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#### Zanthoxylum Fruit is Effective in Controlling Ticks in Cattle

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#### Abstract

Acaricides are used to control ticks in cattle. However, it has negative impact on animal and public health—and contributes to development of resistance to drugs. Herbs and shrubs are being explored as an alternative in controlling ticks. Therefore, we assessed efficacy of fresh and dry *Zanthoxylum* fruits to control ticks in cattle. Sixty cattle were assigned to  $T_1$  (250 g of fresh *Zanthoxylum* fruit),  $T_2$  (250 g of dry *Zanthoxylum* fruit) and  $T_3$  (deltamethrin), and the trial was conducted for 45 days. Both dry and fresh *Zanthoxylum* fruits were effective in controlling ticks in cattle. However, fresh *Zanthoxylum* fruit was more effective than the dry *Zanthoxylum* fruit, and the fresh *Zanthoxylum* fruit had a similar efficacy as deltamethrin.

Keywords: cattle, deltamethrin, drug resistance, tick, Zanthoxylum

#### Introduction

Livestock farming supports the livelihood of farmers in developing countries by providing food and other products such as hide, textiles, draught power, and transportation (Reynolds *et al.*, 2015; Banda & Tanganyika, 2021). Similarly, the dairy farming is the most important

livestock activity in Bhutan, and it contributed about 4% of the Gross Domestic Product and 22% of the rural income (Tshering & Thinley, 2017). However, Bhutanese famers are faced with many diseases in cattle (foot and mouth disease, anthrax, haemorrhagic septicaemia, babesiosis) that affect the productivity of their animals, and many of these diseases are caused by ticks and tick-borne diseases (TTBDs) (Namgyal *et al.*, 2021).

The TTBDs infect over 80% of the world's dairy herd, affecting the productivity and health of herd (De Meneghi *et al.*, 2016) and could probably increase cattle death rate (Phanchung *et al.*, 2002). The TTBDs are expected to remain in the future as the most serious bovine diseases (De Meneghi *et al.*, 2016). Additionally, tick control costs billions of dollars worldwide (Jongejan & Uilenberg, 1994). In Bhutan, cattle infected with *Rhipicephalus (Boophilus)*, *Microplus*, or *Haemaphysalis bispinos*a are

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found across the Indo-Bhutan border (Mushahary *et al.*, 2019). Moreover, cattle are commonly traded across the Indo-Bhutan border areas, posing a greater risk of transmission of multiple TTBDS (Mushalary, 2019).

Acaricides have been used for controlling tick infestation in cattle. However, the use of acaricides have negative effect on environment and human health (e.g., risk to cattle farmers and people who handle cattle, chemical residues in environment, and animal-derived food) (De Meneghi et al., 2016). As a result, the use of plant-based treatment of tick infestation is recommended, and also this plant's (herbs, shrubs) extracts are becoming a key tool for controlling tick infestation in resource-constrained farm settings (Nimbalkar et al., 2020). This strategy gives long-term solutions for establishing environmentally friendly and ensuring optimum animal health. For instance, Zanthoxylum fruits have several medical uses, including insecticidal qualities (Gilani et al., 2010) and is successful used to control trick (Nogueira et al., 2014). However, there are no literature to support the effectiveness of Zanthozylum fruits to control tick in cattle in Bhutan. Therefore, the objective of this study was to assess the effectiveness of Zanthoxylum fruit suspension for ticks' control in cattle, which is reported elsewhere as effective for tick control.

# **Materials and Methods**

## Study area

The trial was carried out at Barshong Gewog, Tsirang Dzonkhag, which has known occurrence of tick infestation (Namgyal *et* al., 2021; Pem *et* al., 2021). The study area is located at 700-1500 meters above sea level (Royal Government of Bhutan, 2018). The gewog has a humid subtropical climate with a maximum annual average rainfall of 2,611.86 mm, while the minimum annual average rainfall is 2,204.88 mm (Flood Engineering & Management Division, 2019). The gewog has a total of 853 cattle, 575 of which are Siri, 17 are Holstein-Friesian cross, and 261 are Jersey cross (DoL, 2019). There are 45 modern cattle sheds, and the cattle farmers commonly use chemicals to treat cattle infested with ticks (Chuwan, personal communications, October 30, 2020).

# Experimental design

Ten cattle dairy farms with a minimum of five animals per farm irrespective of breed and age were selected. As we expected difference in tick infestation to vary by age, cattle were classified into adults and young (2 to 3-year-old). The housing system was evaluated in terms of the presence of modern amenities, such as a cemented floor and drainage, which were found to be absent in traditional housing. Moreover, bedding materials (dry leaves, weeds, and straws) were provided in both the housings for manure purpose. Cattle under modern housing are provided with intensive care such as regular washing and brushing off dirt. In addition, cattle were allowed to graze freely during the daytime and brought back to the shed at night. The participating farmers were explained the treatment protocol and duration of the research.

A total of 60 cattle were assigned randomly (lucky dip) into four groups: T1, fresh fruit *Zanthoxylum*, n = 15; T2, dry *Zanthoxylum*, n = 15; T3, deltamethrin, n = 15; C, control, n = 15.

# Dosage and application

Ripened Zanthoxylum fruits were purchased from the local market to make the suspension to apply on cattle. In order to make dry Zanthoxylum fruits suspension, the fruits were sundried, and powdered by using mortar and pestle. A total of 250 g of powdered dry Zanthoxylum fruits were added in clean container containing 500 ml drinking water. Likewise, the remaining fresh ripened Zanthoxylum fruits (250 g) were crushed into powder and added to a clean container containing 500 ml water to make a suspension. These two suspensions were stored for 12 hours at room temperature, and then the extracts were separated from residues using a fine cotton cloth. The extracted Zanthoxylum-water solute was used in the experiment.

The treatment period was between 01/07-14/08/2020. Before animals were assigned to groups, the number of ticks were counted by removing from the host and considered it as day 0. The solution (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>) was applied to the dewlap areas of cattle because it is the most tick -infested surface (Kabir *et al.*, 2011) by using a clean cotton towel and hair was clipped using scissors to demarcate the area for the experiment. The solutions were applied to cattle on day 0, day 3, day 7, day 14, and day 21.

## Data collection

Ticks from the treated portion of cattle body were counted on the day 3, 10, 24, and 45. Due to the greater incidence of nymphal ticks in June and July (Ostfeld *et al.*, 2001), the area for application of solution was taken at  $5 \times 7$  cm<sup>2</sup> for tick counting.

## Data analysis

Data was entered in Excel, 2016, which was exported to Statistical Package for Social Science (SPSS) software version 22 for analysis. Means, standard deviations, and margin of errors were estimated for tick counts. To compare the means of tick counts of different treatments, one way-ANOVA was used, and the Tukey HSD was used to compare the differences in mean among the treatments (p < .05). We also examined differences in tick infestation for housing, age, sex, breed (exotic and local), and grazing management with an independent two samples *t* test.

## **Results and Discussion**

### Tick counts in cattle

On day zero, before assigning cattle into treatments, no differences was observed among treatments in mean tick counts in cattle,  $(F_{(3, 56)})$ = 1.296, p > .29). However, at day 45, the mean number of ticks on cattle were higher for those cattle not treated (140.33  $\pm$  10.22) than those treated with Zanthoxylum fruits (fresh fruits,  $14.33 \pm 4.98$ ; dry fruits,  $42.87 \pm 22.36$ ) and deltamethrin  $(8.13 \pm 4.47^{b}), F_{(3,56)} =$ 345.849, p < .05. Moreover, the mean tick counts were higher for those cattle treated with dry Zanthoxylum fruits than those cattle treated with fresh fruits at day 3 through day 45 (Table 1). This may be because dry Zanthoxylum fruits when exposed to sun drying may have been contaminated (Sagar and Kumar, 2010), and during sun drying the nutrient contents such as spasmolytic properties of fresh Zanthoxylum bungeanum Maxim. (Z. bungeanum) deteriorates (Jing et al., 2021). So, these factors probably reduced the efficacy of dried fruit Zanthoxylum. No statistically significant difference in mean tick counts in those cattle treated with fresh Zanthoxylum fruits and deltamethrin was found. This probably suggests that fresh Zanthoxylum fruits can be used which would be as effective as deltamethrin in controlling ticks in cattle. This might be due to the spasmolytic properties of Zanthoxylum (Gilani et al., 2017). This present finding of effectiveness of Zanthoxylum was similar to those of Santos et al. (2013) who found that the Zanthoxylum

**Table 1:** Descriptive statistics of tick counts in cattle in four groups of treatment (mean  $\pm$  SD)

Т	Day 0	Day 3	Day 10	Day 24	Day 45
С	$97.53\pm8.79$	$97.67 \pm 8.449 \ ^{a}$	$103.40\pm8.39^{\text{a}}$	$114.93 \pm 5.98 \ ^{a}$	$140.33 \pm 10.22^{a}$
$T_1$	$102.27\pm8.68$	$90.33\pm6.768^{a}$	$45.20\pm8.06^{\text{b}}$	$24.20\pm 6.54^{b}$	$14.33\pm4.98~^{\text{b}}$
$T_2$	$104.47\pm13.46$	$99.40 \pm 13.793^{a}$	$66.13\pm23.79^{\text{c}}$	$50.20\pm21.16^{\text{c}}$	$42.87\pm22.36^{c}$
$T_3$	$99.07 \pm 10.85$	$71.20 \pm 13.476^{\text{b}}$	$33.20 \pm 10.12^{b}$	$16.93\pm4.69^{\text{b}}$	$8.13\pm4.47^{b}$

Values with the different superscripts across the column are significantly different (p<.05).

C - control, T<sub>1</sub>-Fresh Zanthoxylum, T<sub>2</sub>-Dry Zanthoxylum, T<sub>3</sub>-Deltamethrin

*rigidum* fruit extract has 95% efficiency in controlling ticks in Brazil when used at a maximum dose of 40% w/v concentration.

# Tick counts in animal breed, age, sex, and type of housing

The experiment consists of exotic (exotics are Jersey cross and Holstein Friesian cross) and local cattle. The mean tick count was slightly higher in local cattle ( $72.55 \pm 27.09$ ) than those in improved cattle ( $69.41 \pm 28.01$ ), t (58) = .440, p > .05, which was contradictory to Mushahary *et al.* (2019) who reported that crossbred cattle have a slightly more tick prevalence (53.50%) than those of local cattle (49.34%). In addition, Sajid *et al.* (2009) and Ahmed *et al.* (2012) reported that improved cattle because the local cattle are more disease-resistant than the improved cattle (Sajid *et al.*, 2009; De Meneghi *et al.*, 2016).

No difference regarding mean tick count was observed between young  $(73.40 \pm 26.22)$  and adult cattle  $(70.45 \pm 27.87)$ , (t = .342, p > .05), which is in agreement with a study by Kassa and Yalew (2012). However, Kabir *et al.* (2011) and Eyo *et al.* (2014) observed a higher count of ticks in young cattle than in adult cattle. The young cattle (2-3 years) are generally reared in barns and are not allowed to freely graze, which probably explains why tick count in young cattle is low as ticks are found in wood or grassy areas (Anderson & Magnarelli, 2008).

Mean tick counts of male  $(71.92 \pm 28.51)$ and female  $(70.45 \pm 26.81)$  cattle did not differ significantly, (t = -.206, p = .416), which could be due to the time of experiment (i.e., experi-

## ment was conducted in July). July has a hot and humid environmental condition, which is ideal for ticks to multiply (Ali *et al.*, 2020). Similar finding was reported by Kassa and Yalew (2012). Also, Wasihun and Doda (2013) reported that male cattle are more infested with ticks than female, since the males receive less attention from farmers because of their limited role in milk production.

No difference was observed in the mean tick count between modern (69.5  $\pm$  26.24) and traditional shed (72.19  $\pm$  28.48) (t = -.354; p = .45). The observed similarity in tick counts may be attributed to a management system in which cattle are allowed to graze freely during the daytime.

### Conclusion

We aimed to assess the effectiveness of Zanthoxylum fruit suspension for ticks' control in cattle and found that fresh Zanthoxylum fruit have comparable efficacy to deltamethrin in tick control in cattle. Moreover, fresh Zanthoxylum fruit was more effective in controlling ticks in cattle than using dry Zanthoxylum fruit throughout the experiment period. We recommend using fresh Zanthoxylum fruits' extract to control ticks in cattle.

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