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RESEARCH ARTICLE

SILKWORM, BOMBYX MORI (L.) FAT BODY ATPASE'S ACTIVITY ON EXPOSURE TO SUB-LETHAL AND SUB-SUB LETHAL DOSES OF COPPER

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ABSTRACT

Mulberry gardens are being contaminated by various pesticides and fungicides from nearby agricultural practice. Specifically copper containing fungicides utilized in adjacent cultivations where thecrops is being protected by copper fungicides to control the fungal pathogens. Copper besides other heavy metals and pesticides can also alters the activities of ATPase's of silkworm larvae particularly sodium/potassium, magnesium, and total ATPase's. Hence the author aimed to study the effect of copper on silkworm ATPase's activity. In the present study we observed that the ATPase's activity was decreased in 2 days exposure when compared with 4 days exposure of copper in sub-lethal dose than the control worms. However there was a slight decreased ATPase activity in 2 days exposure period at sub-sub lethal dose of copper. Further it is observed that at sub-sub lethal dose on exposure period of 4 days there was no significant change in ATPase's activity. It is concluded that the sub-sub lethal dose (1.75 μg) could not induce toxic effect and silkworm could get recovered on prolonged exposure of four days exposure. This could be due to detoxification mechanism triggered in fat body cells of silkworm during the recovery process.

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INTRODUCTION

Sericulture is being practiced in drought prone areas by the farmers in India along with groundnut crop and grape cultivation. Silkworm is an important economic insect of the order Lepidoptera, and the studies on this silkworm species is an important area of research to improve the sericulture industry in India as well as in other developing countries where sericulture is practiced as a livelihood for the sustainable development of poor and marginal farmers. Copper is known to be an essential trace element and is an integral part of various enzymes from lower to higher level organism. Copper fungicide especially in liquid form rich in copper source are effective in controlling many fungal infection and sometimes certain quantity of copper is normally required for healthy growth and development of plants. The aerial application of copper rich fungicide and certain insecticide find its way into mulberry cultivation and contaminate them along with several other pollutants causing a major threat to Bombyx mori larvae and affecting its silk yield. Similar threats are observed in mulberry plantation due to mismanagement of fungicide and pesticide in Japan and China where sericulture is extensively practiced (Hayakawa *et al*., 1976; Chen and Wu, 1994; and Nath, 1997).

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However, there are many reports that copper at an excessive quantities alter the biochemical parameters of several organism including silkworm (Flemming and Trevors, 1989; Eisler, 1998; Nath, 2000; Kabala and Singh, 2001, andPourakbar et. al., 2007). Adenosine triphosphatase (ATPase) is mostly associated with cell membrane of living organisms. This intrinsic protein enzyme is found in cells which are involved in primary active transport of sodium (Na^+) and potassium (K^+) ions (sodium-potassium pump). ATPase enzyme hydrolyze ATP molecule in to ADP and phosphate ion. They play a major role in import and export of metabolites necessary of regulating the cell metabolism and also counter act on toxins, wastes and solutes majorly hindering the cellular process (Lehninger, 1993). Hence the author is aimed to study the effect of copper at sub-lethal and sub-sub lethal doses in the silkworm *Bombyx mori* fat bodies ATPase activity.

MATERIALS AND METHODS

Silkworm eggs ($PMXCSR_2$) were procured from state sericulture department grainage Hindupur, Andhra Pradesh. Silkworm rearing was conducted at the Dept of Ecology and Environmental Sciences, Pondicherry University as per the standard rearing practice suggested by Krishnaswamy (1978). Fifth instar silkworm larvae were selected for the present investigation. Lethal dose of copper was estimated as 17.54

μg/kg body weight of larvae as reported by Kalai Mohan and Vijaya Bhaskara Rao (2017). The sub lethal dose of copper (3.5 μg/kg`bw) and sub-sub lethal dose(1.75 μg/kg.bw) was exposed after the moulting of fourth instar larvae for the first 2 days and some worms were exposed for 4 days. Silkworm fat body homogenate was prepared according Nath (2003) homogenate was centrifuged at 1,000×*g* for 10 min and in the resulting supernatant protein content was determined by folin-phenol method of Lowry *et al.* (1951).Estimation of total ATPase activity was followed bythe method described by Tirri *et al.* (1973). Na^+/K^+-ATPase activity was calculated from the difference between total ATPase activity and Mg^{2+}-ATPase activity (Na^+/K^+, Mg^{2+}-dependent ATPase). The inorganic phosphorus (*pi*) liberated was assayed according to the method of Fiske and Subbarow (1925). Duncan multiple range test (Duncan, 1955) was used for statistical analysis.

RESULTS

Table 1: *Bombyx mori* fat bodies total ATPase activity (μmol Pi/h×mg protein) on exposure to sub-lethal and sub-sub lethal dose of copper

	Sub lethal dose	Sub-sub lethal dose
Control	5.77 [c]	5.59 [a]
	± 0.43	± 0.19
2 days	2.85 [a]	2.41 [b]
	(50.60)	(47.49)
	± 0.15	± 0.32
4 days	3.62 [b]	5.42 [a]
	(37.26)	(1.52)
	± 0.21	± 0.02

Each value is mean of eight estimation, ± S.D and value followed by the same letter are not significantly different from each other by Duncan's multiple range test (DMRT). Values in parenthesis indicate percent change over control. The data related to the amount of total ATPase activity in fat bodies of silkworm on exposure to sub lethal and sub-sub lethal doses of copper is showed in table 1. Between the control groups and the copper treated groups the amount of ATPase activity was more in the fat bodies of control groups. The fat bodies at sub lethal dose (3.5 μg/kg`bw) of copper exhibited maximum decrease in on all exposure periods (2 and 4 days). However, at sub-sub lethal (1.75 μg/kg`bw) dose of copper the fat bodies exhibited maximum increase in ATPase activity when compared to sub lethal dose. Further the increase in total ATPase activity was not showed significant difference when compared to control group of silkworm larvae.

Table 2. Bombyx mori fat bodies Na^+/K^+-ATPase activity (μmol Pi/h×mg protein)on exposure to sub lethal and sub-sub lethal doses of copper

	Sub lethal dose	Sub-sub lethal dose
Control	5.21 [c]	5.19 [a]
	± 0.23	± 0.70
2 days	1.96 [b]	3.52 [b]
	(62.38)	(40.24)
	± 0.64	± 0.16
4 days	2.85 [a]	4.97 [a]
	(45.29)	(4.23)
	± 0.47	± 0.32

Each value is mean of eight estimation, ± S.D and value followed by the same letter are not significantly different from each other by Duncan's multiple range test (DMRT). Values in parenthesis indicate percent change over control.

The Na^+/K^+ ATPase activity in the fat bodies of silkworm on exposure to sub-lethal and sub-sub lethal doses of copper is showed in table 2. Among the control group the amount of Na^+/K^+ ATPase activity was more in the fat bodies at all exposure periods (2 and 4 days). At sub lethal dose (3.5 μg/kg`bw) $Na^+/K^+$ ATPase activity decreased, however on 4 days of exposure at sub-lethal dose $Na^+/K^+$ ATPase activity increased when compared to 2 days exposure period in the silkworm. In sub-sub lethal dose (1.75 μg/kg`bw) at 4 days of exposure period exhibited increased Na^+/K^+ ATPase activity in fat bodies when compared to sub lethal exposed group.

Table 3. Bombyx mori fat bodies Mg^{2+}ATPase activity (μmol Pi/h×mg protein) on exposure to sub lethal and sub-sub lethal dose of copper

	Sub lethal dose	Sub-sub lethal dose
Control	5.17 [a]	5.81 [a]
	± 0.61`	± 0.17
2 days	1.98 [c]	4.12 [b]
	(61.70)	(29.08)
	± 0.39	± 0.33
4 days	3.37 [b]	5.75 [a]
	(34.81)	(1.03)
	± 0.11	± 0.14

Each value is mean of eight estimation, ± S.D and value followed by the same letter are not significantly different from each other by Duncan's multiple range test (DMRT). Values in parenthesis indicate percent change over control. The Magnesium,Mg^{2+}ATPase activity in the fat bodies of silkworm on exposure to sub-lethal and sub-sub lethal doses of copper is presented in table 3. In the control group the amount of Mg^{2+}ATPase activity was more in the fat bodies at all exposure periods (2 and 4 days) than the copper treated groups. At sub lethal dose (3.5 μg/kg`bw) $Mg^{2+}$ATPase activity decreased, however on 4 days of exposure at sub lethal dose $Mg^{2+}$ ATPase activity increased when compared to 2 days exposure period in the silkworm. In sub-sub lethal dose (1.75 μg/kg`bw) at 4 days of exposure period exhibited increased Mg^{2+}ATPase activity in fat bodies when compared to sub lethal exposed group of larvae.

DISCUSSION

Adenosinetriphosphate (ATP) is the prime linking intermediate compound in energy yielding and energy requiring chemical reactions in cells. ATP is called as a high-energy phosphate compound and releases its energy on hydrolysis by ATPase enzyme. ATPases are served for the maintenance of membrane permeability and energy production (Jowett *et al*., 1981). Further the ATPases regulate the oxidative phosphorylation, ionic transport, and muscle contraction. ATPases are specifically found in the plasma membrane (Lehninger, 1982). Among the ATPases the Mg^{2+}ATPases are present in all types of cells whereas the Na^+/K^+ATPases are present primarily in nerve cells. The mitochondrial Mg^{2+}ATPase play an important role during oxidative phosphorylation (Abrams *et al*., 1972). The mitochondrial ATPase involves in the lysis of the ATP and plays significant role in the initiation of the ATP synthesis (Lehninger, 1993). This enzyme is found in association with both Na^+, $K^+$and sodium dependent NH_4^+ ATPase in fishes and it is associated with the transport of ions across the gill epithelium (Isaia and Masoni, 1976). In our study we observed that copper altered the ATPase activity leading to various physiological changes in fat bodies of silkworm larvae. The integrity of the cellular membrane, intracellular cements and the stabilization of branchial permeability is under the control of Mg^{2+} ATPase (Pottos and Fleming, 1971; Motais and Isaia, 1972). Further it is known that Na^+/K^+ ATPase is an energy dependent enzyme which maintain ionic gradient critical for metabolic transport, osmotic gradient regulation and the maintenance of cell volume in gills of aquatic invertebrates. The active transport of the Na and K ions against concentration gradient is a very vital process in maintaining the electrochemical gradient across the muscle cells (Thomas, 1972). An increase in permeability of ions is observed in plants which are exposed to copper stress eventually lead to membrane damage in cell (Murphy and Taiz, 1997). The plasma membrane in plants can be considered as the first target site for heavy metal toxicity reactions (Janicka-Russak *et al*., 2012).Boyle *et al*, (2013)reported an increase in the expression of membrane transporting Na^+/K^+-ATPases and aquaporin (AQP1) activities in marine invertebrate crabs and calms when exposed for 96h to sub lethal concentrations of copper. Similar results were observed in our study indicting toxic effect at sub-lethal dose whereas sub-sub lethal dose could not induce any toxic effects. In addition, they have interpreted that membrane transporters can be playing a key role to remove the toxic effect of copper and on their transport mechanism are involved in ions and volume regulation in gill cells on copper exposure. Hence sub-sub lethal dose can be considered as a safe and further research is needed to know its beneficial effects on the silkworm growth and development. In the present study inhibition of total ATPase, Na^+/K^+-, and Mg^{2+}- ATPase activity were noted at sub lethal doses. However in sub-sub lethal dose all the ATPase activity were found to be in elevated levels when compared to sub lethal exposed group of silkworm larvae. According to Lakshmidevi and Yellamma (2013) Zinc chloride exposed silkworm exerted an elevation in the levels of Na^+/K^+-, Mg^{2+}-, Ca^{2+}-, and total ATPase activity indicating that Zinc has potential influence on maintaining ion gradients across biological membrane of silkworm fifth in star larvae. Similarly, in the present study copper a heavy metal at sub-sub lethal dose could have exert an elevation of Na^+/K^+-, Mg^{2+}-, and total ATPase activity, maintaining cellular integrity in *Bombyx mori*.

Conclusion

In the study we investigated the effect of copper on total ATPases, Na^+/K^+-, and Mg^{2+}- ATPases in fat bodies of silkworm and found that copper at a higher dosei. e sub-lethal dose (3.5 µg/kg`bw) exhibited toxic effects by altering the ATPase activity and at lower dose i.e. sub-sub lethal (1.75 µg/kg`bw) it could not induce any significant alteration in ATPase activity to the silkworm. It is noted that copper at lower dose could be beneficial to silkworm as a trace element nutrition. Further studies are needed to know the exact nutritional benefit of copper at sub-sub lethal dose to the silkworm on its economic characters.

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