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RESEARCH ARTICLE

Ectoparasite Control in Dairy Focusing Lice: Appraisal of an All-Inclusive Approach

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ABSTRACT

Lice are blood feeding parasites and vectors of many diseases of animals. This study focused on recognizing patterns of epizootiology of lice in dairy animals (small holders and mixed herds). This facet of ecto-parasite control is often overlooked since the most prevalent vector of these animals are at the minimum levels. However, the presence of lice and other ectoparasites within the seasons other than bloom time for ticks (summer-monsoon) is crucial. The dairy animals had an overall prevalence of lice as 51.734 % (in winters) as most prevalent, followed by fleas (14%) and prevalence of 41.86 % and 8.46 % in spring seasons from three different ecology. Resistance to chemical pediculicides has led to development of eco-friendly, biological alternatives. This study reports the use of entomopathogenic fungus (*Bauveria bassiana)* at laboratory conditions. Doses of $1x10^1$, $1x10^3$, $1x10^5$, $1x10^7$, $1x10^9$ conidia/mL) were tested for efficacy against short nosed louse of cattle. A 100% mortality was recorded within 48h of exposure to highest tested concentration against adult lice. LC_{50} ($1.6x\ 10^4$) was calculated using mortality/ survival data. The study forms the first report of using *Bauveria bassiana* against cattle louse that could serve as a baseline for lice control based on eco-friendly and sustainable means. Additionally, this paper signposts the need to devise and implement a consolidated ecto-parasite control for dairy animals.

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INTRODUCTION

Lice (Phthiraptera) can be a health risk to human, livestock and pets not because of blood feeding only, but they play a key role in spread of different diseases (Nafstad and Grønstøl, 2001). They are classified into two morphologically distinct groups i.e. chewing lice and sucking lice. Many lice have host specificity and target one host species only (Coroian *et al.*, 2024). Lice also show site specificity and parasitize specific parts of body like sheep foot louse, sheep face louse (Mckiernan *et al.*, 2021). Diseases like relapsing fever, typhus, brucellosis, rickettsiosis, anaplasmosis, filariasis, bovine dermatomycosis may be vectored by lice. Apart from clinical symptoms and diseases vectored, lice also damage the skin/hide of hosts. Keeping in view the overall impact that the lice have, efforts have been made for years to control them.

Pediculicides have been in use for decades with some success. Dichlorodiphenyltrichloroethane (DDT) and Lindane were the first organochlorine insecticides used. Use of natural Pyrethrins and synthetic pyrethroides has also been documented. However, physiological resistance to lindane in head and body louse was observed widely that marred its effectiveness. Drugs like benzyl benzoate have been effective against louse, but carry side effects like erythema, ocular irritation, pruritis etc. along with them. Ivermectin, a Macrocyclic lactone, has been

reported very effective and is being used widely to control ectoparasites and nematodes in animals.

Currently, ivermectin resistance has limited its use and urged scientists around world to search some alternative better options. Endosymbionts are microorganisms living in body of head louse. These microbes produce nutritional products essential for lice. Drugs resistance and side effects of chemicals have urged scientists to look for more safe, durable and environmentally friendly methods. Use of plant extracts, essential oils, entomopathogenic fungi, entomopathogenic bacteria, entomopathogenic nematodes, etc. have been widely applied against pest arthropods (Abdel-Raheem *et al*., 2017; Fite *et al*., 2019; Beys-da-Silva, 2020).

MATERIALS AND METHODS

Collection and identification of ecto-parasites: A total of n=469 lice were collected from dairy cattle. The approach of sampling was based on convenience method i.e. we sampled dairy cow farms during the winter and spring season. No special traps were used for this study indicative of the ease of collection in highly prevalent ecto-parasites around animals even in the winters. Collected ecto-parasites were transferred to autoclaved glass collection vials. Lice and fleas were identified under stereomicroscope, following identification keys (Kim *et al.*, 1986). *Haematopinus eurysternus* (short nosed cattle louse) were incubated at 27˚C for bioassays. At least 5 lice were subject to different concentrations of fungal spores. The control group was subjected to distilled water as treatment.

Detection of EPF: The isolate of *Bauveria bassiana*, confirmed with the help of morphological and microscopic features was used in the study (Kidd *et al*., 2016). Neubauer chambers were used for determination of quality and dosage of fungal spores. The germination of conidia was done on potato dextrose agar plates.

Adult mortality assays: Five groups, each containing five lice were subject to adult bioassays *in vitro*. Four out of five groups were subject to different doses ($1x10^9$,$1x10^8$, $1x10^7$, $1x10^6$ conidia/mL) of fungi, keeping one as control. Doses of were sprayed over insects. The mortality data was recorded 10 days' post treatment.

Statistical analysis: Mortality data was corrected using Abbott's formula (Abbott, 1925). Data was subject to one-way ANOVA, analyzing different treatments as principal factor. Probit analysis was used to detect 50% mortality for concentration (LC_{50}). All analyses were done using Minitab 18 software (Fite *et al*., 2019).

RESULTS

Lice in the study were collected from dairy cattle during winter season. The lice (51.73 %) were among most predominant ecto-parasites collected from study animals. It was observed that the lice had highest prevalence in winter season (51.73%) (Figure 3), totally in contrast with the tick prevalence (reported in our work submitted for publication). Moreover, lice were more frequently collected from buffaloes (53.8%) compared to the cattle (46.2%). Important genera collected were: *Haematopinus*, *Bovicola* and *Linognathus*. Stereo-micrographs from louse samples have been shown in Figure.1. Based on ocular points near antennae, dark colored plate on ventral side of thorax, distinct para-tergal plates, and legs of uniform size, the majority of collected lice were identified as *Haematopinus eurysternus* (Fig. 1).

Fig. 1: Stereomicrographs (Dorsal and ventral view) of lice collected from study animals (Magnification:4x10X)

Few animals of <3 years' age had infestation with Siphoneptera (Flea: Ctenocephalides) (14%; 28/198) as shown in Fig. 2. Winter lice prevalence was recorded to be highest in the bovines located at Faisalabad (54.2%) followed by bovines at Islamabad (51.7%) and lowest at Bahawalpur (49.3 %) (Fig. 3).

Beauveria is a widely used biocontrol fungal specie, having a facultative growth pattern. Its growth in vitro took place slowly (more than 10 days) on potato dextrose agar, keeping the conditions of incubator at 21˚C and humidity maintained at 70-85%. Shape of conidia was ovoid as shown in Fig. 4. This study reports the use of *Beauveria* sp. for control of lice *in vitro* at LC_{50} as low as $1.6x10^4$ conidia/mL at 95% confidence intervals. A 100% mortality was observed using $1x10^9$ conidia/mL spray within 48 hours of treatment. Mortality of 18.20%, 31.57%, 42.60%, 79.87%, 99.9% was recorded on use of $1x10^1$, $1x10^3$, $1x10^5$, $1x10^7$, $1x10^9$ conidia per mL respectively (Fig. 5).

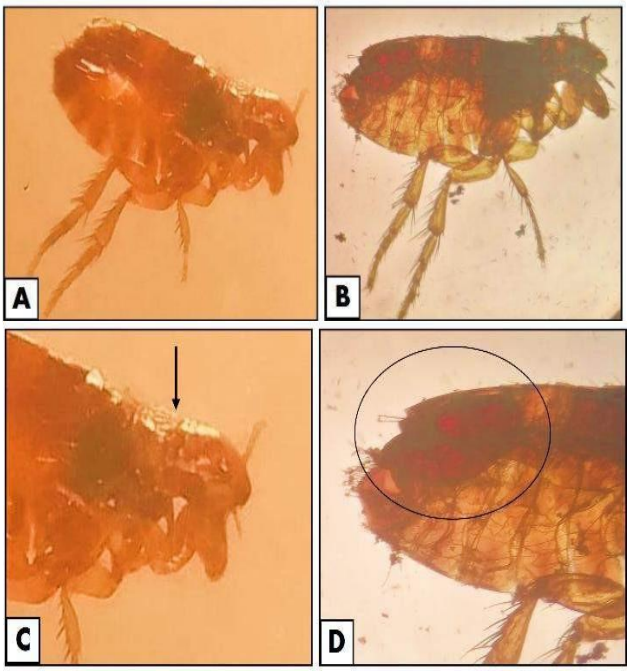

Fig. 2: Lateral view of cat flea as seen from A) Stereomicroscope (4x10X), B) Compound microscope (10X). C) Arrow shows the pronotal combs of cat flea, the acute angle of fons could be seen. D) Encircled: Spermatheca in female flea.

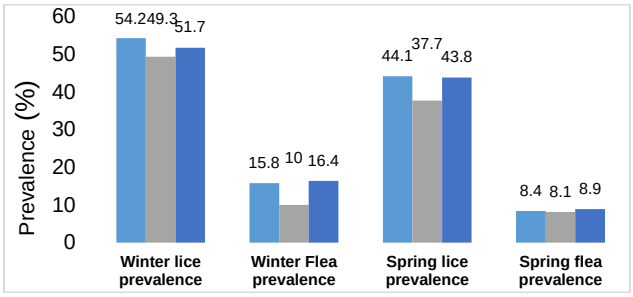

Fig. 3: Prevalence patterns of Lice and Fleas in bovines located at 3 different ecological zones (Faislabad, Bahawalpur and Islamabad-Rawalpindi respectively)

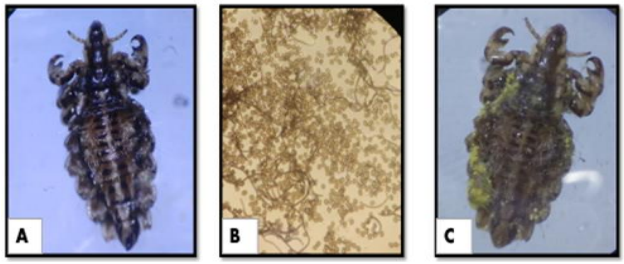

Fig. 4: (A) Haematopinus eurysternus adult from control group (B) Conidia of Beauveria bassiana (C) Louse mortality due to entomopathogenicity.

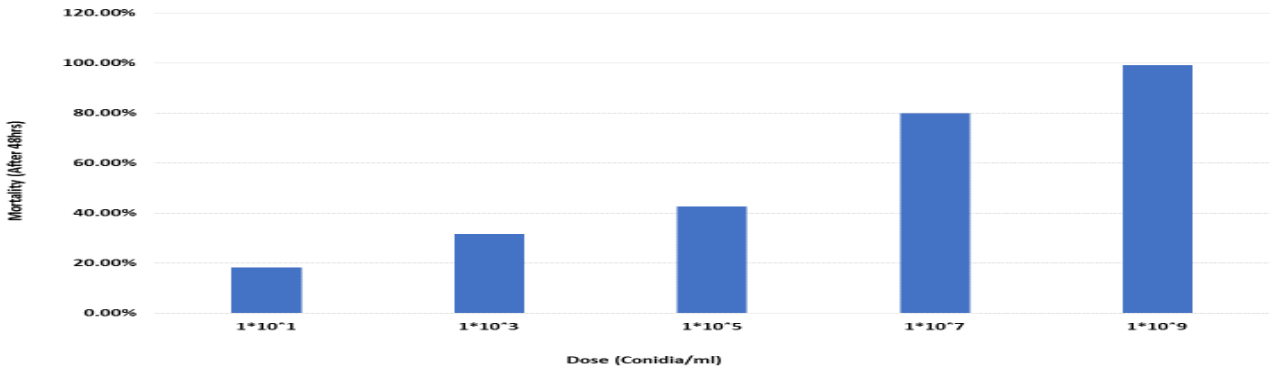

Fig. 5: Record of mortality by various doses of conidia/ ml.

DISCUSSION

Keeping in view the decrease in production and performance patterns of farm animals, the need for efficient lice control is warranted (Nafstad and Grønstøl, 2001; Mckiernan *et al.*, 2021). Biological control of insects is not a new concept owing to the natural abundance of entomopathogenic fungi in several eco-systems. Biopesticides such as *Bacillus sphearicus* and *Bacillus thuringensis* have long been used as larvicidal. EPF is being used to control agriculture pests since old times (Araújo and Hughes, 2016). *Beauveria* was first described by Agostino bassi in 1830 to control larval silkworms. Fungi are being produced at mass scale (high quality commercial and industrial grade products) for use in plants like Mycotrol against aphids. The most important facet of biological control using fungi is the fact that these species of fungi are naturally occurring and have high degree of adaptability when it comes to various climatic conditions (Bara and Laing, 2020). Also, it is favorable to employ the species having natural occurrence that might not be a future threat in terms of non-target effects.

The lice (51.73%) were among most predominant ecto-parasites collected from study animals. Previous studies from Punjab have indicated the prevalence of lice on bovines. Studies from southern Punjab (Multan) and central Punjab (Faisalabad) reported 92% and 18% prevalence of lice on buffaloes, respectively (Hussain *et al.*, 2006; Tasawar *et al.*, 2008). Lice from cattle of Dera Ghazi Khan in a study were reported only 8%, as per calculations of point prevalence in a particular season (Ramzan *et al.*, 2008) which is line with current study.

A study on domestic cattle located in Toba Tek Singh of Punjab province revealed a prevalence of 23.23% by a multi-stage clustered, cross-sectional study. Principal species of lice found were: *Damalinia bovis*, *Haematopinus eurysternus* and the *Linognathus vituli* (Iqbal *et al.*, 2014). Similarly, the

flea collected from cows belonged to *Ctenocephalides canis* and *Ctenocephalides orientalis,* which may be due to presence of stray or pet cats around the bovines (Deak *et al.,* 2022). Hornok and co-workers have reported molecular detection of Rickettsia and *Anaplasma* from *Haematopinus spp.* (Hornok *et al.,* 2010). It signals the importance of lice as vectors of significant livestock diseases.

Beauveria is a widely used biocontrol fungal specie, having a facultative growth pattern (Khachatourians and Qazi, 2008). The arrangement of conidia was brachiform, showing sympodial proliferation (Kidd *et al*., 2016). These findings are following many groups of studies conducted on different insects, using *Beauveria* sp. (Cafarchia *et al*., 2015; Abdel-Raheem *et al*., 2017; Ullah *et al*., 2018).

The appendages of lice on exposure to EPF became brittle and fell- off after the mortality in lice. The exact mechanism of toxicity led by EPF is still under investigation. However, it is speculated that fungal spores become attached to host cuticle. After growth, hyphae enter into the insect body with the help of certain enzymes like chitinases and proteases. The insect exoskeleton is largely composed of chitin, so these enzymes rapidly degrade exoskeletons and internalize the soft organs of insect (Cito *et al*., 2016). Blastospores secrete toxins like beauvericine, basianolidess, beauverolides and destruxin (Lakshmi and Kaur, 2010). It decomposes host internal organs, leading to mortality within shorter time.

In animals, pruritus, restlessness, low weight gain, anemia, low production and reproduction, skin scabbing and hide damage are key damages owing to ecto-parasites other than ticks (Gazafi *et al.,* 2023). Some lice act as vectors of filarial nematodes. In farm animals, lice infestation results in, anemia, loss of production, bovine anaplasmosis, bovine dermatomycosis etc. In a world of pediculicide resistance, the use of entomopathogenic fungus is less toxigenic, eco-friendly and efficient method of control for lice (Beys-da-Silva *et al*., 2020). It is safer to use as humans/animals have not been found as a host to *Beauveria* sp. There is need to further investigate the influence of environmental conditions on efficacy of EPF against lice. Parameters like temperature and humidity may influence the sporulation and germination of fungi and thus commercialization in sub-tropical and tropical climates. Also, the concept of integrated vector management further directs the combined use of different control options against disease vectors (Alkhaibari *et al*., 2018).

Conclusions

The dairy industry relying on high production of animals faces millions of losses annually owing to ecto-parasites infestations, their treatment and control measures. The study forms a report of employing biological control intended for the control of lice (highly prevalent) in winters. It is imperative to focus on a holistic type of ecto-parasite control when it comes to ecto-parasites other than ticks in winter seasons (seasons of low/minimum tick presence).

Author contributions

TZ, AB and MI conceptualized the study, designed the experiments, conducted data analysis, and wrote the first draft of the manuscript. All authors equally contributed.

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