Acta Parasitologica Globalis 5 (3): 198-205, 2014
ISSN 2079-2018

DOI: 10.5829/idosi.apg.2014.5.3.85137

Evaluation of *in vitro* and *in vivo* Effectiveness of Diazinon Against *Damalina ovis.*

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Abstract: An experimental randomized controlled trial were used to assess *in vitro* and *in vivo* acaricidal effect of Diazinon against sheep lice *Damalinia ovis* on sheep kept under semi-intensive production system, in sheep farm of Adama University School of Agriculture, Asella campus, from November, 2011 to January, 2012. Thirty naturally infested sheep with *D. ovis* were purposely selected for the *in-vivo* test according to the inclusion criteria for the study and were randomly allocated in to treatment and control group. Each selected animal were visually examined for lice infestation and lice from individual animal were counted in 24 hrs before Diazinon was applied. One hundred twenty live and motile *D. ovis* were manually collected for the *in vitro* test from the sheep that had been selected for the *in vivo* test and were immediately taken to the laboratory. The lice were then allocated to treatment and control group (60 lice for each group). The result of the present study showed that the *in vitro* and *in vivo* efficacy of diazinon to be 100%. There was a significant variation in percentage mortality of lice between diazinon treated and untreated group throughout the study period ($P< 0.001$). Sex and body condition of animals do not have significant effect ($P>0.05$) on the effectiveness of the tested acaricide. However, there was statistically significant variation in mean lice burden between animals with short and animals with long wool ($P<0.05$) post exposure up to the second round insecticide spray on day 14. Finally, Diazinon was demonstrated to be highly effective against sheep lice *D.ovis*. Thus, sheep producers could use it at recommended dose to treat their sheep infested with this parasite.

Key words: Diazinon · *D. ovis* · Sheep · Assela

INTRODUCTION

Ethiopia is one of the African nations rich in their livestock resources. Small ruminants are among the major economically important livestock constituting about 30% of the total livestock population of Ethiopia [1]. Small ruminants are important contributors to food production in Ethiopia, providing 35% meat consumption and 14% of milk consumption [2]. Among exports of livestock products, skins and hides have the largest share of exports followed by live animals [3].

The current utilization of hides and skins is estimated to be 77.3% for cattle hides, 58.4% for goats skin and 39.7% for sheep skin with expected off take rate of 33%, 35% and 7% for sheep, goats and cattle respectively. Even though small ruminants are important components of the farming system in Ethiopia, their contribution are far below the expected potential. This is because; small ruminant production in Ethiopia is confronted by the several factors like; disease, poor feeding and managements [4, 5].

Ectoparasitic skin disease of small ruminants caused by lice, keds, ticks and mange mites are among the major disease causing serious economic loss to small holder farmer, the tanning industry and the country as a whole. Infestation with ectoparasites is responsible for blood loss, irritation which results in downgrading and rejection of skins, poor growth, decreased production and reproduction and mortality [5]. The major observed economic loss due to mites, lice and keds is associated with skin damage.

About one million hides and nine million skins are processed every year in the eighteen tanneries located in different parts of the country. However, about 50% the sheep skins processed are rejected because of cockle or Ekek [6]. Cockle, otherwise known as Ekek in Amharic, is an allergic dermatitis caused by lice and keds and is

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characterized by discoloration of the skin or formation of small nodules on the dermis which are about 0.5 to 5 mm in diameter. These nodules may rapture and leave scars when they heal. Both these scars and the discoloration formed due to the allergy do not take dyes during tanning which cause rejection of the leather products [6].

The solution for such skin problem (i.e.cockle) is controlling the causative agents, which are ectoparasites, especially, lice and keds by using effective low residue insecticides. One of the insecticides currently on use widely in Ethiopia is diazinon. It is used throughout the world to control ectoparasites such as sheep scab, blow fly strike and sheep lice [7]. The chemical actives that are currently available are all that we are likely to have for the foreseeable future and thus, they must be used more effectively [8].

The continued use and inefficient application of insecticide has been implicated in the development of resistance in sheep lice [9]. If the continued use and inefficient application of the insecticide occurs, the frequency of the resistant individuals will increase until they dominate the population and as a result, an altered response to a treatment occurs. This suggests that resistance in a population is the inevitable outcome of the widespread use and inefficient application of insecticide [10]. In the period from July 1988 to June 1990 insecticide was applied as a backline treatment on 62% of Western Australian flocks, with 38% treated by shower dipping [11]. In 34.7% of the flocks that were infested, treatment did not eradicate lice. Among flocks treated in a shower dip, 68.4% using coumaphos, 37.8% using diazinon and 41.5% using cyhalothrin had infestations following treatment [12]. Insecticide resistance triggers a chain reaction, which, through deteriorated efficacy, leads to more residues and finally becomes an obstacle to world trade, particularly when maximum residue levels (MRLs) are exceeded [13]. Considering the reckless use and inefficient application of the acaricides, the situation in our country is presumed to be worse than the previously indicated countries. In connection, no previous study has been undertaken in this regard. Therefore the objective of this study was to evaluate the *in vitro* and *in vivo* acaricide effectiveness of diazinon in killing the sheep lice *D. ovis*.

MATERIALS AND METHODS

Description of the Study Area: The study was conducted in Oromia region, Arsi zone, Asella town, which is located in south east of Ethiopia within 6° 59' and 8°49' latitude and 40°44' E longitudes and is located at 175 km south east of Addis Ababa. Asella town and the areas around it have an altitude ranging from 1650-3000 m.a.s.l. [14]. Asella has a mild subtropical weather with average annual temperature ranging from 10-22.6°C. It has a daily maximum temperature reach up to 28°C and minimum temperature of 10°C, an annual rainfall that ranges from 700-1658mm and annual average humidity ranging from 43-60 %. The town has a bimodal rainfall occurring from March to April (A short rainy season) and from July to October (Long rainy season).

Study Design: An experimental randomized controlled trial were used to assess *in vitro* and *in vivo* acaricidal effect of Diazinon against sheep lice *D. ovis* on sheep kept under semi-intensive production system, in sheep farm of Adama University School of Agriculture, Asella campus, from November, 2011 to January, 2012.

Study Populations: Local breed of Sheep kept under semi-intensive production system belonging to sheep farm of Adama University School of Agriculture Asella campus.

Inclusion and Exclusion Criteria: Non lactating, naturally *D. ovis* infested sheep, with lice burden of greater or equal to 100 were included in the study by counting the lices dividing all the body parts of the sheep by demarcation in to five parts Neck, Shoulder, Wither, Flank and Ramp where as sheep that are not infested as well as that have been recently treated with any kind of acaricide were excluded.

Sample Size Determination: The sample size for binary outcomes were determined by using the following formula which is described by Schulz and Grimes [15] by assuming α=0•05, power=0•90 (table value = 10.51) and equal sample sizes were used in the two groups (i.e. Treatment and Control). The overall expected efficacy of Diazinon was considered to be 100% according to Thrusfield [16].

$$n = \frac{\text{Power } [(R+1)\text{-}p2\ (R^2+1)]}{p2\ (1\text{-}R)^2}$$

Where;

n = the sample size in each of the groups
p1 = event rate in the treatment group
p2 = event rate in the control group
R = risk ratio (p1/p2).

Thus, the total sample size was 30 sheep.

Sampling Strategy and Experimental Animals' Allocation: Thirty naturally *D. ovis* infested sheepwere purposely selected for the study and randomly allocated in to two groups (Treatment and control) each group with fifteen sheep. Each selected animal were visually examined for lice infestation and lice from individual animal were counted in 24 hrs before treatment (Spraying with Diazinon) and recorded on a recording format for *in vivo* evaluation.

Experimental Animal Management: All the sheep were ear tagged, housed separately (Control and experimental group) in a shed with a cement floor, fed with hay ad-libtum and supplemented wheat bran. They had free access to water. Their house was sprayed with acaricide, wool length was determined visually and their body condition score was recorded according to method developed by Hamito [17].

Description of the Trial Acaricide: The test acaricide, Vetazinon (Diazinon 60% EC) with an active ingredient of 600g/L Diazinon, was obtained from Adami Tulu Pesticides processing share company, Addis Ababa, Ethiopia. It was manufactured in October 31, 2011 with batch number A-1456-233 and has two years of shelf life from date of manufacture.

Examination of Sheep for Lice Infestation and Determination of Lice Burden: Animals were visually examined for lice infestation. The total individual louse count was conducted on the body surface by direct examination with naked eyes [18]. Lice from each sheep were counted using parting method, i.e. five body parts: neck, shoulder, wither; flank and rump were marked with 4 parings done per site, on both sides of the body [18]. Thus twenty sites on each side of the body were examined by partning the fleece about 10 cm and counting all live lice seen. The total count from 40 sites constitutes the body count for each animal. The sites examined were spaced so that they are representative of the full area of the body covered by the fleece on each side of the sheep. The total louse count per animal was estimated by summation of the louse number at each site.

Collection of Lice for *in vitro* Acaricidal Activity: Sufficient number of live and motile *D.ovis* (From which 120 lice selected under dissecting microscope) manually collected from naturally infested sheep of Adama University School of Agriculture Asella campus sheep farm that had been included in an *in vivo* test and were immediately taken to the laboratory for *in vitro* test. The lice were then allocated to treatment and control group (60 lice for each group).

***In vitro* Acaricidal Activity:** After careful selection under dissecting microscope, 10 lice were placed on whatman filter paper disk of each petri dish (10lice/dishes) according [19]. Next, diazinon was diluted in water according to the manufacturer recommended (1:1000) and lice in the treated group were immersed completely in 0.5ml of this solution (0.1% diazinion 60 EC) for 1min [20]. While the control lice were immersed in 0.5ml of distilled water. After 1minute contact time, the solution was soaked and dried using filter paper. Six replicates were conducted for each treatment. The lice were then examined after 10, 30, 60 and 120 min as well as after 6 and 12 h under dissecting microscope [19].

The Vital State of Each Louse Were Classified According to the Following Categories: vital lice; fully active lice with normal movement, lice with major vital signs; walking but unable to walk in a progressive fashion or no right reflex when rolled onto the back, lice with minor vital signs; not walking but presence of internal (gut) movements, movements of antennae, or leg movements (with or without stimulation by forceps), lice with no vital signs at all even when stimulated by forceps [20].For the calculation of mortality, highly stringent criteria were used and lice only judged as dead if they are in the categories 3or 4 (i.e. no or minor vital signs observable) [20].

***In vivo* Acaricidal Activity:** The experimental group comprising of 15 animals were sprayed with (0.1 %) diazinon 60 EC and the same number of control group were left untreated. Lice count was conducted on day-0 prior to treatment, on day 7 and at 7day intervals then after until study termination day 28 according to Holdsworth [18] and the number of lice on each inspection was recorded.

Data Management and Statistical Analysis: All the collected data were entered to Microsoft excel 2007 spreadsheet then transferred to SPSS version 16 for analysis. Descriptive statistics such as mean and standard deviation were computed. Independent sample t-test was used to compare the mean lice burden between the treated and control group. All analysis was performed at 95% CI and 5% significance level. After treatment, acaricide activity was assessed using arithmetic mean louse count

which was calculated for treated and control group and the percentage reduction in mean louse count was determined according to CVMP [21] as follows:

$$\%\ efficacy = \frac{C - T}{C} \times 100$$

Where:

C = mean number of ectoparasites/animal in the control group

T = mean number of ectoparasites/animal in the treated group

RESULTS

In Vitro Assay: Using the strict criteria for mortality, all lice treated with 0.1% diazinon 60 EC didn't show any vital signs at 720 minutes giving a mortality of 100%. All lice in the untreated group survived during the observation period except one louse that did not show any vital sign at 360 min. The study showed that the tested acaricide killed 5% of lice at 10 min, 6% at 30 min and 10% at 60 min, 28.33% at 180 min, 45% at 360 min and 100% at 720 min. There is a significant variation in percentage mortality of lice between diazinon treated and untreated group throughout the study period ($P<0.001$). The highest mortality was seen within the treated group at 720minute. The overall *in vitro* efficacy of diazinon was calculated to be 100%. *In vitro* post treatment mean number of dead lice per time is shown in Figure 1. The mean number of dead lice in the treated group was slightly increased from 10 to 60 minute. Very high mortality rate was observed between 360 and 720 minute. There was also high mortality from 60 and 360 minute. However, almost there was no any change in the untreated group throughout the study period.

In Vivo Assay: Analysis of lice burden reduction on treated and untreated animals before and after treatment Mean number of lice burden reduction on 0.1% diazinon 60 EC treated and untreated animals is shown in Table 1. The test showed that, even though very slight infestation was observed on day 7 and 14, post treatment; there was a significant reduction in mean lice burden on the treated animals ($P<0.001$) as compared to the untreated animals. After the second round diazinon spray on day 14, all animals were found to be free of lice infestation while mean number of lice burden on untreated animals was slightly increased. The overall *in vivo* efficacy of diazinon was also calculated to be 100%.

Invivo mean lice burden on animal per days of inspection is shown in figure 2. Before treatment, there was approximately equal mean lice burden on treated and untreated animals. Significant reduction in mean lice burden from animals treated with 0.1% diazinon 60 EC was observed from day of treatment up to study termination. However, untreated animals were found to be more infested.

Association Between Lice Burden and Sex of Animals Before and after Treatment: Lice burden reduction in relation to sex and days of treatment was computed and was summarized by (Table 2). The result shows that there was statistically insignificant variation in lice burden reduction between male and female animals throughout the study period ($P>0.05$).

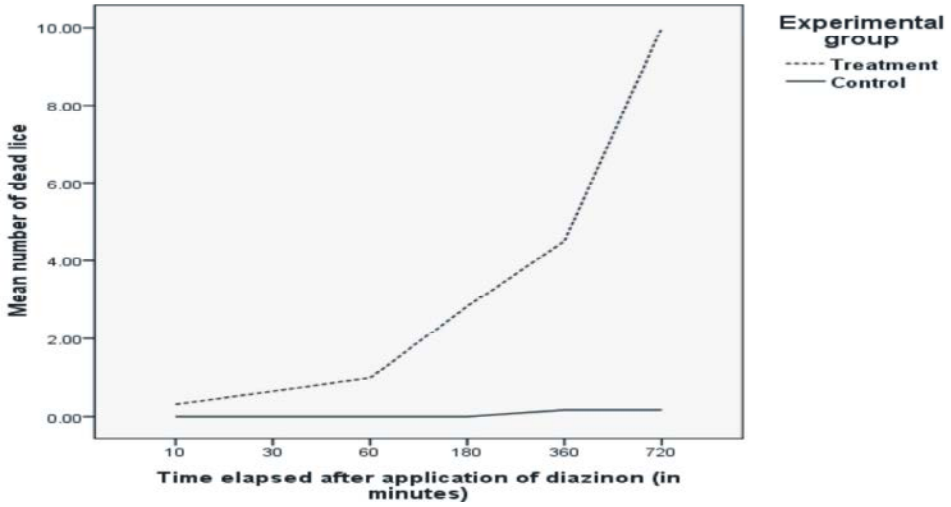

Fig 1: *in vitro* post treatments mean number of dead lice per time

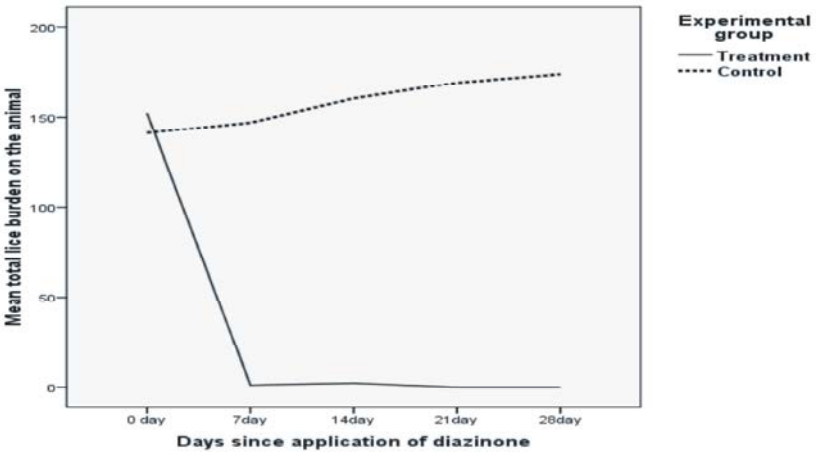

Fig 2: *In vivo* mean lice burden on animal per days of inspection.

Table 1: Comparison of mean lice burden on treated and untreated animals before and after treatment

	Mean number of lice burden ± SD				
	Experimental group				
Treatment days	Treated, N=15	Untreated, N=15	t	df	P-value
Day 0	152.33±49.19	141.07±43.69	0.66	28	0.51
Day 7	1.20±1.15(0.80%)	147.00±42.90(104.20%)	-13.16	14.02	0.000
Day 14	2.27±1.53(0.83%)	160.33±46.84(113.62%)	-13.06	14.03	0.000
Day 21	0.00±0.00	169.27±48.88(119.99%)	-13.41	14	0.000
Day 28	0.00±0.00	174.07±50.90(123.39%)	-13.46	14	0.000

Table 2: Comparison of mean number of lice burden on male and female animals (Pre and post treatment)

	Mean number of lice burden ± SD				
	Sex				
Treatment days	Male N=8	Female N=7	t	df	P-value
Day 0	131.38±34.74	176.29±54.61	-1.93	13	0.08
Day 7	1.12±1.13(0.85%)	1.29±1.25(0.73%)	-0.26	13	0.80
Day 14	2.38±1.30(1.81%)	2.14±1.86(1.21%)	-0.28	13	0.78
Day 21	0.00±0.00	0.00±0.00			
Day 28	0.00±0.00	0.00±0.00			

Table 3: Comparison of lice burden on poor body conditions and good body conditions animals (Pre and post treatment)

	Mean number of lice burden ± SD				
	Body condition				
Treatment days	Poor N=9	Good N=6	t	df	P-value
Day 0	169.33±51.34	126.83±35.45	1.76	13	0.10
Day 7	1.56±1.13(0.92%)	0.67±1.03(0.53%)	1.54	13	0.15
Day 14	2.44±1.42(1.44%)	2.00±1.80(1.52%)	0.54	13	0.60
Day 21	0.00±0.00	0.00±0.00			
Day 28	0.00±0.00	0.00±0.00			

Table 4: Comparison of lice burden (pre and post treatment) based on body wool length

	Mean number of lice burden ± SD				
	Wool length				
Treatment days	Short N=7	Long N=8	t	df	P-value
Day 0	138.71±47.45	164.25±50.61	-1.00	13	0.33
Day 7	0.57±O.97 (0.41%)	1.75±1.04(1.07%)	-2.26	13	0.04
Day 14	1.86±1.68 (1.34%)	2.62±1.41 (1.60%)	-0.97	13	0.04
Day 21	0.00±0.00	0.00±0.00			
Day 28	0.00±0.00	0.00±0.00			

Association Between Lice Burden and Body Conditions of Animals Before and after Treatment: Mean number of lice burden on animals with poor body condition and animals with good body condition pre and post exposure to 0.1% diazinon 60 EC, is shown in Table 3. The test showed that there was no significant variation in lice burden between animals with poor body condition and animals with good body condition (P>0.05).

Association Between Lice Burden and Wool Length of Animals Before and after Treatment: Mean number of lice burden on animals with short and animals with long wool pre and post exposure to 0.1% diazinon 60 EC, is shown in Table 4. The study revealed that after treatment, there was statistically significant variation in mean lice burden between animals with short and animals with long wool (P<0.05) up to the second round insecticide spray on day 14.After second round diazinon spray, both groups were found to be free of lice infestation.

DISCUSSION

An experimental randomized controlled trial study conducted to evaluate the effectiveness of diazinon against *D. ovis*, revealed that the *in vitro* and *in vivo* efficacy of diazinon to be 100%. The efficacy of specific compounds can vary against target species and tolerance to insecticides may develop in specific locations, especially with product use, however, our finding was in line with [16] who stated that diazinon is considered to be effective when its efficacy is 100% for lice. Moreover, the result of this study was also substantiated by another study done by Aziz *et al.* [22].Who suggested the efficacy of diazinon on lice to be 100%.The present study result was demonstrated diazinon to be killing all lices of the treatment group *in vitro* after 12 hours and *in vivo* after 21 days of exposure. In contrast, all lice in the untreated replicates of *in vitro* study were survived during the whole observation period except one louse that did not show any vital signs at 360 min which might be due to desiccation. The tested acaricide killed 5% of lice at 10 min, 6% at 30 min, 10% at 60 min, 28.33% at 180 min and 45% at 360 min *in vitro* and 99.2% on day 7 and 92.17% on day 14 *in vivo* (P<0.05). The result showed that the longer duration of time, the higher knock down effect of the tested acaricide. The slight infestation that was observed on day 7 and day 14 after first round treatment of *in vivo* trial might be due the fact that diazinon does not affect the eggs of lice [23]. Hence, the eggs were hatched. The killing effect of diazinon is that it binds to the enzyme that is normally responsible for breaking down acetylcholine Ach after it has carried its massage across the synapse. When an insect has been poisoned by cholinesterase inhibitors, the cholinesterase is not available to help break down the acetylcholine Ach and the neurotransmitter continues to cause the neuron to fire or send its electrical charge. This causes over stimulation of the nervous system and the insect dies [24]. Mean number of lice reduction on treated animals in relation to sex, body condition and wool length was also computed. The result showed that there was statistically insignificant variation (P>0.05) in lice burden reduction between male and female as well as between poor and good body condition animals throughout the study period. This shows that sex and body condition of animals do not have any effect on the effectiveness of the tested acaricide. On the other hand, statistically significant variation (P<0.05) was observed in the mean lice burden between animals with short and animals with long wool on day 7 and day 14 post treatment up to the second round insecticide spray. The possible explanation for the difference in the effectiveness of diazinon in relation to the wool length could be due to the possibility of the lices to be less exposed to the tested acaricide as they might be hidden more deeper. However, after the second round of diazinon spray, both groups were found to be free of lice infestation.

CONCLUSION

The overall *in vitro* and *in vivo* efficacy of diazinon was 100%.Diazinone was demonstrated to be killing all lices of the treatment group *in vitro* after 12 hours and *in vivo* after 21 days of exposure. Sex and body condition of animals do not have any effect on the effectiveness of the tested acaricide. Statistically significant variation was observed in the mean lice burden between animals with short and animals with long wool on day 7 and day 14 post treatment up to the second round of insecticide spray.

Based on the above conclusion, the following points were recommended:

- As 0.1% diazinon 60EC which is produced at Adamitulu pesticides processing Share Company is highly effective against sheep lice *D. ovis*, sheep producers could use it at recommended dose to treat their sheep infested with this parasite.
- To completely eradicate *D. ovis* infestation from animals, diazinon should be sprayed within two rounds; the second round diazinon spray should be two weeks after the first treatment. This would greatly assist the sheep producers in controlling and eradicating sheep lice.
- If a practical method of applying the insecticide to the tip of wool can be developed, then this technique should provide an effective means of eradicating lice in sheep with long wool.
- It is recommended to undertake such studies in long term interval to monitor the development of acaricide resistance so as to take corrective measures on time.

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