12 min using the kinetics menu. A unit of enzymatic (GST) activity was described as the quantity required to catalyse the

conjugation of 1 mol/L GSH to CDNB/ min/mg of protein. GST activity was measured in terms of U. mg $^{-1}$ protein (Ramkumar and Shivakumar, 2015).

2.5.3. Esterase assay

Esterase activity was determined using a procedure modified slightly from Kranthi (2005). A 6-ml reaction solution (0.1 ml of 0.3 mM –napthyl acetate and 0.2 ml of enzyme source) was pre-incubated for 20 min at 30 °C without light before adding a blending of 1.0 ml fast blue BB salt and sodium dodecylsulfate (SDS) solution with the ratio of 2:5. A Systronics 2205 UV–vis. spectrophotometer was used to measure absorbance at 590 nm after the content was blended. A standard curve was used to calculate the activity of -napthyl acetate (Ramkumar and Shivakumar, 2015).

2.5.4. Peroxidase assay (POX)

The modified methodology of Reddy et al. (1995) was used to determine POX activity using a Systronic UV-vis. spectrophotometer at 430 nm by stimulating the oxidation reaction in the occurrence of H_2O_2 as the substrate. One unit of POX activity was described as the amount of substrate to catalyzed 1 mg substrate/min/ mg of protein. POX activity was expressed as U. mg $^{-1}$ protein.

2.5.5. Catalase assay (CAT)

The percentage of H_2O_2 decomposition by catalase was used to quantify this enzyme activity using a UV-vis. spectrophotometer (Luck, 1971). A unit of catalase activity was described as the amount of H_2O_2 decomposition/sec/ g of protein. The catalase activity was measured as the U/g^{-1} of protein.

2.6. Non-enzymatic assays

2.6.1. Total reactive oxygen species (ROS) determination

The NBT assay was performed to assess total ROS by following Beauchamp and Fridovich (1971) procedure, with minor modifications, by simply adding 1 ml 2 M KOH and 1 ml of DMSO towards the sample and reading the absorbance at 630 nm. The OD results were compared with a reference curve constituted with NBT, and ROS production was calculated as μM NBT equivalent/10 mg tissue.

2.6.2. Nitrite content determination

The nitrite content was determined by following the protocol described by Ding et al. (1988) by applying Griess reagent. The nitrate reductase converted nitrate to nitrite and absorbance was measured at 540 nm. The quantity of azo chromophore precisely includes the level of nitric oxide in samples. The concentration of nitrite (M/mg) was calculated by employing sodium nitrate as a reference.

2.6.3. Ascorbic acid levels determination

Roe and Kuether's (1943) methodology was used to assess ascorbic acid content by combining dehydro-ascorbic acid with 2,4-Dinitrophenylhydrazine (DNPH) and treating the resulting byproduct with H_2S0_4 to produce a red colour that absorbs adequately at 520 nm. The enzyme activity was measured and expressed in μM/mg of protein.

2.6.4. Protein carbonyl content determination

The protein carbonyl concentration was determined by that interact with DNPH, resulting in the generation of acid hydrazones, and was measured at 370 nm, according to Levine et al. (1990).

2.6.5. Determination of the thiobarbituric acid reactive substances (TBARS)

Janero (1990) lipid peroxidation methodology was used to determine the malondialdehyde content (MDA). The lipid peroxidation was typically measured by assessing MDA, which was developed by interacting with thiobarbituric acid (TBA) to produce a red colour including an adsorption optimum at 532 nm. MDA content was measured in nanomoles of MDA obtained per mg of protein.

2.7. Bioassay

Toxicity testing was performed on 4th instar larvae exposed to Nitro-PAH for 24 h and 1 h UV-B light exposure. Following pretreatment, the larvae were placed in each insecticide solutions (750 μl/L, 250 μl/L, or 250 μl/L of imidacloprid, permethrin, or temephos) prepared in distilled water. A total of 25 larvae were used for each treatment and three replicates kept for each dose. Control larvae received only distilled water (WHO, 2005, 1998). For each insecticide, triplicates were kept at each concentration. After 24 h of treatment to each insecticide, larval death was measured. The mosquito larvae fatalities were calculated using Probit analysis (Abbott's, 1925), to generate the LC_{50} and LC_{90} values for a 24 h exposure.

2.8. Difference between UVA, UVB and UVC

UV radiation is classified into three categories based on wavelength, each with a unique biological activity and ability to penetrate human skin. When UV radiation has such a shorter wavelength (100–400 nm), it is more harmful to mosquito larvae. UV radiation with shorter wavelengths, on the other hand, has a lower ability to penetrate the skin. UVA (315–400 nm), UVB (280–315 nm), and UVC (200–280 nm) are the three bands that make up the UV region (IARC, 2012). While UVC is most harmful type of UV rays, it is completely blocked by the atmosphere and never reaches the earth's surface. While fewer medium-wavelength UVB rays penetrate the Earth's ozone layer, those that do are extremely biologically active and can harm organisms than UVA light (Chang et al., 2010). While UVA has a significantly longer wavelength and accounts for nearly 95 percent of UV rays that reach the Earth's surface, it is not particularly harmful to biological organisms (Gibson, 2006; IARC, 2012).

2.9. Statistical analysis

The findings were presented as mean and standard deviation (±SD). One-way ANOVA was used with a post-hoc Dunnett's multiple comparison test were performed to determine whether there were considerable variations ($P < 0.05$). SPSS version 13 software was used for statistical analysis. The enzyme data was analyzed using the Graph Pad- PRISM statistical package software (Version 5.0).

3. Results

3.1. Nitro-PAHs and UV-B induced cell death

The exposure of *C. quinquefasciatus* larvae to Nitro-PAHs + UV-B light resulted in the formation of crystals in the head and abdomen, which is an indicator of cell viability (Figure 1a and b). Overall, exposing larvae to these Nitro-PAHs + UV-B light increased larval tolerance to insecticides and induced cell death with a more pronounced effect observed with higher concentrations of xenobiotics.

3.2. UV-B and Nitro-PAHs on enzymatic activities

Larval exposure to Nitro-PAH + UV-B led to significant modifications of their GST, P450 and esterases activities, as measured using model substrates. We found notable increase in esterase, CytP450, CAT, GST, and POX enzymes ($p < 0.05$) in *C. quinquefasciatus* larvae exposed to Nitro-PAH + UV-B exposure (Figures 2a, 2b, 3, 4, 5 and 6). While no significant changes were observed after exposure to UV light.

3.3. UV-B and Nitro-PAHs on non enzymatic activities

The influence of UV-B and PAHs on known antioxidant enzymes involved in insecticide metabolization, including ROS, carbonyl content of proteins, Nitrite, Ascorbic acid and Thiobarbituric acid levels were

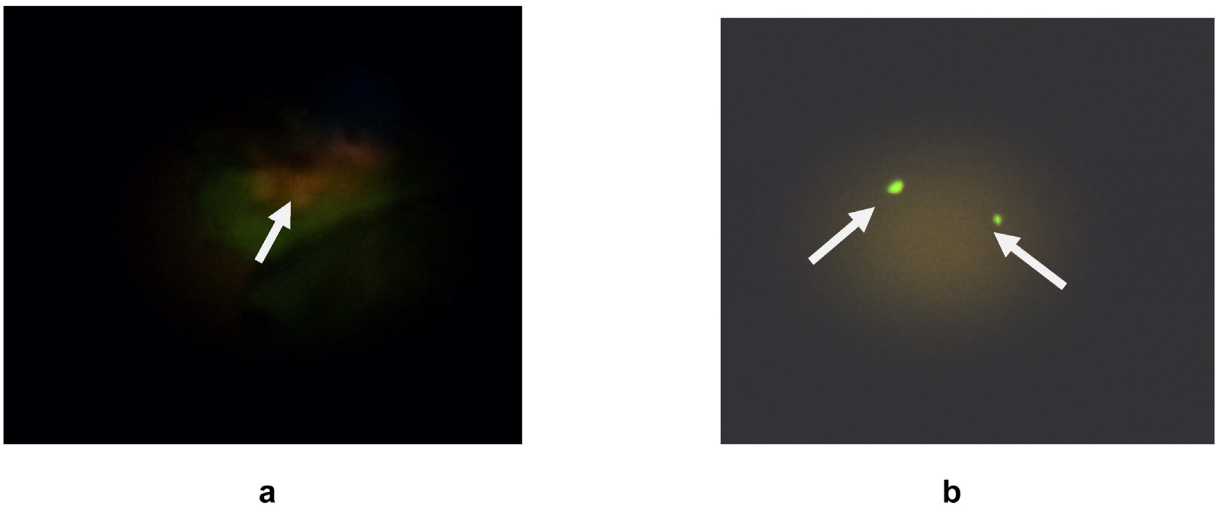

Figure 1. Effect of UV-B and NPAH exposure on cell and tissue mortality in the posterior region of the larvae. (a) Localization of NPAHs in mosquito larvae (b) diffused green color in head specifically indicates the presence of soluble NPAHs.

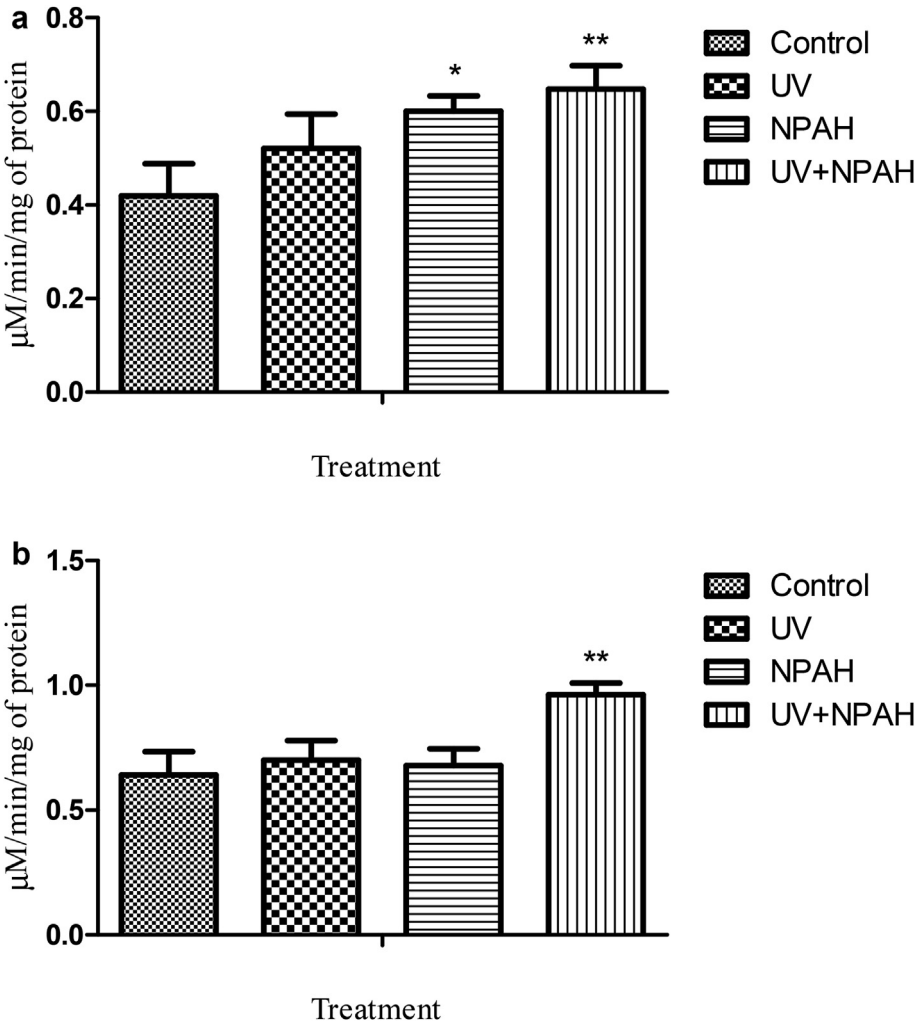

Figure 2. (a) Effects of NPAH and UV-B light on α-esterase activity of *Culex quinquefasciatus* larvae. Values are mean ± S.D. (n = 250). Asterisk designates statistically significant difference (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). (b) Effects of NPAH and UV-B light on β-esterase activity of *Culex quinquefasciatus* larvae. Values are mean ± S.D. (n = 250). Asterisk designates statistically significant difference (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

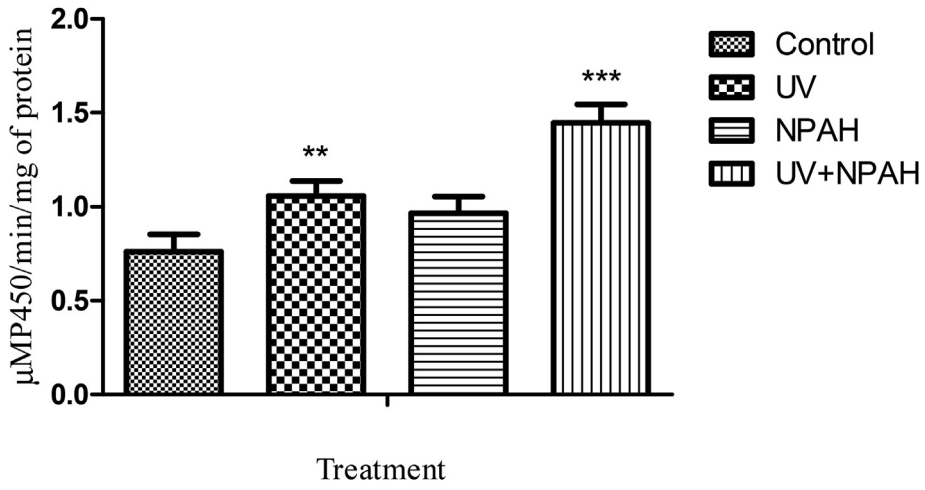

Figure 3. Effects of NPAH and UV-B light on cytochrome P450 activity of *Culex quinquefasciatus* larvae. Values are mean ± S.D. (n = 250). Asterisk designates statistically significant difference (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

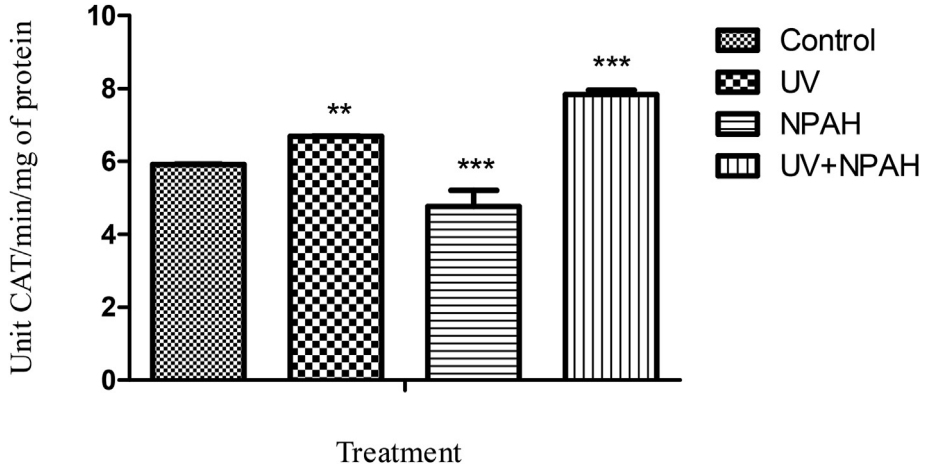

Figure 4. Effects of NPAH and UV-B light on CAT activity of *Culex quinquefasciatus* larvae. Values are mean ± S.D. (n = 250). Asterisk designates statistically significant difference (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

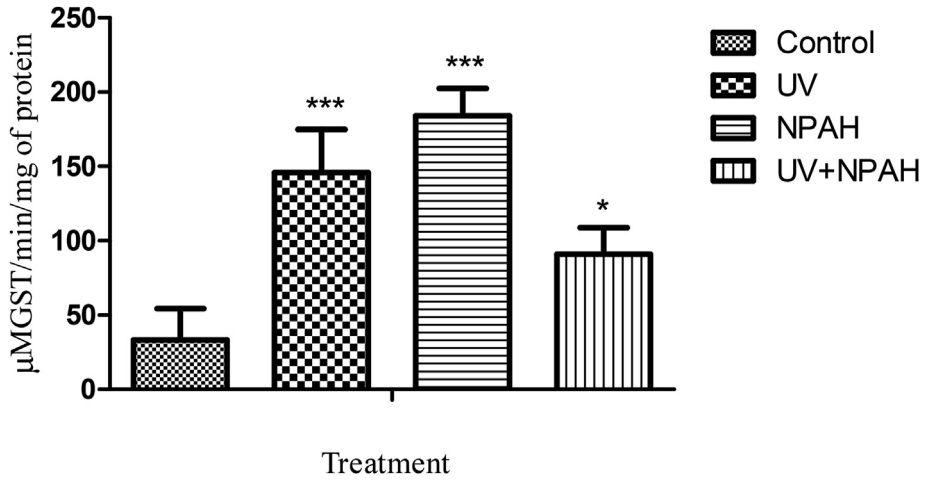

Figure 5. Effects of NPAH and UV-B light on GST activity of *Culex quinquefasciatus* larvae. Values are mean ± S.D. (n = 250). Asterisk designates statistically significant difference (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

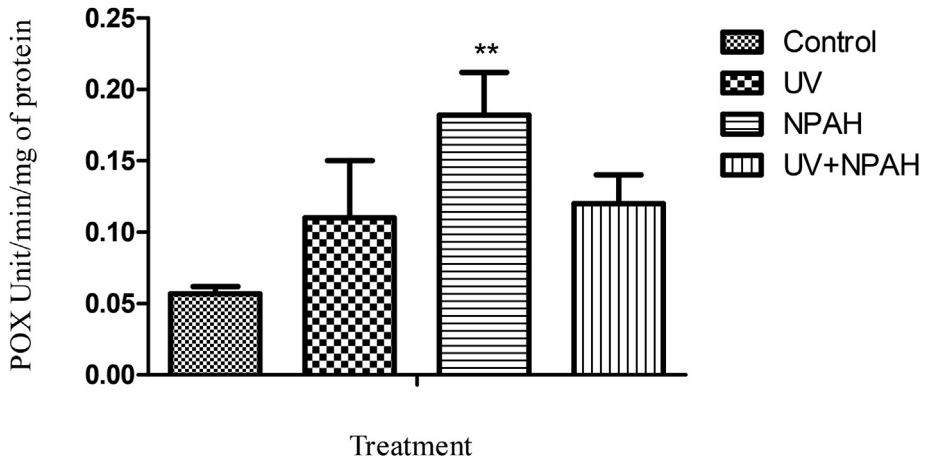

Figure 6. Effects of NPAH and UV-B light on POX activity of *Culex quinquefasciatus* larvae. Values are mean ± S.D. (n = 250). Asterisk designates statistically significant difference (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

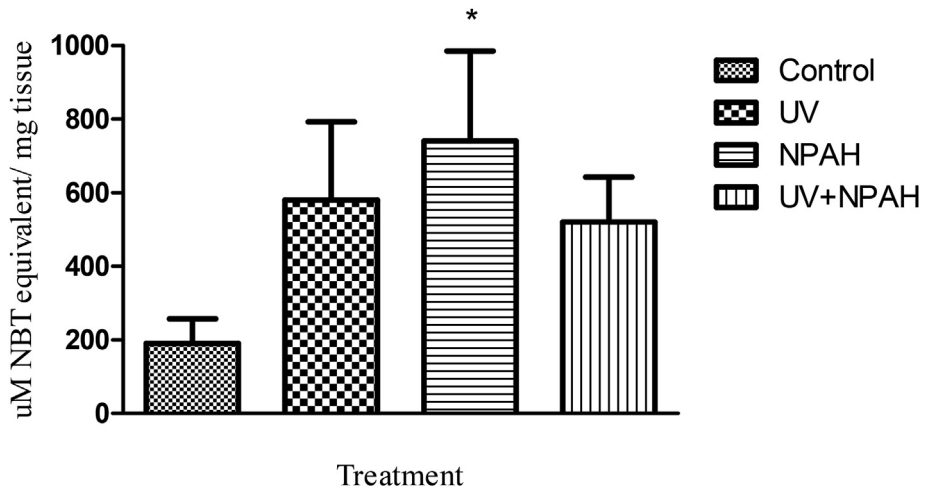

Figure 7. Effects of NPAH and UV-B light on Total ROS levels of *Culex quinquefasciatus* larvae. Values are mean ± S.D. (n = 250). Asterisk designates statistically significant difference (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

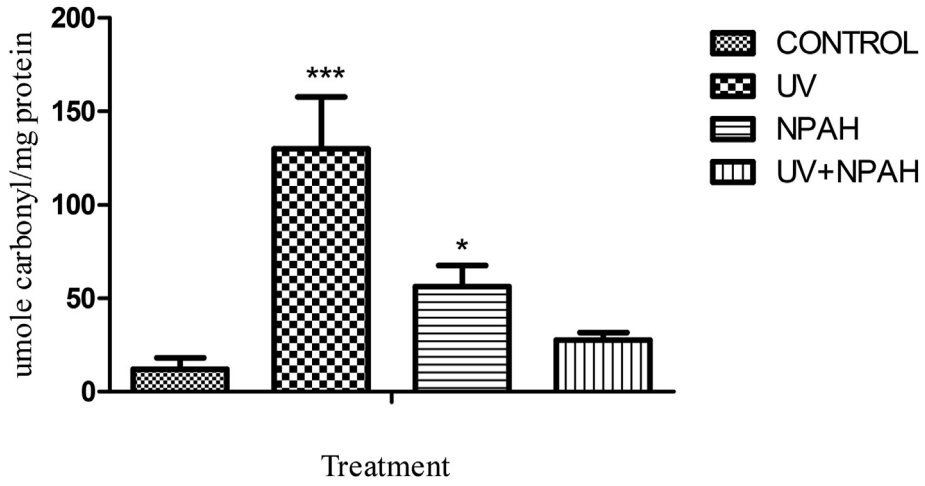

Figure 8. Effects of NPAH and UV-B light on Protein carbonyl content of *Culex quinquefasciatus* larvae. Values are mean ± S.D. (n = 250). Asterisk designates statistically significant difference (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

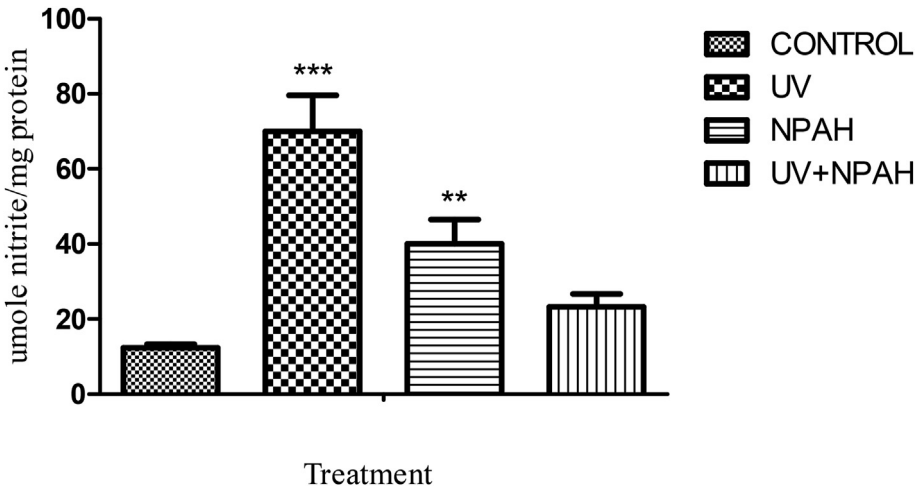

Figure 9. Effects of NPAH and UV-B light on Nitrite levels of *Culex quinquefasciatus* larvae. Values are mean ± S.D. (n = 250). Asterisk designates statistically significant difference (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

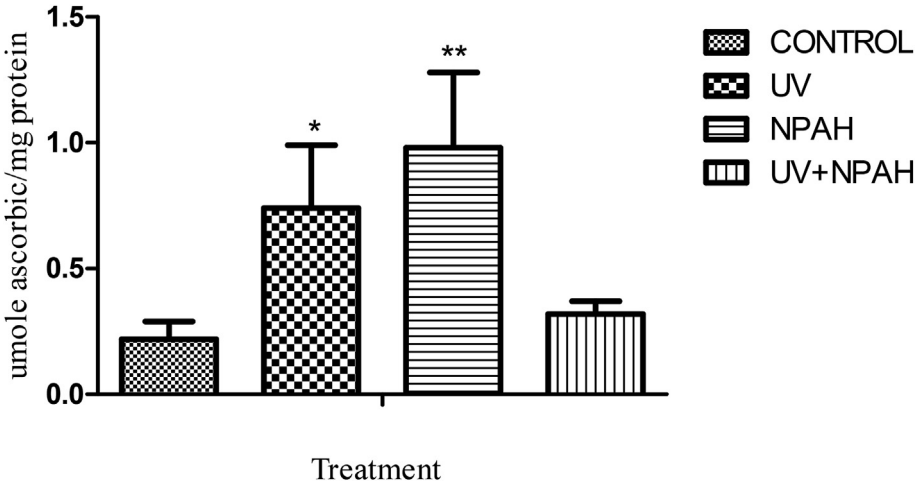

Figure 10. Effects of NPAH and UV-B light on Ascorbic acid levels of *Culex quinquefasciatus* larvae. Values are mean ± S.D. (n = 250). Asterisk designates statistically significant difference (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

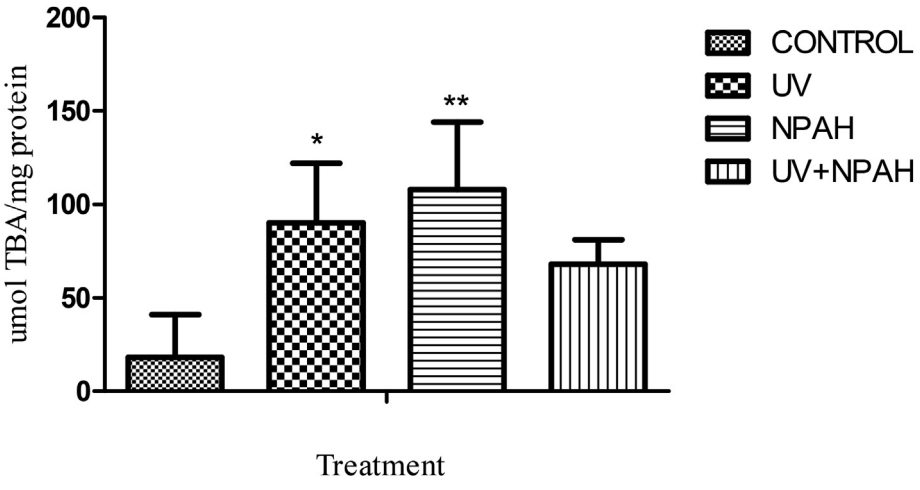

Figure 11. Effects of NPAH and UV-B light on Thiobarbituric acid levels of *Culex quinquefasciatus* larvae. Values are mean ± S.D. (n = 250). Asterisk designates statistically significant difference (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

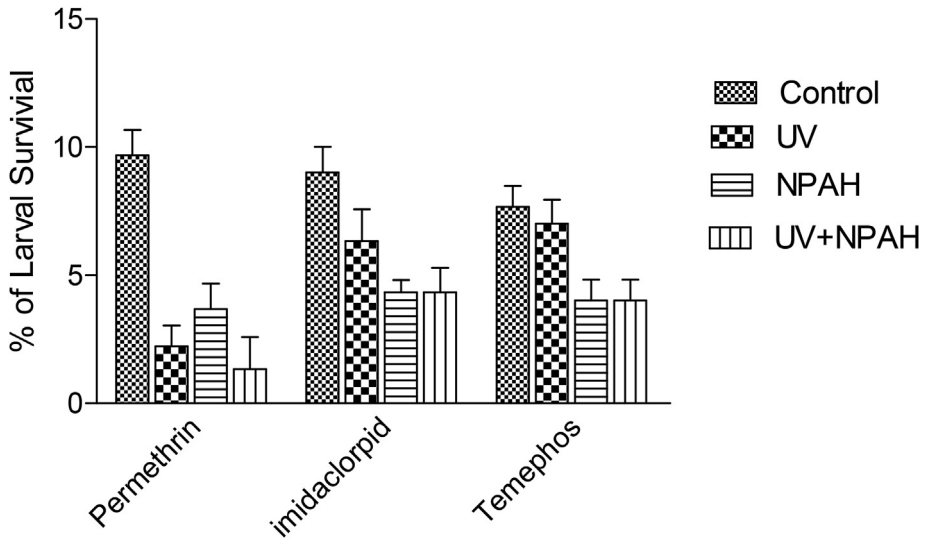

Figure 12. Insecticide susceptibility of mosquito larvae exposed to Effect of UV-B and NPAH exposure on insecticide susceptibility of mosquitoes: The values are expressed Mean ± SD.

significantly reduced after exposure to Nitro-PAH + UV-B (Figures 7, 8, 9, 10, and 11).

3.4. UV-B and Nitro-PAHs on mosquito tolerance to insecticide

The vulnerability to three different insecticides was evaluated with Nitro-PAH + UV-B exposed larvae. Nitro-PAH + UV-B exposure did not affect mosquito larvae vulnerability to temephos, but it significantly increased mortalities due to permethrin sensitivity upon Nitro-PAH + UV-B exposure. Under these conditions, of the three insecticides, permethrin was the only one that was effective in killing the larvae with low concentrations (LC_{50} = 0.65145; LC_{50} = 1.74348; mg/ml), respectively (Figure 12).

4. Discussion

According to our findings, UV-B + Nitro-PAH crystals formed in the heads of larval mosquitoes. The crystallization could be caused by a detoxification system activity saturation which results in the deposition of UV-B + Nitro-PAHs in the head cavity. Surprisingly, in the absence of UV-B, Nitro-PAH had no effect on cell deaths in larvae, whereas the addition of UV-B resulted in significant cell fatalities. Two distinct but related manifestations may be at work: (1) after being exposed to UV-B, PAH becomes photosensitized, causing significant cell damage; and (2) photosensitized PAH is thought to stimulate the formation of selectively toxic reactive oxygen species (Fu et al., 2012).

Numerous studies have found that xenobiotics can stimulate insect detoxifying enzymes and that there is a link between increased detoxifying enzyme activity and tolerance to synthetic insecticides (Fu et al., 2012). Our findings show that exposing mosquito larvae to both UV-B and Nitro-PAHs reduces their tolerance and capacity for detoxification of insecticides, as well as the antioxidant enzymes developing resistance to synthetic insecticides. Our study confirmed that the detoxification situation quickly biotransforms merged UV-B and Nitro-PAHs into proximate carcinogenic metabolites and PAH-diols (Benzo[*a*]pyrene-4, 5-oxide, Benzo[*a*]pyrene-7, 8-diol-9, 10-epoxides, Benzo[*a*]pyrene radical cations) and that the metabolites cause cell death (Shimada, 2006). When larvae were exposed to nitro-PAHs (Nitro-anthracene) and UV-B light, their cytochrome P450 and esterase activities increased, their ROS levels decreased, and their larval mortality to chemical insecticides increased (Ramkumar and Shivakumar, 2015).

Furthermore, we investigated how Nitro-PAHs and UV-B exposure affected the function of detoxifying enzymes, which are involved in toxic compounds decontamination and oxidative stress response. Nitro-PAHs and UV-B irradiation both increased esterase activity. Co-exposure to Nitro-PAHs and UV-B accelerated this initiation. UV-B has earlier been used to stimulate esterase activities in mosquitos (Tetreau et al., 2013), but the exact mechanism involved is unknown. This pattern has also been observed in aquatic organisms and it appears to be caused by a significant relationship between ROS on P450 enzyme activity both in-vivo and in-vitro (Marionnet et al., 2006). The majority of GST activity aids in the management of ROS produced by such an oxidative agent (Yang et al., 2010), which explains how GST activity increases in response to UV-B exposure. Regarding the impact of Nitro-PAH disclosure on GSTs, our findings are in accordance with research that reported that exposing arthropods and fish to PAHs did not improve GST activity (Jebali et al., 2013; Poupardin et al., 2012). Ultimately, we demonstrated unique characteristics of insecticide vulnerability in this study only after exposure to both Nitro-PAHs and UV-B. The concurrent exposure of Nitro-PAHs and UV-B increased mosquito sensitivity to permethrin insecticides, resulting in a 90 % increase in mosquito larvae mortality.

Nitro-PAHs and UV-B light are environmental toxins that have an impact on living organisms such as mosquito larvae. In the current study, mosquito larvae exposed only to NPAHs and UV-B alone were less sensitive to permethrin insecticide. However, when UV-B light and NPAHs were combined, they increased sensitivity to chemical toxins, implying that combining these two treatments with a chemical insecticide in mosquito breeding sites could improve mosquito control efforts. Finally, UV-B and NPAHs are abiotic characteristics that can affect mosquito physiology and insecticide tolerance. However, more research is needed to understand the specific mode of action for these complex interactions.

Declarations

Author contribution statement

Govindaraju Ramkumar: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Ranganathan Muthusamy: Analyzed and interpreted the data; Wrote the paper.

Mathiyazhagan Narayanan: Contributed reagents, materials, analysis tools or data.

Rajendran Dhanapal; Chinnannan Karthik; MS Shivakumar; Govindhan Malathi; Kariyanna: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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