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SOME BEHAVIOURAL RESPONSES OF *LIMICOLARIA AURORA* EXPOSED TO GRAMOXONE

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Summary: The effects of 0.5 , 1.0 , 1.5 and 2.0 % of combined oral and dermal single-application of 0.1 M concentration of gramoxone (contact herbicide, paraquat chloride) on *Limicolaria aurora* were determined and compared with control using *Amaranthus sp.* as bait. Responses were measured through normal feeding and crawling, head retraction into the shell, swelling, excessive mucus secretion, lack of response to mechanical stimuli (mortality). Results showed no effects on controls. Snails exposed to gramoxone fed less as contamination increased. Gramoxone is molluscicidal, it can also result in behaviour–modifying observations in snails which are non-target organisms in the ecosystem.

***Key Words**: African giant land snail, gramoxone, ecotoxicology.*

Introduction

Gramoxone is a contact herbicide. It is non-selective and quickly destroys green plant tissues (absorbed by foliage and green bark with little or no translocation). It has essentially no residual activity in soil [MAF, 2003]. Molluscs are therefore of interest not only to farmers and the pesticide industry but also to ecotoxicologists as monitor species for environmental pollution. They encounter toxic materials either by contact or during feeding. If they crawl over surfaces recently sprayed with pesticides, on toxic molluscicide baits or in heavily polluted substrates, the skin is the first point of contact [Triebskorn *et al*, 1996].

African giant land snails are delicacy in diets of rural communities of Niger Delta [Ebenso 2002a, Ebenso 2004a]. Gramoxone may threaten food security and rural income as many snails consumed are gathered from wild, with some indirect implications for the health of unsuspecting gatherers. Gramoxone also alter predator-prey relationship and functioning of surrounding natural ecosystems. This paper concerned African giant land snail *Limicolaria aurora* exposed to gramoxone, under controlled laboratory conditions.

Materials and Methods

Fresh uncontaminated leaves were collected from healthy *Amaranthus sp.* growing on vegetables gardens in Uyo, within the Cross River basin (flood control) wetland ecosystem of guinean forests of Niger Delta, Nigeria (with 1500 mm rainfall, 90 % relative humidity and a 12: 12 h light: dark photoperiod), where snail consumption is not a taboo [Ebenso, 2003a]. Leaves were transported in sterile plastic bags to laboratory. Gramoxone is a common herbicide used in market gardens in Itu. Gramoxone (paraquat chloride) of analytical grade was used in this study, dissolved in 0.1 M, pH 7.4, 1% contained 0.276g active ingredient.

Live samples of adult *L. aurora* (12$\pm$ 0.05g) were collected locally. Snails were quarantined for 4 days to adapt them to experimental conditions and finally starved for the last 24 h during adaptation according to methods of Ebenso and Okafor [2002], Ebenso [2002b, 2003a, 2003b, 2003c, 2004], Thompson and Cheney [1996]. The snails were reared in plastic boxes of 12 x 12 x 6 cm^3 with mosquito netting on the lid of each box within temperature of 26^oC under laboratory conditions which imitates field conditions stated above. Moistened loam soil up to 0.02 m was spread on the floor of each box. Following methods of Ebenso and Okafor [2002], freshly chopped green pawpaw *(Carica papaya)* leaves were fed *ad libitum* during adaptation period.

During experimentation, 15 *L. aurora* from the boxes were randomly allotted by completely dipping snail skin (foot) for 60 s in gramoxone solution , indicating dermal contact, with 3 snails into each of 5 treatments of 0 (control) , 0.5 , 1.0 , 1.5 and 2.0 % concentrations. These snails were transferred back into 5 boxes representing each treatment group. Freshly chopped green *Amaranthus sp.* were presented to the snails for consumption (orally). These leaves were used as both food for the controls and as bait for the ingestion of gramoxone, according to methods of Ebenso [2004b] . These leaves were contaminated with gramoxone by completely dipping them in the 5 respective treatment solution of 0 (control) , 0.5 , 1.0 , 1.5 and 2.0 % concentrations , and dried at 27^{o}C for 5 mins before consumption by snails in boxes representing respective treatment groups.

An amount of green leaves (fed before and during this study) equivalent to 2 % of the snail body weight was fed to snails as described by Ejidike [2002] , at 1700 h. The snails were watered each day at 1600 h.

The mass of gramoxone contaminated leaves ingested and the quantity of gramoxone orally consumed were calculated according to methods of Triebskorn and Kunast [1990]. Physical observation of snails feeding patterns, that is, behavioural responses, using defined criteria of head retraction into shell, swelling, excessive mucus secretion, mortality (died , due to lack of mechanical stimulus), actively feeding and crawling normally. These observations were recorded daily for 5 days, according to methods of Ebenso [2004b]. Data were analysed using descriptive statistics according to methods of Steel and Torrie [1980].

Table1 : Amount of ingested food by L. aurora containing various concentration of gramoxone and calculated values for absolute quantity of active substance per gram wet weight.

Concentration (%)	Mass of ingested plant per snail (mg/g wet weight)	Mass of active substance ingested (µg/g wet weight)
2.0	98. 00 ± 30.21	5409.60
1.5	120.23 ± 42.31	4977.50
1.0	122.25 ± 61.89	3374.10
0.5	128.13 ± 52.91	1768.20
Control	144.22 ± 42.66	-

Table 2: Response of L. aurora** to gramoxone application*

	0.24 h	48 h	72 h	96 h	120 h
Concentration %	n Response	n Response	n Response	n Response	n Response
0.5	1 died	1 head retracted	1 died	1 swollen	1 excessive mucus
1.0	1 excessive mucus	1 died	1 abnormal movement	1 abnormal movement	2 excessive mucus
1.5	1 excessive mucus	1 head retracted	2 died	1 head retracted	1 head retracted
2.0	1 excessive mucus	1 died	1 head retracted	1 head retracted	1 head retracted

**The 3 snails (control) which were not exposed to gramoxone and others unaffected by exposure, fed and crawled normally with no other observable reactive, are not shown in table, as all survived to the end of 120 h period of observation.*
***n = 3*

Results and Discussion

The response of *L. aurora* snails to different concentrations of combined dermal and oral single-application of gramoxone are recorded in Table 2. Observations of the present study indicate excessive mucus

production, especially in 24 h of gramoxone application.

This agrees with Triebskorn and Ebert [1989] ; Triebskorn *et al* [1996] ; Port and Port [1986] that environmental chemicals not only irritate mucus cells in the skin, but also in the digestive tract if taken up via oral ingestion , in order to form a protective barrier preventing direct contact between the toxin and the epithelia. On the other hand, the mollusc risks dehydration due to high water content of the extruded mucus [Triebskorn *et al* [1996], Triebskorn *et al* [1998]. Additionally, they lose large amounts of energy [Denny 1980, Cottrell *et al* 1993] which may leave them motionless. Lying on one side, snail head retraction was noticed after 48 h. This observation is in agreement with Triebskorn and Ebert [1989] that after three hours slugs lie almost motionless on one side.

Abnormal movement due to poisoning from gramoxone, which are neurotoxic, may result in alternations of locomotion [Wedgwood and Bailey 1988] and feeding behaviour [Wright and Williams 1980, Wedgwood and Bailey 1986, Bourne *et al* 1988, Bailey 1989, Bailey *et al* 1989]. As gramoxone contamination in this study increased in leaves presented to the snails, consumption decreased (Table 1). This compares with Wright and Williams [1980]. These authors suggested that the observed reduction in feeding, as toxic concentration increased, is due to a paralysing effect on the digestive tract walls after ingestion. In this study, one snail foot was swollen at 96 h. This observation compares with report of Triebskorn [1989] that swelling of the interior body and the anterior body and the posterior flattening of slug occurred when sub-lethal dose of chemical was fed.

Gramoxone is toxic and molluscicidal to *L. aurora* with 41.6 % mortality in population recorded within 72 h of study. Higher concentrations deter feeding [Bailey 2002].

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