

Bioassays and Susceptibility of Adult Ticks (Acarina: Ixodidae) to Diazinon Dimpylate

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Abstract

Engorged adults of the species *Amblyomma variegatum*, which commonly infested cattle in Anambra State, southern Nigeria, were used in a simple laboratory bioassay to evaluate the effectiveness of Diazintol[®] (Diazinon dimpylate) and estimate the unknown strength of samples of Diazintol[®] used by herdsmen, on the ticks, in the area. Adult ticks were found on cattle in a Male: Female ratio of 1.4:1. LC₅₀ of Diazintol[®] determined for engorged females was 0.08% while that for the males was 0.078%. Diazintol[®] concentration of 0.067% which caused about 38% mortality in adult *A. variegatum* was estimated by extrapolation from the graphs of Diazintol[®] concentration against Average mortality (%) of adult ticks. Sexual dimorphism observed in this species was thought to be responsible for the significant difference ($P < 0.05$) in the response of engorged adult *Amblyomma variegatum*, to Diazintol[®].

Keywords: Diazintol[®], *Amblyomma variegatum*, Susceptibility, Bioassay.

Introduction

Amblyomma variegatum (Acarina: Ixodidae) is a major factor in the epidemiology of bovine dermatophilosis in humid tropics (Koney, 1996; Ikpeze, 2002). The infection, which caused damage to the skin of cattle accounted for an economic loss of nearly 45% of the expected annual proceeds from Nigeria's hide (Bida, 1975; Bida and Dennis, 1976). Systemic antibiotic chemotherapy was not economically feasible in the treatment of dermatophilosis in Africa and Australia (Stewart, 1972) where acaricides have been used as suitable alternative for the control of ticks on cattle. Direct spraying of acaricides on cattle is the usual method of tick-control in Nigeria, but it has been reported that the emergence of resistant strains of ticks had rendered the commonly used proprietary acaricides such as Gamatox[®], Asuntol[®], Delnay[®] ineffective for field use (Onyali *et al.*, 1989).

The Petroleum Trust Fund (PTF) of Nigeria had facilitated the importation into the country of Diazintol[®], an emulsifiable concentrate containing 162 mg/ml (16.2% W/V) of Diazinon dimpylate. Effective program of tick control with Diazintol[®] would therefore reduce the incidence of dermatophilosis in Nigeria and elsewhere.

The aims of this study are to (a) Determine the efficacy of Diazintol[®] against *Amblyomma variegatum* that commonly infests cattle in the study area. (b) Estimate the samples of unknown concentrations of Diazintol[®] used by herdsmen in the study area, and (c) Provide field data that would serve as reference for monitoring acaricide susceptibility on ticks in the study area.

Mention of any proprietary product will not constitute its recommendation and endorsement for use nor would it imply its registration under the National Agency for Food and Drug Administration and Control, (NAFDAC).

Materials and Methods

The area of study is Anambra State, southern Nigeria. The vegetation is derived guinea savanna, characterized by short and tall grasses, bushy shrubs, and patches of forest. The tributaries of Anambra River drain the area. Climatic and ecological conditions prevalent in this area are conducive for cattle, the development of ticks, and the spread of dermatophilosis (Ikpeze, 2003).

Cattle from a nomadic cattle settlement at Umunya on the outskirts of the commercial city of Onitsha provided the ticks used in this study. Ticks were removed from 25 cattle, selected randomly at the settlement every week. 500 cattle were sampled between June and October 2003. Engorged adult ticks, identified as *Amblyomma variegatum* (Ikeme, 1976a) provided the subjects and replicates given the same treatment. 0.05, 0.06, 0.07, 0.08, 0.10 and 0.15% concentrations of Diazintol[®] were prepared according to manufacturer's recommendation (vol./vol. basis) by mixing one ml of the emulsion with the appropriate volumes of tap water in labeled glass jars. Tap water was used as the control. Samples of unknown concentrations of Diazintol[®] were also collected from herdsmen in the area.

The modified "tea-bag" technique of bioassay (Ikeme, 1976b) was used to ascertain the mean mortality percentages of engorged adults of both sexes of the ticks observed after three minutes immersion in unknown concentrations of diazinon when compared with similar recorded percentages using the different concentrations of Diazintol[®] under study. Engorged adults enclosed in muslin bags (about 50 male or female ticks per bag) were immersed into each of the different concentrations to mimic field exposure of infested cattle passing through a conventional acaricidal dip. After excess fluid was absorbed with coarse filter paper, each bag was enclosed in a similarly labeled Petri dish for about 45 minutes. There were three replicates per treatment.

For each group tested, subjects were immersed in tap water and served as adequate control. Dead ticks were counted and recorded. Ticks were presumed dead if they did not respond to human breath or move normally when prodded. Using similar subjects (e.g., engorged adult ticks of the same sex) per treatment reduced nuisance variables, which usually occur in experimental biology. ANOVA and Student's *t*-test at 0.05% level of significance were used for statistical analysis.

Results

A. variegatum detached from cattle during the period of study were categorized in Table 1. Male: Female attachment ratio was observed to be 1.4:1. Susceptibility of engorged adult *A. variegatum* to different concentrations of Diazintol® is shown in Tables 2 and 3 in which males were observed to be generally more susceptible than the females. Manufacturer's recommended concentration of 0.10% caused between 63% to 65% mortality among the ticks. Control mortality was 0% in all assays.

Table 1: *Amblyomma variegatum* from cattle in the study area.

Year 2003	Adult <i>Amblyomma variegatum</i>			Attachment ratio Male: Female
	Males	Females		
June	811 (400)*	574 (450)		1.4:1
July	811 (400)	409 (300)		1.1:1
August	784 (400)	539 (450)		1.5:1
September	431 (300)	314 (300)		1.4:1
October	827 (450)	482 (450)		1.7:1
Total	3216 (1800)	2317 (1950)		1.4:1

* Values in parenthesis were used for the trials

Table 2: Susceptibility of engorged adult female *Amblyomma variegatum*

Diazintol Concentration (%)	Female mortality				Average (%)	Average (%)
	Replicates					
	i	ii	iii	iv		
Control	0	0	0	0	-	-
0.05	14	15	15	14	14.5	29
0.06	17	16	16	17	16.5	33
0.07	21	19	18	20	19.5	39
0.08	24	22	25	26	24.25	48.5
0.10	32	33	31	30	31.5	63
0.15	37	34	36	35	35.5	71
A	18	20	17	18	18.25	36.5
B	22	18	19	21	20	40

A and B = Unknown concentrations

Table 3: Susceptibility of engorged adult male *Amblyomma variegatum*

Diazintol Concentration (%)	Male mortality				Average (%)	Average (%)
	Replicates					
	i	ii	iii	iv		
Control	0	0	0	0	-	-
0.05	15	17	14	16	15.5	31
0.06	19	16	18	15	17	34
0.07	21	19	21	20	20.25	41
0.08	23	29	27	25	24.75	49.5
0.10	33	34	31	25	32.5	65
0.15	38	37	35	36	36.6	73
A	19	20	18	16	18.25	36.5
B	21	22	20	18	20.25	40.5

A and B = Unknown concentrations

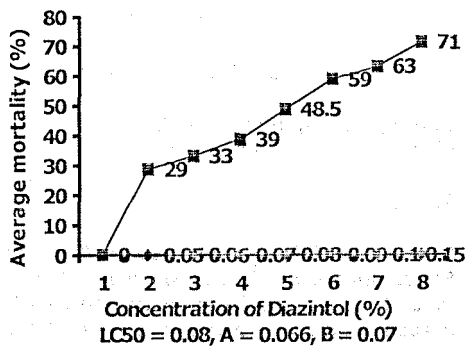


Fig 1: Concentration of Diazintol against average mortality of engorged female *A. variegatum*

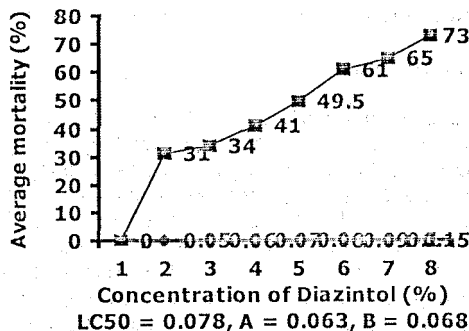


Fig. 2: Concentration of Diazintol against average mortality of engorged male *A. variegatum*

Dose-response curves for engorged adult female and male ticks are shown in Figures 1 and 2. LC₅₀ of Diazintol® extrapolated from the curves were 0.08% concentration for the female and 0.078% concentration for the male ticks. Samples A and B of Diazintol® of unknown concentrations, which caused between 36.5% and 40.5% in the ticks, were similarly estimated at about 0.067%. Analysis of variance and student's *t*-test at 5% level of significance showed significant difference (*P* < 0.05) in the response of male and female *A. variegatum* to Diazintol®.

Discussion

Male: Female attachment ratio of 1.4:1 (see Table 1) was an indication that more males than females were found on the host. Perhaps the males attached for a longer period on the host in anticipation to mate with the females. Since the residual action of diazinon was about six weeks (Lapage, 1968), tick control with Diazintol® should be programmed in the study area, more often in the dry than the wet season in order to prevent rainwater from washing the active ingredients from the body of treated animals. The observation in this study that males were generally more susceptible than females to Diazintol® was in line with the

findings of Mount *et al* (1982) in a related species, *Amblyomma americanum*, which infested dogs in the United States. These authors attributed this difference in susceptibility to sexual dimorphism in the genus, *Amblyomma*, in which the males are smaller than the females. LC_{50} 0.08% and 0.078% reported in this study for engorged females and males, respectively, were also closely related to the findings of Koch and Burkwhat (1984), who reported LC_{50} 0.067% and 0.047% for the unfed female and male *A. americanum*, respectively.

In our opinion, natural population of ticks from natural infestations used in this study might have been subjected to several environmental pressures, which may include prior experiences with other acaricides. The result from this study would therefore be more closely correlated with field conditions than the results that may be obtained with ticks reared in the laboratory, which had not been challenged to environmental pressures.

Dosage-mortality data from this study would therefore provide useful reference for monitoring acaricide susceptibility in the study area. The result also revealed that herdsmen in the area used about 0.067% concentration of Diazintol[®], which was less than the mean LC_{50} of $0.079 \pm 0.001\%$ (0.078% for females and 0.08% for males) determined in this study. Substandard dosage application of acaricides by herdsmen, as shown in this study, may not be unconnected with the reported emergence of resistant strains of ticks in Nigeria (Onyali *et al.*, 1989). The result of this study also indicated that an area control technology against ticks in which the present batch of PTF-sponsored Diazintol[®] was used could achieve up to 65% mortality among engorged adults of *A. variegatum*, which play a very major role in the epidemiology of Dermatophilus infection in Nigeria (Ikpeze, 2004). The method employed in this study may be used to determine the strength of other acaricides as well as that of any sample of acaricide of unknown concentrations, as demonstrated in this study.

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