

Effect of Bisphenol S on Duration of Stages of Life Cycle and Selected Biochemical Parameters in *Culex quinquefasciatus* Say

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ABSTRACT: *Culex quinquefasciatus* Say, is the principal vector of filariasis. Bisphenol A- substitute, Bisphenol S (BPS) is a xenobiotic used in plastic products, which leach out to the environment. The present studies focuses on the effect of BPS on the duration of stages of life cycle and select biochemical parameters in *C. quinquefasciatus* at 0.5, 1 and 2 ppm. A significant reduction in the duration of life cycle was observed in egg rafts reared in BPS. An increase in the total protein content along with a reduction in total free amino acid was observed in the 4th instar larvae. The content of uric acid showed a sharp increase. An elevation in the level of aspartate aminotransferase and alanine aminotransferase were observed. The present study revealed that xenobiotic, BPS which is now widely used as a substitute for endocrine-disruptor BPA facilitated rapid moulting in *C. quinquefasciatus* mosquitoes.

Keywords: Bisphenol S, *Culex quinquefasciatus*, Full life cycle test, aminotransferases, total protein, uric acid.

INTRODUCTION

Synthetic polymers known as epoxy resins contain among their components, Bisphenol A (BPA) or its derivatives, which are used to improve the durability of inner varnishes of food cans and in the manufacturing of different kinds of food containers, such as baby bottles, microwaveable containers and plastic dairy containers. Endocrine disruptors such as Bisphenols and Phthalates have harsh effects on the human reproductive system (Roy *et al.*, 2009). The Bisphenols also are associated to endometrial hyperplasia and endometrial cancer in human beings. Several reports have shown the migration of BPA from cans and baby bottles to foods (Cao and Corriveau 2008; Yonekubo *et al.*, 2008).

Bisphenol-S [bis (4-hydroxyphenyl) sulfone (BPS)], has two phenolic rings are linked by a sulphur dioxide (SO₂) group with chemical formula, C₁₂H₁₀O₄S. BPS is of interest for the preparation of high temperature resistant thermosetting thermoplastic polymers. After health concerns associated with Bisphenol A grew in 2012, BPS began to be used as a replacement. There has been less research on BPS than on BPA, but preliminary studies have shown that it also possesses hormone-mimicking properties (Delfosse *et al.*, 2012; Grignard *et al.*, 2012; Kitamura *et al.*, 2005; Kuruto-Niwa *et al.*, 2005).

Culex quinquefasciatus is a mosquito vector for *Wuchereria bancrofti*, the filarial worm that causes filariasis, which is one of the six important tropical diseases. Approximately 80 million people are infected globally, and of these approximately 30 million chronic

cases manifest as typical elephantiasis. The disease is highly prevalent in developing countries such as India. Moreover, Japanese encephalitis virus has been isolated from field-collected *C. quinquefasciatus*. This makes *C. quinquefasciatus* an important vector species (Pidiyar *et al.*, 2004).

Actions of Bisphenol A and Bisphenol S on the reproductive neuroendocrine system during early development in Zebra fish were examined (Qiu *et al.*, 2015). Studies have also revealed that the BPA-substitute Bisphenol S alters the transcription of genes related to endocrine, stress response and biotransformation pathways in the aquatic midge *Chironomus riparius* (Herrero *et al.*, 2018). Studies have been conducted on the effect of environmental pollutant, Bisphenol A on life cycle and protein turn over in *Culex quinquefasciatus* (Gayathri, 2019). Embryonic and larval developments were shortened in *C. quinquefasciatus* on full life cycle exposure to Bisphenol A, where it acted as a developmental agonist (Valsala and Asirvadam 2022). Typical breeding sites of *C. quinquefasciatus* include polluted waters rich in organic matter and plastic wastes. While the impact of Bisphenol S related endocrine disruption in humans and vertebrates have been extensively studied, its effects on invertebrates have not been elucidated.

Against this background, the present study was undertaken and focuses on the effects of BPS on the duration of stages of life cycle and selected biochemical parameters *C. quinquefasciatus*.

MATERIAL AND METHODS

Egg rafts of *Culex*. sp. of mosquitoes were collected by keeping 3 litres of water mixed with active yeast powder in buckets placed at damp corners of the College campus. Mosquitoes started laying eggs after 2-3 days, once foul smell emanated from the bucket of water sample and egg rafts were collected in the mornings during 2021-2022. Soon after the collection of *Culex* egg rafts, two rafts each were placed in containers (20cm diameter and 8.5cm depth) filled with 850 ml dechlorinated tap water and 150 ml solution from the medium used to collect the *Culex* egg (provided as a source of food). Even at high concentrations of 200 ppm, 100 % mortality of larval instars was not obtained. Hence the efforts to determine LC50 and rearing with sub lethal concentrations were discarded. Environmentally relevant concentration of BPA was found to be 1 ppm (Gayathri, 2019). Hence in the case of BPA- substitute, BPS, concentrations were selected above and below environmentally relevant concentration of BPA (1 ppm), along with 1 ppm.

Accordingly, experimental containers had select concentrations of 0.5, 1 and 2ppm of BPS. Every third day fresh BPS was added to maintain the concentrations at 0.5, 1 and 2 ppm. Rafts placed in containers without BPS served as control. For study of selected biochemical parameters, 4th instar larvae were used, identified by standard taxonomic keys (Barraud, 1934; Tripathy and Dash 1998). Experiments were conducted in the research laboratory of Zoology Department, University College.

Full life cycle test (eggs are allowed to develop up to adult in select concentrations of BPS) was performed to determine the effect of select concentrations of BPS on life cycle duration of *C. quinquefasciatus*. Protein estimation was done by using Folin's reagent (Lowry *et al.*, 1951). Total free amino acids were estimated by the method of Spies, 1957. Uric acid was estimated by Uricase/PAP method by Trinder (1969). Activity of

AST was assayed by Reitman and Frankel's method, 1957. Activity of AST was also assayed by Reitman and Frankel's method 1957.

All the experiments were repeated six times. The kit for estimation of uric acid and aminotransferase were from Biolab (Mumbai). All other chemicals were purchased from Sigma-Aldrich (USA).

The data was subjected to One way Analysis of Variance (ANOVA) using SPSS 24.0 software (IBM, USA). Data was considered to be statistically significant, if $P \leq 0.05$. When F values obtained in ANOVA indicated significant difference, Duncan's Multiple Range Test (DMRT) was employed to discern specific difference among the control and tests. The groups that were not significantly different in DMRT were considered as homogeneous.

RESULTS AND DISCUSSION

Despite the fact that invertebrates represent more than 95% of known species in the animal kingdom and are extremely important regarding ecosystem structure and function, to date there is little information on the effects of BPS in these organisms. The lack of information is a major concern, given that BPS is a ubiquitous pollutant that can be found in almost any environmental compartment, is able to interact with numerous invertebrate species, and thus may disrupt trophic chains and alter the natural balance of ecosystems (Herrero *et al.*, 2018). Fourth instar larvae appeared in 1 ppm and 2 ppm. Pupa appeared in 0.5 ppm and 1ppm treatments on the seventh day and in 2 ppm on sixth day, compared to ninth day in control. Adult mosquitoes appeared on the eighth day in 1 ppm and 2 ppm treatments compared to eleventh day in control. In terms of hours, adult emerged in 2 ppm, 80 hours earlier than in control (Fig. 1).

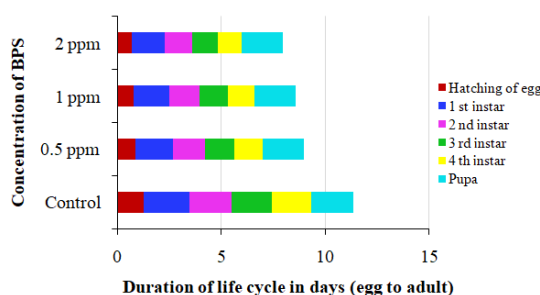


Fig. 1. Duration of life cycle stages (in days) of *C. quinquefasciatus* larvae reared in BPS.

The study was conducted by rearing *Culex quinquefasciatus* in BPS at 0.5ppm, 1ppm, and 2ppm concentrations. It was observed from this study that, the exposure of *C. quinquefasciatus* to BPS at three select concentrations resulted in shortening of the duration of life cycle. Reduction in duration of life cycle in test larvae agreed with the observations made in *C. quinquefasciatus* exposed to organophosphate insecticide and temperature stress (Mpho *et al.*, 2001).

A similar result is obtained in the study conducted on *Culex* mosquitoes using temperature as a stress factor. With increase in temperature a gradual decrease in life cycle duration was observed (Ciota *et al.*, 2014).

Shortening of life cycle was more significant from the hatching of egg up to 4th instar stage in BPS exposed egg rafts. No significant change was observed in pupal duration in control and BPS reared larvae. Shortening of life cycle span is an adaptive response shown by the

insect to escape the stress created by the xenobiotic, BPS. Its effect is mainly enabled through endocrine regulation, especially by accelerating the rate of moulting. Shortening of life cycle duration in *Culex* larvae exposed to environmentally relevant concentrations of Bisphenol A has also been observed. Reduction in the duration of various stages of life cycle has been attributed to upregulation of genes regulating production of 20-Hydroxyecdysone, 20-E, active form of moulting hormone (Valsala and Asirvadam 2022).

The total protein content in 4th instar *C. quinquefasciatus* larvae reared in select concentrations of BPS were found to be significantly higher than that of the control larvae. A concentration dependent increase in the level of protein in body tissue of 4th instar larvae of *C. quinquefasciatus* (Fig. 2) Values are mean (n=6) ± S.E.M (Standard Error of Mean). Mean values of different superscript letters (a, b, c) were significantly different (p ≤ 0.05).

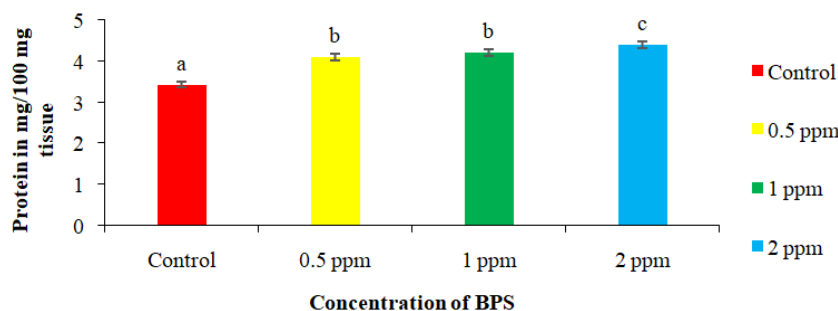


Fig. 2 Total protein in 4th instar larvae of *C. quinquefasciatus* reared in select concentrations of BPS.

The total free amino acid content of the 4th instar larvae of *C. quinquefasciatus* reared in select concentration of BPS was significantly lower than that of the control group. Minimum concentration of total free amino acid

was shown by 4th instar larvae reared in 2 ppm BPS (Fig. 3). Values are mean (n=6) ± S.E.M (Standard Error of Mean). Mean values of different superscript letters (a, b, c) were significantly different (p ≤ 0.05).

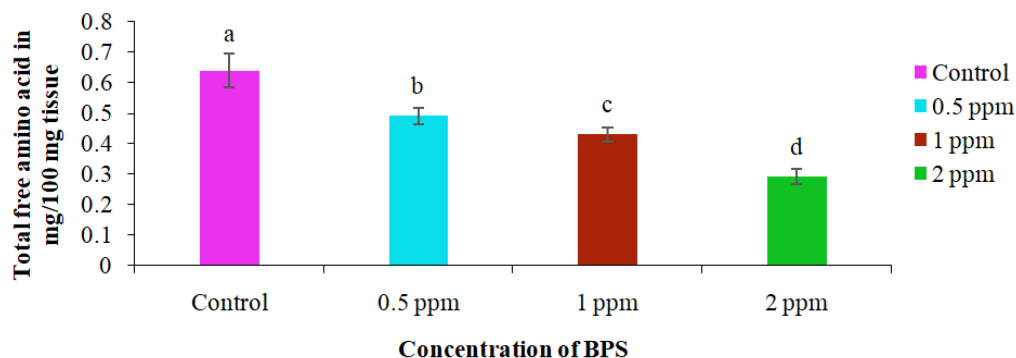


Fig. 3. Total free amino acid in treated larvae of *C. quinquefasciatus*.

The total protein content and total free amino acid content of the treated 4th instar larvae were examined. The total protein content showed a significant concentration-dependent elevation while a significant concentration-dependent decrease in the level of amino acid was observed. Similar results were obtained from studies conducted on *C. quinquefasciatus* were the larvae adapt to temperature shock through changes in protein turn over and amino acid catabolism (Gayathri and Evans 2018).

The amount of uric acid present in BPS treated 4th instar larvae of *C. quinquefasciatus* was significantly elevated than that of the control group. The uric acid concentration increased gradually with the increase in concentration of BPS (Fig. 4). The uric acid content in

4th instar *C. quinquefasciatus* larvae reared in BPS showed a significant elevation. The amount of uric acid in control was less when compared to those larvae reared in BPS. Elevation of uric acid in test larvae agreed with observations made in *Oryctes rhinoceros* larvae exposed to cow dung experimentally contaminated with *Bacillus thuringiensis israelensis* spores (Nayar *et al.*, 2010). Exposure of adult male *Periplaneta americana* to electromagnetic radiation (EMR) emitted by mobile phones also resulted in an increase in uric acid content (Syalima *et al.*, 2017).

Values are mean (n=6) ± S.E.M (Standard Error of Mean). Mean values of different superscript letters (a, b, c) were significantly different (p ≤ 0.05).

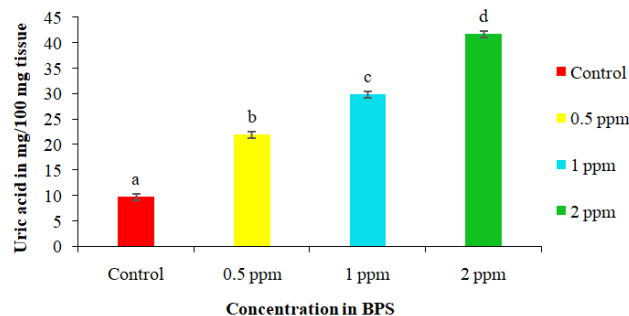


Fig. 4. Uric acid in treated larvae of *C. quinquefasciatus*.

The elevation in uric acid level is a clear indication of increased catabolism of total free amino acids. Hyperuricemia together with decrease in total free amino acid have pointed out another target in the host body, the DNA. Increased catabolism of nucleotides may also lead to hyperuricemia.

The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) showed a significant

elevation in BPS reared larvae compared to control. The activity of transaminases expressed as ALT/AST ratio showed a significant reduction in *C. quinquefasciatus* larvae exposed to BPS (Fig. 5).

Values are mean (n=6) ± S.E.M (Standard Error of Mean). Mean values of different superscript letters (a, b, c) were significantly different ($p \leq 0.05$).

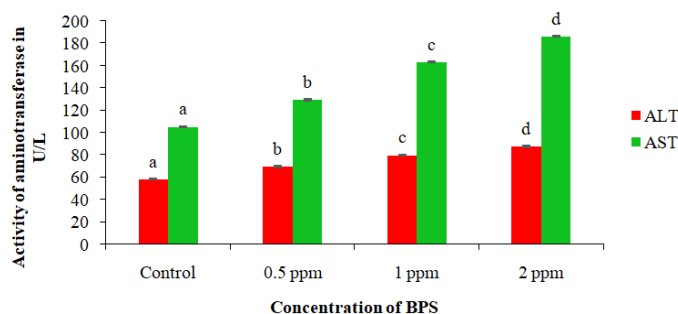


Fig. 5. Activity of aminotransferases in 4th instar *C. quinquefasciatus* larvae reared in select concentrations of Bisphenol S.

In the present study, the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), showed a significant elevation in *C. quinquefasciatus* larvae exposed to BPS. The findings are also in accordance with the reports in *Oryctes rhinoceros* larvae exposed to *Bacillus thuringiensis israelensis* spores (Nayar *et al.*, 2010) and *Periplaneta americana* exposed to toxic concentrations of fenvalerate (Reddy and Yellamma 1991).

CONCLUSIONS

Widespread awareness on endocrine-disrupting properties of BPA has created a huge influx of its substitutes in to the market and finally to the ecosystem. The present study is an eye-opener due to the fact that BPA substitute BPS is also having similar effects on the aquatic ecosystem, especially on *Culex* mosquitoes in which they reduce the life cycle duration producing more number of generations within a brief time. Plastic waste management itself can act as a vector control strategy through which release of mosquito developmental agonist in the ecosystem can be minimized.

FUTURE SCOPE

Further studies are needed to study the effect of BPS on expression of genes regulating metamorphosis especially 20- Hydroxyecdysone(20-E). Investigations

on the effect of similar plastic leachates on the life cycle of mosquito larvae will give us a comprehensive picture whether shortening effect on life cycle is due to synergism among the xenobiotics.

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Conflict of Interest. None.

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