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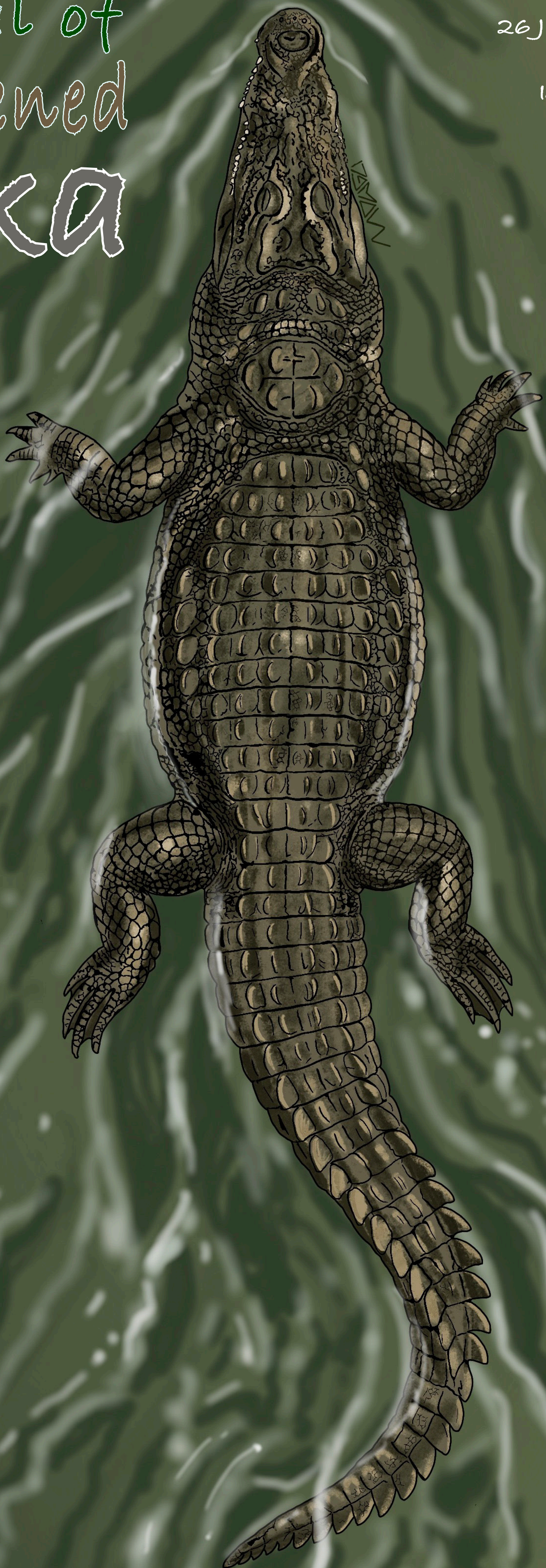

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Cover: Nile Crocodile *Crocodylus niloticus* regulating body temperature on a warm day. Digital art on Procreate by © Aakanksha Komanduri.

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ARTICLE

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Efficacy of 5% neem seed kernel extract against ectoparasites in six captive wildlife species at Rajiv Gandhi Zoological Park, India

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Abstract: This wildlife study aimed to assess the prevalence of ectoparasites and evaluate the in vivo efficacy of a 5% Neem Seed Kernel Extract (NSKE) insecticide in captive wildlife species at Rajiv Gandhi Zoological Park and Wildlife Research Centre (RGZPWRC), Katraj, Pune, Maharashtra, India. Ectoparasites were collected non-invasively from six wildlife species, namely, Black Buck *Antelope cervicapra*, Spotted Deer *Axis axis*, Leopard *Panthera pardus*, Tiger *Panthera tigris*, Elephant *Elephas maximus*, and Sloth Bear *Melursus ursinus* using insect traps in animal shelters and night houses, alongside combing and visual inspection. A total of 865 ectoparasites were documented in the entire study duration of 19 days, of which, 662 ectoparasites were documented before and 203 after the application of 5% NSKE, each over a nine-day period. Flies comprised 93.99% of the total ectoparasites found (865) during the entire study, followed by lice at 5.55% and fleas at 0.46%. The overall relative prevalence was 76.53% pre-treatment and 23.47% post-treatment. By species, Black Buck showed the lowest relative prevalence at 6.24%, and Elephants the highest at 21.73%, with an average relative prevalence of 16.67%. Leopards, Tigers, and Elephants had values of 21.62%, 19.66%, and 21.73%, respectively. Taxonomically, flies represented 96.30% in Black Buck, 63.91% in Spotted Deer, 98.93% in Leopards, and 100% in both Elephants and Sloth Bears. The estimated prevalence exceeded 100% in some species due to multiple parasites per host based on average parasitic load calculations. During treatment, shelters and night houses were washed and sprayed with 5% NSKE. Re-sampling after nine days showed a marked reduction in ectoparasite counts. Efficacy of 5% NSKE against flies, fleas, and lice was 69.29%, 100%, and 66.67%, respectively, with an overall efficacy of 69.33% ($P < 0.001$) with a 95% confidence interval ranging from 65.70% to 72.90%. The highest efficacy was observed in Elephants at 81.76%. Use of 5% NSKE is hence strongly recommended as an insecticide or repellent in captive wild animal housing systems. These findings support the integration of botanical insecticides into integrated pest management programs for captive wildlife.

Keywords: Acaricidal activity, alternative pest management, animal welfare, *Azadirachta indica*, Azadirachtin, botanical biopesticides, Katraj Zoo, parasite control in zoos, Pune, veterinary parasitology.

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INTRODUCTION

India's tropical climate creates favourable conditions for the growth and survival of parasites and vector populations, which increases the risk of ectoparasitic infestations in both humans and animals. There are only limited or sporadic studies available on the prevalence of ectoparasites in captive and wild animals in Indian zoological parks and wildlife sanctuaries (Moudgil & Singla 2021). As noted by Moudgil et al. (2015), the understanding of parasitic diseases in wild animals is still developing. Difficulties in tracking and sampling wildlife, especially in their natural habitats, have made it challenging to generate reliable data on host distribution and the spread of zoonotic parasites (McCallum & Dobson 1995). Ectoparasites found in wild mammals include lice (Phthiraptera), ticks (Order: Acarina), fleas, biting flies (Diptera), mites, and bot flies. Among these, species like *Haemaphysalis cuspidata*, *H. kinneari*, and *Amblyomma hebraeum* have been reported from big cats like leopards and tigers in various zoo-based studies. Infestations with *Sarcoptes scabiei*, *Ctenocephalides felis*, and the dog chewing louse *Trichodectes canis* have also been recorded in zoo and wild animal species (Gaurava & Singh 1999; Nashiruddullah & Chakraborty 2001; Islam 2010; Moudgil et al. 2015). These ectoparasites are known to cause significant effects such as blood loss, skin irritation, hypersensitivity, transmission of diseases, and behavioural disturbances. However, the impact and control of these parasites in captive zoo animals, especially in Indian zoological parks, remains a poorly researched area (Samuel et al. 2001). Without sufficient data on ectoparasite prevalence, it becomes difficult to implement focused health management, vector control, and conservation strategies in zoo settings. Recently, there has been an increasing focus on using safer, eco-friendly alternatives to chemical insecticides for controlling ectoparasites. Among these, botanical insecticides such as neem seed kernel extract (NSKE) have shown promising results. Given the growing concerns around pesticide resistance and chemical residues, NSKE is being considered as a potential alternative for use in zoological environments, where animal safety and environmental sustainability are key concerns. Among various botanical formulations explored for ectoparasite control, NSKE has emerged as a widely studied and promising alternative. It is known for being biodegradable, non-toxic to non-target organisms, and free from harmful residues, making it suitable for application in sensitive environments such as zoological parks. NSKE contains bioactive compounds like azadirachtin, which have shown broad-spectrum efficacy against multiple ectoparasitic arthropods, particularly ticks and fleas. Previous studies have reported a significant reduction in ectoparasite load following its application, highlighting its potential in practical field use (Webb & David 2002; Albarrán-Rodríguez et al. 2019). In addition to its ectoparasitic properties, factors such as local availability, cost-effectiveness, and alignment with animal welfare practices contribute to its increasing relevance in current ectoparasite control strategies, especially in captive wildlife settings. The objectives of this study were:

1. To determine the prevalent ectoparasites in the six wildlife species, namely, Black Buck, Spotted Deer, Leopard, Tiger, Elephant, and Sloth Bear housed at the Rajiv Gandhi Zoological Park and Wildlife Research Center (RGZPWRC), Katraj, India.
2. To calculate the efficacy of 5% NSKE as a botanical insecticide.

This study tested the efficacy of 5% NSKE as a botanical insecticide.

MATERIALS AND METHODS

Site and duration of the research: The research work was conducted from January–March 2025. The ectoparasites were collected from six wildlife species namely, Black Buck *Antelope cervicapra*, Spotted Deer *Axis axis*, Leopard *Panthera pardus*, Tiger *Panthera tigris*, Elephant *Elephas maximus*, and Sloth bear *Melursus ursinus*, housed at RGZPWRC, Katraj (Table 6). All the necessary permissions and approvals were procured from the principal chief conservator of forests (PCCF) wildlife, Maharashtra State Biodiversity Board and RGZPWRC, Pune. Also, the ethics committee for animal experiments from KNP College of Veterinary Science, Shirwal approved the research vide minutes of the 24th Institutional Animal Ethics Committee (IAEC) meeting, Item No. 3, Sr. No. 08, protocol number: IAEC/08/24/KNPCVS/2024.

Collection of ecto-parasites: A pre and post-treatment design was used for evaluating the efficacy of 5% NSKE as an insecticide used in vivo strictly in the animal shelters (not topically) and to study the relative prevalence of ectoparasites. Non-invasive sampling methods such as light traps (Image 1), glue-based traps (Image 2 & 3), Chemical cue-based (slow CO_2-releasing) insect luring traps (Image 4) (Smallegange et al. 2010; Schoenthal 2015; Aldridge et al. 2016; Sukumaran et al. 2016; Gibb & Oseto 2019; Schilling et al. 2022)

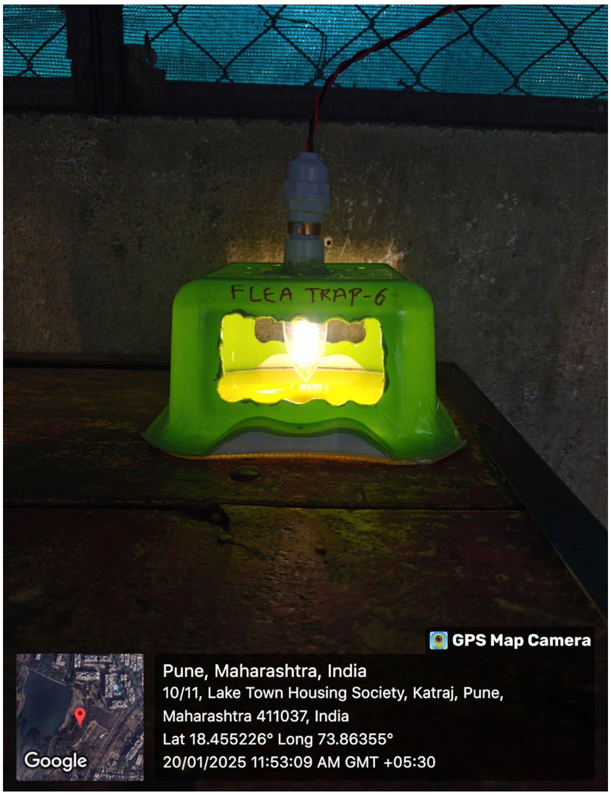

Image 1. Light based insect luring trap (Flea trap).

Image 2. Glue based insect traps (rolls).

Image 3. Glue based insect traps (boards).

Image 4. Carbon dioxide based insect traps.

were implied for the collection of ectoparasites from the animal shelters, along with noninvasive physical screening methods. Animal handling was strictly avoided in this study, and the ectoparasite samples were collected only using the traps. These samples were then assessed and considered indicative of the parasitic load in the given wildlife species.

Those ectoparasites that could not be separated from the insect traps due to their strong adhesion with the glued insect traps or due to the chances of disruption of the parasite morphology were digitally collected by photographically capturing them using 10x and 20x optical lenses mounted on a mobile phone camera lens for enhanced magnification and a clearer image capture (Image 5 & 9). The screening and collection of ectoparasites were carried out for nine days each in the pre-treatment and post-treatment phases. A nine-day sampling was conducted to minimize the study duration so as to assess the single use efficacy of NSKE. Samples were to be collected from various wild animal species, including herbivores and carnivores, herd animals, as well as individual animals. Hence, all the animals housed in every species were included in the study.

Condition of the animal housing studied: All six species of animals studied had separate night houses

Image 5. Digital collection of fragile ectoparasites.

Image 6. Commercially available neem seed kernel extract.

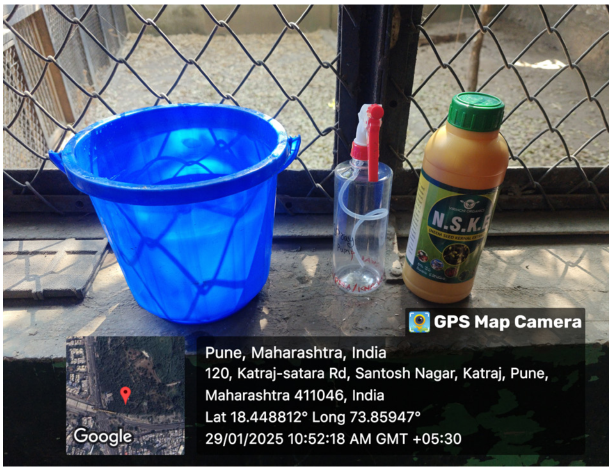

Image 7. Preparation of 5% NSKE solution.

Image 8. Washing of animal housings with 5% NSKE.

and enclosures. This study focused on setting up traps in the night houses at a fixed position and particular distance so as to avoid harm to the animals while resting and ensure the closest proximity for luring ectoparasites into the traps. The night houses had assigned caretakers who ensured regular washings and periodic disinfection to avoid infections and repel insects while the animals rested at night. The commonly used practices at every night house included foot baths of potassium permanganate, washings of the floor with salt solution, cleaning of biowastes and drying with the help of fans.

Application of 5% NSKE in animal shelters of the zoo: On the 10th day of the study, a 5% solution of NSKE was prepared using tap water and commercially available concentrate of NSKE, as per the manufacturer's guidelines and applied once in all the six wild animal shelters by spraying, washing and avoiding a direct contact of the solution with the animals in the enclosures (Image 6, 7, & 8). A single application of NSKE ensured that the efficacy is calculated with respect to the minimal use of NSKE, and the frequency of application could therefore be increased in further studies and on field applications as per the requirements.

Processing, identification and ectoparasite prevalence calculation: The collected samples were preserved using 5% glycerinated 70% alcohol solution until processing and identification based on morphological features (Soulsby 1982). The ectoparasite specimens were classified accordingly into the five major ectoparasite categories (and sub-categories wherever required) of interest which are; flies, fleas, ticks, mites and lice. The processed flies were identified to genus level using keys mentioned in (Soulsby 1982) (Image 10, 11, 12, 13, & 14). Mosquitoes were identified on the basis of morphology and mouth parts (Image 15 & 16). Fleas were processed to observe the comb and classified to species level on the basis of morphological characteristics. Similarly, lice were processed, identified, recorded and classified accordingly (Image 17 & 18). The data obtained from the processing and identification was recorded for calculation of species specific prevalence, relative prevalence and estimated prevalence of ectoparasites in the six selected wildlife species at the zoo.

Formulae used for calculations: The values of 'average parasitic load per infested host animal' ($\overline{Z}$) were compiled (Table 1) from various studies which recorded and calculated them as the mean / average count of an ectoparasite usually occurring in a given species of infested host (Griffiths 1978; Lehmann 1994; Rózsa et al. 2000; Krasnov 2008; Sarkar et al. 2012; Eads et al. 2015; Razali et al. 2018; Zajac et al. 2021; Oliver & Eckerlin 2022). These values were then used for calculation of the estimated number of infested hosts in the given population of wildlife species by using the formula:

Estimated number of infested hosts (H_{inf}) = Total ectoparasite count ÷ $\overline{Z}$

Further, the H_{inf} value was used for the calculation of the estimated prevalence of an ectoparasite in a given wildlife species by using the formula:

$$\text{Estimated Prevalence (\%)} = \text{Estimated no. of infested hosts } (H_{inf}) \div \text{Total number of animals (N)} \times 100$$

The estimated prevalence itself defines the estimation of prevalence in scenarios where the calculation of actual prevalence is not possible or may result in false positives. In this study, using non-invasive sampling for ectoparasite collection in the captive wildlife species and calculation of actual prevalence values was impossible as observation and allotting of procured ectoparasites to a part of the population studied becomes impossible. So, as an equally efficient alternative, the relative prevalences were calculated, which gave an idea about the proportion of one type or species of ectoparasite among the total ectoparasites collected. As a part of this, the estimated prevalence gave an idea about the probability of the number of animals infested with a given ectoparasite within the studied population. Also, the values of the estimated prevalence should not be solely interpreted or treated as actual prevalence values.

Calculations for the efficacy of 5% NSKE as an insecticide: The efficacy of 5% NSKE was calculated on the basis of reduction in the ectoparasite count, by comparing the mean ectoparasite count before and after the application of 5% NSKE, by using modified Abbott's Formula (Webb & David 2002; Tabassam et al. 2008; Narladkar 2018) which is:

$$\text{Efficacy (\%)} = (C_{pre} - T_{post}) \div C_{pre} \times 100$$

Where, C_{pre}: Mean ectoparasite count before treatment of 5% NSKE.

T_{post}: Mean ectoparasite count after treatment of 5% NSKE.

Statistical analysis of the data: Ectoparasite counts recorded before and after treatment were summarized as frequencies and percentages. Treatment efficacy was calculated as the percentage reduction in ectoparasite counts following application of the insecticidal formulation. The recorded data was analyzed with the help of the Statistical Package for Social Sciences (SPSS-20). Descriptive statistics that include frequency and means were used for the analysis. Non-parametric statistical test; chi-square test (χ^2) was used with one degree of freedom. The 95% confidence intervals (CI) for efficacy estimates were calculated using the Wilson score method, which provides robust interval estimation for binomial proportions. A p-value < 0.05 was considered statistically significant. All statistical interpretations were based on standard biostatistical methods.

RESULTS

During the nine-day pre-spraying phase, a total of 662 ectoparasites were collected from animal shelters before the application of 5% NSKE. Before treatment, flies constituted the majority at 93.95% (622/662), followed by lice at 5.45% (36/662), and fleas at 0.60% (4/662), making them the least prevalent. In the following nine days after treatment, a total of 203 ectoparasites were collected, with flies making up 94.09% (191/203), followed by lice at 5.91% (12/203), which were the least observed during this phase (Figure 1). Overall, 865 ectoparasites were recorded collectively during the pre and post-spraying phases across various wild animal species. The overall relative prevalence of ectoparasites was lowest in Black Buck (6.24%) and highest in Elephants (21.73%), followed by Leopard (21.62%), Tiger (19.66%), Sloth Bear (15.38%), and Spotted Deer (15.37%). The average relative prevalence of ectoparasites was 16.67%, indicating higher ectoparasite burden in Leopards, Tigers, and Elephants compared to the overall average (Table 2) (Figure 2). Out of the total 865 ectoparasites collected during the study, 76.53% were recorded before and 23.47% after the application of 5% NSKE. This reflects a highly significant difference in the distribution of the ectoparasite among the six wildlife species studied, with chi-square test

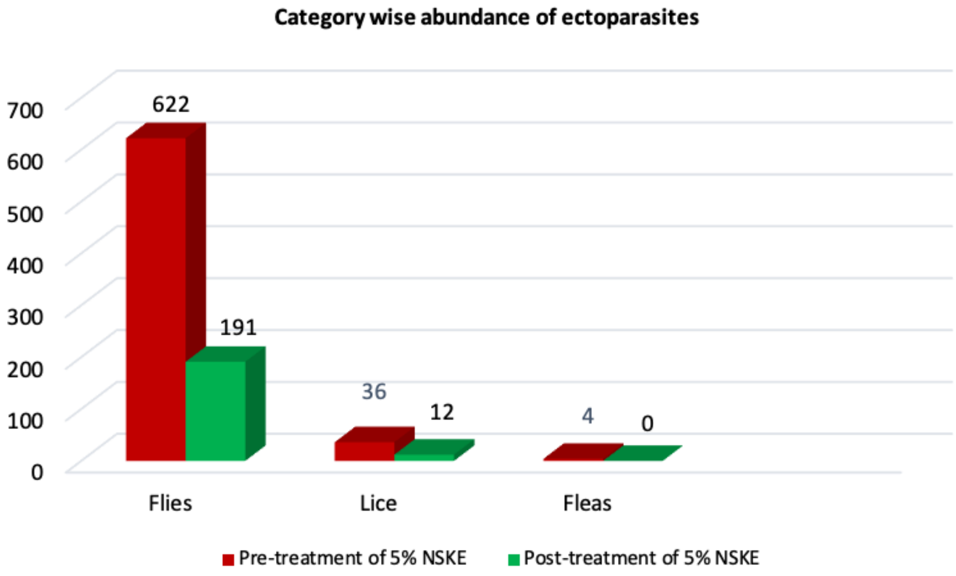

Figure 1. Category wise abundance of ectoparasites.

Table 1. Values of 'average parasitic load per infested host animal ($\overline{Z}$)' in various wildlife host species (in captivity).

Wildlife species (Binomial name)	$\overline{Z}$ values for the ecto-parasite species/category					
	Muscid flies	Midges	Mosquitoes	Lice	Fruit flies	Fleas
Black Buck *Antelope cervicapra*	3–4	10	8	15–20	5	7
Spotted Deer *Axis axis*	4	12	10	20	6	8
Leopard *Panthera pardus*	3	6	5	10	3	4
Tiger *Panthera tigris*	2	7	6	10	4	5
Elephant *Elephas maximus*	5	15	12	1	4	4
Sloth Bear *Melursus ursinus*	2	5	4	4	3	3

values of 78.628 and 28.340 before treatment and after treatment, total ectoparasite counts with P<0.001 (Table 2). Among the six wild animal species studied, the highest estimated prevalence of flies was observed in Sloth Bear (475.00%), followed by the Leopard (362.66%), Elephant (261.11%), Tiger (223.68%), Black Buck (4.26%), and Spotted Deer (2.55%). For a better understanding here, the estimated prevalence simply gives an idea about the probability of the number of animals infested with a given ectoparasite within the studied population. (Note: estimated prevalence values > 100% indicates that the ectoparasite population exceeds the capacity of individual hosts based on average parasitic load values, suggesting environmental accumulation in housing structures). For example, the estimated prevalence reported as 475% in sloth bear signifies that the current prevalence of flies in the animal housings of sloth bear is 4.75 times 100% or 4.75 times greater than the average number of flies a single infested Sloth Bear can harbor. Bovicola spp. lice were found only in Spotted Deer (2.78%). Fleas (*Ctenocephalides* spp.) showed the highest prevalence in Leopard (16.66%) and a low level in Black Buck (0.63%), while no fleas were detected in the other species (Table 2). The present study finds the highest estimated prevalence of ectoparasites in Sloth Bears (475.00%) and the lowest estimated prevalence of ectoparasites in Black Buck (4.89%), the most prevalent taxa being the flies, followed by the fleas, and lastly the lice as per the estimated prevalence values in various wildlife species studied at RGZPWRC (Table 3). Also, no ticks and mites were observed and recorded from the wildlife host species studied at the zoo. Estimated prevalence values exceeding 100% also indicate that the calculated number of infested hosts was too low to account for the total ectoparasites found. This is calculated using the average parasitic load values for each category of ectoparasite in different hosts, from previous studies. Therefore, estimated prevalence alone

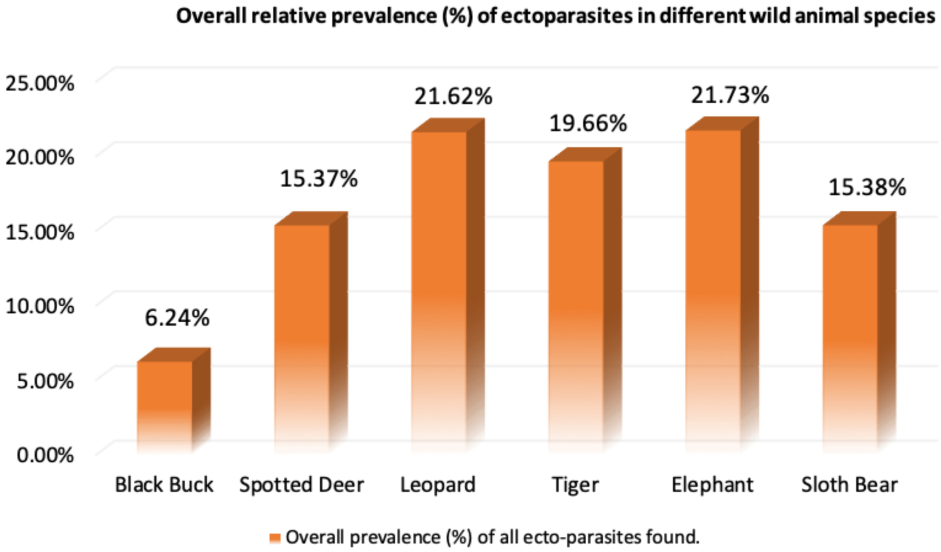

Figure 2. Overall relative prevalence (%) of ectoparasites in different wild animal species.

Table 2. Host species wise prevalence of ectoparasites observed in the wildlife species at Katraj Zoo:

	Wildlife species (Binomial name)	Total no. of wild animals observed	Total no. of ectoparasites observed w.r.t the application of 5% NSKE (Prevalence % in the host species)		Total no. of ectoparasites observed [A+B] (Overall prevalence %)
			Before [A]	After [B]	
1.	Black Buck *Antelope cervicapra*	45	43 (79.63)	11 (20.37)	54 (6.24)
2.	Spotted Deer *Axis axis*	104	93 (69.93)	40 (30.07)	133 (15.37)
3.	Leopard *Panthera pardus*	03	147 (78.61)	40 (21.39)	187 (21.62)
4.	Tiger *Panthera tigris*	04	118 (69.41)	52 (30.59)	170 (19.66)
5.	Elephant *Elephas maximus*	02	159 (84.57)	29 (15.42)	188 (21.73)
6.	Sloth Bear *Melursus ursinus*	02	102 (76.69)	31 (23.30)	133 (15.38)
Total		**160**	**662**	**203**	**865**
χ^2 value:			78.628**	28.340**	

*—Significant at P < 0.05 | **—Highly significant at P < 0.01 | NS—Non-significant.

should not be interpreted to assess the prevalence and should always be interpreted alongside relative or actual prevalence data (Eads et al. 2015; Klepeckienė et al. 2020; Smith et al. 2023). But it surely does give us an idea about the existing condition of parasitic infestations in situations where individual host monitoring is difficult or impossible, and hence, actual prevalence cannot be calculated.

Efficacy of 5% NSKE as an insecticide/insect repellent: It was observed that the reduction in the prevalence of the ectoparasites in various wild animal species was highly significant (Table 4). The efficacy was calculated to be as high as 81.76% in the Elephant. The average efficacy of 5% NSKE was calculated to be 69.33% with a 95% confidence interval that ranged 65.70–72.90%. (Table 4, Figure 3), which indicates a highly significant decrease in the overall ectoparasite counts in all six wildlife species studied. This is supported by highly significant decreases in flies (χ^2 = 228.49, df = 1, P < 0.001) and lice (χ^2 = 12.00, df = 1, P < 0.001), complete elimination of fleas (given that sample size was very small), and an overall significant reduction in total ectoparasites (χ^2 = 243.562, df = 1, P < 0.001) (Table 5). The efficacy of 5% NSKE was noted as the most effective against the fleas, which was found to be 100%, followed by the efficacies against flies, which was found to be 69.29% and lice, which was found to be 66.67% (Table 5). In the species-wise analysis, the efficacy of the treatment ranged from 55.93% in tigers to 81.76% in elephants. This large variation in efficacy is mainly due to differences in the animal housing

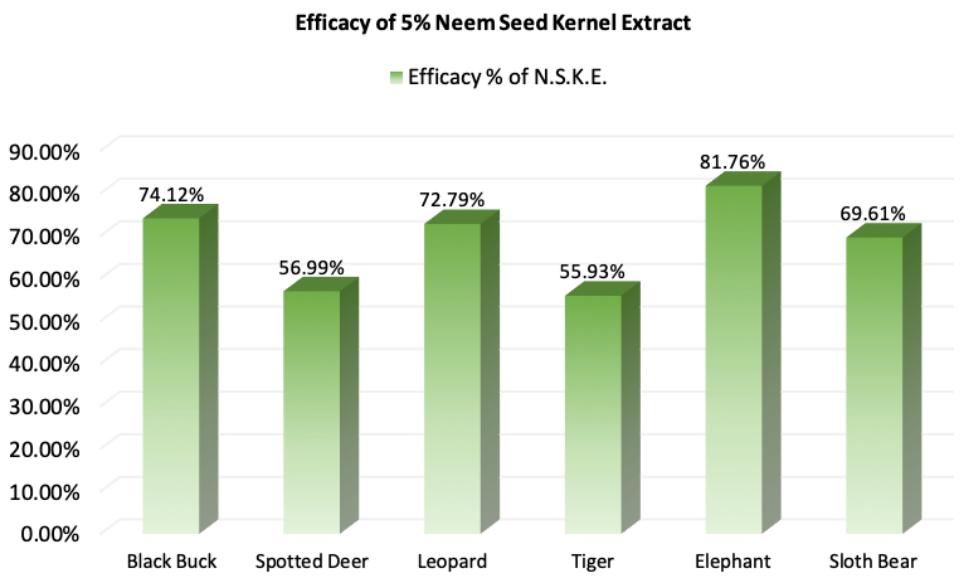

Figure 3. Efficacy of 5% neem seed kernel extract.

Image 9. Digitally collected midge.

Image 10. Processed *Drosophila* spp. fly (male) (4X).

conditions, sample size and micro-environmental factors. All the species showed statistically significant reductions in parasite counts, with chi-square values above the critical limit at P < 0.01. The 95% confidence intervals showed that elephants (74.86–87.43 %) and leopards (64.84–79.79 %) had the most consistent results with narrower ranges. Overall, the species-wise analysis revealed a statistically significant reduction in ectoparasite infestation following application of 5% NSKE across all species, including Black Buck (χ^2 = 18.96, df = 1, P < 0.001), Spotted Deer (χ^2 = 21.12, df = 1, P < 0.001), Leopard (χ^2 = 61.23, df = 1, P < 0.001), Tiger (χ^2 = 25.62, df = 1, P < 0.001), Elephant (χ^2 = 89.89, df = 1, P < 0.001), and Sloth Bear (χ^2 = 37.90, df = 1, P < 0.001), with an overall highly significant reduction in total ectoparasite load (χ^2 = 243.562, df = 1, p < 0.001) (Table 4). The treatment also showed different levels of effectiveness against the observed ectoparasites. For flies, the count reduced from 622 before treatment to 191 after, giving an efficacy of 69.29% with a 95% confidence interval between 65.67% and 72.92%, indicating a consistently high reduction rate. In case of fleas, the count dropped from four before treatment to zero after, showing 100% efficacy with a 95% confidence interval of 39.80% to 100%, thus achieving complete control in the study animals. For lice, the count came down from 36 before

Image 11. Processed *Drosophila* spp. fly (female) (4X).

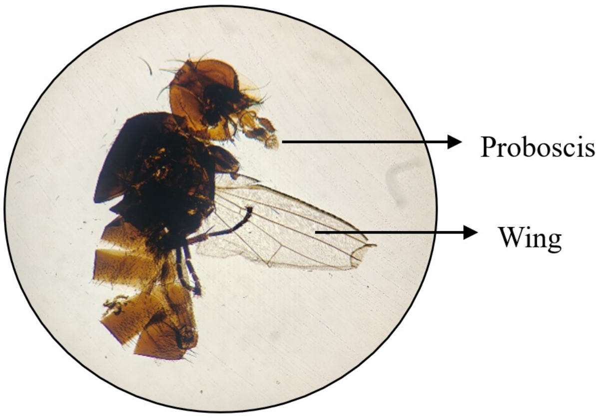

Image 12. Processed *Musca* spp. fly (male) (4X).

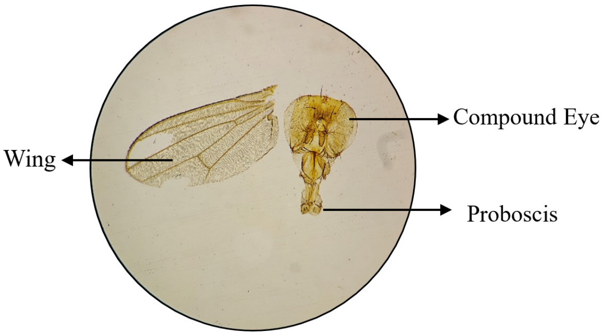

Image 13. Wing and mouth parts of *Drosophila* spp. fly (4X).

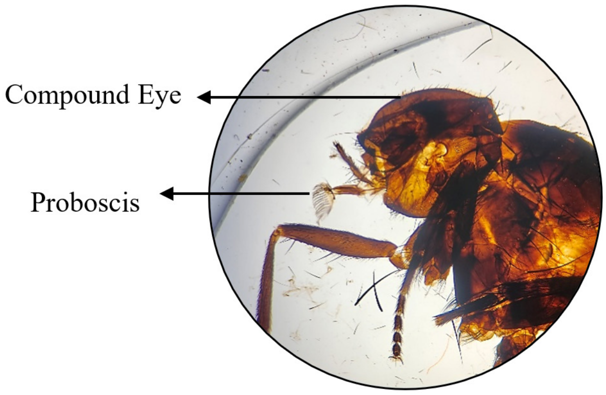

Image 14. Mouthparts of *Musca* spp. fly (4X).

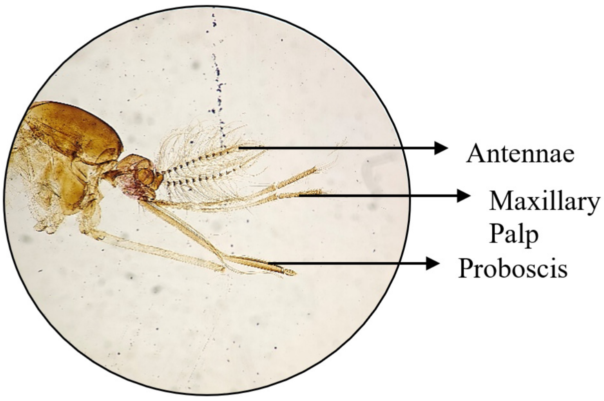

Image 15. Mouthparts of *Anopheles* spp. mosquito (10X).

Image 16. Mouthparts of *Culex* spp. mosquito (10X).

treatment to 12 after, giving an efficacy of 66.67% with a 95% confidence interval ranged from 51.27% to 82.07%. Though the reduction was considerable, the wider confidence interval indicates some variation, possibly due to the smaller number of lice cases observed. Overall, the treatment was found to be most effective against fleas (mathematically), followed by flies and lice (Table 5).

Table 3. Overall estimated prevalence of various ectoparasites in wildlife species at Katraj Zoo.

Species	Flies		Fleas		Lice	
	H_{inf}	P_{est} (%)	H_{inf}	P_{est} (%)	H_{inf}	P_{est} (%)
Black Buck	1.92	4.26	0.28	0.63	0	0.00
Spotted Deer	2.65	2.55	0.00	0.00	2.9	2.78
Leopard	10.88	362.66	0.50	16.66	0	0.00
Tiger	8.94	223.68	0.00	0.00	0	0.00
Elephant	5.22	261.11	0.00	0.00	0	0.00
Sloth Bear	9.5	475.00	0.00	0.00	0	0.00

*—The H_{inf} values are mathematical estimates and not actual host count.
**—The estimated prevalence values > 100% indicates the ectoparasite population exceeds the capacity of individual host.
H_{inf}—Estimated number of infested hosts | P_{est}—Estimated prevalence value.

Table 4. Overall reduction of ectoparasite count and efficacy of 5% neem seed kernel extract against ecto-parasites in various wild animals at Katraj Zoo.

Species	Ecto-parasites observed w.r.t. application of 5% NSKE		Reduction of ecto-parasites (A-B)	Efficacy (A-B / A) × 100 (%)	Total no. of ecto-parasites observed in the study	χ^2 value	CI (%) range
	Before (A)	After (B)					
Black Buck	43	11	32	74.42	54	18.963**	58.82 – 86.48
Spotted Deer	93	40	53	56.99	133	21.120**	46.30 – 67.21
Leopard	147	40	107	72.79	187	61.225**	64.84 – 79.79
Tiger	118	52	66	55.93	170	25.624**	46.49 – 65.06
Elephant	159	29	130	81.76	188	89.894**	74.86 – 87.43
Sloth Bear	102	31	71	69.61	133	37.902**	59.71 – 78.32
Total:	**662**	**203**	**459**	**69.33**	**865**	**243.562****	**65.70 – 72.90**

*—Significant at P < 0.05 | **—Highly significant at P < 0.01 | NS—Non-significant.

Table 5. Overall efficacy of 5% neem seed kernel extract in various taxa of ectoparasites.

Taxa	Ectoparasites observed w.r.t. application of 5% NSKE		Reduction of ectoparasites [A-B]	Efficacy [A-B / A] × 100 (%)	Total no. of ectoparasites observed in the study	χ^2 value	CI (%) range
	Before [A]	After [B]					
Flies	622	191	431	69.29	813	228.488**	65.67 – 72.92
Fleas	4	0	4	100.00	4	--	39.80 – 100.0
Lice	36	12	24	66.67	48	12.000**	51.27 – 82.07
Total:	**662**	**203**	**459**	**69.33**	**865**	**243.562****	**65.70 – 72.90**

*—Significant at P < 0.05 | **—Highly significant at P < 0.01 | NS—Non-significant.
Note: The 100% efficacy against fleas is mathematical result of small sample size.

DISCUSSION

Safety data of NSKE used as an insecticide

Comparisons show organic concentrates of NSKE are more potent and present higher host risk, whereas aqueous extracts and Neem seed oil formulations are generally less hazardous (Isman 2006). The reported systemic toxicity in lab mammals mainly follows oral or chronic exposure, not single topical use. The current literature has limits; most of the 'no adverse effects' reports are observational, without haematology, biochemistry, or histopathology, so subclinical effects

Image 17. *Ctenocephalides* spp. flea (10X).

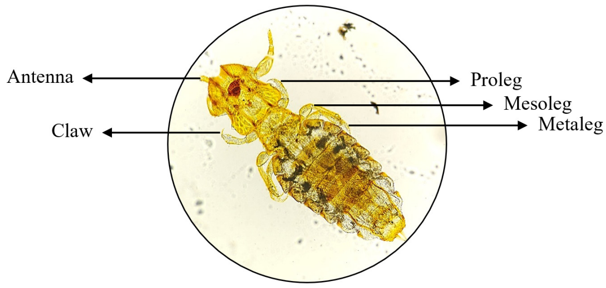

Image 18. *Damalinia* (*Bovicola*) spp. louse (10X).

Table 6. Details of animals studied and their categorization.

Wild animal	No. of animals	Category	Year
Black Buck *Antelope cervicapra*	45	Least Concern (LC)	IUCN 2016
Spotted Deer *Axis axis*	104	Least Concern (LC)	IUCN 2016
Leopard *Panthera pardus*	03	Near Threatened (NT)	IUCN 2023
Tiger *Panthera tigris*	04	Endangered (EN)	IUCN 2022
Elephant *Elephas maximus*	02	Endangered (EN)	IUCN 2024
Sloth Bear *Melursus ursinus*	02	Vulnerable (VU)	IUCN 2023

Note: Conservation status was assigned according to the IUCN Red List assessments (IUCN 2016, 2022, 2023, 2024). (Duckworth et al. 2015; Williams et al. 2020; Dharaiya et al. 2020; Goodrich et al. 2022; Steinmetz et al. 2023; Shivakumar et al. 2025; Stein et al. 2025)

cannot be excluded (Cotticelli et al. 2023). As controlled or published trials do not exist for large zoo species such as tigers, leopards, elephants, sloth bears, etc. so extrapolation requires caution. Available evidence supports topical 5% aqueous NSKE as a safe zoo-use candidate when: (a) only aqueous preparations are used, (b) treatment frequency is minimised, (c) pregnant or neonatal animals are treated cautiously, and (d) clinical and laboratory safety monitoring is included in protocols (Boeke et al. 2004; Isman 2006).

Taxa-wise dominance of ectoparasites

The present study demonstrated a pronounced taxa-wise dominance of flies, which constituted 93.99% of the total ectoparasite count (865), while lice and fleas occurred in comparatively lower proportions. This dominance may be attributed to the high reproductive rate and mobility of flies, along with favourable enclosure conditions such as the presence of organic matter and moist substrates. The smart trapping approach employed, particularly CO_2-based, light, and glued traps, was more efficient in capturing volant ectoparasites, thereby enhancing fly detection relative to host-dependent parasites like lice. Additionally, the study period from January–March, corresponding to late winter and early summer, provided optimal conditions for fly activity, while being less conducive for flea and lice proliferation. Seasonal variation is likely to influence taxa-wise patterns, with monsoon conditions potentially increasing ticks, fleas and lice prevalence due to higher humidity, and summer months further intensifying fly dominance, whereas cooler periods may result in reduced overall ectoparasite abundance.

Calculation of estimated prevalence using average ectoparasite load values

Average ectoparasite load values ($\overline{L}$) used in this study were synthesized from published reports describing parasite counts or mean intensity in the same or ecologically comparable wildlife species and were applied as standardized reference estimates for analytical calculations. These values were not intended to represent exact infestation levels under the present zoo conditions, but to provide a pragmatic proxy in the absence of site-specific quantitative ectoparasite enumeration, which is often constrained in large captive wildlife. Although ectoparasite loads may vary with environmental conditions, host management, and season, the uniform application of literature-derived values across species ensures internal consistency, and such variability is unlikely to affect the comparative outcomes or validity of the calculations.

Efficacy rates of commonly used synthetic insecticides as compared with NSKE

In wildlife ectoparasite management, synthetic insecticides such as deltamethrin, cypermethrin, permethrin, and amitraz have demonstrated high efficacy rates, often exceeding 90% against a wide range of ectoparasites, including ticks, fleas, and flies. In contrast, 5% NSKE generally provides moderate but meaningful efficacy. While synthetic compounds offer rapid knockdown and extended residual action, NSKE's slower action is counterbalanced by its excellent safety profile, biodegradability, and lower risk of resistance development, making it a suitable eco-friendly alternative in zoo and conservation programmes where chemical load reduction is critical (Isman 2006). Although synthetic insecticides such as pyrethroids and amitraz exhibit higher immediate efficacy, their use in zoological settings is constrained by concerns related to animal safety, environmental contamination, residue persistence, and resistance development. In contrast, 5% NSKE, despite its comparatively moderate efficacy, offers a favourable cost–benefit balance by providing adequate ectoparasite control while ensuring low toxicity, biodegradability, and minimal ecological impact. NSKE is economically viable, locally available, and suitable for repeated environmental application without imposing chemical stress on captive wildlife or their surroundings. Esspecially in zoo and conservation programmes where long-term sustainability, safety of non-target organisms, and reduction of chemical load are priorities, NSKE represents a pragmatic and responsible alternative to high-efficacy synthetic insecticides rather than a direct replacement.

Environmental impact of NSKE

Due to azadirachtin, NSKE has a substantially lower ecological impact compared to many synthetic insecticides. Studies have shown it to be far less toxic to fish and aquatic invertebrates than pyrethroids such as deltamethrin, and its biodegradability limits long-term persistence in the environment (Stark 2001). While effects on non-target organisms can occur at high concentrations, mesocosm studies indicate minimal disruption to aquatic communities at realistic exposure levels, with most toxicity linked to formulation additives rather than azadirachtin itself (Kreutzweiser et al. 2004). This positions NSKE as a more eco-compatible option for ectoparasite management in wildlife habitats.

Overall outcome of the study and economic analysis of NSKE used in the study

The prevalent ectoparasites recorded during the study at RGZPWRC, Katraj, Pune, were flies, fleas, and lice. The overall efficacy of 5% NSKE applied in animal shelters and night houses across the studied wildlife species was 69.33%, showing a highly significant reduction in ectoparasites ($P < 0.001$). Hence, the use of 5% NSKE as an insecticide/insect repellent was seen to be highly effective especially in wildlife settings where veterinarians are restricted to use chemical or synthetic insecticides. Also, the total quantity of concentrated solution of 5% NSKE used for a single washing of all the animal housings was 1600 millilitres, which cost INR 400.00 (at discounted rate of INR 250 per L on wholesale purchase) against INR 1200.00 (at INR 750 per L at retail price). The total area of animal housing washed was 1000 m^2. Hence, a single washing or application of 5% NSKE in all the animal housings costed between INR 0.4 (per m^2 of area) to INR 1.2 (per m^2 of area) if purchased at retail price. Hence, NSKE proved to be a cost-efficient and safer alternative to chemical insecticides for its preventive use in captive wildlife settings.

Limitations of the study

This study was conducted as a post-graduation dissertation work with limited permissions and a restricted environment with respect to time and technical clearances. Also, the short duration of this study allowed it to merely test the effect of the use of NSKE in wildlife as an ectoparasiticide rather than a comparative study of different treatments of ectoparasiticides. Although being one of the very few directly conducted studies on the efficacy of NSKE in captive wildlife, it was limited only to using NSKE in the animal housing structures as an in vivo assessment and not as a direct treatment to the captive wild animal species, which limited the assessment of NSKE a preventive insecticide rather than as a treatment option. A better study design could not be implemented due to the inclusion of wild animals with contrasting food (carnivores, herbivores and omnivores) and living habits (herd, pair or solitary living) in the study. This is a major factor for ectoparasite management and also affects prevalence calculation and estimation due to differences in study designs. Implementation of different types of designs for different categories of wild animals could have been highly time-consuming and may have resulted in errors while comparing efficacy calculated through different study designs.

Comparison with previous studies

The results of the present study are in agreement with earlier findings that report moderate but consistent efficacy of neem-based formulations against ectoparasites. Studies in livestock have shown that NSKE achieves a 65–72% reduction in ectoparasite burden, values comparable to the 69.33% overall efficacy observed in the present investigation (Akhtar & Isman 2013; George et al. 2014). In contrast, synthetic insecticides such as deltamethrin, cypermethrin, and amitraz frequently demonstrate > 90% efficacy, but their repeated use is associated with toxicity concerns, environmental persistence, and resistance development, particularly in sensitive settings like zoological parks (Isman 2006). Wildlife ectoparasite studies have also reported flies as the dominant taxa, largely influenced by environmental conditions and sampling techniques, supporting the taxa-wise dominance recorded in this study (Miller et al. 2014). Compared to earlier works, the present study is distinctive in adopting a smart, non-invasive digital trapping approach, providing ethically sound surveillance while reinforcing the practical applicability of 5% NSKE as an eco-friendly ectoparasite control option in captive wildlife.

Recommendations for future studies

a. Tailored study designs should be planned for different categories of wildlife, taking into account their food habits, living habits, and other characteristic features.

b. Species-specific studies should be conducted, with one study focusing exclusively on a single animal species to obtain the most accurate results. This approach would provide detailed, species-wise variations in efficacy, aiding further analysis.

c. Comparative studies between captive and free-ranging wildlife should be undertaken to determine if natural behaviours or environmental factors in free-ranging animals protect them from ectoparasitic infestations. Any such factors identified could be adapted for use in captive management.

d. Optimised study designs should be developed for postgraduate research and short-duration studies to save time and minimise trial-and-error approaches.

e. In vitro investigations on ectoparasites and their life cycle stages should focus specifically on parasites collected from the host species being studied.

f. Pharmacokinetic and safety studies on biological insecticides should be conducted to assess absorption, metabolism, and elimination in host species. This would help detect and prevent subclinical effects before any serious issues arise from long-term use.

g. Long-term efficacy trials of 5% NSKE, in comparison with synthetic and other botanical insecticides, should be carried out to identify the most effective and sustainable ectoparasite control measures.

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Author contribution: Dr. S.B. Kendre has designed the study of this research such that the objectives are accomplished by noninvasive sampling and with minimal animal handling. He designed the parasite traps exclusively. All the photographs and images for this research were captured by him. Field and laboratory work involved in this study was conducted as well as monitored by him. Dr. P.D. Pawar is the major advisor of this research. He planned, monitored and executed this entire research. He is the main coordinator of this study. Dr. R.V. Jadhav is the advisory committee member and Director of the institute / research center where the research was conducted. He co-ordinated the on field research work and supervised the wild animal handling, parasite collection and application of the 5% NSKE in animal enclosures. He actively secured the necessary permissions from the Maharashtra State Zoo Authority for commencement of this research study. Dr. U.M. Tumlam is the advisory committee member and contributed in the research with her expertise and guidance in research management and execution. Dr. A.Y. Doiphode is the advisory committee member in this research. He actively developed a special study design that favored sample collection in wildlife conditions, with minimal biases. Dr. V.G. Nimbalkar handled the raw data sets of this study and carried out all the statistical analysis. With her expertise in statistics, she converged all the raw numerical observations of this study into meaningful and statistically sound conclusions. Dr. P.K. Bhangale helped in the field execution of this research, monitored sample collections, insecticide application and collected photographic evidences at every step of the research. Dr. V. C. Priyal obtained all the prerequisite permissions and necessary authorizations required to conduct the study smoothly. With her knowledge in wildlife, she closely monitored samples, study results and data sets for corrections if any. Dr. S.M. Meshram contributed in conduction of the study by collecting, preserving, processing and storing the parasite samples. She kept the phase wise records of all the samples, animal enclosures and animal species studied in this research.

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