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A Study of the Toxicological Effects of a Sesbania Seed Extract for Controlling the Hairy Grain Beetle, and a Comparison of its Efficacy with the Insecticide Cypermethrin

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ABSTRACT

Objective: *This study aims to evaluate the effect of oil extracted from sesban seeds using petroleum ether as an organic solvent, and to compare its efficacy with that of the chemical insecticide cypermethrin, which has been used to control the hairy grain beetle (Khabrā).* ***Method:*** *The experiments were conducted by exposing the insect to different concentrations (0.5 per cent and 1 per cent and 1.5 per cent) at a temperature of 38 °C. The control rates were calculated after 24 and 48 hours.* ***Results:*** *The results showed a direct relationship between increasing concentration and exposure time, leading to a higher control rate. The extract achieved a control rate of 90 per cent at the highest concentration of 1.5 per cent after 48 hours, whilst the chemical pesticide recorded a control rate of 100 per cent at the same concentration and over the same time period.* ***Novelty:*** *Ultimately, the study's experiments confirmed the success of the extract as an alternative for controlling this insect pest in stored grains.*

INTRODUCTION

Cereal crops belonging to the Gramineae family are of great importance for food, industrial and fodder purposes. The area under cultivation with major cereals is estimated at approximately half of the world's cultivated land. Cereal crops dominate global agricultural production because cereals and their products provide humans with large quantities of carbohydrates as well as protein, [1] , Howe (1965) highlighted the economic damage caused by storage insects, including changes to the nature of stored materials, weight loss, contamination by their various chemical waste products and secretions, and the transmission of pathogens , The hairy grain beetle (Trogoderma granarium) (Everts) is a grain storage insect; this insect primarily infests stored grains and their products, oilseeds- particularly field pistachios and pulses and their products- as well as dried animal feed, in addition to infesting empty sacks and other materials, causing significant economic losses. Grains infested with any species of the genus Trogoderma must not be consumed, particularly damaged grains, as their hairy larvae may cause serious carcinogenic lesions [2] ,The hairy grain beetle is widespread in a number of warm, tropical countries; most of its larval stages take a long time before it transforms into the pupal stage, as it enters a long period of dormancy; it is therefore considered one of the insects with the most complex life cycles and the most difficult to control [3]. Consequently, one or more methods must be employed to prevent insect infestations by killing the insects or minimising the damage they cause to stored materials [4]. Hence, the need arose to protect this important food source and control the pests that affect it. As a natural consequence of global development, humankind has found itself tasked with finding effective means to curb the spread of these pests and minimise, as far as possible, the extent of losses to food stocks, so they had no choice but to resort to the use of chemical pesticides with rapid results [5].

Aim of the study

1- To investigate the lethal effect of sesban seed extract on adult Khabara beetles at different concentrations and at a temperature of 38 ± 2 °C
2- To study the lethal effect of the chemical pesticide 5% Alpha-cypermethrin on the adults of the hairy grain beetle at different concentrations and at a temperature of 38 ± 2 °C.

Theoretical section

A brief history of the insect

The khapra beetle (Trogoderma granarium) is a storage insect; it is one of the most dangerous storage insects and one of the most resistant to extreme environmental conditions and, at times, to pesticides [6]. Its discovery in California in 1953 led to its complete eradication following control efforts that lasted until 1966, costing the US government approximately 15 million dollars (Kerr, 1984). Parashar (2006) noted that the larvae of the hairy grain beetle are among the most dangerous life stages of the insect, as the larvae begin by feeding on the seed endosperm, emptying the seed of its contents and leaving only the husk; and these grains infested by the larvae become filled with excrement and moulted skins, leading to a deterioration in the quality of the grain. The damage caused is difficult to control due to the larvae’s ability to survive without food for long periods and their capacity to feed on low-moisture substrates; They are capable of entering small cracks and deep holes and remaining there for extended periods, and they also possess a relative tolerance to many insecticides and fumigants; it is therefore important to prevent the introduction of the grain weevil into uninfested areas [7].

Prevalence and geographical distribution of the insect

The grain weevil T. granarium was first recorded in India; recent reports indicate that this insect is present in more than 35 countries worldwide (Pasek, 2004) . The Indian grain weevil, native to India, has spread to warm, dry, tropical regions (How and Burges, 1956). Numerous infestations by this insect have been observed in the tropical and subtropical regions of Asia and Africa [8]. It is found across vast areas of the world, ranging from 35° north latitude to the equator, and from southern India to Europe (Anonymous, 1981). The hairy grain beetle (Khabra) spread to parts of the United States, and after these areas had been cleared of the insect, it was discovered in warehouses in New Jersey, Michigan and New York [9].

Nomenclature and Classification:

The scientific name for the grain weevil (Al-Khabra), T. granarium, was established by the scientist Everts in 1899. The complete classification of this insect is as follows.

Kingdom: Animalia

Subkingdom: Bilateria

Superphylum: Panarthropoda

Phylum: Arthropoda

Subphylum: Mandibulata

Superclass: Panhexapoda

Class: Insecta

Subclass: Dicondylia

Order: Coleoptera

Suborder: Polyphaga

Family: Dermestidae

Genus: Trogoderma

Specific name: granarium

Scientific name: Trogoderma granarium (Everts) [10].

The common name for this insect is the khapra beetle; in Hindi, this word means 'gnawer' [11].

Life cycle of the insect

The female of the grain weevil T. granarium lays her eggs individually amongst stored grains without secreting any adhesive substance during the egg-laying process, The egg is cylindrical in shape and white in colour (Figure 1, a), measuring 0.27 mm in length and 0.25 mm in width; it turns yellow over time [12]. The larva is yellowish-white in colour, except for the head and body bristles, which are reddish-brown (Figure 1.b) (EPPO/CABI, 1997) . The larvae reach the fully grown stage and transform into free-living pupae (Figure 2) near the surface of the seeds (Al-Azawi and Mahdi, 1983). Adult hairy grain beetles are characterised by their ovoid, somewhat elongated shape, measuring 1.6–3.0 mm in length and 0.9–1.7 mm in width (Figure 3). The head is usually tilted towards the ventral side (Figures 4 and 5) The thorax is dark brown, whilst the rest of the body is brown; the female is larger than the male and has a glossy colour (Haines, 1991). Adult insects are unable to fly, despite possessing wings; they occasionally extend their elytra and hind wings whilst walking, but do not fly [13].

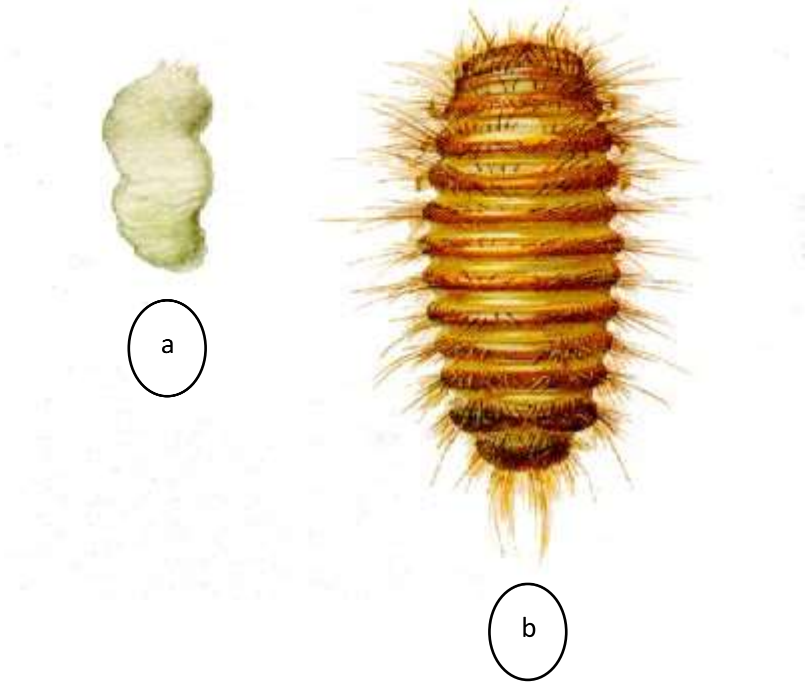


Figure 1. (a Egg of the khabara beetle b. . Larva of the khabara beetle)

Figure 2. Nymphs of the Khabra beetle.

Figure 3. Dorsal view of a whole Khabra beetle

Figure 4. Front view of a fully grown Khabra beetle

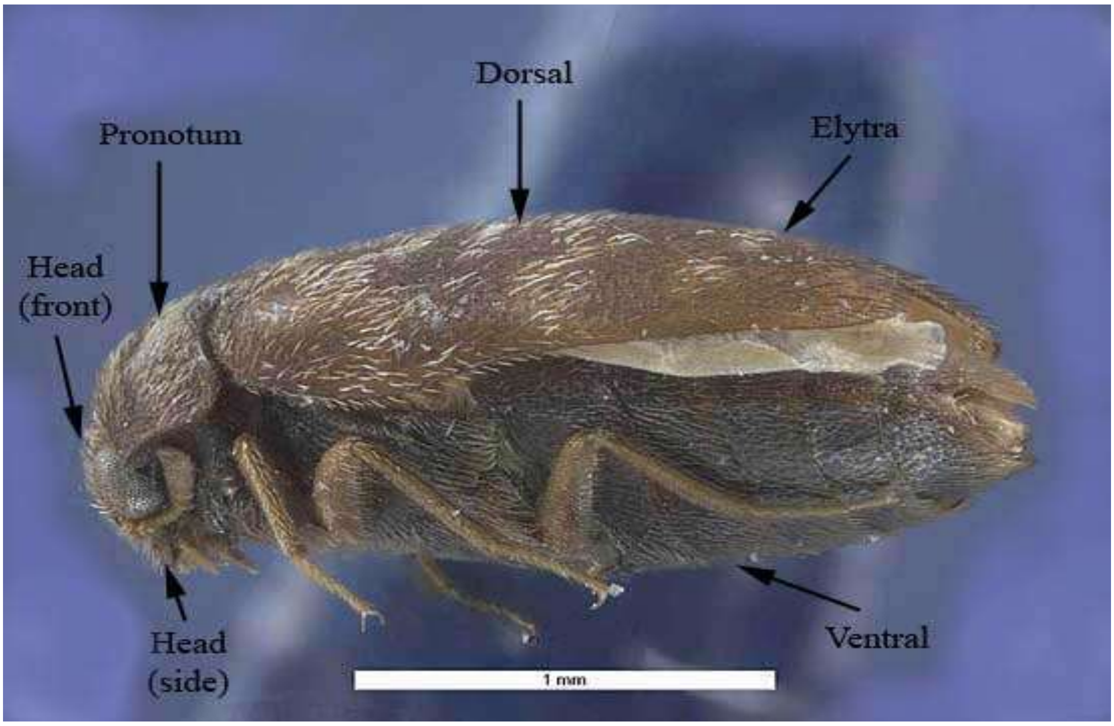


Figure 5. Side view of a fully grown Khabra beetle

Figure 6. Wheat infested with the khabara beetle.

The use of plants in pest control

The ancient Egyptians were renowned for their skills in medicine and healing, as evidenced by their inscriptions, in which they left recipes for combating lice and fleas and repelling mosquitoes. Numerous images have also been found in their inscriptions depicting a number of herbs they used to treat their ailments, which are still in use today **[14][15][16]** noted that the peoples who lived in Mesopotamia were familiar with many plants that were lethal to insect pests, as evidenced by inscriptions found on storehouse tablets written in cuneiform script; Thirty-three medicinal recipes were discovered in the city of Mosul, which were found to be derived from ancient Babylonian and Assyrian sources, used seed oils from a number of plants (peanuts, cotton, maize and soya beans) to combat the grain weevil Sitophilus granarius. noted that the plant kingdom contains numerous chemical compounds used to protect stored crops from insect infestation. Nawrot and colleagues (1986) isolated 49 compounds from plants of the Compositae and Umbelliferae families, which were tested on stored-product insects (the grain weevil, the red flour beetle and the khabara beetle). These extracts exhibited feeding-inhibitory effects on adults of the grain weevil (Sitophilus granarius) and the red flour beetle (Tribolium castaneum), as well as on the larvae of both the khabara beetle and the red flour beetle; treatment during the larval stage resulted in morphological deformities in the resulting adults. noted the existence of 39 plant extracts used in the control of storage insects; as the study, conducted on nine plant species, demonstrated an anti-feeding effect on both the larval and adult stages of the false flour beetle and the grain weevil, and on the larval stage only of the khabara beetle. tested the effects of both acetone and petroleum ether extracts from the leaves of Cassia occidentalis. The seeds were treated with four concentrations, and their effects on the grain weevil were observed; it was found that they inhibited egg-laying as well as reducing the number of adults produced [17-20].

The use of chemical pesticides in pest control

A pesticide is any natural or synthetic biological or chemical substance that reduces pest populations, either by killing them- in which case it is called a 'pesticide'— or by preventing their reproduction and reducing their numbers-in which case it is called a 'pestistatic'. used the pesticides chlorpyrifos-methyl, actellic and malathion in varying proportions to control certain stored-product insects and on yellow maize; the first and second pesticides provided excellent protection for 12 months of storage, whilst the third pesticide was also effective but failed to prevent a significant increase in insect numbers during the final month of storage. Dohorey and his colleagues (1981) reported that when the floors and walls of storage warehouses were sprayed with Actellic, malathion and pyrethrins to protect grain from infestation by storage insects, Actellic was found to be more effective than malathion and pyrethrins, In another study, found that when comparing the effect of kaolin and phatos-natural inert substances-with that of pyrethrin from manufactured pyrethroid insecticides and phytothion from manufactured organophosphate insecticides, the former, at high concentrations, resulted in a 100 per cent kill rate after 4 days in the case of treated rice grains, whilst at high concentrations they produced the same mortality rate as at low concentrations after 7–9 days for adult Khabara beetles [21-24]. differences in the susceptibility of Khabra beetle larvae, reared on different food sources and at different temperatures, to the pesticides; larvae reared on sesame were more susceptible to the pesticides Ficam and Permethrin compared with larvae reared on wheat and cowpeas. The study also showed that larvae reared at a temperature of 25°C ◦ were more responsive to both insecticides than those reared at temperatures of 30°C and 35°C.

Sesbania sesban

Classification of the plant

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta - Seed plants
Division	Magnoliophyta - Flowering plants
Class	Magnoliopsida - Dicotyledons
Subclass	Rosidae
Order	Fabales
Family	Fabaceae - pea family
Genus	Sesbania
Species	Sesbania sesban (L.)

(Evans, 1990).

There are 11 species of Sesbania within the Fabaceae family, and these species are:

1- S. herbacea
2- S. tomentosa
3- S. exaltata
4- S. arborea
5- S. hawaiiensis
6- S. hobdgi
7- S. molokainsis
8- S. aegtiaca

9- S. canabania
10- S. macrocarpa
11- S. sesban (L.)
The common name for this plant is 'Ohai' **[25]**.

Characteristics of the plant

It is a tree reaching a height of (2–4) metres, bearing pinnate leaves with thin, oblong leaflets (Figure 7). It has yellow flowers and a long, narrow, horn-shaped fruit (Figure 8) which contain a number of seeds. It grows well in soil with little water and few nutrients, tolerates high salinity and reproduces by seed (Figure 9) [26]. It grows and flowers in late spring or during the summer and cannot tolerate low temperatures or frost [27].

Geographical distribution

The native range of the sesban plant is North and East Africa and the United States of America [28].

Economic importance of the Fabaceae family

This family is one of the most important families of flowering plants, as it provides various foodstuffs for humans and animals, as well as being a source of oils, dyes (including haematoxylin), gums and resins; hundreds of species are cultivated for ornamental purposes. Among its important food products are bazalia, beans, cowpeas, broad beans, lentils, chickpeas, soya beans and groundnuts; for fodder, alfalfa and clover; and other important species include carob, tamarind and sesbania. Ornamental plants include camel thorn, tree wormwood, ficus and cassia [29].

Figure 7. Sesbania sesban (L.)

Figure 8. Flowers of the sisban plant with seed pods

Figure 9. Seeds of the sesbania plant

Fava-Cypermethrin 5% insecticide

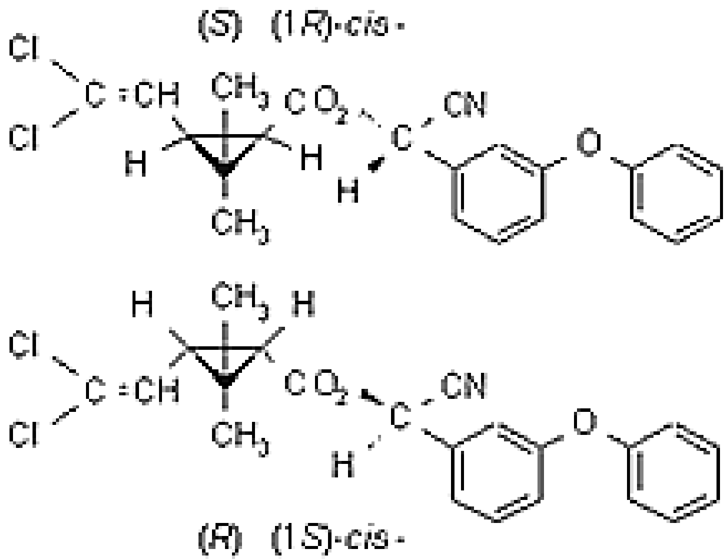
This is a synthetic pyrethroid insecticide, first produced in 1974 and authorised for use in 1977. It is an effective insecticide for controlling a wide range of pests in agriculture and the home. Alpha-cypermethrin 5% is an emulsifiable insecticide that acts by contact and has both an intestinal effect and an effect on the insect's central and peripheral nervous systems. It is used for the widespread control of insects with chewing and sucking mouthparts [30].

Chemical structure of the insecticide alfa-cypermethrin:

Generic name: alfa-cypermethrin

Chemical name: (S)(α-Cyano-3-phenoxybenzyl) IR-cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate.

Chemical formula: $C_{22}H_{19}Cl_2NO_3$ as shown in the following figure [31].



The development of synthetic pyrethroid compounds followed the discovery of natural pyrethrins extracted from certain plants; whilst they are largely similar, pyrethroids are more stable on treated surfaces [32].

Uses of the insecticide fasipermethrin

Fasipermethrin is used in several fields, including agriculture, where it is employed for the control and treatment of a number of agricultural pests such as cotton, fruit and vegetable crop mites; it is also used in other areas such as the eradication and control of insects in warehouses, factory buildings, ships and vehicles used to transport food and grain; In the field of livestock production, it is used to control external parasites in all farm animals; it is considered a preventative treatment for insects that infest horses [33]. In 1983, cypermethrin was used for the first time to control Homoptera, at a concentration of 30–5 g per hectare after being dissolved in water; it was also used to control Lepidoptera, and its rapid effect on these insects was observed, Consequently, the insecticide was used to control numerous insects via spraying, and was also used against a number of insects after being mixed with organophosphate or carbamate insecticides at specific concentrations to enhance its lethality (Fisher et al. 1983, Worthing 1991, Hance). It has also been mixed with numerous pesticides such as Sherpa, Ripcord, Politrin, Mastor, Cyperkill and Cymbush [34].

Distribution of the pesticide alpha-cypermethrin

The pesticide is released into the air via spraying; however, it breaks down or degrades rapidly in the air upon exposure to sunlight and other compounds present in the air, and usually persists for only one or two days before degradation. Rain and snow help to remove it, The pesticide degrades photochemically with a half-life of 8–16 days, producing 3-phenoxybenzoic acid and 3-phenoxybenzyldehyde; furthermore, soil microorganisms are capable of breaking down the pesticide into the same compounds under atmospheric conditions [35].

Toxic effects of the insecticide fipronil

Fipronil is considered to be moderately to acutely toxic to rodents, with a median lethal dose (LD50) – the dose that causes the death of 50 per cent of test subjects under specific conditions – in rats administered orally is 399.8 mg/kg body weight, whilst the median lethal dose via dermal exposure is > 2000 mg/kg body weight.

Practical Section and Methods

Rearing the insect and preparing the food medium

The Khapra beetle (Trogoderma granarum) (Coleoptera; Dermestidae) (Everts) was used in this study. This insect was obtained from infested grains from Silo Tekrit. Fifteen colonies were established, and clean, sterilised glass jars were prepared. (Everts). These beetles were obtained from infested grain from Silo Takrit. Fifteen colonies were established, and clean, sterilised 800-millilitre glass bottles were prepared; 250 grams of wheat (Abu Ghraib variety). The mouths of the bottles were covered with muslin and securely sealed with rubber bands, and the colonies were placed in an 'Incubato' incubator (Fisher Scientific model) at a temperature of 35 ± 2 °C and a relative humidity of 65 ± 5 per cent [36]. The cultures were continuously refreshed to obtain adult insects and to remove moulted exoskeletons.

Materials used:

Seed collection

Sesban seeds were obtained from commercial outlets in the city of Samarra; they were cleaned of impurities and dust and placed in clean plastic bags until required for the experiments.

The chemical pesticide Alpha-cypermethrin 5%

The chemical pesticide, Alpha-cypermethrin 5% (locally known as Supertak-5), was obtained from local markets. It is manufactured by Queen River (China), was produced in January 2014 and is valid until January 2015.

Preparation of the plant extract

Preparation of the sesban seed extract

In the present study, the seed extract was prepared according to the method described by [37] in the laboratory of the Department of Life Sciences, Faculty of Education. Following cleaning of the sesban seeds, they were ground using an electric grinder, and petroleum ether was used as the organic solvent. Forty grams of the seeds ground in the electric grinder were weighed and placed in an extraction vessel (manufactured by the British company Whatman). The extraction process was carried out using a Soxhlet extractor; 400 millilitres of petroleum ether were used, and the mixture was heated to 50°C [38]. Heating was continued until the yellow colour of the seeds had disappeared, and this process was repeated until the required quantity of extracted oil was obtained. The solvent containing the oil was then evaporated to separate the oil from the solvent, which evaporates rapidly at 55°C using a vacuum oven (vacuum oven) (a

French-made Napco model). The extracted oil was then transferred to 50-millilitre screw-capped glass bottles and stored at 4°C until used in the study [39-40].

Preparation of concentrates for seed extract (oil) experiments

Various concentrates of sesban seed oil were prepared to test their toxic effect on adult Trogoderma granarum beetles (Trogoderma granarum) (Everts). Four concentrations of the extracted seed oil were prepared, namely 0.5 per cent, 1 per cent and 1.5 per cent, respectively, and were prepared as follows: The extracted oil has a concentration of 100 per cent, as the above solutions were prepared using 50 millilitres of distilled water and according to the following equation:

$C1 \times V1 = C2 \times V2$, where

C1 denotes the concentration of the oil, which is 100 per cent.

V1 denotes the volume of oil required to prepare the solutions.

C1 denotes the concentration to be used (0.1, 0.5, 1 and 1.5 per cent).

C2 denotes the volume of the solvent, which is distilled water (50 millilitres).

The oil was dissolved in distilled water by adding Twin 80 (Polysorbate), which was added dropwise to the mixture (distilled water and oil) until the mixture became a suspension that was easy to mix and spray. Various concentrations of 5 per cent fipronil were prepared to test their toxic effect on adult hairy grain beetles (Xabira). Four concentrations of 5 per cent fipronil were prepared, namely (0.1, 0.5, 1 and 1.5 per cent). These were prepared according to the method described for the preparation of sesbane seed oil concentrations.

Food used

Three food media were used: wheat (Abu Ghraib variety), which was clean and free from insect infestation; it was thoroughly cleaned and stored separately in clean, sterilised plastic containers until used in the tests.

Effect of temperature on the hairy grain beetle (Khabrā)

A temperature of 38 ± 2 °C was used. Sterilised plastic Petri dishes were prepared, and 30 grams of the food medium (wheat, maize and bulgur). Ten whole insects were then placed in each Petri dish, which were maintained at a temperature of 38±2°C. The life cycle was monitored, with the dishes cleaned regularly to remove moulted skins, until the life cycle was complete and the results were recorded.

CONCLUSIONS

Fundamental Finding: It is observed that the effect of different concentrations of the plant extract used varies, and that only a specific concentration is effective and has a greater impact than the others. **Implication:** There is a direct relationship between the exposure factor to the extract and the pesticide and the kill rate. **Limitation:** The study is limited by the variation in effectiveness across different concentrations, as only a specific concentration demonstrated a greater effect on the kill rate. **Future Research:** Future research should investigate additional concentrations and exposure conditions to determine the optimal concentration and further clarify the relationship between exposure duration and kill rate.

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