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Research Article

ASSESSMENT OF *IN VITRO* EFFICACY OF DELTAMETHRIN, FLUMETHRIN AND FIPRONIL ON BUFFALO LICE (ANOPLURA: HAEMATOPINIDAE)

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ABSTRACT

Deltamethrin, flumethrin and fipronil were used in the in vitro treated contact bioassay to determine the susceptibility/resistance of buffalo lice, *Haematopinus* spp. Lice were exposed to serial dilution of acaricides and rate of mortality was calculated to determine the LC_{50} and LC_{95} by probit regression. The LC_{50} values of deltamethrin, flumethrin and fipronil were determined as 1.36, 0.19, 0.139 ppm, respectively and LC_{95} were 14.45, 12.59, 2.90 ppm, respectively, with 95% confidence limit. The coefficients of determination (R^2 values) of estimations were more than 80%, indicating a good fitting of the data in the probit model. The higher slope values recorded for deltamethrin and fipronil indicating a high drug response in concentration gradient manner on lice. The slope of mortality was 1.6 ± 0.34, 0.90 ± 0.15 and 1.39 ± 0.18, respectively, for deltamethrin, flumethrin and fipronil whereas, the value of goodness of fit (R^2) was 0.88, 0.93, 0.94, respectively. Among the acaricides used, fipronil was most effective followed by flumethrin and deltamethrin. The study appears to be first in India to demonstrate the susceptibility of buffalo lice against commonly used acaricides by *in vitro* bioassay.

Key words: Acaricides, Buffaloes, Bioassay, Efficacy, *Haematopinus* spp.

Haematopinus spp. is host-specific to water buffaloes (*Bubalus bubalis*) and is the main ectoparasite of the species. Lice infestation leads to skin irritation (Coles *et al.*, 2003), anorexia, restlessness, reduced productivity (Butler, 1985), moreover these insects can also act as vectors for infectious disease like anaplasmosis (Da Silva *et al.*, 2013). Generally, heavily infested animals have low erythrocyte numbers and hemoglobin ratios, indicating anemia (Campbell *et al.*, 2001). In India, lice control mainly relies on chemical acaricides like organophosphates (OP), synthetic pyrethroids (SP), amidines and phenylpyrazole, which are available in different trade names over-the-counter throughout the country. The large selection pressure induced by indiscriminate use of conventional acaricides has led to the emergence and spread of resistance which makes ectoparasite control complicated (FAO, 2004). Resistance to acaricides may induce treatment failures, which will result in chronic infestations requiring additional and episodic treatments (Sinha *et al.*, 2010). The present study was aimed at evaluating the efficacy of deltamethrin, flumethrin and fipronil against buffalo lice, *Haematopinus* spp. by *in vitro* bioassay.

MATERIALS AND METHODS

Collection of lice

Live lice were collected from severely infested 10 buffaloes (tail and dewlap) from a private farm in Banaskantha district of Gujarat, India, in May, 2012. The lice were collected in vials, closed with muslin cloth to allow air and moisture exchange, brought to the Entomology Laboratory, Department of Veterinary Parasitology, Sardar Krushinagar Dantiwada Agricultural University, Sardar Krushinagar. The lice were identified, kept in glass petri dishes with closed lids together with a piece of thick cotton cloth and kept in incubator at 25± 1°C and 70-80% relative humidity.

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Acaricides

Serial dilutions of synthetic pyrethroids, deltamethrin (1.25%) (5 ppm, 15 ppm, 30 ppm, 50 ppm, 70 ppm in distilled water), flumethrin (1%) (10 ppm, 25 ppm, 50 ppm, 100 ppm, 200 ppm in trichloroethylene) and phenylpyrazole compound, fipronil (0.25%) (1 ppm, 5 ppm, 8 ppm, 16 ppm, 20 ppm, 25 ppm in acetone) were used to determine the susceptibility/resistance of buffalo lice. Each dilution was tested in triplicates.

Bioassay

The *in vitro* treated surface (contact) bioassay was performed according to Levot and Hughes (1990) with minor modifications. Three 60x60 mm cloth squares were prepared for each acaricide dilution and labelled (in pencil) with the relevant dilution. One ml of each dilution is pipetted onto each cloth rectangle and allowed to dry at room temperature for 24 hours. Cloths impregnated with distilled water, trichloroethylene and acetone, respectively, were prepared similarly as control. The impregnated cloths were inserted into labeled glass tubes using forceps. The live lice were placed into each tube, sealed the glass tubes with muslin cloth and kept in desiccators placed in BOD incubator maintained at 25±1° C and 70-80% relative humidity for 5 hours. Mortality was recorded every 15 min for up to 5 hrs after the acaricide exposure. The relative

numbers of dead or live lice were counted. The immobile lice showing signs of desiccation were taken as dead, while live lice walk away normally. Results were analysed by probit regression (Bany *et al.*, 1995) and the lethal concentration was calculated. Dose response data were analyzed by probit method (Finney, 1962) using Graph Pad Prism 4 software.

RESULT AND DISCUSSION

The effect of different concentration of deltamethrin, flumethrin and fipronil on *Haematopinus* spp. is presented in table 1. The mortality of lice was found to increase with increasing concentrations of acaricides. In most tests, 50% mortality was recorded at the first reading (15 min) and 100% mortality after 1 hr. Maximum mortality of lice was recorded at 70 ppm for deltamethrin, 200 ppm for flumethrin and 20 ppm for fipronil. Analyzing dose response data, the LC_{50} values of deltamethrin, flumethrin and fipronil were determined as 1.36, 0.19, 0.139 ppm, respectively while, the LC_{95} values were estimated to be 14.45, 12.59, 2.90 ppm, respectively (Table 2). The regression graph of probit mortality of lice plotted against log values of progressively increasing concentrations of acaricides are shown in Fig. 1, 2, 3. The dotted lines in the regression curve represented the 95% confidence limits. The slope of mortality was 1.6 ± 0.34, 0.90 ± 0.15 and 1.39 ± 0.18, respectively, for deltamethrin, flumethrin and fipronil whereas, the value of goodness of fit (R^2) was 0.88, 0.93, 0.94, respectively. In present study 100% of mortality was observed at the concentrations depicted in table 1. However, much higher concentration of these acaricides are being advocated by commercial manufacturers for control of ectoparasites. The recommended dosage of deltamethrin, flumethrin and fipronil are 12.5 ppm (for lice), 75 ppm (for ectoparasites) and 1mg/kg of body weight, respectively for control of ectoparasites. This indicated susceptibility of lice against these accaricides. The *in vitro* bioassay has showed fipronil, flumethrin and deltamethrin are highly efficacious even at the lower concentration, against *Haematopinus* spp. However, fipronil (LC_{95} = 2.90 ppm) was found to be most effective followed by flumethrin (LC_{95} = 12.59 ppm) and deltamethrin (LC_{95} =14.45 ppm). Similarly, in an *in vivo* study at C.C.S. Haryana Agricultural University, Gupta *et al.* (2000) reported flumethrin to be highly effective against *Haematopinus* spp. in adult buffaloes. This represents the first empirical data in India, on the susceptibility of buffalo lice against commonly used acaricides by bioassay. However, Singh *et al*. (2015) reported development of deltamethrin resistance in buffalo lice from Punjab. This may be due to the intensive use of chemical acaricides for agriculture pest in Punjab. Because of the convenience of topical application, chemical acaricides have gained wide acceptance by livestock owners for use in ectoparasite control programs (Skogerboe *et al.*, 2000). However, the application of the acaricides is poorly supervised, resulting in economic problems produced by phenomenon such as

Table 1
Dose dependent response of acaricides against *Haematopinus* spp.

Acaricides	Concentration (ppm)	Mortality (%)
Deltamethrin	5	86.7
	15	93.3
	30	96.7
	50	100
	70	100
	Control	0
Flumethrin	10	93.3
	25	96.7
	50	100
	100	100
	200	100
	Control	13.33
Fipronil	1	90
	5	96.7
	8	100
	16	100
	20	100
	25	100
	Control	6.67

Table 2
Slope, LC_{50}, LC_{95} with 95% confidence limit (CL) values of acaricides against *Haematopinus* spp.

Acaricides	LC_{50} (95% CL)	LC_{95} (95% CL)	R^2	Slope ±SE
Deltamethrin	1.36 (1.05-5.15)	14.45 (9.41-39.26)	0.88	1.6 ± 0.34
Flumethrin	0.19 (0.10-0.36)	12.59 (5.89-73.96)	0.93	0.90 ± 0.15
Fipronil	0.139 (0.09-0.20)	2.09 (1.42-6.54)	0.94	1.39 ± 0.18

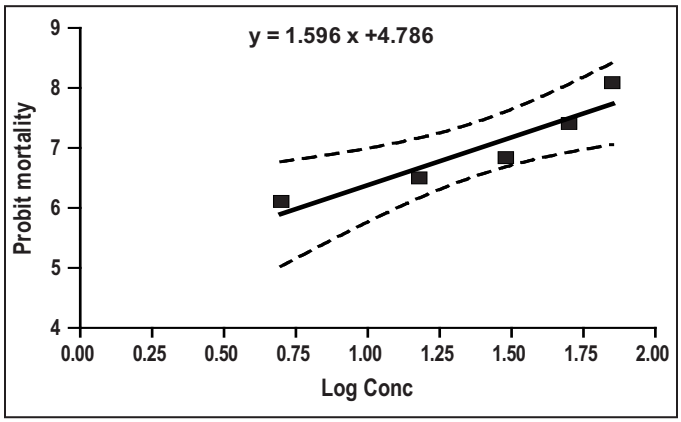

Fig. 1. Dose mortality curve of *Haematopinus* spp. against deltamethrin

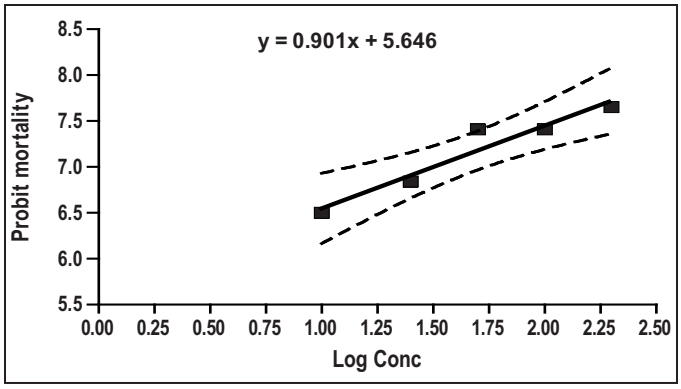

Fig. 2. Dose mortality curve of *Haematopinus* spp. against flumethrin

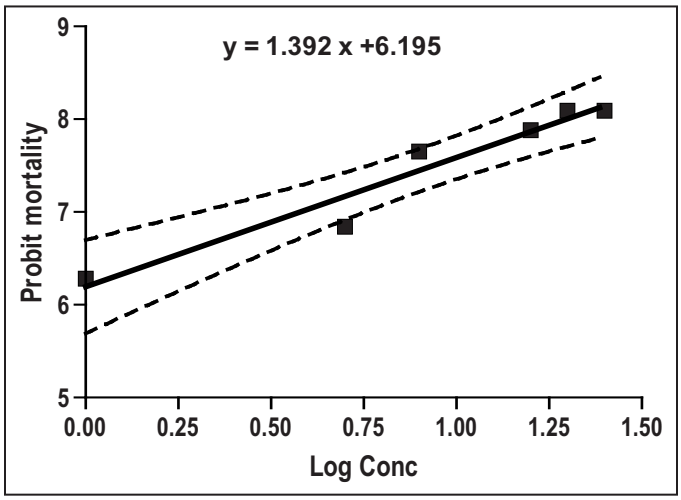

Fig. 3. Dose mortality curve of *Haematopinus* spp. against fipronil

acaricide resistance (Rodriguez Vivas *et al.*, 2007). Many laboratory bioassays have been conducted to study the acaricide resistance pattern in Indian isolates of *Rhipicephalus* (*Boophilus*) *microplus* (Shyma *et al.*, 2013; Shyma *et al.*, 2015) and *Hyalomma anatolicum* (Shyma *et al.*, 2012) ticks which revealed higher degree of resistance against regularly used chemical compounds. The report compelled people to think that the same might happen with the lice even, but the information about resistance is rarely available (Durand *et al.*, 2012).

The acarines control practices in India strongly favour the development of resistance, although experimental data regarding resistance in lice are still lacking. Infact, it is not that louse infestation is not a problem in Indian livestock but due to lack of systemic study on determination of acaricide resistance in lice. However, in present study of applied drugs at given population of lice did not show any sign of resistance. This needs to be supported with further study in lice collected from wider area and with more acaricides formulations.

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