IN VITRO EFFICACY OF ALPHACYPERMETHRIN ON THE BUFFALO LOUSE *HAEMATOPINUS TUBERCULATUS* (BURMEISTER, 1839)

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ABSTRACT

In Italy buffalo farms adopted intensive breeding techniques, however the high density of animals in intensive breeding favours the diffusion of ectoparasites, such as louse.

The aim of this study was to determine the *in vitro* efficacy of the insecticide alphacypermethrin (ACYP) against the buffalo louse, *Haematopinus tuberculatus*. The study was performed by using louse collected from animals in a commercial buffalo farm located in the Campania region of Southern Italy. Lice (adults and nymphs) were collected from highly infested buffaloes. The ACYP was diluted with physiological solution to different concentrations: 1.5%, 0.75%, 0.37%. A volume of 600 µl of the diluted sample was spread evenly over a filter paper held in the lower half of Petri dish. Ten adult lice and ten nymphs were placed on the top of each filter paper disc. The control groups were treated with physiological solution. Seven replicates were used for each concentration. The louse vitality was assessed at different time intervals: 1, 2, 4, 8, 10, 15, 20, 30, 40, 50, 60 minutes, after every 10 min until 240 min or at the louse death. After 240 min the louse vitality was examined each 60 min until 540 min. *In vitro* bioassays revealed that the lousicidal efficacy of ACYP improved as the concentration and the exposure time increased. The results of this *in vitro* study confirm that ACYP at 1.5% concentration can also be used in buffalo for the control of lice, as already in use in cattle. Further field trials will need to be conducted to confirm the safety, the dosage and the *in vivo* parasitological efficacy of this drug on buffaloes.

Keywords: Alphacypermethrin, water buffalo, *Bubalus bubalis*, louse, *Haematopinus tuberculatus*

INTRODUCTION

The sucking louse *Haematopinus tuberculatus* (Burmeister, 1839), Phylum Arthropoda, Class Insecta, Order Phthiraptera, Suborder Anoplura, Family Haematopinidae, is a specific louse of water buffalo (*Bubalus bubalis*), being the principal ectoparasite which attaches this species (Bastianetto *et al.*, 2002); it has been reported on water buffalo in Asia, Africa,

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Australia, and South America (Meleney and Kim, 1974). In Europe it has been reported in Albania, Macedonia, France, England and Italy (Veneziano *et al.*, 2003). Cattle, camels and American bison are also susceptible to infection (Chaudhuri and Kumar, 1961). Louse infestation often leads to skin irritation, anemia, anorexia, restlessness and loss of body condition. The itch caused by *H. tuberculatus* is responsible for the low milk and meat productivity (Bastianetto and Leite, 2005).

The buffalo louse infestation needs to be controlled, particularly if the general condition of animals is affected (Veneziano *et al.*, 2004). On water buffalo, several formulations marketed for cattle have been tested in field trials against *H. tuberculatus* in particular macrocyclic lactones such as ivermectin (Lau and Sing, 1985), avermectin, doramectin (Bastianetto *et al.*, 2002) and eprinomectin (Veneziano *et al.*, 2004).

Alphacypermethrin (ACYP) is a synthetic pyrethroid insecticide, containing more than 90% of the most active enantiomer pair of the four *cis* isomers of cypermethrin as a racemic mixture. ACYP acts on insect's axons in the peripheral and central nervous systems by interacting with sodium channels. This compound is effective against a wide range of pests of many crops and is also used for the control of various veterinary and public health insects, including lice. In Italy ACYP is marketed as a pour-on formulation for use in cattle, with zero milk-withdrawal time. Therapeutics, such as antiparasitic compounds, are often administered to buffaloes based on dosage and intervals recommended for cattle because very few drugs have buffalo-specific label indications (Veneziano *et al.*, 2004).

This is why the literature lacks information on the use and efficacy on buffaloes of most insecticides. Because there is no data on the *in vitro* efficacy of the different group of insecticide on buffalo louse, the aim of this study was to determine, for the first time, the *in vitro* efficacy of the ACYP at different concentrations against the buffalo louse, *H. tuberculatus*.

MATERIALS AND METHODS

The study was performed by using louse collected from animals in a commercial buffalo farm located in the Campania region of Southern Italy. The buffaloes had naturally lice infestation. In order to carry out species identification, 50 louse were collected 5 days before the beginning of the trial from 5 randomly selected adult buffaloes. Louse specimens were studied by using the following routine procedure: *in vitro* mounting, examination under optical and dissection microscopes, and comparison of the resulting morphometric data with those reported in the literature. Species determination was based on the keys proposed by Chaudhuri and Kumar. (1961) and Meleney and Kim. (1974). The buffalo louse *H. tuberculatus* (nearly 600) was collected by 5 researchers from 5 buffaloes highly infested.

A commercial preparation of ACYP (Renegade™, pour on, 1.5%, w/v, Pfizer Animal Health, Italy) was used to prepare three different working concentrations of ACYP (1,5%, 0.74%, 0.37%).

The method used to assess the lousicidal activity of ACYP was adapted from the World Health Organization (WHO, 1981) protocol and according to the methodology from Priestley *et al.* (2006). Bioassays were performed at 27 °C and 75% relative humidity (RH). The direct contact assay was carried out as follows. The ACYP was diluted in physiological solution to different

concentrations: 1.5%, 0.74%, 0.37%. Three drops of Tween 80 were added as emulsifier. A volume of 600 µl of each concentration was properly distributed over a 9 cm diameter filter paper held in the lower half of a 9 cm glass Petri dish. The liquid was allowed to evenly spread for 15 min until the filter paper was totally soaked with the insecticide.

Ten adult lice and ten nymphs of *H. tuberculatus* were placed in two Petri dishes for each concentration. Additional adult and nymphs stages treated with the same volume of physiological solution and Tween 80 only served as untreated control and were placed in two separate plates. Seven replicates were used for each concentration.

Louse vitality was assessed by stereomicroscopy (Leica EZ4 HD) at different time intervals: 1, 2, 4, 8, 10, 15, 20, 30, 40, 50, 60 min and then every 10 min until 240 min or at the louse death. After 240 min, the vitality of lice has been measured at 60 min intervals up to 540 minutes, i.e. the time when all treated parasites were found to be dead. Lice showing no movements of legs and intestine and unresponsive when stimulated with an entomological pin were considered as dead (Priestley *et al.*, 2006).

The efficacy (%) of ACYP was calculated at 1, 2, 4, 8, 10, 15, 20, 30, 40, 50, 60, 70- 240 minute (at ten minutes' interval) and 240-540 minute (at sixty minutes' interval), by using a modified Abbot's formula (1925):

Efficacy = 100 X [n live lice in control plate - n live lice in treated plate/n live lice in control plate]

Differences in louse count were analysed using an ANOVA test for comparison of the treated with the control group. *P* values<0.05 were considered significant.

RESULTS AND DISCUSSION

The morphometric data regarding the lice collected during this study were closely corresponded to those reported for *H. tuberculatus* (Figure 1).

The results demonstrated that ACYP was effective against both adults and nymphs at all concentration levels. *In vitro* bioassays revealed a higher efficacy of ACYP on lice with increasing concentrations and exposure times. The results have been summarized in Figure 2 and Figure 3, showing the knockdown activity of the ACYP against *H. tuberculatus*. The Table 1 shows the efficacy values of all concentrations at different time intervals, expressed as percent reduction.

ACYP was effective against the buffalo louse at all concentration levels. The 1.5% concentration has shown the fastest parasiticidal effect on adults lice, with a complete knockdown activity achieved within 70 min. On the contrary, while a parasiticidal activity was observed at concentrations 0.75% and 0.37%, the death of all lice occurred at 200 and 540 min, respectively. Moreover, a significant difference ($P<0.05$) between 1.5% and lower concentrations was reported as early as 4 min post-treatment, indicating the important role of drug dilution. At lower concentrations (0.75 to 0.37%), no significant differences were observed, although the death of lice occurred more rapidly at 0.75%. The nymph stages have shown higher susceptibility to ACYP than adults, with a overall knockdown activity being achieved earlier. The 1.5% concentration of ACYP displayed a complete knockdown activity after 50 min post-treatment, and mortality in nymphs was significantly higher ($P<0.05$) than in adults as early as 8 min post-treatment. Similarly, mortality in nymphs was found higher

Figure 1. *Haematopinus tuberculatus* at different stages.

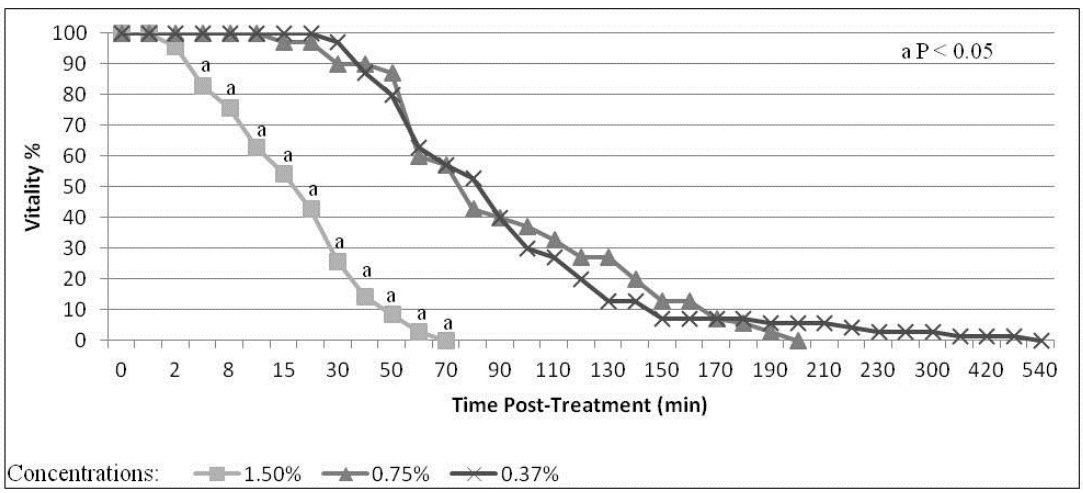

Figure 2. Knockdown activity of Alphacypermethrin on adult stages of *Haematopinus tuberculatus* at different concentrations.

(P<0.05) than adults also at concentration 0.75% as early as 8 min from the start of treatment. At 0.37%, no increase in mortality has been observed for the nymphs as compared to adults.

A full knockdown activity of ACYP at different concentrations has been indicated on nymphs after 50 min at 1.5%, and after 190 and 360 min at 0.75% and 0.37%, respectively. Although 1.5% was statistically the most effective concentration as compared to lower dilutions (as early as 2 min post-treatment) also on nymphs, 0.75% concentration was more effective than 0.37% from 4 to 110 min (P<0.05). Thereafter, these two concentration levels induced similar reductions of lice.

There are limited data available on *in vitro* efficacy of drugs against the buffalo louse, the results observed in the present study are comparable only to data reported by Khater *et al.* (2009). The authors showed, in a similar *in vitro* bioassay, that all treated lice were killed within a minute after treatment by using some essential oils. While the *in vitro* treatment of *H. tuberculatus* with different concentrations of d-phenothrin determined the 100% mortality within 20 minutes.

In relation with the lousicidal efficacy, according to WAAVP guidelines (Holdsworth *et al.*, 2006), an *in vivo* ectoparasiticide drug should provide a reduction in the parasitic population of at least 95% to demonstrate its efficacy in louse control. Highly efficacy values have been recorded within 60 min on adults and from 30 min on nymphs at 1.5% concentration. The concentration of 0.75% the efficacy value ≥ 95% has been achieved starting 190 and 180 minutes on the adults and on the nymphs, respectively. The times were longer at the concentration 0.37%, 220 minutes and 190 minutes on adults and on nymphs, respectively.

The preliminary results of this *in vitro* study confirm that ACYP may be used in buffaloes, as well as in cattle, for the control of sucking lice at a 1.5% concentration.

This is the first study to evaluate the *in vitro* efficacy of ACYP against the buffalo louse *H. tuberculatus*. Further field trials are required to confirm safety, dosage and *in vivo* parasitological efficacy of this insecticide on water buffalo.

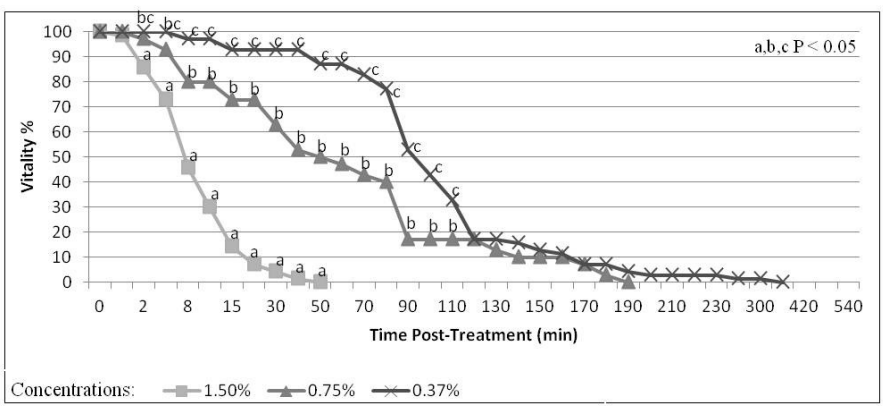

Figure 3. Knockdown activity of Alphacypermethrin on nymph stages of *Haematopinus tuberculatus* at different concentrations.

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Table 1. Lousicidal efficacy of different concentrations of Alphacypermetrin at different time intervals.

C = Control T = Treated

Concentrations	1.5%						0.75%						0.37%					
	N° Adults			N° Nymphs			N° Adults			N° Nymphs			N° Adults			N° Nymphs		
Time (minutes)	C	T	Efficacy (%)	C	T	Efficacy (%)	C	T	Efficacy (%)	C	T	Efficacy (%)	C	T	Efficacy (%)	C	T	Efficacy (%)
0	70	70	-	70	70	-	70	70	-	70	70	-	70	70	-	70	70	-
1	70	70	-	70	69	1.4	70	70	-	70	70	-	70	70	-	70	70	-
2	70	67	4.3	70	60	14.3	70	70	-	70	68	2.9	70	70	-	70	70	-
4	70	58	17.1	70	51	27.1	70	70	-	70	65	7.1	70	70	-	70	70	-
8	70	53	24.3	70	32	54.3	70	70	-	70	56	20.0	70	70	-	70	68	2.9
10	70	44	37.1	70	21	70.0	70	70	-	70	56	20.0	70	70	-	70	68	2.9
15	70	38	45.1	70	10	85.7	70	68	2.9	70	51	27.1	70	70	-	70	65	7.1
20	70	30	57.1	70	5	92.9	70	68	2.9	70	51	27.1	70	70	-	70	65	7.1
30	70	18	74.3	70	3	95.7	70	63	10.0	70	44	37.1	70	68	2.9	70	65	7.1
40	70	10	85.7	70	1	98.6	70	63	10.0	70	37	47.1	70	61	12.9	70	65	7.1
50	70	6	91.4	70	0	100.0	70	61	12.9	70	35	50.0	70	56	20.0	70	61	13.0
60	70	2	97.1				70	42	40.0	70	33	52.9	70	44	37.1	70	61	13.0
70	70	0	100.0				70	40	42.9	70	30	57.1	70	40	42.9	70	58	17.1
80							70	38	45.7	70	28	60.0	70	37	47.1	70	54	22.9
90							70	28	60.0	70	12	82.3	70	28	60.0	70	37	47.1
100							70	26	62.9	70	12	82.3	70	21	70.0	70	30	57.1
110							70	23	67.1	70	12	82.3	70	19	72.9	70	23	67.1
120							70	19	72.3	70	12	82.3	70	14	80.0	70	12	82.9
130							70	19	72.3	70	9	87.1	70	9	87.1	70	12	82.9
140							70	14	80.0	70	7	90.0	70	9	87.1	70	11	84.3
150							70	9	87.1	70	7	90.0	70	5	92.9	70	9	87.1
160							70	9	87.1	70	7	90.0	70	5	92.9	70	8	88.6
170							70	5	92.9	70	5	92.9	70	5	92.9	70	5	92.9
180							70	4	94.3	70	2	97.1	70	5	92.9	70	5	92.9

Table 1. Lousicidal efficacy of different concentrations of Alphacypermetrin at different time intervals. (Cont.)

C = Control T = Treated

Concentrations	1.5%						0.75%						0.37%					
	N° Adults			N° Nymphs			N° Adults			N° Nymphs			N° Adults			N° Nymphs		
Time (minutes)	C	T	Efficacy (%)	C	T	Efficacy (%)	C	T	Efficacy (%)	C	T	Efficacy (%)	C	T	Efficacy (%)	C	T	Efficacy (%)
190							70	2	97.1	70	0	100.0	70	4	94.3	68	3	95.6
200							70	0	100.0				70	4	94.3	68	2	97.1
210													70	4	94.3	68	2	97.1
220													70	3	95.7	68	2	97.1
230													70	2	97.1	68	2	97.1
240													70	2	97.1	68	1	98.5
300													70	2	97.1	68	1	98.5
360													70	1	98.6	68	0	100.0
420													70	1	98.6			
480													69	1	98.6			
540													69	0	100.0			