Effect of Kerosene and Diesel Contamination on the Hatchability of *Achatina achatina* Eggs

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Abstract
The Giant African land snail, Achatina achatina, is widely consumed by locals in many parts of Nigeria, especially during the rainy season when they are picked from forest floors. A number of factors influence the hatchability of their eggs thereby limiting their population growth. In this research, the effect of the commonly used petroleum products, kerosene and diesel, on the hatchability of the snail eggs was evaluated. Different volumes of the products (25cm^3, 50cm^3) were homogenously mixed in 600g of garden soil to form four treatments, each having three replicates. Five eggs were buried in each treatment/replicate. After about 23 days, hatchlings began to emerge but only from the control group which was free from both petroleum products. It was concluded that the indiscriminate use of kerosene and diesel especially in villages should be discouraged due to their harmful effect on snails and possibly other life forms.

Key words: *kerosene, contamination, Achatina achatina, eggs.*

Introduction

Achatina achatina is one of the three Giant African Land Snails; others being *A. fulica* and *Archachatina marginata*. *Achatina achatina* and *Archachatina marginata* are more common in Nigeria than *A. fulica* (Ani *et al*., 2013). According to Cobbinah *et al*. (2008), the snail species can lay eggs about two or three times per season, and egg laying, hatching and development in the wild are seasonally influenced by temperature, humidity and moisture content of the soil (Ejidike *et al*., 2002; Ebenso, 2006; Ugwu *et al*., 2011).

Dedi *et al*. (2011) stated that *Achatina fulica* adapts very well to all kinds of environments, being able to modify its biological cycle according to local conditions. They lay small yellowish white eggs about 4mm in diameter, which hatch within 18 to 30 days. However, the percentage of unhitched eggs could be because they are parasitized, desiccated or not fertilized (Mateo, 2006), contaminated by fungi (Dedi *et al*., 2011), or contaminated by some chemical pollutant.

Nigeria is one of the major petroleum producing countries of the world and the environmental impact associated with its exploration and exploitation has been a popular area of experimental research in the last three decades (Odjegba and Sadiq, 2002). *Achatina* species have been shown to bioaccumulate heavy metals (Ebenso and Ologhobo, 2008a,b; Edorh *et al*., 2009) with mild to deleterious effects. For instance, Nechev *et al*. (2002) investigated the effect of diesel fuel treatment on the lipid profile of snail *Rapana thomasiana*, and reported that the diesel fuel treatment significantly changed its fatty acid composition.

Kerosene and diesel are two of the components of petroleum commonly used by households in Nigeria, and are commonly found to be contaminating the environment. Kerosene is also normally applied on small ponds of water to kill the larvae of the *Plasmodium*-carrying *Anopheles* mosquitoes. Thus, it is readily introduced into the environment in which snails lay eggs. This article is focused on examining the effect of kerosene and diesel contamination on the hatchability of *A. achatina* eggs.

Materials and Methods

i. Collection of Mature Snail Specimens

Twenty (20) mature specimens of *Achatina achatina* snails were bought from the Mile One market in Port Harcourt on the 4th of April, 2014, for the production of eggs. They were picked from Nembe, Port Harcourt Local Government Area of Rivers State, and brought to the market by rural women who earn their living from selling the species. They were weighed using a portable weighing balance OHAUS LS200 and were found to be of mean weight (±S.E) of 191.5g±1.817.

ii. Housing and Feeding of the Snail Specimens

The mature specimens were reared in plastic pens; four (4) snails were kept in each of five plastic containers measuring 34cm x 53cm x 35.5cm. Humus soil (20kg) was introduced into the plastic snaileries up to a depth of 4cm in order to create enough depth for the snails to dig in to hide or lay eggs. A shallow trough was used to serve water to the snails. This was cleaned daily and fresh tap water put in it for the snails. The specimens were fed *ad libitum* by 5.00pm everyday with pawpaw leaves and uneaten food was removed every day in order to prevent the growth of microorganisms which could cause harm to the species.

iii. Collection of Eggs

The pens were checked every two days for eggs. In order to do this, the snail specimens were removed, uneaten food removed and the soil was gently turned out to check for eggs. When eggs were found, the number of eggs was noted along with the date of collection.

The snails were kept for a period of 21 days for this purpose. The eggs were kept in one large, transparent container with a perforated cap, irrespective of when they were collected. This was to guide against bias in introducing the eggs into the hatcheries. Egg hatchability of snailets was calculated using the formula,

$$\% \text{ Hatchability} = \frac{\text{Number of eggs hatched}}{\text{Total number of eggs incubated}} * \frac{100}{1}$$

iv. Hatchery Pens

In order to test the effect of both petroleum products on the hatchability of the eggs, smaller transparent, plastic snaileries (11cm x 15cm x 11cm) were obtained. They were filled with humus soil to a depth of 4.0cm height.

600g of soil was used in all the pens. Five eggs were randomly selected, from the container with all the eggs that were collected over the period, and introduced into those used for the hatchery research.

v. Purchase of Petroleum Products

Kerosene and diesel were obtained from a petroleum filling station located at Rumuomasi, Obio-Akpor Local Government Area in Rivers State.

vi. Experimental Design

The completely randomized design was used and there were three replicates for each treatment. Three replicates were used due to the number of eggs collected.

The petroleum products used were kerosene and diesel which were measured out using a measuring cylinder calibrated in cm^3.

In the control, treatment A, 600g of unpolluted soil was used. In treatments B and C, $50cm^3$ of diesel and kerosene, respectively, were thoroughly mixed in 600g of garden soil. In treatment D, $25cm^3$ of kerosene was thoroughly mixed in 600g of garden soil. Five eggs were introduced into each of the treatments on 29thof April, 2014. There were three replicates for each treatment which were set up in the Department of Animal and Environmental Biology, University of Rivers State, Port Harcourt, and watched daily for hatchlings.
Both the eggs and soil specimens were weighed using a portable weighing balance OHAUS LS200.

Results and discussion
At the end of 21 days of keeping the mature snails, 45 eggs were collected. Figure 1 displays the number of eggs collected on specific days.

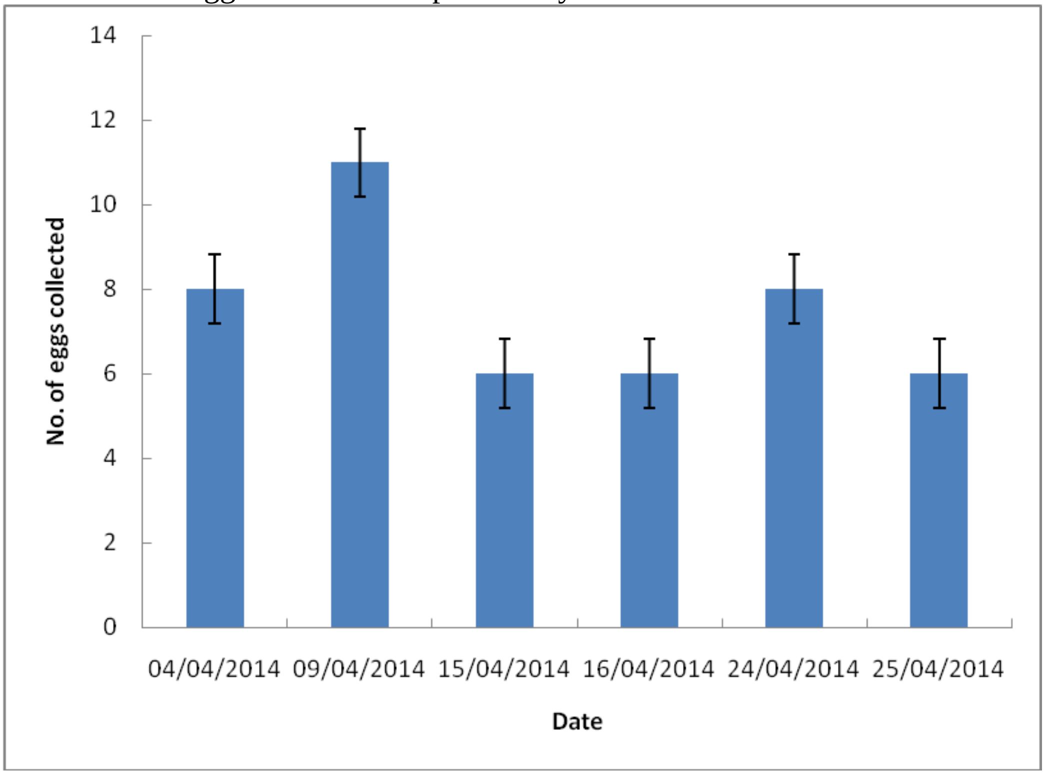

Figure 1: Number of *A. achatina* eggs collected on specific days
The eggs had mean weight 1.44mg ±0.01 and mean length, 9.533mm±0.08.

i. Record of Hatchlings
The first set of hatchlings appeared in replicates 1 and 2 of the control treatment on the 22nd of May, 2014. That was after 23 days from date of introduction of eggs into treatment groups. A total of ten hatchlings only were recovered from replicates 1-3 of the control, Treatment A (Figure 2). There was no hatchling from the other treatments. The result of this research indicated that diesel and kerosene could inhibit the hatchability of *Achatina achatina* eggs. In the treatments with the petroleum products, no hatchling was observed, even in the lowest concentrations applied. As such, total hatchability was found to be quite low, 22.22%, because the petroleum products used prevented eggs in the treatments other than the control from hatching. However, hatchability of eggs in the control group was good, being computed to be 66.67%.

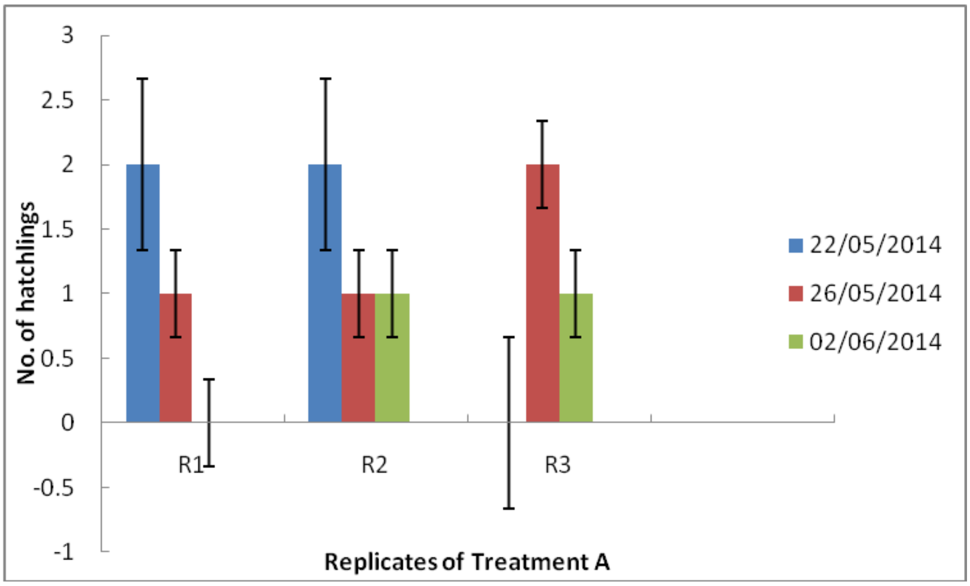

Figure 2: Number of hatchlings recovered from replicates 1-3 of Treatment A

Douglas *et al*. (2003) studied the effects of water-soluble fractions of crude oil and crude oil dispersants on the hatchability of eggs of *Clarias gariepinus*. They observed that delays in hatching followed a pattern whereby the time taken to hatch increased with an increase in the concentration of the water soluble fraction of crude oil. In the treatment with crude oil and dispersant, no hatchling was observed, even in the lowest concentrations applied. This implied a complete inhibition of hatching in crude oil dispersant mixtures; it also confirmed an earlier work by Bhattacharyya *et al*. (2003), that chemical dispersants and cleaners may not be well suited for cleaning up oil spills or near freshwater marshes.
Hatchability of eggs depend solely on the soil/incubating medium, temperature and composition. Provided the right condition is given, snailet or hatchling will emerge from the soil/incubating medium within 18-30 days (Omole *et al*., 2000).

When they were brought out of the plastic snail pens and examined, the egg shells appeared burnt and when the eggs were broken, it was observed that development of the embryo had actually started but was stopped at an early stage, preventing further development and resulting into the death of the embryo.
This is of interest as kerosene and diesel are some of the constantly used crude oil fractions that find entry into the environment.
Hatchability of eggs of the Giant African Land Snails (GALs) depends on soil, temperature, soil humidity and composition (Ebenso, 2006), while those that can inhibit hatchability have been stated to include parasitism, desiccation, lack of fertilization (Mateo, 2006), and contamination by fungi (Dedi *et al*., 2011). The same garden soil was used for all the treatments, which were kept under the same conditions of room temperature and humidity. So, differences in soil factors can be eliminated as been the cause for the non-hatching of eggs from the other treatments except for the control. Parasitism was not responsible for the results also, as all the unhitched eggs were present after the experimental period. Apart from the petroleum products used, the only other factor which could be implicated for the unhitching of the eggs was probably lack of fertilization. However, eggs were randomly picked for the treatment groups and with a replication of times three, all the eggs used for the contaminated soils could not have been unfertilized. It is therefore, thought that the limiting factor which hindered hatching in the treatment groups was the present of the contaminants, kerosene and diesel.

The results therefore, showed that even with a concentration as low as 25cm^3 kerosene/600g of garden soil, could hinder the hatchability of *Achatina achatina* eggs.

Conclusion

This research has shown that petroleum compounds like diesel and kerosene have an inhibitory effect on the eggs of *A. achatina*, thereby preventing them from hatching into snailets. The effects of these hydrocarbons on the eggs could lead to a reduction in the population of the species, if the environment is not managed properly and precautionary measures are not taken to curtail pollution by petroleum products.

Recommendation

It is recommended that citizens are educated on the harmful effects of indiscriminate use and disposal of petroleum products on the ecosystem on which man relies. Snail farming is also encouraged in order to sustain the production the giant land snails.

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