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Toxicological Studies of Arsenic on the Growth of a Short Horned Grasshopper

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Abstract: A study was conducted to observe the long term effects of food, treated with arsenic (sodium arsenate) on the development of a short horned grasshopper *Oxyavelox* (Fabricius, 1787). Paddy leaves treated with two sublethal concentrations of Sodium arsenate [0.0125 mg.l^{-1} (d1) and 0.025 mg.l^{-1} (d2) of water] were provided as food to newly hatched nymphs until they reached adult stage. It was evident that growth was significantly retarded along with the elevated doses of As in adult. Moreover, thetotal rearing time (TRT) also decreased significantly in dosed grasshopper than the control.

Key words: Short Horned Grasshopper · Sodium Arsenate · Growth Rate · Body Weight

INTRODUCTION

Grasshopper is considered as primary consumer in the food chain and many animals present in the ecosystem depends upon this insect for their food. It is the most abundant among ground-dwelling insects, representing up to 20 to 30 per cent of arthropod biomass [1]. The species spectrum of a contaminated ecosystem would be changed depending upon the toxicity level of the heavy metals [2]. Montaser *et al.* [3] has been reported that the problem of metal pollution is considered among the most serious one that facemankind in the twenty-one century. When *Aiolopusthalassinus* (Fabr.) adults were fed with mercury, cadmium and lead contaminated diets and it was found that various toxicological symptoms were appeared in the body [4]. The study also revealed that when mercury concentration in food increased, the total rearing time for each instars of *Oxyafuscovittata* increased, whereas, adult body weight and life span significantly decreased [5]. Experiments on bioaccumulation of arsenic in the branchial tissue of *Sabellaspallanzanii* confirm the environmental origin of this element [6]. In most arsenic affected area of India and Bangladesh the ground water used for irrigation is As contaminated, which leaving a risk of the accumulation of this toxic element in soil and eventually transfer to different trophic level through food chain [7]. It was also observed that grasshoppers could be regarded as the bioindicators of heavy metal pollution as well as heavy metal concentration in the environment [8]. This investigation was conducted to study the influence of arsenic (As) on growth pattern of short horned grasshopper *Oxyavelox* as the effect of this heavy metal on growth pattern of acrididswas not conducted so far.

MATERIALAND METHODS

Adult acridids of interest *Oxyavelox* (Fabricius, 1787) was collected from field near Amtala, Howrah. The field was selected because this area is not considered as arsenic affected in the state of West Bengal, India. Plastic jars of 5 liters capacity containing 3.0 cm thick sand at the bottom were taken as the rearing cage. The open portion of the cages was covered with nylon net in order to maintain the air supply properly. Rearing was carried out in laboratory conditions, the temperature ranging between 13.9°C and 36.3°C and relative humidity ranging between 60% and 98%. After copulation, the female laid eggs in the sand. After approximately 30 days of oviposition the first instars hatched out from the eggs. The first instars and their successive stages including the adult insects were also reared following the same procedure.

Conical flask of 50 ml capacity containing food plant *Oryza sativa* Lin. was placed in the rearing jar for providing food to the insects. To study the effects of As, fresh leaves of *Oryza sativa* Lin. was collected from the cultivated field in the college campus and dip in the dosed distilled water treated with 0.0125 mg.l^{-1} (d1) and 0.025 mg.l^{-1} (d2) Sodium arsenate for twelve hours.

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To study the effect of As on the metamorphosis in the acridids of interest at different doses, nymphs and adults were fed with dosed paddy seedlings and for control plants grown in As free water [9]. Food was changed every 24 hours. Body weights have been measured with electronic digital balance (KERN –KB-120, Germany) to take linear measurements, etherized insects were observed under binocular and length of body and femur and antenna were taken with scientific scale.

RESULTS

The study revealed that in the non-treated *Oxyavelox* (Fabricius, 1787), the body weight in 1st instars 0.008± 0.0004 (SE) g. and adult female was 0.461±0.006g. and male 0.376±0.003g (Table 1). Whereas 0.0125mg.l^{-1} arsenic salt treated first instars showed average body weight of 0.011±0.0002g. and adult female was 0.411±0.002g. and male was 0.359±0.003g. (r=0.51, t=2.52, p<0.05, y= -0.019+0.366x, F=13.625, p<0.05) showed significant effect of Arsenic on the body weight in comparison to the normal one (Table 2). *Oxya* sp. treated with 0.025mg.l^{-1} showed also similar body weight up to 3rd instars but a significantly decreasing trend of body weight was observed from the 4th instars to adult [4th instars female has body weight 0.117±0.002g. (r= 0.85, t= 6.89, p<0.05, y= 0.188+0.082x, F= 100.79) and 4thinstars male 0.085±0.001g. (r= 0.47, t=2.26, p<0.05, y= 0.149+0.070x, F= 10.822), 5thinstars female has body weight of 0.225±0.004g. (r= 0.93, t= 10.90, p<0.05, y= 0.042+0.115x, F= 252.260) and male 5thinstars has 0.172±0.004g (r= 0.93, t= 10.99, p<0.05, y=0.143+0.134x, F= 256.813) and the body weight of adult female 0.431±0.007g. (r= 0.46, t= 2.19, p<0.05, y= 0.165+0.355x, F= 10.012) and adult male 0.336±0.006g. (r= 0.71, t= 4.30, p<0.05, y= 0.360+0.200x, F= 39.512)].

The length of the body in normal *Oxya* sp. was studied. The study revealed that in untreated condition the length of different instars and adults are 1st instars 0.345±0.011(SE) cm, 2nd instars 0.55±.0.014 cm, 3rd instars 1.12±0.043cm, 4th instars female 2.58±0.076 cm, male 1.85±0.028 cm, 5th instars female 3.53±0.020 cm, male 3.03±0.023 cm and adult female 4.13±0.022 cm, male 3.7±0.042 cm. 0.0125 mg.l^{-1} treated *Oxya*sp. revealed almost similar pattern of body length in 1st, 2nd and 3rd instars whereas in 4th instars female body length was 2.15±0.032 cm(r=0.64, t=3.53, p<0.05, y=0.079+1.947x, F=26.323) and male 1.69±0.023 cm (r=0.59, t=3.09, p<0.05, y= -0.034+1.754x, F=19.934). In 5th instars length of the female body was 3.115±0.020 cm, (r=0.92, t=9.99, p<0.05, y= -0.175+3.73x, F=210.818) and 5th instars male 2.765±0.018 cm (r=0.83, t=6.24, p<0.05, y= -0.045+2.9x, F=81.482). So the treated 4th and 5th instars *Oxya*sp. showed significance decrease in length in relation to normal. When *Oxya*sp. was treated with 0.025 mg.l^{-1} of arsenic salt it was found that there was significant decrease in body length in 4th instars female: 2.155±0.021cm (r=0.65, t=3.64, p<0.05, y= -0.010+2.181x, F=28.082) and in 5th instars female: 2.94±0.026 cm (r=0.95, t=12.38, p<0.05, y= -0.190+3.611x, F=320.704) and 5th instars male: 2.545±0.015 cm (r=0.94, t=12.01, p<0.05, y=-0.084+2.8x, F=306.640) and also in adult female: 3.545±0.021 cm (r=0.95, t=13.21, p<0.05, y=0.236+2.569x, F=369.97) and male: 3.1±0.022cm (r=0.90, t=8.70, p<0.05, y= -0.162+3.699x, F=159.070) (Table 3).

The study revealed that length of the femur in 2nd instars in all normal and treated grasshopper were almost same but from the 4th instars the treated grasshopper exhibit significant decrease in size than the normal instars (Tables 2 and 3).

Length of antenna in treated and untreated 1st instars showed no significance difference and but maintained a decreasing pattern of growth up to adult. The important output of this study was that arsenic has prominent effect on the growth and development of *Oxyavelox*. There was significant decrease in body weight with the increase of dose of the arsenic. Moreover the body of this grasshopper becomes thinner and glossy color of the body did not observe.

DISCUSSION

Arsenic is distributed in nature like other heavy metals, but in the last few decades increasing concentrations in the water in different parts of the world including West Bengal in India is creating the threats for many plants and animals including human being in our ecosystems [10]. Arsenic has become a serious problem, because of its acute toxicity;and causing a chronic disease of a serious nature developing over a number of generations or years. In the present work, we have investigated some of the effects of elevated As concentration on body weight, body length, femur and antenna of the short horned grasshopper *O. velox*. From the present experiment it was evident that growth was significantly retarded along with the elevated doses of As in adult. The body weight wasdecreased with the elevated doses of As.In a similar observation it was found that weight of freshly hatched adult of *Aiolopusthalassinus* was decreased than the control

Table 1: Normal growth pattern in *Oxyavelox*

Character	1ST Instar	2nd Instar	3rd Instar	4th Instar		5th Instar		Adult	
				Female	Male	Female	Male	Female	Male
Body Weight (g)	0.008±SD=0.002	0.015±SD=0.004	0.045±SD=0.015	0.19±	0.095±	0.347±	0.268±	0.461±	0.376±
	SE=0.0004	SE=0.0009	SE=0.003	SD=0.029	SD=0.013	SD=0.020	SD=0.019	SD=0.027	SD=0.015
				SE=0.006	SE=0.003	SE=0.004	SE=0.004	SE=0.006	SE=0.003
Body Length (cm)	0.345±SD=0.051	0.55±SD=0.061	1.12±SD=0.193	2.58±	1.85±	3.53±	3.03±	4.13±	3.7±
	SE=0.011	SE=0.014	SE=0.043	SD=0.342	SD=0.124	SD=0.091	SD=0.103	SD=0.098	SD=0.189
				SE=0.076	SE=0.028	SE=0.020	SE=0.023	SE=0.022	SE=0.042
Femur Length (cm)	0.115±SD=0.037	0.235±SD=0.049	0.48±SD=0.095	1.125±	0.835±	1.51±	1.16±	1.92±	1.64±
	SE=0.008	SE=0.011	SE=0.021	SD=0.085	SD=0.075	SD=0.072	SD=0.076	SD=0.083	SD=0.067
				SE=0.019	SE=0.017	SE=0.016	SE=0.017	SE=0.019	SE=0.015
Antenna Length (cm)	0.098±SD=0.004	0.13±SD=0.047	0.275±SD=0.072	0.66±	0.445±	0.95±	0.76±	1.14±	0.96±
	SE=0.0009	SE=0.011	SE=0.016	SD=0.050	SD=0.051	SD=0.051	SD=0.051	SD=0.050	SD=0.050
				SE=0.011	SE=0.011	SE=0.011	SE=0.011	SE=0.011	SE=0.011

SD = Standard deviation, SE= Standard error

Table 2: Growth pattern in *Oxyavelox* treated with 0.0125 mg.l^{-1}arsenic salt

Character	1st Instar	2nd Instar	3rd Instar	4th Instar		5th Instar		Adult	
				Female	Male	Female	Male	Female	Male
Body Weight (g)	0.011 ±0.0002(SE)	0.022±0.001	0.052±0.002	0.154±0.002	0.102±0.003	0.261±0.003	0.198±0.003	0.411±0.002	0.359±0.003
	r=0.82, t=6.14*	r=0.62, t=3.34*	r= 0.31, t=1.38	r=0.60, t=3.18*	r=0.28, t=1.24	r=0.94, t=11.38*	r=0.91, t=9.44*	r=0.79, t=5.41*	r=0.51, t=2.52*
	Y=-0.057+0.012x	Y=0.196+0.019x	Y=0.042+0.05x	y=-0.147+0.181x	y=0.027+0.099x	y=0.239+0.178x	y=0.287+0.121x	y=0.129+0.351x	y=-0.019+0.366x
Body Length	0.39±0.016	0.6±0.015	1.04±0.024	2.15±0.032	1.69±0.023	3.115±0.020	2.765±0.018	4.075±0.019	3.74±0.020
(cm)	r=0.35, t=1.59	r=0.38, t=1.74	r=0.24, t=1.05	r=0.64, t=3.53*	r=0.59, t=3.09*	r=0.92, t=9.99*	r=0.83, t=6.24*	r=0.29, t=1.29	r=0.14, t=0.60
	y=0.182+0.327x	y=0.143+0.521x	y=-0.003+1.043x	y=0.079+1.947x	y=-0.034+1.754x	y=-0.175+3.73x	y=-0.045+2.9x	y=0.137+3.508x	y=-0.074+4.012x
Femur Length	0.13±0.011	0.315±0.015	0.675±0.020	1.045±0.011	0.79±0.014	1.25±0.011	1.045±0.011	1.68±0.017	1.44±0.015
(cm)	r=0.18, t=0.78	r=0.57, t=2.95*	r=0.73, t=4.55*	r=0.50, t=2.46*	r=0.32, t=1.43	r=0.91, t=9.11*	r=0.66, t=3.72*	r=0.84, t=6.53*	r=0.83, t=6.29*
	y=0.039+0.125x	y=-0.011+0.318x	y=-0.058+0.703x	y=-0.018+1.065x	y=0.066+0.735x	y=0.306+0.788x	y=0.005+1.040x	y=0.061+1.564x	y=-0.327+1.975x
Antenna Length	0.0995±0.0004	0.15±0.011	0.365±0.023	0.65±0.011	0.45±0.011	0.985±0.018	0.735±0.015	1.05±0.015	0.85±0.011
(cm)	r=0.23, t=1	r=0.20, t=0.87	r=0.50, t=2.39*	r=0.10, t=0.43	r=0.05, t=0.21	r=0.26, t=1.14	r=0.17, t=0.73	r=0.61, t=3.26*	r=0.74, t=4.69*
	y=-0.063+0.106x	y=-0.238+0.181x	y=0.538+0.217x	y=0.208+0.513x	y=-0.303+0.585x	y=-0.3+1.27x	y=-0.172+0.865x	y=-0.110+1.175x	y=-0.625+1.45x

*p<0.05

Table 3: Growth pattern in *Oxyavelox* treated with 0.025 mg.l^{-1} arsenic salt

Character	1st Instar	2nd Instar	3rd Instar	4th Instar		5th Instar		Adult	
				Female	Male	Female	Male	Female	Male
Body Weight (g)	0.009 ±0.0002(SE)	0.022±0.002	0.054±0.002	0.117±0.002	0.085±0.001	0.255±0.004	0.172±0.004	0.431±0.007	0.336±0.006
	r=0.51, t=2.52*	r=0.43, t=2.02	r= 0.35, t=1.58	r=0.85, t=6.89*	r=0.47, t=2.26*	r=0.93, t=10.90*	r=0.93, t=10.99*	r=0.46, t=2.19*	r=0.71, t=4.30*
	Y=0.028+0.009x	Y=-0.682+0.032x	Y=0.196+0.045x	y=0.188+0.082x	y=0.149+0.070x	y=0.042+0.115x	y=0.143+0.134x	y=0.165+0.355x	y=0.360+0.200x
Body Length	0.375±0.01	0.565±0.015	1.14±0.05	2.155±0.021	1.825±0.016	2.94±0.026	2.545±0.015	3.545±0.021	3.1±0.022
(cm)	r=0.31, t=1.38	r=0.12, t=0.51	r=0.06, t=0.26	r=0.65, t=3.64*	r=0.13, t=0.56	r=0.95, t=12.38*	r=0.94, t=12.01*	r=0.95, t=13.21*	r=0.90, t=8.70*
	y=0.051+0.358x	y=0.071+0.526x	y=0.266+0.843x	y=-0.010+2.181x	y=0.017+1.793x	y=-0.190+3.611x	y=-0.084+2.8x	y=0.236+2.569x	y=-0.162+3.699x
Femur Length	0.125±0.01	0.29±0.018	0.495±0.023	0.945±0.011	0.775±0.01	1.35±0.011	1.065±0.017	1.645±0.011	1.45±0.011
(cm)	r=0.13, t=0.56	r=0.40, t=1.85	r=0.08, t=0.34	r=0.80, t=5.61*	r=0.45, t=2.14*	r=0.80, t=5.61*	r=0.52, t=2.59*	r=0.90, t=8.68*	r=0.85, t=6.77*
	y=0.098+0.114x	y=0.154+0.254x	y=-0.128+0.556x	y=-0.018+0.965x	y=0.071+0.716x	y=0.408+0.734x	y=0.169+0.870x	y=0.015+1.616x	y=-0.058+1.546x
Antenna Length	0.099±0.0007	0.14±0.011	0.175±0.016	0.545±0.011	0.35±0.011	0.83±0.011	0.665±0.011	1.1±0.016	0.835±0.015
(cm)	r=0.14, t=0.60	r=0.10, t=0.43	r=0.58, t=3.03*	r=0.76, t=4.95*	r=0.69, t=4.04*	r=0.78, t=5.30*	r=0.68, t=3.93*	r=0.31, t=1.38	r=0.73, t=4.56*
	y=0.188+0.081x	y=-0.333+0.183x	y=-0.128+0.210x	y=0.125+0.463x	y=0.101+0.305x	y=0.4+0.45x	y=-0.232+0.840x	y=-0.584+0.453x	y=0.375+0.475x

*p<0.05

insect when treated with chlorides of mercury, cadmium and lead [9]. The study also revealed that the 2nd and 3rd instars of treated *Oxya* sp. were almost maintained a growth rate similar to the untreated insects. But the rate of decrease in body weight along with other body parts with increasing doses of toxicant indicating that grasshopper could not recover from the stress of As after the 3rd instar. All these adverse effects may be observed due to accumulation of arsenic in the insect body, as found in *Oxyafuscovittata* treated with Cd [11]. Mathew and Al- Doori [12] has been studied the effect of heavy metals on the *Drosophila melanogaster* and observed similar type of growth reduction during their development.Another important finding of the present study is that TRT decreased significantly in dosed grasshopper than the control. Study revealed that TRT in

controlled *Oxyavelox* was approximately sixty days, whereas, it was one month for both d1 and d2. So it may be concluded that As is toxic to virtually every system in the body and causes retardation of growth as well as morphology.

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