Evaluation of the Effectiveness of Diazinon against *Amblyomma variegatum* (Acarina: Ixodidae)

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Abstract

The evaluation of the effectiveness of acaricides against ticks (Acarina: Ixodidae) using a modified tea bag technique is described. The tests involved natural populations of *Amblyomma variegatum* from natural infestations on cattle at the slaughterhouse Onitsha, southern Nigeria. The tests carried out between November 2002 and May 2003 mimics the exposure of ticks to freshly prepared differing concentrations of the acaricide Diazintol® (containing 162mg/ml or 16.2% W/V of Diazinon dimpylate). Fifty-percent lethal concentrations (LC_{50}) of diazinon for larvae, nymphs, adult males and females of *A. variegatum* were determined (from the plots of concentration^{-1} against mortality) as 0.047, 0.056, 0.07, and 0.075 %, respectively. Treatment was highly significant ($P < 0.01$).

Key words: *Diazinon, Effectiveness, Amblyomma variegatum, Cattle, Nigeria.*

Introduction

Amblyomma variegatum (Acarina: Ixodidae) is an important ectoparasite of the domesticated ruminants throughout its range in the humid tropics (Walker and Koney, 1999). Heavy infestations of ticks on cattle can cause massive tissue destruction (Stachursky *et al*, 1988). An associated has been established between *Amblyomma variegatum* and Dermatophilosis in cattle (Koney, 1996). Wet season prevalence of dermatophilosis in cattle in Northern Nigeria is estimated at 12% (Bida, 1975). An annual economic loss of about 312.40 million (when $1.5 = 31) due to the rejection of damaged hides represents nearly 45% of the total proceeds from sales of Hides at the Nigerian Export Market (Bida and Dennis, 1976).

Systemic antibiotic chemotherapy has not been economically feasible for the treatment of dermatophilosis in Africa and Australia (Stewart 1972). Direct spraying of acaricides on cattle is the usually method of tick-control in Nigeria but emergence of resistant strains of ticks has rendered commercially available acaricides such as Gammatox®, Asuntol®, Delnay® etc., ineffective for field use in the country (Onyali et al., 1989). This paper intends to evaluate the effectiveness of the acaricide, Diazintol®, (Diazinon dympylate, 162mg/ml or 16.2 %W/V) imported into the country from Holland. Different methods so far used to evaluate the effectiveness of acaricides against different species of ticks on dogs include the tea-bag techniqueGladney *et al* (1972), Gladney and Dawkins (1976), Barnard *et al* (1981), Drummond (1981), and disposable pipette method (Koch and Burkwhat (1984). These methods generally involved ticks reared in the laboratory and then used to infest dogs artificially under laboratory conditions. Results from such experiments may not necessarily correlate with results from field trials. Koch *et al* (1985) however described a new method that involved natural populations of ticks on dogs. Because of the task involved in handling cattle, the method used in the present study is a modified tea-bag technique using natural populations of ticks from natural infestations; and designed to mimic natural exposure of ticks on slaughter-cattle to fresh acaricidal preparations.

Materials and Methods

Ticks used in the study: Cattle for slaughter at Onitsha were sampled for ticks used in this study. Slaughter Cattle in the

State were usually bought from Ugwuoba and Amansea Cattle Markets located near the Veterinary Control Posts at the boundary of Enugu and Anambra States Nigeria. Cattle sold at the markets were however derived from Northern States of Nigeria.

Amblyomma variegatum was the commonest species of ticks identified (using the keys of Hoogstraal, 1956) from cattle at this location. The population sample of *A. variegatum* were processed and separated into four groups (larvae, nymphs, adult males and females) which provided the subjects and replicates for the tests.

Acaricide for the study: A commercially available acaricide, Diazintol® (containing 162mg/ml or 16.2%W/V of diazinon dimpylate), manufactured by Alfasan International B.V. of Holland for Animal CareServices Konsult (Nig) Limited was used in the study.

Concentrations of diazinon: Differing concentrations of diazinon were prepared according to label recommendation (vol./vol. basis). 1ml (162mg of diazinon dimpylate/ml) of the emulsion was mixed in each of the measured volumes 1999, 1749, 1499, 1249, and 999 ml of tap water to obtain 0.0585, 0.0668, 0.0753, 0.08, and 0.1% concentrations (approx.) of diazinon, respectively.

Experimental design: 500ml each of the different concentrations of diazinon were poured into five different Jam jars labeled A, B, C, D, E corresponding to 0.0585, 0.0668, 0.0753, 0.08, and 0.1% concentrations, respectively. A Jam jar labeled F contained only tap water and served as adequate control.

A batch (25 subjects per batch) from each group of *A. variegatum* were enclosed in muslin bags (tea bag - type envelopes) and immersed for 3 minutes in each differing concentrations of diazinon. One batch from each group was dipped for 3 minutes in tap water that served as adequate control. Treatment was repeated three times with each group. On removal from the "dip", excess fluid was absorbed from the bags with coarse tissue paper and then covered in similarly labeled Petri dishes for about 50 minutes. Ticks were considered dead if they did not exhibit natural locomotor activity 60 minutes after exposure to diazinon.

Dead ticks were counted and mean mortality percentages computed as recorded (Table 1).

Table 1: Responses of *A. variegatum* to concentrations of diazinon.

Replicates	Concentrations and mortality*				
	A	B	C	D	E
L I	13	13	16	18	20
II	14	16	15	16	18
III	12	13	16	17	21
Mean	13	14.5	15.6	17	19.6
Mean%	**52**	**58**	**62.4**	**68**	**78.4**
N I	12	13	14	16	19
II	12	14	13	17	18
III	11	12	15	15	19
Mean	11.6	13	14	16	18.6
Mean %	**44.4**	**52**	**56**	**64**	**74**
M I	11	12	14	14	18
II	9	11	12	15	17
III	10	10	10	14	17
Mean	10	11	12	14.3	17.3
Mean %	**40**	**44**	**48**	**57.2**	**69.2**
F I	8	11	11	12	17
II	9	9	13	12	15
III	9	10	10	11	16
Mean	8.7	10	11.3	13	16
Mean %	**34.8**	**40**	**45.2**	**52**	**64**

**Control mortality = 0% in all trials, L = larvae, N = nymphs, M = adult males, F = adult females*

The graphs of reciprocals of diazinon concentrations (%) against mean mortality (%) of the life stages of *A. variegatum* are plotted in Fig. 1. Fifty-percent lethal concentrations (LC_{50}) of diazinon for the life stages of *A. variegatum* were determined by extrapolation from the graph. Statistical analysis of variance (ANOVA) and Student's t-test were used to determine the levels of significance (Table 2).

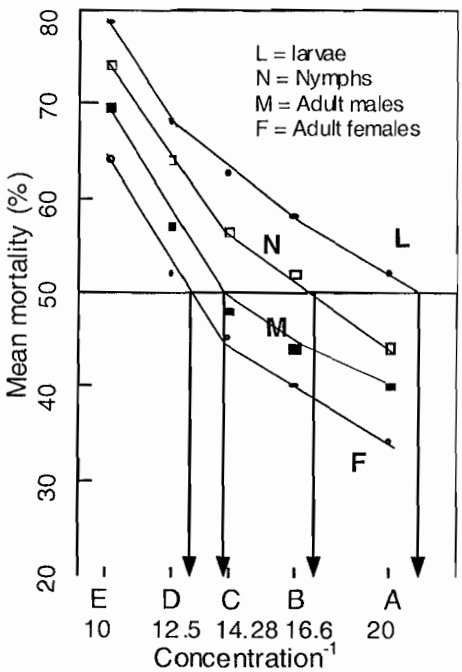
Results and Discussion

The results of the 7-months study from November 2002 to May 2003 are presented in Tables 1. Mean mortality percentages of larvae, nymphs, adult males and females of *A. variegatum were* observed to increase as the concentration of diazinon increases. LC_{50} of 0.047% for larvae, 0.056% for nymphs, 0.07% for adult males, and 0.075% for adult females determined from Fig 1 show that

Table 2: Statistical analysis of data for the completely randomized block design experiments

	Block Treatments						
	A	B	C	D	E	Sum (∑)	Mean (χ)
L	52	58	62.4	68	78.4	318.8	63.76
N	46.4	52	56	64	74.4	292.8	58.56
M	40	44	48	57.2	69.2	258.4	51.68
F	34.8	40	45.2	52	64	236	47.2
∑	173.2	194	211.6	241.2	286	1106	
χ	43.3	48.5	52.9	60.3	71.5		

susceptibility of diazinon for *A. variegatum* was in the order Larvae > Nymphs > males > females. This finding is in line with the observations of Mount *et al* (1982).



LC_{50} for Larvae $= 21^{-1} = 0.047\%$
LC_{50} for Nymphs $= 17.8^{-1} = 0.056\%$
LC_{50} for Adult Males $= 14.2^{-1} = 0.007\%$
LC_{50} for Adult Females $= 13.25^{-1} = 0.075\%$

Fig. 1: Diazinon (conc^{-1}) against mean mortality (%) of *A. variegatum*

These authors had observed in *A. americanum* which infests dogs in the United States, that larvae were 98 fold more susceptible to diazinon than were nymphs and adults, and that the males were generally more susceptible than females, perhaps due to the smaller size of males. They also observed that male and female *Rhipicephalus sanguineus,* which are nearly equal in size, did not follow this pattern.

Koch and Burkwhat (1984) also reported that LC_{50} of diazinon for unfed adult males and females of *A. americanum* were 0.047 and 0.067% respectively, another indication that the males of the Genus *Amblyomma* were more susceptible to diazinon than females. Data analysis in showed significant differences ($P<0.05$)in the responses of the various stages and sexes of *A. variegatum* to diazinon (Table 2).

Conclusion: The acaricidal efficacy of diazinon against *A. variegatum* has been determined from this part of the country. *A. variegatum* exhibits sexual dimorphism and the various life stages differ significantly from each other. The rate of mortality in each life stage increased as the concentration of diazinon increased. Diazinon may be employed for the control of A. *variegatum, which* plays a major role in the epidemiology of Dermatophilus infection in Nigeria. The mention of some proprietary products in his study neither implies their endorsement nor registration by the National Agency for Food and Drug Administration and Control, NAFDAC.

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