Original article

The Toxicity of plant material, *Drimia Altissima* (*Urginea Altissima),* Against the Field Rat, *Arvicanthis Abyssinicus:* A potential non-synthetic rodenticide.

Million Teshome[1], Hailu Kassa[2], Keil Charles[3]

Abstract

Background: Rodents are important pests of public health and agricultural importance, capable of transmitting diseases to humans and causing crop damage. The present rodent control strategy depends primarily on synthetic rodenticides, which are highly toxic, affect non-target species, and are expensive. Naturally produced organic pesticides may be more desirable as they are less toxic to non-target animals and are economically sustainable.

Objective: The objective of the study is to explore the toxicity and palatability of the bulbs of *Drimia altissima* against the field rat, *Arvicanthis abyssincus* with the aim of developing locally based organic rodenticides.

Method: This is a laboratory study for evaluating the toxicity of *D. altissimia* bait against the field rat, *Arvicanthis abyssincus*. In the study, field rats were randomly assigned to treatment and control groups. The treatment groups received different concentrations of *D. altissimia* poison bait prepared in the lab. The control groups received the plain bait. Mortality was recorded during the test period.

Results: Of the treatment groups, 80%-100% of the rats receiving the poison bait died, while none of the rats offered a choice between the plain bait and the poisoned bait died. It is estimated that 8% of the powdered bulb would produce 50% mortality.

Conclusion: The powdered bulb of *D. altissima* resulted in rat mortality in the test but not in the control group. The liberation of the toxic substance from the poisoned bait might have been slowed by the presence of the plain bait in the choice test. The result of this study suggests that further work is needed for understanding the toxicological properties of the active agents in the bulb. [*Ethiop. J. Health Dev.* 2010;24(3):175-179]

Introduction

Agricultural pests and zoonotic diseases are among the factors responsible for the chronic food shortages and for the high mortality rates from infectious diseases. In Ethiopia, the agriculture sector rodent depredations are a matter of concern in grain, fruit, and vegetable crops, as well as in reforestation projects (1). The unstripped grass rat, *Arvicanthis abyssinicus*, is the most widely distributed and economically important rodent pest in Ethiopia. This species is well adapted to live in areas inhabited by humans and is commonly found in open fields close to villages. It is a major pest of agricultural and public health significance. In Ethiopia, although data showing the level of crop loss due to rodents is lacking, in some areas it could reach as high as 10-25% during outbreak season (2). Rodents caused disease outbreaks, some like plague with historical significance, have been well documented in many countries (3). However, due to the lack of resources, underdeveloped human capacity and infrastructure, rodent caused diseases have not been documented in Ethiopia (2).

In order to decrease rodent caused crop losses, government sponsored rodent control programs are carried out during outbreak seasons. The control strategy includes trapping, habitat alteration, and poisoning using zinc-phosphide and anticoagulant rodenticides (2). However, these rodenticides pose significant threat both to the environment and to public health. Acute poisonings with pesticides including rodenticides account for 300,000 death worldwide (4). Zinc-phosphide releases phosphine gas, which is a nervous system toxicant. Inhalation of phosphine gas released from inorganic phosphides used as fumigants was responsible for a death of a 6 year old boy and for two adults suffering from severe toxicity (5). In addition zinc phosphide is toxic to non-target animals including wildlife (6). Ingested zinc phosphide was found in carcasses of poisoned rodents and animals eating these carcasses might be at risk of secondary poisoning (7).

In additional to zinc-phosphide, 1st-generation anti-coagulant rodenticides have also been used in rodent management program in Ethiopia. Compared to zinc-phosphide, anticoagulant rodenticides require multiple feeding, and as a result are less toxic to humans as well as to non-target animals. Furthermore, vitamin K can be used as antidote in case of accidental poisoning with anticoagulants. However, many rodent species have developed resistance to 1st generation anticoagulants (8)

[1]Ministry of Agriculture and Rural Development, Crop Protection Department, Addis Ababa Ethiopia;
[2]Department of Public and Allied Health, Bowling Green State University, Bowling Green Ohio, 43403, FAX: 419-372-2400, Phone: 419-372-9615, Author for Correspondence, hkassa@bgsu.edu;
[3]Department of the Environment and Sustainability, Bowling Green State University, Bowling Green, Ohio, 43403, ckeil@bgsu.edu

and they are no longer used in rodent management program in Ethiopia (9). 2nd-generation anticoagulants were introduced in Ethiopia in recent years, but they still have environmental limitations. Like zinc phosphide, 2nd generation anticoagulants are single-feed rodenticides, and as a result are potentially hazardous to non-target animals (10, 11). Furthermore, rats have developed resistance to 2nd generation anticoagulants (12, 13). Therefore, due to their high cost, environmental limitations and resistance, the use of 2nd generation anticoagulants has been curtailed in Ethiopia (2).

Globally, there is a great deal of interest in developing and using naturally occurring plant-based chemicals for the management of pests of public health and agricultural importance. The added benefit of using naturally occurring pesticides is that they are sustainable, economically viable, and environmentally friendly. There are many native plant species in Africa with known chemical toxicity to animals that can be developed as pesticides. In the high land region of Ethiopia, farmers have been using the bulbs of a plant known as *D. altissima* for controlling rats (2). Bulbs of *D. altissima* contain acute cardiac glycosides known as bufadienolides (14), which affect primarily the cardiac system, but also affect the gastro-intestinal, respiratory, and nervous systems (15). Livestock poisoning in southern Africa has been linked to the consumption of the bulbs of *D. altissimia* during the dry season (15). The bufadienolides are typically polyhydroxy C-24 steroids with a pentadienolide ring at C-17, and have been extracted from bulbs of *D. altissima* in Ethiopia (16) and from a related species in South Africa (17).

A preliminary study was conducted to evaluate the toxic effect of *D. altissima* against the field rat, *A. abyssinicus*, in a non-choice test. Exposures to 20% concentration of D. *altissima* preparation resulted in complete mortality of the test animals (2). So far there is no published study showing the toxicity of bulbs of *D. altissimia* against rodent pests. The purpose of the current study was to determine the rodenticidal properties (toxicity and palatability) of *D. altissima* preparation against the field rat, *A. abyssinicus* in a choice and non-choice tests.

Methods

The test rats were collected from pastoral lands and agricultural fields using live traps baited with bread spread with peanut butter. In the lab, the rats were kept individually in a metal cage measuring 37cm x33cm x21cm and maintained on a diet of whole wheat pellets and water *ad libitum*. After three weeks of acclimatization, each rat was sexed, weighed and returned to the cage for the test. Pregnant females, rats appearing sick and juveniles were excluded. The remaining rats were then randomly assigned into treatment and control groups. The treatment groups were divided into a choice and no-choice test groups. In the choice test the rats were given a choice between untreated and treated baits. In the non-choice test while the rats were given only the treated bait; the control rats were given the untreated bait.

Bulbs cut from *D. altissima* were chopped into small pieces, allowed to soak in water for 24 hours and then washed. The chopped bulbs were immersed for a short time in 98% ethanol heated on a steam bath and afterwards air dried. The dried bulbs were crushed and ground in an electrical grinder until a powdery form of the material was produced. The test baits were prepared by mixing measured amounts of the powdered bulbs into a base containing 90% wheat flour, 5% vegetable oil, and 5% sugar. In this manner, treated baits containing 20%, 10%, 5%, and 0% of the powdered bulbs were prepared and offered to the rats in a choice and non-choice situation.

In all test groups, 10 animals, 5 males and 5 females were used per dosage level. In the no-choice test, each animal received 20 grams of the treated bait *ad libitum* for 2 days , while in the choice test, each animal received 20 grams of the treated bait and 20 grams of the untreated (plain) bait water *ad libitum* in separate bowls. The positions of the bowls were alternated daily to avoid feeding position bias. The duration of the choice test was 4 days. The duration of the no choice test was 2 days. Ten rats of (5 males and 5 females) were used as control and were given 20 grams of the untreated bait water *ad libitum* for 4 days. In acute toxicity test animals are normally exposed to poison baits for a period of 1 to 2 days (18). However, in this case since this plant material has never been evaluated for its rodenticidal properties, we decided to extend the duration of the non-choice and the choice test to 2, and 4 days respectively.

The rats were observed daily for the duration of the test period. In the control group the rats were observed only for signs of illnesses and the amount of the untreated bait they consumed was not recorded. The amount of baits consumed and the mortality patterns were recorded daily for the duration of the experimental period. The amount of bait consumed was calculated after adjusting for spilled food and for variation in daily moisture content. Each day, unspoiled single use paper towel was placed under each cage to facilitate the collection of spilled foods. Any spilled food would be carefully separated from feces, weighed and added to the unconsumed bait weight. The variation in bait weight due to moisture was determined by placing in separate cages 20 grams each of the treated and untreated baits under a similar laboratory condition and determining if there were daily variations in weight.

Results

None of the control animals died during the test period. They appeared healthy and were actively moving inside their cages. The exact amount of the untreated bait each control animal consumed was not recorded, but was visually estimated at approximately 50% or more of the original amount presented. There was no daily variation

in laboratory temperature and humidity. There was no gain or loss in weight due to moisture variation. There was no bait spillage during the test period.

The exposure and mortality results are presented in Table 1. None of the animals that had a choice between plain food and food with the bulb mixture died.

Table 1: **Experimental Data Results**

Treatment	Gender	N	Mean body mass (g)	Test Duration (day)	% powdered bulb in Bait	Mean daily bait intake (g)	Dose (mg/kg/day)	Mean plain food intake (g)	Powdered bulb (%) in total daily food intake	Mortality (%)
Choice	Male	5	68.09	4	0	0	0	nr	0	0
		5	82.6	4	5	0.42	254	5.34	0.4	0
		5	85.1	4	10	0.34	400	6.61	0.5	0
		5	89.23	4	20	0.42	941	7.27	1.1	0
	Female	5	54.22	4	0	0	0	nr	0	0
		5	79.33	4	5	0.4	252	6.87	0.3	0
		5	97.03	4	10	0.42	552	7.59	0.4	0
		5	76.63	4	20	0.4	1044	7.06	1.1	0
No Choice	Male	5	78.16	2	0	0	0	nr	0	0
		5	90.95	2	5	0.61	335	0	5	100
		5	87.88	2	10	0.65	740	0	10	80
		5	86.37	2	20	0.79	1829	0	20	100
	Female	5	72.08	2	0	0	0	nr	0	0
		5	58.04	2	5	0.57	491	0	5	100
		5	60.48	2	10	0.64	1058	0	10	100
		5	75.9	2	20	0.57	1502	0	20	40

nr: not recorded

The amount of treated bait and plain food consumed was recorded and is shown in Table 1 along with calculations of the dose for each group. The results do not follow the typically expected dose-response pattern. Rats in the 10% and 20% free choice groups had doses (mg/kg/day) higher than those in the 5% no choice-group, yet displayed no mortality. However, in the non-choice group the same dosage level produced mortalities. As an alternative measure of exposure, the amount of the powdered bulb as a fraction of total daily food intake was calculated. These results, also shown in Table 1, provide a different view of the response to the bulb powder. When the powdered bulb was 1.1% of the total daily food intake or less, there was no mortality. Observed mortality began when the powdered root bulb was 5% or more of total daily food intake.

Figure 1 illustrates the exposure response curve when the mass of powdered bulb as a percentage of total daily food uptakes is used as a measure of dose. Figure 1 combines the male and female rat data. Since many of the mortality responses are zero using probit regression was not possible. A linear line is plotted through the data points which indicated to powdered bulb of approximately 8% of daily diet would produce 50% mortality.

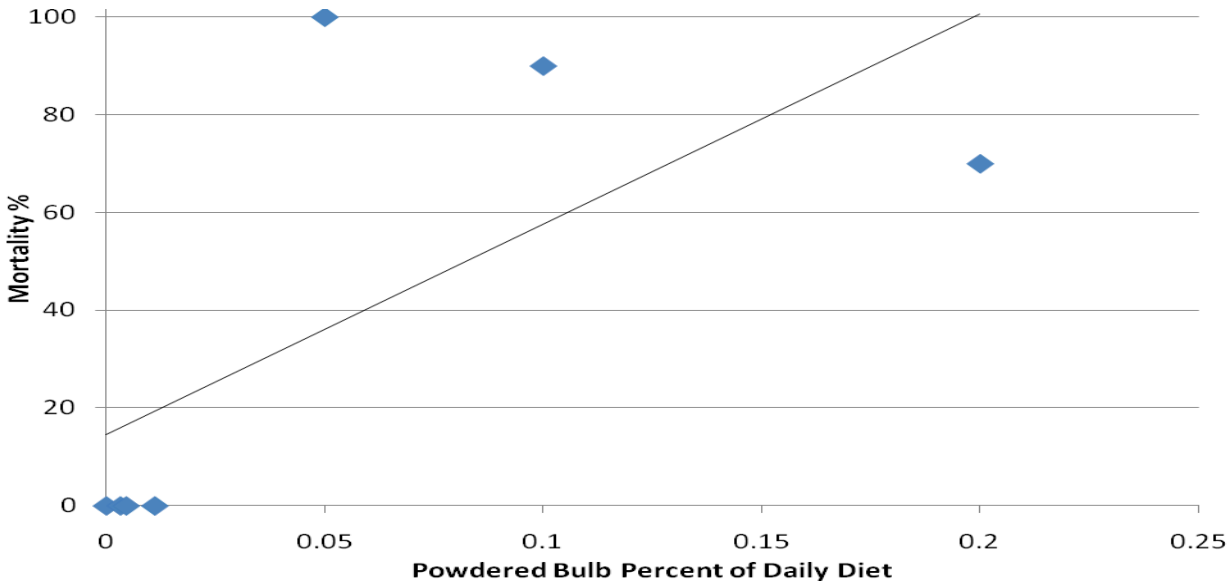

Figure 1: **Mortality Results for all Rats as a Function of the Percentage of the Daily Diet that was Powdered Bulb.**

Discussion

Since the bait was poisoned with the powdered bulb of the plant rather than the active ingredient itself, the mobility of the active ingredient may change with different amounts of total food intake. Perhaps the digestive enzymes of the rat are better able to liberate the active poison when there is a smaller amount of food in the digestive system. At present there is no scientific information concerning the toxicokinetics of bufadienolides extracted from the bulbs of *D. altissima*. Information exists for bufadienolides extracted from other poison plants. For instance the disposition kinetics of Cotyledoside, a bufadienolide found in an African poisonous plant (*Tylecodon wallichii*) fits a 1-compartment model. It has a short half-life and rapid clearance (19). If the bufadienolides found in *Drimia* species also display 1-comaprtment kinetics, they will have short residency time and rapid clearance. In that case, a sufficient dose of the active ingredient should reach the target organ within the duration of exposure to cause death. We believe that the lethal dose might not have been liberated from the poison bait due to the presence of the unpoisoned bait in the rat digestive system. The rats in the choice test consumed 17 times more unpoisoned bait compared to the poisoned bait.

The disposition kinetics of the bufaddienolide glycosides found in *D. altissima* will have to be explored in future work. One option for future developments is to develop the bulb processing procedure to extract the active agent from the root bulb and using the active agent directly when making poisoned bait. Having the active agent directly available for uptake would likely increase the potency if our hypothesis about the kinetics is correct.

There clearly was a problem with the acceptance of the *D. altissima* bait. When given a choice, the rats consumed an average of 17 times more plain food than poisoned bait. In the non- choice test, the rats only ate slightly more poisoned bait than the rats that had a choice between poisoned bait and plain bait. Their total consumption of food was on average only 9% that the rats in the choice group consumed. As of now we do not know if it is the taste of the powdered bulb or the taste of the poison that affects the acceptability of the bait. Bait aversion has always been a problem with many rodenticides. Palatability of poisoned baits may be influenced by the composition of the inactive ingredients such as the base bait. The base bait is normally a grain that field rodents are more likely to encounter in the environment and using such base baits improves palatability. For example, in rice fields using rice as base bait substantially increased the uptake of the rodenticides, zinc pohsphide and warfarin (20).

Extracting and using the active agent might help with the acceptability of the bait. If the active agent were extracted and used directly, smaller amounts might produce more palatable poisoned bait. Another option is changing the formulation of the base bait itself to overcome animal aversion to the treated baits. Improving the palatability of rodenticides is an important concern for control programs.

Conclusions

Due to cost and environmental toxicity of current rodent management approaches, an integrated pest management approach incorporating natural pesticides should be considered as an alternative to the exclusive use of pesticides as pest management tools. Ethiopia has a number of indigenous plants that are toxic to animals and

traditionally used for pest control purposes. The plant species, *D. altissima*, found in the highland regions of Ethiopia is one of these plants that can be developed as natural pesticide against rodents of agricultural and public health importance. However, additional studies using large scale should be conducted to determine the palatability, toxicity and safety of chemicals extracted from the bulbs of *D. altissima*.

The powdered bulb of *D. altissima* can result in rat mortality given certain conditions. These results suggest that further studies be done to understand the toxicological properties of the active agents in the bulb. Next steps should include extracting and isolating the active agents, using the active agents in toxicity tests and improving the palatability of the poisoned bait.

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