SUMMARY

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Screening study infer that pesticides intoxication may induce oxidative stress (OS) more in lower organism. However, the effect of sub-chronic exposure of pesticides on livestock leading to OS requires further evaluation at molecular level. After an acute exposure, the risk assessment should be performed to quantify the exposure of humans to that particular contamination.

The research work was targeted to explore toxic impacts of azadirachtin (AZT) at physiological, biochemical and molecular levels of a common agri-field organism, *Spathosternum prasiniferum prasiniferum*. Subsequently, protective potential of antioxidant enzymes was also investigated against AZT induced toxicity in this species.

The present work dealt with mortality values of AZT in the both sexes adult of *S. pr. prasiniferum* were exposed to different concentrations of AZT and corrected mortality was calculated according to Abbott method. On the basis of the evaluated mortality (%), different concentrations (1, 5, 10, 15 and 20 ppm in water and control where grasses without pesticide are used) were selected for subsequent experiments to assess toxicity of AZT in *S. pr. prasiniferum*. This study was based on exposure to AZT for a period of 48-hrs (in-vivo) and 6-hrs (in-vitro). Five treatment groups were designed for the study in compared to control.

Decline in physical activities of both female and male adults were observed after initial toxicity. Reduced physical activities and changes in behaviour were seen in intoxicated adult insects. Therefore, the above results suggested that, besides antifeedant property AZT is toxic at the physiological level of *S. pr. prasiniferum*.

Insects reared in control food showed well organized body with uniform appearance. Interestingly, insects exposed to different concentrations of AZT for 48-hrs mani-

fested deformed body architecture. Ecdysis deformity was also evident in AZT treated adults.

Effect of AZT at physiological level of *S. pr. prasiniferum* was discussed in this study. Azadirachtin exposure was detected to alter normal nutritional-indices of this acridid species. Food ingestion, digestion and consumption index (CI) which are necessarily increased in higher doses, whereas the approximate digestibility (AD) is not significant increased though efficiency of conversion of digested-food (ECD) and efficiency of conversion of ingested-food (ECI) are reduced after treatment of AZT.

Next, impact of AZT on this species, specifically at molecular level was investigated. Post-treatment increase in activities of antioxidant enzymes were noticed in female. Such increase in enzyme activities indicated an induction of central detoxification system of the body. In addition, increased activities of endogenous antioxidant enzymes like superoxide-dismutase (SOD)/catalase (CAT) revealed abundance of reactive oxygen species (ROS) and OS within cellular environment female than male. Reactive oxygen species mediated lipid peroxidation (LPO) and protein degradation were also evident in the form of higher malondialdehyde (MDA) and non-protein-soluble-thiols (NPSH) contents in tissue samples of treated species. Molecular chaperones are essential for maintaining protein homeostasis.

The DNA-strand breaks were evident through DNA-fragmentation gel electrophoresis assay in AZT exposed insect. Overall, findings of this work indicated that, exposure to higher concentrations of AZT altered/modulated cellular environment of *S. pr. prasiniferum*.

In cellular level response were analysed in this species following exposure of AZT. Survival assay of several cell types from different organs were carried out. In-vivo

and in-vitro biochemical assay of different organs revealed decreased defence system in treated male in comparison to female.

Since the alimentary tract confronts major amount of pesticide entering through oral route, OS was performed to observe any tissue damage in the gut of AZT exposed insect. Antioxidant markers revealed damages in various parts of whole gut and MDA significantly increased with an impairment of soluble-thiols in male. The antioxidant-enzymes were variably impaired resulting in tissue-damages more in male than female.

At brain level, there are small amounts of enzymatic and non-enzymatic antioxidants. Elevation of thiobarbituric-acid-reactive-substances (TBARS) and oxidative redox parameters like SOD/CAT in female brain suggests that female can respond in better way to overcome the OS against such pesticide toxicity.

Haemocytes are the key players of innate immunity in insect's body that are present in haemolymph. Hence, effect of exposure of AZT on haemolymph of insects was analysed to detect immunotoxic potential of the AZT. Therefore, these findings confirmed the cytotoxic potential of AZT in *S. pr. prasiniferum*.

The gonads are the primary reproductive organ of animals. This experiment suggests that AZT increased the major biomarkers with decreasing antioxidant enzyme activities resulting in more free radicals (FRs) related threat in adult male gonads. More toxicity in male than female finally ends up in less net reproducibility of the species in toxicity.

Antioxidant activities of in instar-II decreased significantly in comparison to instar-IV and that evidently shows that AZT impairs the instar specific juvenile tissue. This suggested that, SOD/CAT actively scavenged FRs thereby minimizing the stress in treated instar-IV.

Study at molecular level indicated that, AZT caused excessive ROS production that produced adverse effects in specific stages of the life cycle and of specific gender.

Hence, the study was focused towards endogenous antioxidant enzymes to observe any protective potential against AZT induced toxicity in these insects.

Acute exposure to AZT enhanced antioxidant enzyme activity within the adult female body which indicated reduced cellular stress. Instar-IV showed a sharp increase in activities of endogenous antioxidant enzymes (SOD/CAT) and influenced nutrient-metabolizing-enzymes. For a sustained/chronic exposure, the risk assessment is important at community level with a focus on its long-term ecological and environmental impact.