

Evaluation of Snake Repellents against the Principal Venomous Snakes of India in Laboratory Condition

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Abstract

Efficacy of two snake repellent formulations namely shoo snake, liquid fence and a chemical eugenol was evaluated in a laboratory condition in a closed wooden chamber. The principal venomous snake species of India namely spectacled cobra (*Naja naja*), common krait (*Bungarus caeruleus*), Russell's viper (*Daboia russelii*) and saw-scaled viper (*Echis carinatus*) were caught from wild and exposed to different substrates like plywood, cloth and filter paper sprayed with snake repellents both in day time as well as night time. Results showed that cobra exhibited repellency of 83.33 % against plywood coated with shoo snake during daytime while saw-scaled viper exhibited 100 % repellency during night time. Krait exhibited 75 % repellency against cloth coated with same concentration of shoo snake during nighttime. Saw scale viper exhibited 100% repellency against plywood coated with shoo snake during night time and eugenol sprayed on cloth during day time. Liquid fence treated on wood repelled 75 % of saw scale vipers during daytime.

Both snake repellents shoo snake, liquid fence and the chemical eugenol exhibited varied degree of repellency towards all the four species of snakes on different substrates. Among the tested chemical formulations, shoo snake and eugenol exhibited higher repellency in closed cages under laboratory conditions. Since the entire four snake species are likely to occur in same geographical area, the snake repellents applied on any substrate are expected to repel all snake species both in day as well as night time. Based on the present study, shoo snake may be considered as a snake repellent for field use in addition to the existing normal precautionary safeguard measures.

Introduction

Snake bites are a common problem in many parts of the world especially South Asia is the most heavily affected region. Reasons may be its high population density, widespread agricultural activities and prevalence of numerous venomous snake species etc. India has the highest number of deaths due to snake bites in the world with 35,000-50,000 people dying per year according to World Health Organization [1,2]. Snake bites happen mostly in late evenings and at night hours when the snakes are unintentionally stepped on or when snakes are picked up while rummaging through leaf litter, scrap materials etc. Snake bite is an important occupational injury affecting farmers and plantation workers. Open-style habitation and practice of sleeping on the floor also expose people to bites from nocturnal snake species.

There are some 2,700 described species of snakes in the world, of which India has 276 species; 62 of them are venomous snakes whose bites can be fatal or life-threatening to man. But the majority of snakebite deaths in India is mainly due to four species, collectively called 'The Big Four' also described as the 'medically important venomous snakes of India'[3,4]. Details of the snakes are given in (Figure 1) Spectacled Cobra *Naja naja*, (Figure 2) Common krait *Bungarus caeruleus*, (Figure 3) Russell's viper *Daboia russelii* and Figure 4 Saw-scaled viper *Echis carinatus*.



Figure 1: Spectacled cobra

The Big Four are commonly found throughout India and occur in a variety of habitats from forests to countryside including desert areas, arid tracts and even well-populated towns, army barracks and cantonments. The Indian army soldiers serving in the border areas, desert regions, deep jungles, remote mountain areas are facing life-threatening situations due to bites from all these poisonous snakes. Bites from sea snakes (Hydrophiidae), pit vipers (Crotalinae) and king cobra (*Ophiophagus hannah*) also result in some mortalities or life-threatening consequences, but chances for human encounter with these snakes is limited because of their restricted geographic range like fishermen in respect of sea snakes and plantation workers in respect of pit vipers, and king cobra. The Polyvalent Snake Antivenin Serum



Figure 2: Common krait

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Figure 3: Russell's viper



Figure 4: Saw-scaled viper

I.P.(ASVS) produced by M/s Haffkine Bio-Pharmaceutical Corporation Ltd, Mumbai in India is a mixture that will address the bites of all the four species of medically important venomous snakes of India namely, Cobra, Krait, Russell's viper and Saw scale viper.

Man-snake conflict is increasing due to deforestation and urbanization in India and it is becoming dangerous. Snakes are known to be the best biological control agent for controlling the rat populations which damage crops and stored materials in go downs. Snakes are valued for their venom in production of antivenin and their skin. Hence, measures are very much taken to protect both men as well as snakes. In some part of the world, answers to man-snake conflicts have been sought in several ways, like involving mechanical and chemical methods. Usage of chemicals and natural oils to repel snakes has been tried in Guam Island, located in the pacific ocean north off the Australian coast, against the brown tree snake (*Boiga irregularis*) [5-9] and in parts of Japan against the Okinawa habu (*Trimeresurus flavoviridis*) [10,11]. But in Indian context, [12] Renapurkar et al. reported the repellency of hexane extracts of five species of plants and oil extracts of four species of plants, against venomous snakes non venomous snakes of India.

Use of methyl bromide as fumigant for brown tree snakes and other aerosolized essential oils were reported as snake repellents [13-16]. In U.S.A and Australia, formulations to repel snakes are available in the market. The pre-marketing trials with these had been conducted on some species of snakes native to those countries. Although these repellents have, to some extent, been tested in Australia and USA, the manufacturers admit that the extent of repellency varied from species to species. Hence, the results from these chemicals cannot be extrapolated to Indian species of venomous snakes. Those trials conducted elsewhere were not against the poisonous snakes found in India.

Aim of the Study

In India there is no snake repellent is available in the market. Hence, the efficacy of commercially available two snake repellent formulations namely shoo snake from Australia, liquid fence from USA and a chemical eugenol (smells like shoosnake) applied on different substrates (plywood, cloth and filter paper) and evaluated against the Big Four venomous snakes of India under laboratory conditions in closed cages both daytime as well as night time.

Materials and Methods

Evaluation of snake repellents against the principal venomous snakes of India were conducted at the Chennai Snake Park in a laboratory, under controlled conditions. Suitable safeguards were ensured in respect of the health of the individual snakes chosen and the time lapsed after their last feeding, their being not gravid. It was also ensured that the snakes had already shed their skins recently so that this process would not interrupt the trials or affect snake behaviour. Studies on snake behaviour [17-22] have several limitations as compared to other animal taxa. Presence of the observer may influence and alter the behaviour of study-subjects [23-27]. Hence, video graphic analysis was used in this experiment involving unmanned, automatically-functioning video cameras. The snake behaviour was monitored by close circuit cameras and the computerized data has been recorded in hard disk and stored in compact discs.

Snake repellent evaluation chamber

The efficacy of snake repellents against venomous snakes of India was evaluated as per the method described by Clark (2007) with slight modification. The snake repellent evaluation chamber has T-maze with two large chambers at the ends of two diverging arms, chambers being large enough to contact proximal part of main chamber; was designed and constructed with plywood. This enclosure contained three chambers and paths (Figure 5). The main chamber A of size 120 X 40 X 60 cm, gave way to the centre path of 15 cm width, which was flanked on both the sides by smaller chambers B and C, measuring 65 X 52 X 75 cm. Paths, diverged from the distal end of the centre path and terminated into the respective chambers B and C. The top of the chamber was covered with wire mesh so as allow air ventilation and to avoid escape of snakes from the chamber.

To quantify the results and also as vouchers of behavioural sequence data, video recording of the experiment was done following [28]. Video recording was done by using nine, movement-triggered, infra-red video cameras (Sony Hi-Focus Colour CCD Camera) installed inside the chamber that were switched on during the snake repellent evaluation period of 30 minutes time. During exposure of snakes to chemicals, cameras get activated in such a way that snakes are covered by two, opposite-facing cameras and that of every path by one camera fixed at the terminal, so that there was minimal imagery overlap and comprehensive video-coverage of movement of snakes in the whole experimental arena. Outputs of all such videos were stamped with important parameters like date, time, humidity, temperature, name of the facility and camera number. Some movements were also photographed with still camera. The whole enclosure was kept in a room where the intensity of light was maintained at minimum during trial run and room temperature was maintained at 27 ± 2 °C.



Figure 5: Snake repellent evaluation chamber (before and after fitting CCD cameras) as viewed from above

Chemicals and formulations of snake repellents

Shoo snake (M/s DTP Sales, Australia), liquid fence (M/s Liquid Fence Company Inc., Broadheadsville, U.S.A) and eugenol (99% pure M/s Aldrich USA) were evaluated used for their efficacy as snake repellents. [9] Clark and Shivik reported eugenol as a potent irritant for brown tree snake (*Boiga irregularis*) which resembled the smell of shoosnake. Shoo snake and liquid fence were dissolved in distilled water, while eugenol was dissolved in hexane. In the case of shoo snake dilution was adopted as per manufacturer’s recommendation (125 ml in 10 litre water). 1.25 ml of shoo snake was added to 9.75 ml distilled water. In the case of liquid fence, the dilution was the same as that of shoo snake. One ml of eugenol was mixed with 9 ml hexane for treating the different substrates. Solvent hexane was used as control. The surface area of different substrates used in this experiments include plywood, cloth and Whatman No.1 filter paper as shown in Figure 6 were treated with snake repellents using micropipette for evenly spraying the solutions. It was calculated that plywood and cloth required 10 ml of test solution while filter paper required only 5 ml.

Snakes

All the snakes used in this study were caught from the wild. Twelve individuals of each species were captured. The Big Four principal venomous snakes of India namely the spectacled cobra (*Naja naja*), the common krait (*Bungarus caeruleus*), the Russell’s viper (*Daboia russelii*) and the saw-scaled viper (*Echis carinatus*) were identified and used. Only normal, healthy, adult individuals, that were not fed very recently, not in ecdysis and not gravid, were used. After every trial nearly two month intervals were given for next use of the same snake. Details on the length and weight of all the snakes are given in Table 1. After completion of the repellent studies, the snakes were maintained separately and their health was monitored regularly. Frequent and repeated usage of the same individuals was minimized.

Experimental procedure for snake repellent evaluation

Before starting the experiments, the length and weight of the snakes, were measured. The snakes were introduced into the chamber



Figure 6: Details of plywood used for treatment of snake repellents (30cm X 15 cm)

Serial No.	Name of the snake	Length in meter (Min. to Max.)	Weight in grams (Min. to Max.)	Total number of snakes used
1.	Spectacled Cobra (<i>Naja naja</i>)	0.75 to 1.68	250 to 1125	12
2.	Common krait (<i>Bungarus caeruleus</i>)	0.45 to 1.52	50 to 400	12
3.	Russell’s viper (<i>Daboia russelii</i>)	0.90 to 1.68	500 to 1150	12
4.	Saw-scaled viper (<i>Echis carinatus</i>)	> 0.3	> 25	12

Table 1: Details of the length, weight and total number of wild caught snakes used in the evaluation of snake repellents

(A) of the experimental enclosure for acclimatization for 2 hours. After complete air drying, the treated substrate was introduced into the test enclosure at a distance of 1 to 2 feet away from the snake that was resting in the chamber. Experiments were also carried out with solvent hexane used for dissolving eugenol as blank trials. Repellency behaviour of the snake was determined by the time required within a period of 30 minutes by the snake to move away from the chamber where snake repellent-treated substrate was kept to the other chamber where no chemical was present. As soon as the experiment was initiated, video recording was also switched on so that the behaviour and movements of snakes was recorded in the hard disk of the computer using the software (Timhillone software) specially designed for this experiment (M/s. Kavva Soft, Chennai). These experiments were carried out during daytime from 06.00 hrs to 18.00 hrs and nighttime from 18.00 hrs to 06.00 hrs.

Results & Discussion

A total of 323 trials were carried out on the evaluation of snake repellents against all the four species of snakes during the period from February 2009 to August 2010. Details of the trials conducted during day time as well as night time with snake repellents shoo snake, liquid fence and a chemical eugenol treated on different substrates against the four principal venomous snake species of India is given in Table 2. The details on the effect of shoo snake, liquid fence and eugenol sprayed on plywood, cloth and filter paper are given (Figure7- 9) respectively.

The repellent studies on shoo snake showed that cobras were

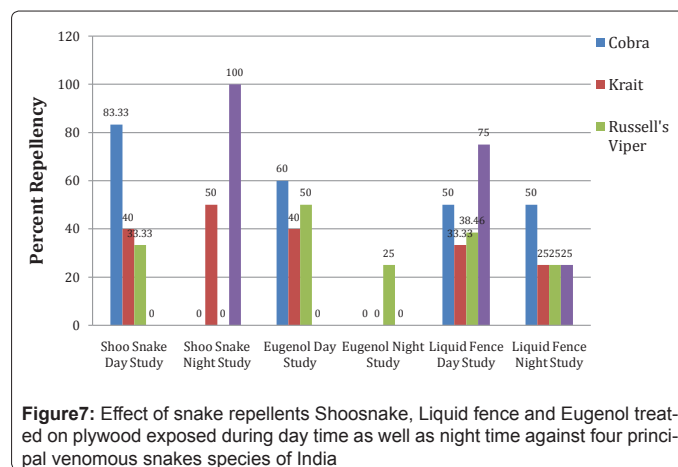


Figure7: Effect of snake repellents Shoosnake, Liquid fence and Eugenol treated on plywood exposed during day time as well as night time against four principal venomous snakes species of India

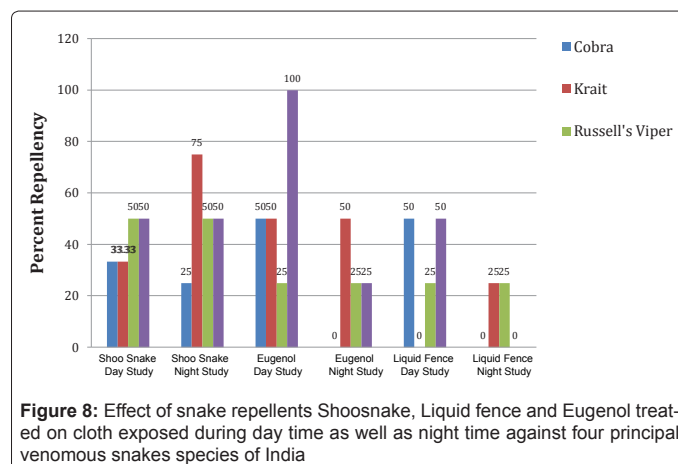


Figure 8: Effect of snake repellents Shoosnake, Liquid fence and Eugenol treated on cloth exposed during day time as well as night time against four principal venomous snakes species of India

Snake species	Snake repellent formulation	Substrate and repellency values											
		Wood				Cloth				Filter paper			
		Day		Night		Day		Night		Day		Night	
		No. of snakes repelled/exposed	% Repellency	No. of snakes repelled/exposed	% Repellency	No. of snakes repelled/exposed	% Repellency	No. of snakes repelled/exposed	% Repellency	No. of snakes repelled/exposed	% Repellency	No. of snakes repelled/exposed	% Repellency
Cobra	Shoo snake	5/6	83.33	0/3	0.00	2/6	33.33	1/4	25.00	0/5	0.00	0/4	0.00
	Eugenol	3/5	60.00	0/4	0.00	2/4	50.00	0/4	0.00	0/4	0.00	0/4	0.00
	Liquid fence	2/4	50.00	2/4	50.00	2/4	50.00	0/4	0.00	1/5	20.00	0/4	0.00
Krait	Shoo snake	2/5	40.00	2/4	50.00	2/6	33.33	3/4	75.00	3/6	50.00	1/3	33.33
	Eugenol	2/5	40.00	0/4	0.00	2/4	50.00	2/4	50.00	0/4	0.00	2/4	50.00
	Liquid fence	2/6	33.33	1/4	25.00	0/4	0.00	1/4	25.00	1/5	20.00	0/4	0.00
Russell's viper	Shoo snake	1/3	33.33	0/4	0.00	2/4	50.00	2/4	50.00	0/4	0.00	2/4	50.00
	Eugenol	2/4	50.00	1/4	25.00	1/4	25.00	1/4	25.00	0/4	0.00	0/4	0.00
	Liquid fence	5/13	38.46	1/4	25.00	1/4	25.00	1/4	25.00	2/8	25.00	1/4	25.00
Saw-scaled viper	Shoo snake	0/8	0.00	4/4	100.00	2/4	50.00	2/4	50.00	1/5	20.00	0/4	0.00
	Eugenol	0/6	0.00	0/5	0.00	4/4	100.00	1/4	25.00	0/4	0.00	1/3	33.33
	Liquid fence	3/4	75.00	1/4	25.00	2/4	50.00	0/4	0.00	1/6	16.67	1/4	25.00
	Total	69		48		52		48		60		46	
	Grand total											323	

Table 2: Details of the trials conducted during day time as well as night time with snake repellents shoo snake, liquid fence and a chemical eugenol treated on different substrates against the four principal venomous snake species of India

repelled by 83.33 % on plywood, 33.33 % on cloth and no repellency on filter paper during daytime and 25 % repellency on cloth and no repellency on plywood and filter paper during night. In kraits, 40 % repellency was observed on plywood, 33.33 % on cloth and 50 % on filter paper during daytime and 50 % were repelled on plywood, 75 % on cloth and 33.33 % on filter paper during night. Russell's vipers, showed 33.33 % repellency on plywood, 50 % on cloth and no repellency on filter paper when during daytime and no repellency on plywood and 50 % repellency on cloth and filter paper during night time. Saw-scaled viper showed no repellency on plywood, 50 % repellency on cloth and 20 % repellency on filter paper during daytime and during night time 100 % repellency on plywood, 50 % on cloth and no repellency on filter paper were observed.

Eugenol produced 60 % repellency in cobra on plywood, 50% on cloth and no repellency on filter paper during daytime and during night, no repellency on any substrate was observed. In the case of krait, 40 % repellency was observed on plywood, 50 % on cloth and no repellency on filter paper during daytime and during night, no repellency on plywood and 50 % repellency each on cloth and filter paper. Russell's viper exhibited 50 % repellency on plywood, 25 % on cloth and no repellency on filter paper during daytime and during night, 25 % repellency on plywood as well as cloth and no repellency on filter paper. Saw-scaled viper showed 100 % repellency on cloth, no repellency on plywood and filter paper during day time and no repellency on plywood, 25 % on cloth and 33.33 % on filter paper during night. One set of blank trials were conducted with solvent hexane and one set with no solute showed no repellency by any of the four snakes tested in the trial on all the three substrates.

Liquid fence produced 50 % repellency against cobra on both plywood and cloth, 20 % repellency on filter paper during daytime trials and 50 % repellency on plywood and no repellency on cloth and filter paper during night time. In the case of krait, the repellencies were 33.33 % on plywood, no repellency on cloth and 20 % on filter paper during daytime were observed whereas 25 % repellency on plywood and cloth and no repellency on filter paper was recorded during night time. Russell's viper exhibited repellency of 38.46 % on plywood, 25% on cloth as well as filter paper. Saw-scaled viper showed 75 % repellency on plywood, 50 % on cloth and 16.67 % on filter paper during daytime

but during night time repellency of 25 % on plywood as well as filter paper and no repellency on cloth was observed.

Comparative evaluation of snake repellent formulations Shoo snake, eugenol and liquid fence against the 'Big Four' venomous snake species of India (spectacled cobra, common krait, Russell's viper and saw-scaled viper) shows that cobra showed highest (83.33 %) repellency against plywood coated with Shoo snake during day time; krait showed highest (75 %) repellency against cloth coated with shoo snake normal conc. During night; saw-scaled viper showed highest (100 %) repellency against plywood coated with Shoo snake during daytime as well as against cloth coated with eugenol during night. However, Russell's viper showed only moderate repellency (50 %) against cloth coated with shoo snake exposed during both day and night, on filter paper during night, and against plywood coated with eugenol exposed during daytime. However, these high repellence values were obtained only few times out of several trials conducted.

The snake repellent data obtained from the above studies were subjected to Chi square test and Sigmasat was used for all the statistical analysis. Details of the calculations are given in Table 3. The results showed that only Cobra exhibited significant repellency ($p < 0.01$) during day time as compared to night times. In the case of other snakes including Krait, Russell's viper and Saw scale viper no significant repellency was observed between day studies and night studies. When the both snake repellent formulations and the compound eugenol were compared for their efficacy as snake repellents against all the snakes, only shoo snake could show a significant repellency ($p < 0.05$) during night studies as compared to day time studies.

In the case of studies on snake repellents in India, [12] Renapurkar et al. reported that hexane extracts of five species of plants, namely, garlic (*Allium sativum*), sweet flag (*Acorus calamus*), neem (*Azadirachta indica*), tobacco (*Nicotina tabacum*) and five-leaved chaste tree (*Vitex negundo*) and oil extracts of four species of plants namely sweet flag (*Acorus calamus*), pine, citronella and thyme were found to repel the snakes effectively as calculated based on the repellency index. They concluded that only *Acorus calamus* and pine oil showed an appreciable snake repellent property which required further investigation. Duration of exposure period to plant extracts and oils in the snake repellent experiment was six hours which is longer duration which may of little

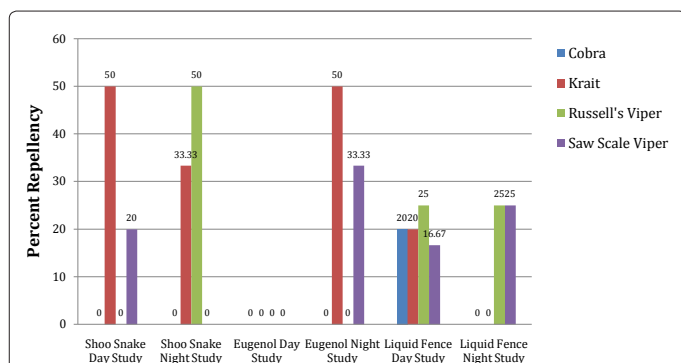


Figure 9: Effect of a snake repellents Shoosnake, Liquid fence and Eugenol treated on filter paper exposed during day time as well as night time against four principal venomous snakes species of India

1. Day studies VS Night studies				
		Repelled	Not repelled	Chi-Square value
Cobra (<i>Naja naja</i>)	Day studies	17	26	$\chi^2 = 8.146, P < 0.01$
	Night studies	3	32	
Krait (<i>Bungaruscaeruleus</i>)	Day studies	14	31	
	Night studies	12	23	
Russells viper (<i>Daboia russelii</i>)	Day studies	14	34	
	Night studies	9	27	
Sawscale viper (<i>Echis carinatus</i>)	Day studies	13	33	
	Night studies	8	26	
2. Formulation Differences				
	Formulations			
Day studies	Shoosnake	20	42	
	Eugenol	16	37	
	Liquid fence	22	45	
Night studies	Shoosnake	17	29	$\chi^2 = 6.384, P < 0.05$
	Eugenol	8	40	
	Liquid fence	9	39	

Table 3: Details of the statistical analysis of data obtained from four principal venomous snake species of India exposed during day time as well as night time to snake repellents (shoo snake, liquid fence and a chemical eugenol) treated on different substrates

use in practical application. [9] Clark and Shivik (2004) followed the method of spraying the aerosols of snake repellents directly on to the snake's head for 2 seconds at a distance of 30mm and observed for any response 5 minutes following aerosol application. Responsiveness of the snakes to the various reagent based aerosols varied widely. Based on the responsiveness the essential oils and compounds were categorized into 2 groups. Even by this method also different essential oils and the aroma products species of snakes responded in a different way. Compounds that are effective against mammals like cinnamic acid and capsaicin were found not effective against snakes. Dr. T's Snake-A-Way a registered product in United States as snake repellent could not repel the Brown Treesnake. Hence, the interpretation of the data from different set of studies has to be done according to the species of the snakes.

Conclusions and Recommendations

Among the two snake repellent formulations and eugenol tried, the best results were obtained for shoo snake. In the case of Liquid fence and eugenol, only moderate results were obtained against all the

snakes in laboratory conditions. Considering the reason that all the four major venomous snakes of India are likely to be found in the same habitat and the snake repellent chosen for field application is expected to protect human beings and 100 % effective. It is recommended that only shoo snake may be tried as an additional safeguard, along with other precautions to be taken against snakebite but not as a sole agent to repel all the snake species. Based on the above studies it is possible to design a chemical formulation that can be a better snake repellent than available ones.

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