PREFACE

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Tremendous use of pesticides during recent years indicates the significant role played by pesticide in modern civilization. They not only formed as an important tools of modern agriculture but also saving life of millions of people in developing and underdeveloped nations suffering from several diseases spread by insect. But the unluckiest fact remains that these pesticides make the life of non-target species hell, amongst them insects are the worst targets.

Besides the use as insecticides, organophosphates (OPs) are used as chemical warfare agents and clinical medicine. Majority of OP compounds share the common feature of being excellent inhibitors of cholinesterase. The action of OPs is known a great deal at the cellular and molecular levels. As there is an urgent need to use non-toxic and biodegradable chemicals derived from plants, azadirachtin (AZT) a neem based pesticide emerged by offering an economic and eco-friendly solution. Even though its persistency is less in the environment, its safety to animals is not well documented. Hence, a study is aimed to understand the toxic potentials of AZT as bio-pesticide on *Spathosternum prasiniferum prasiniferum*.

The findings of the present study are presented in different organs of this specific insect species. First screening of pesticide through study on different in-vitro tissues from common agri-field animals. Second screening of model insect by assessing the availability in common agri-field. Third stage devoted to application of selected pesticide on selected pest to determine the nutritional-indices and oxidative stress (OS) by estimating the levels of stress-markers. Fourth work deals with the antioxidant detoxification mechanism studying selective enzymes. Fifth work devoted with genotoxicity to study DNA-fragmentation changes of gonadal tissues under AZT. The present work was not investigated in deeper way and by no means complete. It represents only a preliminary effort on

the part of the author. The author may be excused for any other lacunae in this present investigation.

Toxicological information of AZT on this species in India is scanty. In this present investigation, I have studied the toxicity of different concentrations of AZT on this acridid species. The functional status of selected tissues of this species was examined under exposures of AZT under controlled laboratory condition. The sensitivity of this species in altered environmental conditions provides an appropriate opportunity to investigate its cellular and biochemical functioning under selected environmental stress. Present information would provide a database for determination of the nature and magnitude of toxicity of this botanical pesticide in this species (Orthoptera: Acridoidea).

ABSTRACT

Human continuously uses pesticides in agri-field to control pest population and weeds for considerable agricultural productivity. Side-by species like grazing animals, are adversely affected by it during the time of pesticide exposure. Pests are either affected by or become resistant to the pesticides. Insects, birds (hen) and mammals (cattle/goat) that are the common dwellers of agri-field represents three distinct phylum having versatile physiological features. Studies on the effect of common pesticides on their DNA-stability and important enzymatic-activities are scanty. Acridids (short-horned-grasshopper) are highly abundant living-organism/major-component in the vegetation's prevailing in grasslands, forests and agri-fields, which are distributed largely, globally. Among the acridids found in West Bengal, *Spathosternum prasiniferum prasiniferum* (Walker 1871) are multivoltine in nature and were considered for the present study. It acts as a dependable bio-indicator species in response to environmental stress. Azadirachtin ($C_{35}H_{44}O_{16}$, AZT) is a tetranortriterpenoids present in neem, which develops antifeedancy/growth-regulation/fecundity-suppression/sterilization/oviposition/repellence and deformity in insect via biochemical/cellular changes/oxidative stress (OS) by the production of free radicals (FRs) and causes their death. Reactive oxygen species (ROS) are cytotoxic factors generated in invertebrates in stress conditions. Antioxidants protect insects by scavenging FRs. Agricultural productivity/quality/eco-sustainability is concerned to this issue. Acridids are bio-indicator of grassland ecosystem and they are highly abundant. During cultivation, insects are exposed (dose/time dependant) to AZT. Antioxidants activity (superoxide-dismutase; SOD/catalase; CAT by gel-zymogram-assay) and DNA-stability (fragmentation assay) in hepatic/gut tissues were studied for screening after in-vitro exposure of commercially available common insecticides like chlorpyrifos, fenvalerate, nimbecidine/AZT to cow/goat/bird-hen/insect-grasshopper. The newly hatched adults

(female:male = 1:1) are exposed to 1 to 20 ppm AZT for 48-hrs and have compared to control and 6-hrs incubation with same concentration of AZT for in-vitro experiments. Here, I investigated the dose dependant AZT toxicity on nutritional-indices/biochemical/physiological influences OS-marker (alkaline-phosphatise/ALP; thiobarbituric-acid-reactive-substances/TBARS; non-protein-soluble-thiols/NPSH; acetylcholinesterase/AChE) and antioxidant enzyme activity (superoxide-dismutase/SOD; catalase/CAT; glutathione-peroxidase/GPx) and enzymes that metabolizes nutrients (amylase/cellulase) of gut, brain, haemolymph, gonads from both sexes and juvenile tissues of this species. Food-weight/insect-weight/excreta-weight were recorded before/after the experiment to evaluate the consumption-index (CI), approximate-digestibility (AD), efficiency of conversion of digested-food (ECD) and efficiency of conversion of ingested-food (ECI). In general, all pesticides were found to impair enzymatic-activities. However lower organisms are affected more than higher vertebrates when treated with AZT. The DNA-fragmentation was found more in insects/birds than that of the mammals in hepatic/gut tissues. Inversely, toxicity/antioxidant marker-enzymes were more responsive in insect gut tissues. However, mitochondrial toxicity has revealed variable effects in different genus/species. From screening of insecticide, it is noticed, chlorpyrifos to be the most toxic pesticide followed by fenvalerate/nimbecidine (azadirachtin, AZT). Nevertheless, comparatively less toxic AZT revealed its higher DNA-destabilizing effects in the field-insects than the other tested animals. Azadirachtin developed restlessness, jerky-movements and swarming movements in the insects. Nutrient-metabolizing-enzymes (amylase/cellulase) were screened. Azadirachtin (>7ppm) decreased ECD and restricted food consumption that increased insect-mortality (50-80%). The gut malondialdehyde (MDA) significantly increased with an impairment of soluble-thiols. The antioxidant-enzymes were variably impaired resulting in tissue damages more in male than female.

Finally, AZT influenced nutrient-metabolizing-enzymes and antioxidant enzymes indicating these parameters to be good stress-markers. It promoted OS-marker in brain/haemolymph in both sexes but female had significantly stimulated antioxidant enzymes to overcome cellular-stress. Increase of brain TBARS, antioxidant-enzymes and decrease in NPSH by AZT indicates OS-induction in this species. The brain-DNA was noticed in several instances. Both in-vivo and in-vitro experiment demonstrated significant influences on oxidative biomarkers with increasing antioxidant enzymatic activities in either sex. The male gonads represents decreasing antioxidant enzyme activities compared to female gonads. Lesser protection by CAT and SOD are noticed in male than female in response to AZT exposure. This experiment suggests that AZT increased the major biomarkers with decreasing antioxidant enzyme activities resulting in more FRs related threat in adult male gonads. Variable dose responses were noticed on ALP, AChE and MDA in either gender suggesting multiphasic action of the pesticides. Higher mortality rate is noticed in male with lower nymphal life span. Moreover, instar-II is more susceptible than instar-IV in-vitro intoxication of AZT. In generally with some prominent adaptive strategies, female insects responded more intensely. Field-insects are highly integrated to their ecosystem and to the local bio-geo-chemical cycle. Nevertheless, some of the sensitive biochemical parameters of this organism may be used as biomarker for pesticide toxicity. Possible life threat of vast representatives of agricultural ecosystem by pesticide should be avoided to maintain different bio-geo cycle and eco-sustainability. This finding might be extrapolated for further evaluation of the ecological impact of AZT on the food chain/web in a composite-ecosystem.

Keywords: pesticides, azadirachtin, agriculture, livestock, grasshopper, gut, brain, haemolymph, gonads, juvenile tissue, nutritional-indices, oxidative stress toxicity, reactive oxygen species, antioxidant defence, DNA-stability