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## Evaluation of neem oil and lemon grass oil as natural fly repellent on lumpy skin disease vector in buffalo farms

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### Abstract

Lumpy skin disease (LSD) is an acute infectious disease of cattle and buffaloes and is endemic in most of the Sub-Saharan African countries. In recent times, it has been reported in many states of India including Karnataka since 2019. It is one of the economically devastating viral diseases causing severe financial loss to farmers and in livestock industries as a result of significant reduction in milk production, body weight, beef loss, hide quality, working efficiency and also causes infertility, abortion and death. Since it is vector borne disease, the present study has shown, *Haematobia* fly could be the major vector for transmission of LSD in and around Bidar due to its high abundance in disease affected farms and during outbreak period. Control of vectors remains one of the major strategies for prevention and control of disease. Hence, organic essential oils against vectors is one of the eco-friendly approach for control of disease. In the present study, organic essential oils Viz., Neem oil and Lemon grass oil as natural fly repellent was evaluated by on-field study and results revealed, Neem oil, once in a week @ 50 per cent and Lemon grass oil, twice in a week @ 5 percent have good repellency against *Haematobia* flies, without any adverse effects on hide of buffaloes and intern may help in reducing the spread of LSD from infected to healthy animals through vectors.

**Keywords:** Lumpy skin disease, *Haematobia* (Horn) flies, neem oil and lemon grass oil

### Introduction

Lumpy skin disease is one of the most economically significant emerging viral diseases. It is endemic in most of the African countries and also expanded to Middle East region (Tuppurinen and Oura, 2011) [22]. It has high morbidity and low mortality rate and affects cattle and buffaloes irrespective of their ages and breeds. It causes significant economic losses to farmers due to reduction in milk production, beef loss, infertility, abortion, loss of condition and damage of the hide. It becomes an important threat to livestock and dairy industry (Birhanu, 2015) [5].

Lumpy skin disease is an acute infectious disease characterized by high fever, flat nodules on the skin, mucous membranes and internal organs, enlarged superficial lymph nodes, emaciation, oedema of the skin, and sometimes death (Radostitis *et al.*, 2006) [18]. The causative agent is a Lumpy Skin Disease virus, classified in capripoxvirus of family poxviridae (Babiuk *et al.*, 2008) [3].

Major risk factors of outbreak of LSD are hot and humid climate conditions and potent abundance of vector populations. Wide ranges of LSD outbreaks are generally related with heavy rain fall and high level of blood feeding insect's activity (Gari *et al.*, 2010) [10]. Haematophagous arthropods include the following taxa: biting flies like horn flies, stable flies (Diptera: Muscidae) and equine flies (Diptera: Tabanidae), mosquitoes (Diptera: Culicidae), *Culicoides* biting midges (Diptera: Ceratopogonidae) and hard ticks (Acari: Ixodidae), etc. (EFSA, 2015) [8].

In India, LSD was first reported in Khairbani, Betnoti and Mayurbhanj and Bhadrak districts of Odisha on 12 August 2019. Subsequently, the outbreaks were reported from other parts part of the country like Chhattisgarh, Madhya Pradesh, Jharkhand, West Bengal, Assam, Andhra Pradesh, Telangana, Tamil Nadu, Maharashtra, Karnataka and Kerala (Srinivas *et al.*, 2020) [20]. Even now the disease is spreading across India, and outbreaks were noticed in other states also.

World Organization for Animal Health (OIE) has classified Lumpy skin disease as a notifiable disease because of its considerable economic significance (Tuppurainen and Oora, 2012) [23]. Due to devastating economic losses occurring to the farmers, control of LSD causing vectors has become necessary to study and is a major strategy for prevention and control of disease in the absence of effective vaccination in India. Hence, in the present study organic essential oils (Neem oil and Lemon Grass Oil) against Haematophagous arthropods are thought to be eco-friendly approach in integrated vector management (IVM).

## Materials and Methods

### Place of study

Present study was carried out at Veterinary Clinical Complex, Veterinary College, Nandi Nagar, Bidar (KVAFSU), Karnataka during the period of January 2020 to December 2020.

### Vector study

Haematophagous arthropods which transmits virus mechanically are, biting flies like horn flies (*Haematobia irritans*), stable flies (*Stomoxys calcitrans*) (Diptera: Muscidae) and horse flies (Diptera: Tabanidae), Ixodidae (Hard ticks), Culicidae mosquitoes and *Culicoides* biting midges *etc.* Different affected farms were selected randomly in and around Bidar for identification of vectors which are predominant in their abundance. Vectors were collected and identified as per the standard protocol based on wings and mouth part (Bhatia *et al.*, 2007) [4].

### Evaluation of essential oils as natural fly repellents

Essential oils selected for the study were Neem oil (*Azadirachta indica*) and Lemon grass oil (*Cymbopogon citratus*). Study was conducted in different buffalo farms for evaluation of repellence property of essential oils at different concentration (5%, 10%, 20% and 50%). For this study, 6 buffalo farms were selected in and around Bidar.

### Animal trial

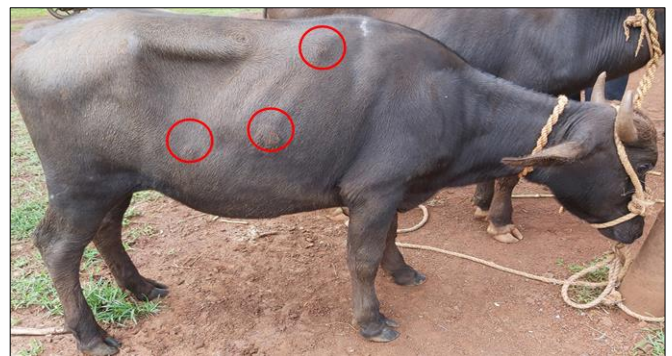
Tests were conducted using 5, 10, 20 and 50 per cent of organic essential oils. Desired concentrations of oils were prepared by diluting it in water. Six animals were selected for each concentration for trial. Later particular concentration of organic essential oil which has shown potent repellency was selected for on-field in different buffalo farms.

#### Procedure

- Animals were selected from affected farms, preferably animals with high infestation of flies for the study.
- Numbers of target flies present on animal body were counted by visual method, given by EFSA, 2017 [7]. Visual observation should be carried out in early morning and 1 min per animal per side was taken to count the number of flies resting on body. Each side can be counted by one operator. It is repeated for 3 days and mean of number of flies was calculated.
- Selected essential oil was prepared at desired concentration (for 500 ml) and sprayed over animal body using hand sprayer.
- Numbers of flies present on animal body were counted again after spraying by visual observation at 24, 48, 72 and 168 (7<sup>th</sup> day) hours.
- Paired t-test was used for the analysis of data (Snedecor and Cochran, 1994).

## Results and Discussion

A total of 90.12 per cent of villages in Bidar district were infected with lumpy skin disease in study period, with the morbidity rate of 6.00 per cent and 0.17 per cent in cattle and buffaloes respectively. Both prevalence and point prevalence study revealed that, the prevalence of lumpy skin disease among the study population was higher in cattle than in buffaloes. The overall mortality rate and case fatality rate recorded in cattle were 1.16 and 6.19 per cent respectively. However, no mortality and case fatality rate were recorded in buffaloes due to LSD during this study period. The findings are in agreement with Ahmed *et al.* (2020) [1] and EFSA (2020) [9]. Buffaloes are less susceptible to lumpy skin disease because of its thicker skin, it prevents the insect bites and reduces the chances of transmission of LSDV (Chihota *et al.*, 2003) [6].



**Fig 1:** Photograph showing lumpy skin disease infected buffalo with nodular lesions

### Vector study

Lumpy skin disease affected farms were visited for identifying most abundant and endemic vectors present in and around Bidar. In all the visited 30 cattle and buffalo farms, most common vectors present were horn flies (*Haematobia irritans*). So, it was assumed that *Haematobia* fly was the major vector for transmission of LSD in and around Bidar. Upon abundance study, *Haematobia* fly abundance increases from July to November, which was coinciding with the LSD outbreaks in animals. This makes suspicion strong on *Haematobia* fly as vector for transmitting LSD between affected and health animal.



**Fig 2:** Photographs showing, *Haematobia* fly, Wing of *Haematobia* fly and mouth parts of *Haematobia* fly

Similarly, during the outbreak period of disease, *Haematobia irritans* were observed in abundance in livestock farms and were assumed of transmitting LSDV in the particular area (Kahana-Sutin *et al.*, 2017) [12].

### Evaluation of essential oils as natural fly repellents

#### Repellent study of neem oil against adult *Haematobia* flies

Neem oil was evaluated at 5, 10, 20 and 50 per cent for its fly

repellency for 03 days. All four concentrations have shown repellent action against *Haematobia* flies. The highest repellency was recorded at 50 per cent concentration, it has

shown 97.65 and 85.89 per cent of repellency on first and third day respectively.

Repellent study of neem oil against adult *Haematobia* flies

Concentration (in percentage)	Average no. of flies before treatment	Repellence after treatment (in percentage)		
		1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day
5%	44.6	21.5%	3.58%	No repellence
10%	15.33	47.8%	21.72%	15.1%
20%	79	50.64%	32.92%	20.26%
50%	85	97.65%	89.42%	85.89%

Repellent study of lemon grass oil against adult *Haematobia* flies

Maia and Moore (2011) [15] stated that higher than 10 per cent concentrations of Lemon grass oil can cause skin sensitivity.

Therefore, only 5 per cent concentration was selected for both animal trial and *on-field* experiment. It has shown 68.82 and 20.12 per cent of repellency without any adverse effects on the skin of animal.

Repellent study of lemon grass oil against adult *Haematobia* flies

Concentration (in percentage)	Average no. of flies before treatment	Repellence after treatment (in percentage)		
		1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day
5%	51.33	68.82%	53.24%	20.12%

Upon preliminary screening of organic essential oils as natural fly repellent against *Haematobia* flies revealed that Neem oil @50 per cent level and Lemon grass oil @ 5 percent level has shown to be effective.

the animal body. The compounds, complex tetranor terpenoid lipids are toxic to insects (Jibrin and Mohammed, 2020) [11].

On-field evaluation of organic essential oils as natural fly repellent

Neem oil (50%)

Animal trial repellence study showed that 50 per cent of Neem oil has better repellent action when compared to other three concentrations. Therefore, 50 per cent concentration was selected for further on-field evaluation.

There was significant decrease in the number of flies on body of buffalo on treatment with 50 per cent neem oil with mean of  $5.16 \pm 1.07$ ,  $12.33 \pm 3.37$ ,  $17.00 \pm 3.88$  and  $44.16 \pm 10.44$  for first, second, third and seventh day after spray respectively ( $p < 0.05$ ).

Number of flies decreased significantly on the body of buffaloes on treatment with 50 per cent neem oil. The percentage of repellency was more on first day when compared to second and third day after spray. These results were also reported by Kumar *et al.* (2013) [13] and Aremu *et al.* (2009) [2] against different insect vectors. In contrast according to Maia and Moore (2011) [15] does not suggest neem oil, as it causes skin irritation and dermal toxicity when used undiluted.

The Mechanism behind action of *Azadirachta* is by blocking the lactic receptor and makes the insect to move away from

Lemon grass oil (5%)

Similarly, 5 per cent concentration was selected since it has shown considerable repellent property. Therefore, 5 per cent concentration was selected for on field study.

There was significant decrease in the number of flies on body of buffalo on treatment with 5 per cent lemon grass oil with mean of  $29.00 \pm 4.28$ ,  $36.00 \pm 5.63$  and  $43.50 \pm 6.64$  for first, second and third day after spray respectively ( $p < 0.05$ ). Whereas on seventh day it was  $68.5 \pm 8.5$  after spray ( $p > 0.05$ ).

Number of flies decreased significantly on the body of buffaloes on treatment with 05 per cent lemon grass oil. The percentage of repellency was more on first day when compared to second and third day after spray. These results are similar to Lachence and Grange (2014) [14] and Oyedele *et al.* (2002) [16] against different insects. In contrast, Tawatsin *et al.* (2001) [21] found that, the lemon grass oil rapidly evaporates loss of efficacy and leave the animal unprotected from insects.

The lemongrass oil has compounds such as terpenoid components (Citral and Geranyl acetate), which damage their activity of orientation and locomotion by disrupting the recognition of host substratum by insects. The odor of oil is repellent to insects (Plata-Rueda *et al.*, 2020) [17].

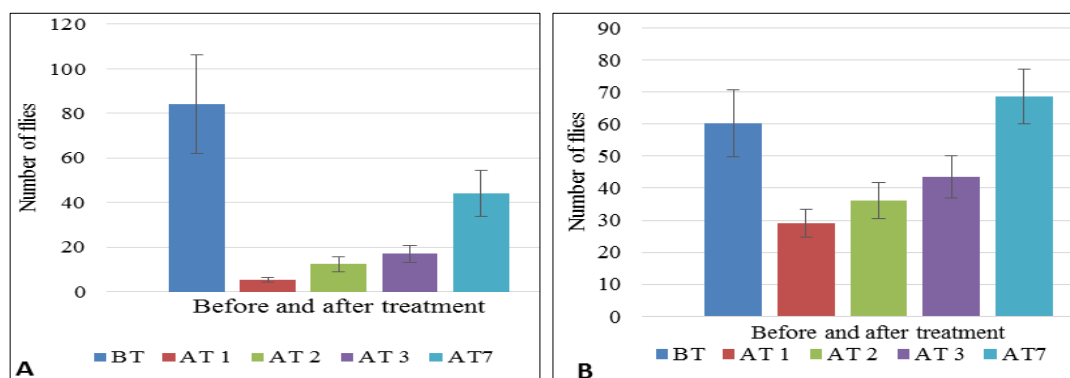


Fig 3: On-field evaluation of (A) Neem oil (50%) and (B) Lemon grass oil (5%) as natural fly repellent against *Haematobia* flies in Buffaloes



### Comparison of organic essential oils as natural fly repellent

In the present study, Neem oil (50%) and Lemon grass oil (5%) were examined for natural fly repellent. Study revealed that both the oils have considerable repellence property at particular concentration. As per cost concern, Neem oil is costlier than Lemon grass oil, but it is easily available to farmers for regular use during outbreak period. However, Lemon grass oil can be used only at lower concentration (5%), as higher concentration causes dermal toxicity on animal skin, whereas Neem oil has no such side effects even at higher concentrations and has good repellence property against biting insects (Maia and Moore, 2011) [15]. As usage of natural fly repellents are eco-friendly approach. Hence, these essential oils may be recommended in integrated vector management (IVM) in control of spread of Lumpy skin disease in cattle.

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