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Assessment of Barium Carbonate Toxicity on the Developmental Stages of *Sarcophaga ruficornis* (Diptera: Sarcophagidae)

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A B S T R A C T

The flesh fly *Sarcophaga ruficornis* is a pest of veterinary importance as it affects various animals reared for livestock including cows, buffaloes, sheep, and goats causing myiasis, which results in huge economic loss to the livestock owners. Various attempts have been made to control this fly using different chemical formulations. Barium carbonate is highly toxic compound upon acute and chronic exposures. Barium compounds have been found to have a toxic effect on different animal models. These were found to be cytotoxic and genotoxic to WTHBF-6 cells; cardiotoxic in rabbits and have been found to show toxic effects on *Rhabditis marina*. As $BaCO_3$ is a known rodenticide, the effect of barium carbonate was observed on the developmental stages of *S. ruficornis* starting from larval stage till the adult emergence has been assessed in the present study so as to check the potency of $BaCO_3$ as an effective control chemical for *S. ruficornis*. 3rd instar larvae of *S. ruficornis* were given an exposure of different graded concentrations (0.01, 0.001, 0.0001 and 0.00001 g/ml) of $BaCO_3$ to observe the toxic effects on larval and pupal stages. To the best of our knowledge, this is the first report on the toxicity of $BaCO_3$ on the different life stages of *S. ruficornis*. Results revealed the mild toxic effect on the development cycle of *S. ruficornis* with pupal mortality and a decrease in adult emergence. Present study confirms a mild yet toxic potential of $BaCO_3$ to hinder the life cycle of *S. ruficornis*. Further short term toxic studies are required so as to claim the efficiency of $BaCO_3$ in controlling fly populations.

Introduction

Barium (Ba) with CAS number 513-77-9 and atomic number 56 is a soft silvery metallic alkaline earth metal. It has a high chemical reactivity and is never found in nature as a free element. It is highly toxic upon acute and chronic exposures (Bhoelan *et al*., 2014). Barium has been tested for its toxicity in different studies (Llugany *et al*., 2000; Langdon, 1994; Akinfieva and Gerasimova, 1984). Some studies have been conducted on rats and mice to assess the toxicity of barium and its compounds (Constant *et al*., 1996; Dietz *et al*., 1992). Various naturally occurring barium compounds include barium nitrate [$Ba(NO_3)_2$], barium chloride ($BaCl_2$) and barium carbonate ($BaCO_3$). It has been found that toxicity of barium compounds depends upon their solubility (Gad, 2014). Water soluble barium compounds are found to be poisonous and more toxic as compared to insoluble forms. Insoluble barium compounds have been found to cause baritosis, a benign condition which occurs because of their accumulation in the lungs.

Barium ions act as muscle stimulant at low doses whereas affect the nervous system at high doses causing cardiac irregularities, tremors, weakness, anxiety, shortness in breath and even paralysis. Barium ions can block potassium ion channels which affect the proper functioning of nervous system thus adding on to its toxicity. Other organs which are damaged by water soluble barium compounds (barium ions) include the eyes, heart, lungs and skin. Heavy exposures may lead to blindness and sensitization of the skin. Barium carbonate, procured from its ore known as with erite, is a chemical compound used as rodenticide. It is insoluble in water but is soluble in gastrointestinal tract, thus showing its toxic effects in humans. Some previous studies also show the toxic effects of barium carbonate (Akinfieva and Gerasimova, 1984; Agarwal *et al.*, 1995). Barium carbonate and barium sulfate cause irritation to mucous membrane of upper airways and can also cause irritation to the skin and eyes. Intragastric administration of barium sulfate to albino rats was not found to produce deaths until the dose reached 25% to 40% of body weight, thus showing a mild toxic effect (Boyd and Abel, 1966). Aqueous solutions of barium hydroxide and barium oxide are strongly alkaline and can cause significant ocular burns and skin irritation. Barium carbonate causes gastrointestinal disturbances (nausea, vomiting and diarrhea); numbness and paraesthesias of limbs; severe hypokalemia resulting in general paralysis of skeletal muscles; dysarthria; dysphagia and ventricular arrhythmias. Barium nitrate has been found to be toxic when ingested or inhaled. Death may result from cardiac or respiratory failure usually within few hours to a few days following exposure to the compound.

The flesh fly *Sarcophaga ruficornis* (Fig. 8) of the family Sarcophagidae is a pest of veterinary and public health importance and is explored for different types of researches (Singh and Kumar, 2015; Pal and Kumar, 2013; Kumar and Khan, 2004). It is a well known species and has medical importance as a myiasis producing fly and it is seen in forensic entomological context (Singh and Singh, 2015). Myiasis is a parasitic infestation of body of a live animal by fly larvae that grow inside the host while feeding on its tissues. They differ from most of the flies in being ovoviviparous and the method of deposition of their eggs on carrion, decaying material or open wounds of mammals. The fly being the vector of various pathogens such as bacteria, viruses and protozoans, is a menace to human as well as livestock (Nouwaratn and Chitapa, 1995). They carry Leprosy bacilli and can transmit intestinal pseudomyiasis to people who eat their larvae. It is also a documented parasite of the toad *Bufo melanostictus* (Roy and Dasgupta, 1977). The fly has been reported to cause myiasis among sheep and cattle resulting into huge economic losses to the livestock owners. Some domestic animals fail to respond to the effects of myiasis which is a severe and persistent problem for livestock industries all over the world, causing them huge annual economic losses. *Sarcophaga* has been reported to cause intestinal myiasis in humans. In rural tropical regions, myiatic flies thrive and often the subject requires medical attention with immediate surgical removal of the parasites. Flesh fly *Sarcophaga* and its larvae feed on carrion such as dead insects, snails, or smaller and larger vertebrate carcasses and faeces. These are also known to eat decaying vegetable matter and excrement. They may be found around compost piles and pit latrines. This fly prevails in many parts of the world including southern Europe, France and Oriental regions including Thailand, Malaysia, Egypt, Myanmar, India, Nepal, Saudi Arabia, Philippines, Indonesia, Japan, Korea, Sri Lanka, Taiwan, China, Australia, Hawaii and USA (Suwannayod *et al.*, 2013).

The principal controlling method of adult population of flies involves insecticidal applications. Organophosphorous, or organochlorine compounds may be used. An alternative preventive method is the SIT (Sterile Insect Technique) where a significant number of artificially reared sterilized (usually through irridation) male flies are introduced which compete with wild bred males for females to copulate and thus females lay batches of unfertilized eggs which cannot develop into the larval stages. Maintenance of hygiene and sanitation can prevent these flies to infest humans. Various chemical formulations have been tested for controlling the population of myiatic flies including *S. ruficornis*. Only a few studies are available on the reports of toxicity of barium compounds on larval infestations (Ricks and Hoskins, 1948). Thus in the present study, an attempt has been made to access the toxicity of barium carbonate on the life cycle of *Sarcophaga ruficornis.*

Materials and Methods

Rearing of larvae and identification

Goat meat in petri dishes was kept in insect cages (Fig. 1) of dimensions 50x50x50 cm. After 5 days, eggs hatched into larvae. 3rd instar larvae of *Chrysomya bezziana*, *Musca domestica* and *Sarcophaga ruficornis* (Fig. 2 and 3) were found. In order to get the larvae of *S. ruficornis* from the mass, permanent mount of taxonomically important larval regions was prepared. Structure of anterior and posterior spiracles were observed and compared by making slides. Following observations were made after observing the slides of anterior spiracles of these three types of larvae. Anterior spiracles of *C. bezziana* had 4-6 lobes, *M. domestica* had 5-7 lobes and *S. ruficornis* had 12-13 lobes (Fig. 4). The structure of posterior spiracles was also used for differentiating the three flies. In *Musca domestica*, tortuous slits were present and the membrane of peritreme was closed. The structure of posterior spiracle in *Musca domestica* was different as compared to other two flies, so larvae of *Musca domestica* were separated and comparison was made between the structure of posterior spiracles of *C. bezziana* and *S. ruficornis* and following observations were made. Number of interperitremal plates was found to be 1 in case of *Chrysomya bezziana* and 2 in case of *Sarcophaga ruficornis*. Peritremes were found to be open in *Chrysomya bezziana* and were hidden in concavity in the case of *Sarcophaga ruficornis.* Oblique slits were present in *Chrysomya bezziana* and more or less vertical in *Sarcophaga ruficornis* (Fig. 5).

Exposure Treatment

Different concentrations of barium carbonate were prepared for the exposure treatment. These concentrations were prepared by dissolving appropriate amount of analytical grade of barium carbonate (Qualigens) in distilled water. Concentrations of 10, 100, 1000 and 10000 ppm were used to assess the toxicity of $BaCO_3$ for the present study.

Experimental Setup

Third instar larvae procured from the goat meat were used for carrying out the experiment. Glass petri dishes were set in replicates with 15 larvae each for all of the four test concentrations. 15 larvae were dipped one by one in each of the concentrations for 30 seconds. After the 30 sec. treatment, the larvae were shifted to a petri dish with lid and the petri dish was marked with appropriate number for the experiment. These marked petridishes were then kept in a dark place and were examined for one month at definite day span. The changes and mortality rates were recorded till the emergence of fly on daily basis.

Observations were recorded and were used to find out the results.

Parameters used

The effects of different concentrations of barium carbonate on the larvae of *S. ruficornis* were evaluated using following parameters: larval mortality, pupation, pupal mortality and adult emergence. For recording the observations, larvae were touched with fine zero grade brush. Pupation was recorded by counting the number of viable, turgid and brown coloured puparia at all the four treatment concentrations.

Results and Discussion

In the present study, *Sarcophaga ruficornis* was taken as a model to access short term toxicity of barium carbonate. Table 1 shows the effect of $BaCO_3$ on the survival and development of *S. ruficornis*. Barium compounds are known for high reactivity causing irritations, burns and various other ailments of heart, eyes and skin. In the present study, no larval mortality was observed on exposure to the different concentrations of $BaCO_3$ but a considerable pupal mortality was observed (Table 1; Fig. 9). The highest concentration viz. 10000 ppm resulted in 26.6% of pupal mortality and 73.3% of adult emergence (Table 1). A concentration dependent pupal mortality was seen (Fig. 10). However the 3rd instar larvae of *Sarcophaga* escaped mortality and pupated normally but most of them did not emerged into adults with all the tested concentrations. In another study, 60-70% and 10-30% of mortality was observed in mice and rats respectively when exposed to the concentration of 4000 ppm of barium chloride dehydrate (Dietz *et al.*, 1992). Barium was also found to decrease the population abundance in the nematode, *Rhabditis marina*, affecting the development time of the nematode from concentration of 400-2000 ppm. Barium at the concentration of 300 ppm affected the development of population whereas the concentration of 3600 ppm caused mortality (Lira *et al.*, 2011). Barium chloride ($BaCl_2$) caused severe ventricular dysrhythmias in conscious rabbits (Mattila *et al.*, 1986). Lethal effects of barite ($BaSO_4$) were also observed in bivalves where introduction of $BaSO_4$ caused 100% mortality (Strachan and Kingston, 2012). The effect of barium compounds was also observed in microorganisms. Barium titanate ($BaTiO_3$) has a significant toxic effect on growth of *Euglena gracilis*, a flagellated euglenoid, even in small concentrations of 1µgm/L $BaTiO_3$ B in 24 hours.

Table.1 Larval and pupal mortality; and adult emergence observed in replicates (R1 and R2) at different test concentrations in S. ruficornis following a 30 second dip treatment

$BaCO_3$ Concentration (ppm)	Larval mortality		Pupation		Pupal mortality		Adult Emergence	
	R1	R2	R1	R2	R1	R2	R1	R2
10000	0	0	15	15	4	4	11	11
1000	0	0	15	15	3	2	12	13
100	0	0	15	15	1	2	14	13
10	0	0	15	15	0	1	15	14

Fig.1 Flesh kept in petri dishes for flies to lay eggs

Fig.2 3rd instar larvae of *Sarcophaga ruficornis*

Fig.3 3rd Instar larvae of *Sarcophaga ruficornis* in petri dish

Fig.4 Slide of anterior spiracle of larvae of *Sarcophaga ruficornis* made for identification purpose

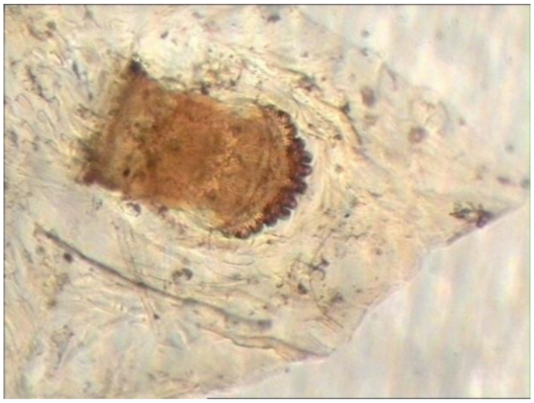


Fig.5 Slide of posterior spiracle of larvae of *Sarcophaga ruficornis* made for identification purpose

Fig.6 A live pupa of *Sarcophaga ruficornis* from which an adult emerged later on (Average length=1 cm)

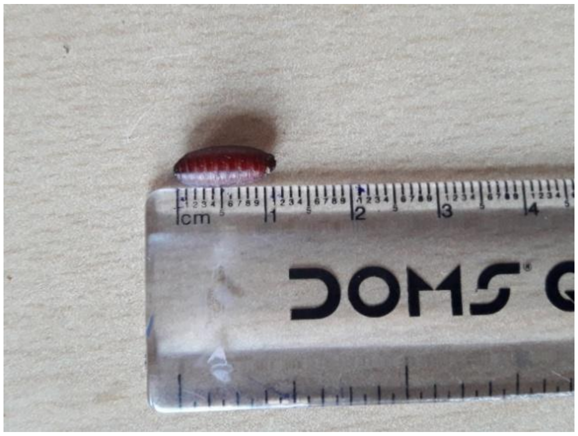


Fig.7 Fly emerging from pupa under controlled conditions

Fig.8 A dead adult fly *Sarcophaga ruficornis*

Fig.9 A dead pupa after larval exposure to 1000 ppm $BaCO_3$ (average length= 5.5 cm)

Fig.10 Pupal mortality (%) at different BaCO3 concentrations (30 sec. exposure treatment)

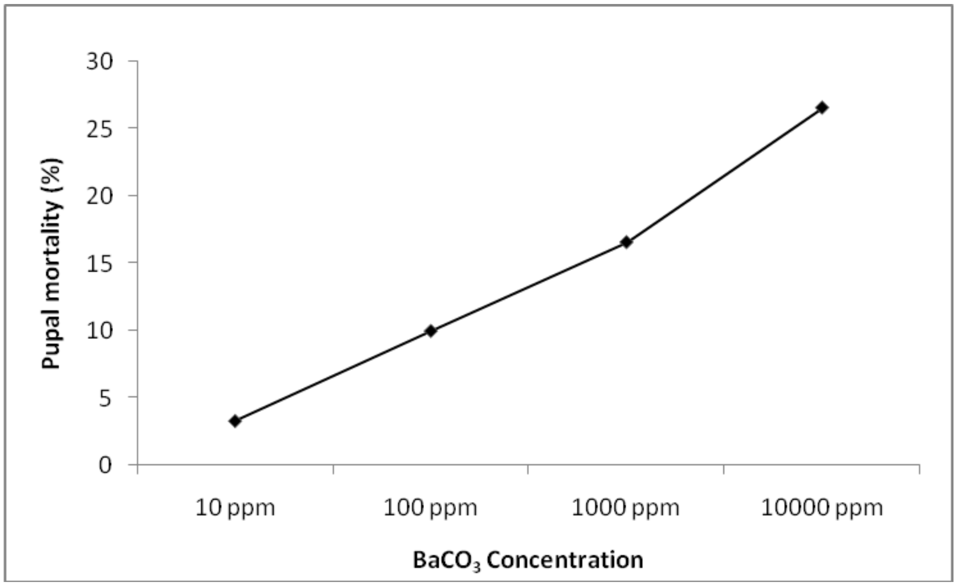
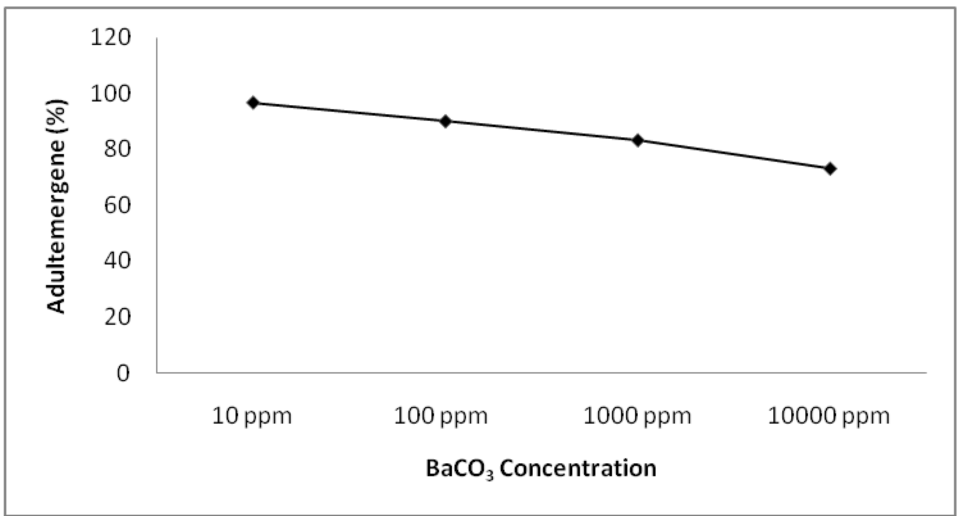


Fig.11 Adult emergence (%) at different BaCO3 concentrations (30 sec. exposure treatment)



In *Anabaena flos -aquae*, a cyanobacteria, the effects were however seen at higher concentrations of ≥75µg/mL for nanoparticles of barium titanate and 100µg/mL for micro particles of $BaTiO_3$ after 96 hours (Polonini *et al.*, 2014). Exposure to sublethal concentrations of barium chloride ($BaCl_2$) for 21 days to adult Zebrafish, *Danio rerio*, affected reproduction, sex steroid hormones and transcription of the genes belonging to the hypothalamic-pituitary-gonadal axis. It resulted in lesser hatching rates and decreased egg production (Kwon *et al.*, 2016). The effect of barium compounds was seen in humans too. In one such case, the suicidal poisoning of barium chloride in a 49 year old man caused death of the subject. The autopsy levels were found to be 9.9 mg/L in blood; 8.8 mg/L in bile and 6.3 mg/L in urine. However, the cause of death was found to be cardiorespiratory arrest due to $BaCl_2$ (Jourdan *et al.*, 2001).

In another study, exposure to 0.01, 0.05, 0.1, 0.5, 1 and 5 μg/cm^2 of barium chromate induced concentration dependent cytotoxicity in WTHBF-6 cells with relative survival of 88%, 74%, 67%, 12%, 3% and 0.1% respectively. With increase in concentration, the amount of chromosomal damage was also observed (Wise *et al.*, 2003). Toxic action of barium chloride was also observed in *Oryza sativa* L. (Rice). Barium chloride affected germination, growth and metabolism of rice plant and caused root growth inhibition. Inhibition of seedlings was observed at 0.1mM. Decline and complete elimination of germination was observed at 1mM and 100mM concentration respectively (Debnath, 1982).

In conclusion, barium carbonate is a highly toxic compound upon acute and chronic exposures. Barium and its compounds have been tested for their toxicity in some of the studies and a very few studies have been found on the toxicological effects of $BaCO_3$ on myiatic flies. The present paper reveals the mild toxicity of $BaCO_3$. 26.6% of pupal mortality was observed at the highest concentration (10000 ppm) with a 30 sec. larval dipped treatment. No obvious larval mortality was found with any of the tested concentrations. A 73.3% adult emergence was also observed following the highest concentration treatment. Conclusively the present study emphases the mild toxic nature of $BaCO_3$, a potent rodenticide on the developmental stages of *S. ruficornis* at the used treatment concentrations.

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