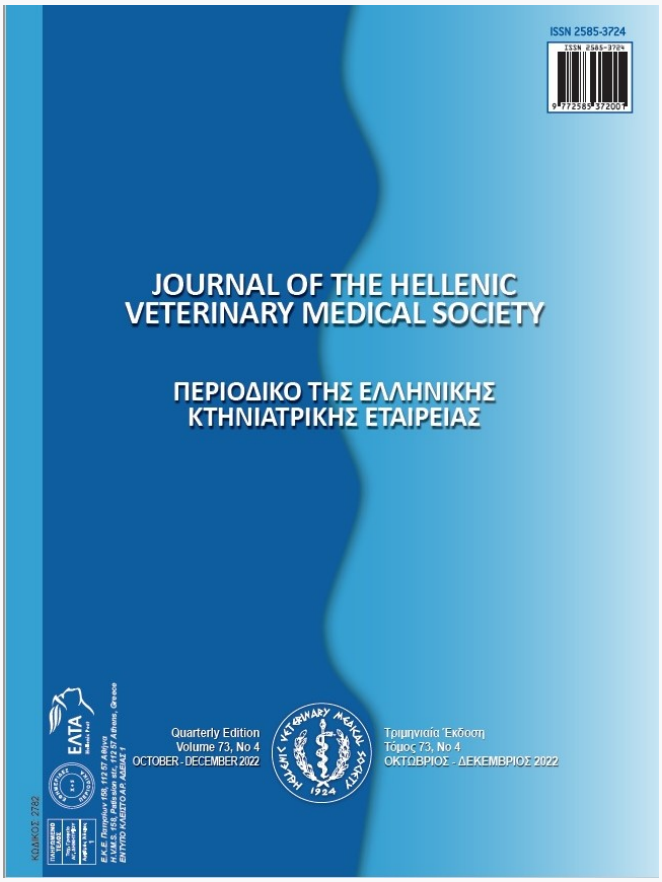
Journal of the Hellenic Veterinary Medical Society

Vol 73, No 4 (2022)



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doi: 10.12681/jhvms.27963

To cite this article:

Azeem, S., Akbar, H., Ahmad, L., Ashraf, M., & Puvanendiran, S. (2023). When used in animals, a neem (Azadirachta indica)-based ectoparasiticide performs better than a synthetic one. *Journal of the Hellenic Veterinary Medical Society*, *73*(4), 4833–4840. https://doi.org/10.12681/jhvms.27963

J HELLENIC VET MED SOC 2022, 73 (4): 4833-4840
ΠΕΚΕ 2022, 73 (4): 4833-4840

Research article
Ερευνητικό άρθρο

When used in animals, a neem (*Azadirachta indica*)-based ectoparasiticide performs better than a synthetic one

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ABSTRACT: Finding alternate ways to relieve animals from the ectoparasites is one of the most important aspects of the One-Health concept. Herbal ectoparasiticides offer a promising alternative to synthetic ectoparasiticides. The present study was designed to evaluate the ectoparasiticide efficacy of Nimboli® (Neem: *Azadirachta indica* extract) in various animal species, keeping in view the objectives of One-Health. The ectoparasiticide efficacy of Nimboli® was also compared with a commercial synthetic ectoparasiticide Tagafon (Hydroxyethyl Phosphoric Acid). Various animals infested with ticks, fleas, lice, flies, and maggots and presented at clinics were treated with Nimboli® (n=36) and Tagafon (n=19). Mean time taken by each ectoparasiticide to knock-down various ectoparasites was recorded. The present study suggested significantly higher efficacy of Nimboli® against ticks, fleas, lice, flies and maggots as compared with Tagafon. The mean time taken by Nimboli® to knockdown various ectoparasites of the different kinds and their larvae was also less compared to the mean time taken by Tagafon. The present study indicated that Nimboli® can be an alternative to synthetic ectoparasiticides. The present study highlights the importance of herbal ectoparasiticides, especially those containing neem in the treatment and control of ectoparasites in animals. The study has special relevance for the control of ectoparasites at organic farms. Moreover, the countries to which neem trees are native such as Sri Lanka, Pakistan, India, Indonesia, Malaysia, Myanmar, Senegal, and Thailand can benefit from this natural ectoparasiticide.

Keywords: fleas, flies, lice, ticks, maggots, neem, larvicidal

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Date of initial submission: 04-09-2021
Date of acceptance: 01-04-2022

INTRODUCTION

Ectoparasites inflict significant damage to domestic and farm animals and cause considerable economic loss to farmers, either through stock loss or the need to finance preventative measures (Tenquist, 1977). Various chemical and herbal ectoparasiticide preparations are available in the market, but with variable efficacy. Resistance against these chemical insecticides has also been reported (Devine et al., 2001). Findings on the clinical trials of Nimboli® (neem extract) against ectoparasites of various species of animals are presented here. The study offers great potential to exploit indigenous plants for the treatment of various ectoparasites of animals, as well as search for alternate means to treat ectoparasites under the One-Health concept.

Parasitic infestation contributes to a significant proportion of animals' diseases in Pakistan. The ectoparasites are one of the major constraints to livestock health and productivity (Muhammad et al., 2021). Furthermore, various ectoparasites act as carriers of various haemoparasites, bacteria and viruses, which further damages the health of animals. According to an estimate, skin damage resulting from ectoparasites in Pakistan leads to export losses of millions of Pakistani rupees (Chaudhry *et al.* 2011). Therefore, the control of ectoparasites is crucial for maintaining the optimum health and productivity of animals.

Various ectoparasiticide preparations are in vogue to mitigate the parasitic infestation in animals. Two types of ectoparasiticide preparations are commonly available in the market: herbal and synthetic. Recent studies comparing herbal and synthetic ectoparasiticides have suggested the superior activity of herbal ectoparasiticides for treating parasitic infestations in animals. Besides, synthetic ectoparasiticides cause severe tissue reactions such as hyperglycemia, engorgement of blood, hemorrhagic and necrotic foci, etc (Patel et al., 1998). Resistance against synthetic insecticides has also been reported (Devine et al., 2001). Comparatively, herbal preparations are equally effective in controlling ectoparasites; yet do not induce inflammatory or other tissue damage in animals (Kulkarni and Bansod, 2001; Vatsya and Singh, 1997). Additionally, a resistance against herbal ectoparasiticidals has not been reported.

The most prominent botanical pesticides in recent years are those derived from neem *(Azadirachta indica*) trees (Adenubi and Akande 2019). Neem is native to India, Indonesia, Malaysia, Myanmar, Pakistan, Senegal, Sri Lanka and Thailand. Additionally, owing to the multifarious uses of neem, the tree has been introduced to various countries in Africa, Australia and South America (Orwa *et al.* 2009). Neem is a source of naturally occurring insecticide, pesticide and agrochemicals (Brahmachari, 1999). The neem seed is composed of a shell and its kernel is used for pest control. The neem products have been obtained from several species of neem trees in the family Meliaceae. Six species of this family have been the subject of herbal pesticide research. They include *Azadirachta indica, A. excelsa, A. siamens, A. Indica var siamensis, Melia azadarach* and *M. volkensii*. The members of family Meliaceae, especially *A. indica* contain at least thirty-five bioactive compounds (Mulla et al., 1999).

Azadirachta indica has major ectoparasiticides in its various parts, especially in seeds and leaves. Herbal combinations containing *A.indica* has been found quite useful and efficacious against ectoparasites such as nymphal ticks, fleas, lice and mites (Abdel-Ghaffar et al., 2008; Bhilegaonkar and Maske, 1997; Habluetzel, et al., 2007; Kilonzo, et al., 2001; Lima de Souza et al., 2017). The aqueous extract of *A. indica* has been shown to have activity against the maturation of parasite oocytes, the oviposition, the reproductive potential and embryonic development of a fish ectoparasite (Banerjee et al., 2014). These neem combinations have been used against natural infestation of *Trichodectes canis* and *Ctenocephalides felis var orientis* of dogs, *Dermanyssus gallinae* (red mite) of poultry, sarcoptic mange in goats, dog and cat fleas, sheep louse as well as fish ectoparasites (Abdel-Ghaffar et al., 2008; Banerjee, 1997; Banerjee et al., 2014; Camarda et al., 2018; Das, 1997). Neem oil alone or in combination with karanj oil, glycerine, camphor and sweet oil is potent against mite infestation in sheep and poultry. This neem oil combination is also effective against dermatitis in crossbred cattle (Camarda et al., 2018; Kulkarni and Bansod, 2001; Hirudkar et al., 1997). Neem seed kernel extract was found to be potentially larvicidal against the flea species *Ctenocephalides felis* and *Xenopsylla brasiliensis* and it could be effectively used for the control of the insects by dusting their breeding sites with appropriate concentrations of the extract (Kilonzo et al., 1991). The residual effect of neem-based preparations, which ranges from a week to two months, is usually sufficient to prevent re-infestation (Bhilegaonkar and Maske, 1997; Camarda et al., 2018).

Of the several compounds in *A. indica,* azadirachtin is the chief active ingredient with activity against ectoparasite (Brahmachari 2004).Azadirachtin and other bioactive compounds in neem products exhibit various modes of action against ectoparasites, such as anti-feeding, growth regulation, fecundity suppression and sterilization, oviposition repellency, etc. A study has reported that azadirachtin treatment @ 2500 mg litre^{-1} significantly reduced feeding activity of tick larvae, prolonged the period of molting to the nymphal stage, and caused a 60% reduction in molting capacity (Al-Rajhy et al., 2003). Another study evaluating the effects of azadirachtin-enriched neem oil on *Rhipicephalus sanguineus* has suggested cell damage and death due to azadirachtin. Both these studies confirm that neem products can be used to control ticks in animals.

The amount of information on the rational and evidence-based use of neem and its products for the control of animal ectoparasites in Pakistan and Sri Lanka is limited. Additionally, most chemical ectoparasiticides available in the Pakistani market are expensive and growing resistance in parasites against these has also been observed. Therefore, the present study was designed to evaluate the ectoparasiticide properties of Nimboli® (Scientific and Technological Development Cooperation of Pakistan—STEDEC, Pakistan), a commercial preparation of neem—against various ectoparasites in livestock and pets of Pakistan. Additionally, the ectoparasiticide efficacy of Nimboli® was compared with a commercial chemical ectoparasiticide: Tagafon.

MATERIALS AND METHODS

A total of 36 animals including canines (n=24), caprines (n=2), bovines (n=4), ovines (n=2) and equines (n=4) were treated with Nimboli® (a registered trademark of STEDEC, Ministry of Science and Technology, Pakistan) for various ectoparasites such as fleas, flies, fly larvae (maggots), lice, mites and ticks. All animals infected with ectoparasites were brought to the clinics operated by the University of Veterinary and Animal Sciences, Lahore, Pakistan. Consent was taken from the owner of the animal before the application of Nimboli® on the clinical cases.

Nimboli® undiluted solution was applied as a topical application on the skin for the treatment of various ectoparasites. The solution was applied to the animal body in caudo-cranial direction using a sponge, cotton, or a piece of cloth. For wounds infested with maggots, Nimboli® was poured inside the wound.

The ectoparasiticide efficacy of Nimboli® was compared with a commercial product Tagafon 98% (Hydroxyethyl Phosphoric Acid) powder; Composition per gram: Trichlorfon 980 mg; Silicon Dioxide 20 mg) manufactured by Star Laboratories Private Limited, Lahore, Pakistan on a separate set of animals. Tagafon was applied on 19 animals; all canines infested with ticks, flies, lice, fleas and maggots. To make a comparison, the same number of animals was taken from both treatments.

Statistical Comparisons

Test Applied on Different Kind of Parasites by Both Medicines

For this purpose, the test applied was about testing a hypothesis of difference of means of two normal populations when $\sigma_1 \neq \sigma_2$ is unknown.

Hypothesis:

$$H_0: \mu_1 - \mu_2 = 0;\ H_1: \mu_1 - \mu_2 < 0$$

Where μ_1 is the time taken by Nimboli® to kill the parasites and μ_2 is the time taken by Tagafon to kill the parasites.

The level of significance was set as α=0.05

Test Statistic:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

$$X_1 = 7.25 X_2 = 10.74\ S_1^2 = 31.7763$$

$$S_2^2 = 23.094$$

$$= \frac{7.25 - 10.74}{\sqrt{\frac{31.779}{20} + \frac{23.094}{19}}}$$

$$= \frac{-3.34}{\sqrt{2.8043}}$$

$$= -2.08$$

Critical Region:

$$t_c \leq t_{\alpha,\upsilon}$$

$$t_c \leq t_{0.05,36}$$

$$t_c \leq -1.6905$$

$$\upsilon = \frac{[S_1^2/n_1 + S_2^2/n_2]^2}{\frac{(S_1^2/n_1)^2}{n_1-1} + \frac{(S_2^2/n_2)^2}{n_2-1}}$$

Where, υ is the degrees of freedom.

RESULTS

Topical application of Nimboli® resulted in the knockdown of most ectoparasites: ticks, lice, mites, fleas and flies within 5 minutes of application. After pouring Nimboli® all maggots were found dead within 5-20 minutes of application and the wounds healed uneventfully (Table 1). Nimboli® prevented the re-infestation of maggots for 7-8 days after its application, suggesting its residual effect.

No harmful reaction such as skin irritation in any of the humans applying or any of the animals treated with Nimboli® was observed suggesting that the agent was well-tolerated by both animal handlers and the animals.

The formation of pustules which usually result after manual removal of dead ticks and subsequent bacterial infection of skin was also prevented by Nimboli®.

Topical application of Tagafon resulted in death of most ectoparasites (Ticks, lice, maggots) within 3 to 25 minutes of application (Table 2).

Test Applied on Different Kind of Parasites by Both Medicines

The results of this comparison are presented in Tables 3 and 4. Since the calculated value falls in the critical region we reject H_0 and conclude that the mean time taken by Nimboli® to knockdown different kinds of parasites is less than the mean time taken by Tagafon.

DISCUSSION

Ectoparasites inflict major damage to livestock health and productivity. The present study was designed to evaluate the ectoparasiticide activity of a Neem-based product: Nimboli® and compare its ectoparasiticide activity with a synthetic product: Tagafon. The study suggested significantly higher efficacy of Nimboli® against ticks, fleas, lice, flies and maggots as compared with Tagafon. The mean time taken by Nimboli® to knockdown various ectoparasites of the same and different kinds and their larvae was also less compared to the mean time taken by Tagafon. These data suggest that Neem-based ectoparasiticides can be used as an alternative to synthetic ectoparasiticides.

The knockdown effect of Nimboli® on dog and cat fleas is consistent with Guerrini and Kriticos (1998). The knockdown effect of Nimboli® on biting louse of sheep and goats is consistent with Heath et al., (1995) and Habluetzel et al., (2007). The knockdown effect of Nimboli® on ticks is consistent with Sivaramakrish-

Table 1. Data collected after using Nimboli®

Sr. No.	Species	No. of animals	Parasite	Time (min.) taken to knockdown the parasite
1	Canine	16	Ticks	5
2	Canine	5	Flies	5
3	Canine	2	Lice	5
4	Canine	1	Fleas	5
5	Caprine	1	Fleas	5
6	Bovine	1	Ticks	5
7	Bovine	1	Ticks	5
8	Bovine	1	Ticks	5
9	Bovine	1	Ticks	5
10	Caprine	1	Maggots	5
11	Ovine	1	Maggots	5
12	Ovine	1	Maggots	5
13	Equine	1	Maggots	10
14	Equine	3	Maggots	20

min: Minutes

Table 2. Data collected after using Tagafon.

Sr. No.	Species	No. of animals	Parasite	Time (min.) taken to knockdown the parasite
1	Canine	1	Ticks	25
2	Canine	1	Ticks	3
3	Canine	1	Ticks	4
4	Canine	1	Lice	9
5	Canine	1	Maggots	9
6	Canine	1	Maggots	7
7	Canine	1	Maggots	9
8	Canine	1	Maggots	14
9	Canine	1	Maggots	11
10	Canine	1	Maggots	16
11	Canine	1	Maggots	10
12	Canine	1	Maggots	12
13	Canine	1	Maggots	6
14	Canine	1	Maggots	11
15	Canine	1	Maggots	9
16	Canine	1	Maggots	13
17	Canine	1	Maggots	12
18	Canine	1	Maggots	10
19	Canine	1	Maggots	14

min: Minutes

Table 3. Data collected after using Nimboli®.

Sr. No.	Species	Parasite	Time (min.)
1	Canine	Ticks	5
2	Canine	Ticks	5
3	Canine	Ticks	5
4	Canine	Ticks	5
5	Canine	Ticks	5
6	Canine	Ticks	5
7	Canine	Ticks	5
8	Canine	Ticks	5
9	Canine	Flies	4
10	Canine	Flies	4
11	Canine	Flies	4
12	Canine	Flies	4
13	Canine	Flies	4
14	Canine	Lice	5
15	Canine	Lice	5
16	Canine	Fleas	5
17	Canine	Ticks	10
18	Canine	Ticks	20
19	Canine	Ticks	20
20	Canine	Maggots	20

min: Minutes

Table 4. Data collected after using Tagafon

Sr. No.	Species	Parasite	Time (min.)
1	Canine	Ticks	25
2	Canine	Ticks	3
3	Canine	Ticks	4
4	Canine	Lice	9
5	Canine	Maggots	9
6	Canine	Maggots	7
7	Canine	Maggots	9
8	Canine	Maggots	14
9	Canine	Maggots	11
10	Canine	Maggots	16
11	Canine	Maggots	10
12	Canine	Maggots	12
13	Canine	Maggots	6
14	Canine	Maggots	11
15	Canine	Maggots	9
16	Canine	Maggots	13
17	Canine	Maggots	12
18	Canine	Maggots	10
19	Canine	Maggots	14

min: Minutes

nan et al., (1996), Borges et al., (2003) and Lima de Souza et al. (2017).

The residual effect of Nimboli® is evidenced by the prevention of maggot infestation for 7-8 days after its application. The residual effect probably lasted longer than a week; however, most animals were not available for observation after a week. The residual effect of Nimboli® is consistent with the findings of Bhilegaonkar and Maske, (1997).

Nimboli supported wound healing consistent with Kulkarni and Bansod (2001), who reported that neem oil has a curing effect in skin diseases by retarding the sloughing process and promoting wound healing. In the present study after application of Nimboli®, no pustules forming occurred after tick removal consistent with the findings of Rao et al. (1986), suggesting antiseptic activity of Nimboli®.

A limitation of the study was that Nimboli® and Tagafon were applied on different animals infected with different ectoparasites at different times.

Overall the study revealed that *Azadirachta indica* has the potential to be used as an alternative to synthetic ectoparasiticides for the control of animal pests. In addition, the use of herbal products to control ectoparasitic infections in animals is one of the best ways to minimize the environmental impact of synthetic ectoparasiticides, supporting the One-Health concept. Moreover, herbal products are safe both for human handlers and the animals treated with these products. Hence using herbal ectoparasiticides to control ectoparasite infestations in animals promotes animal health, without harmful effects on humans and environment health. Herbal ectoparasiticides are especially useful for tribal and nomadic livestock producers and farming communities that have a limited access to chemical insecticides as well as for organic farming communities that cannot use synthetic ectoparasiticides. Future studies focusing on side-by-side comparison of ectoparasiticide activity of neem-based and synthetic products could further elucidate comparative efficacy of these ectoparasiticides. Further research exploring the potential of *A. indica* as an alternative ectoparasiticides is likely to shed light on its usefulness in the vector control programs.

ACKNOWLEDGMENTS

The authors highly acknowledge the cooperation of Mr. Atiq-ur-Rehman and Mr. Naseer Ahmad of ST-EDEC House, Ministry of Science and Technology, Government of Pakistan for their cooperation in acquiring Nimboli®.

CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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