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Acaricidal Efficacy of Three Organophosphates on Different Stages of *Amblyomma variegatum*

[1]A.J. Natala, [2]T.R. Ahembe, [3]F.A. Gberindyer, [4]S. Danbirni and [5]E.O.A. Ubani
[1]Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria
[2]Department of Veterinary Surgery and Theriogeneology,
[3]Department of Veterinary Physiology, Pharmacology and Biochemistry, University of Agriculture, Makurdi
[4]Department of Veterinary Surgery and Medicine, Ahmadu Bello University, Zaria
[5]National Animal Production and Research Institute, Shika

Abstract: The study was conducted to compare the acaricidal effects of three organophosphorous pesticides, chlorfenvinphos, diazinon and coumaphos as Steladone®, Asuntol® and Diazintol® respectively. They were evaluated on different stages of *Amblyomma variegatum* (unfed nymphs, unfed adults and fed adults) using Shaw's bioassay method. Three concentrations of each acaricides (within the recommendation) were tested on all the ticks' stages. The highest mortality rate was observed in chlorfenvinphos treatments in all the stages and concentrations tested while diazinon produced the least effect. At the highest concentrations, all the acaricides produced high mortality rates but at the lowest concentration, diazinon was not effective. Generally, the acaricidal effects were concentration-dependent and the least mortality was recorded in the fed adult stage, depicting the possibility of blood and its constituent's antagonistic interaction with the acaricide. The outcome of this study suggests that chlorfenvinphos and coumaphos could be used interchangeably because of the equivalence in their acaricidal effects. There is also the need for constant screening in order to ascertain Status.

Key Words: Acaricides, *Amblyoma variegatum*, nymp stages, organophosphates, pesticides

INTRODUCTION

Amblyomma variegatum belongs to the family Ixodidae (hard ticks) and the genus Amblyomma. They are largely distributed in tropical and subtropical areas of Africa. This three-host tick feed on different species of mammals, rarely birds while the larvae and nymphs feed mainly on birds, reptiles and small mammals (FAO, 1984). *Amblyomma variegatum* is essentially a tick of herbivores and predominantly found on cattle, sheep and goat (Barre, 1989). The ability of ticks to transmit protozoal, rikettsial, bacterial and viral diseases to livestock constitute the major economic losses in livestock production (Jongejan and Uilenberg, 1994; Jain and Archana, 2006).

Four major ways of controlling ticks and of course, tick-borne diseases are managerial, chemical, biological and immunological (Jain and Archana, 2006). Chemical approach (acaricides) is probably the major method of ticks control in Africa (Dipeolu and Ndungu, 1991). The major acaricides used are organochlorate (dieldrin), organophosphates (coumaphos), carbamates (carbaryl), pyrethroids (pyrethrins) and avermectines (ivermectin) (Hamel and Duncan, 1986). The organophosphate acaricides are commonly available and used in pest control than any other group of acaricide (Gosh, 1989), on Nigerian markets, organophosphate acaricides indeed enjoy high patronage (Natala and Ochoje, 2009). Reason for it may not be far from the fact that they are highly efficacious, reasonable persistence and less undesirable side effects and tissue residues (Eto, 1974).

Other examples of organophosphates widely used are malathion, and dichlorvos (Hill and Wright, 1978; Gosh, 1989). Their mode of action is by irreversible inhibition of acetyl cholinesterase at the postsynaptic junction and subsequent spastic paralysis and death of the tick. The efficacy of different organophosphates differs in different genera and developmental stages of different ticks (Bittencourt *et al*., 1989; Khurana *et al*., 1992). The objective of this study is to compare the acaricidal effects of three organophosphorous pesticides (chlorfenvinphos, diazinon and coumaphos as Steladone®, Asuntol® and Diazintol® respectively) on different stages of *Amblyomma variegatum* (unfed nymphs, unfed adults and fed adults) using Shaw's bioassay method.

MATERIALS AND METHODS

Study area: This study was conducted between July - December, 2010 at the Entomology laboratory,

Corresponding Author: S. Danbirni, Department of Veterinary Surgery and Medicine, Ahmadu Bello University, Zaria

Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria

Collection of amblyomma variegatum: Ticks used were unfed nymphs, unfed adult and fed adult. All were obtained from a colony maintained at the Entomology laboratory, Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria. They were placed in glass tubes covered with muslin and incubated in a dessicator maintained at 72% relative humidity (RH) and 27ºC.

Acaricides tested: The choice of organophosphate acaricides used was based on their commercial availability and patronage by both farmers and veterinary clinics in Kaduna State. Thus, Steladone® (300 g/L of Chlorfenvinphos) manufactured by Norvitis, Switzerland; Diazintol® (162 mg/mL diazinon dimpylate) manufactured by Alfasan international, Holland and Asuntol® (200 mg/mL coumaphos) manufactured by Bayer were selected for this study.

Preparation of the acaricide solution: The reconstitution of the acaricides was done incognizance to the manufacturers recommended concentrations to be used on infested animals using distilled water. The indicated concentration range for Steladone® and Diazintol® is 0.02-0.05 while that of Asuntol® is 0.02-0.008. The formula, $V_1C_1 = V_2C_2$ was used to prepare the various concentrations of acaricides where V_1 and V_2 are the volume of the acaricide to be drawn from the stock product and the final volume after reconstitution respectively, C_1 and C_2 are the stock product concentration and the required final concentration after preparation respectively. For all the preparations, the final volume was 500mL. Three concentrations of each acaricides, 0.03-0.05 (Steladone® and Diazintol®) and 0.008-0.02 (Asuntol®) as recommended by the various manufacturers were prepared to test their acaricidal effects on the ticks.

Test for acaricidal effect: The bioassay technique used was the filter paper dip method described by Shaw (1966). Briefly, 3 mls of each concentration of acaricides was measured unto double layered filter paper discs (No. 11) in a Petri dish (10×100cm). 10 ticks each (unfed nymphs, unfed adults or fed adults) were then introduced into this preparation. This set up was then covered and incubated in a desicator at 27ºC and 72% RH and the mortalities were assessed 24 hours after incubation. Five replicates were used for each concentration and stage of tick as described above. Distilled water (3 mls) was used as a control on the unfed nymphs, unfed and fed adults.

Efficacy of the acaricides was based on the mortality rate after 24 h of incubation. Ticks were considered alive if when breathed upon or agitated exhibit paddling of the legs or general movement. Those that were unable to maintain the usual upright posture or do not respond to the above test were considered dead.

Statistical analysis: All values were reported as mean±SEM. Difference between means were obtained by one- way analysis of variance (ANOVA) using Tukey's test. Values were considered to be significantly different at $p<0.05$

RESULTS

The results showing mortality rates of varying concentrations of chlorfenvinphos (steladone®), diazinon (diazintol®) and coumaphos (asuntol®) on different stages of *Amblyomma variegatum* 24 h following application are shown in Table 1, 2 and 3, respectively. Table 4, 5 and 6 compare the effects of the three organophosphates on the unfed nymph, unfed adult and fed adults of *A. variegatum* respectively after 24 h when the prescribed maximum and minimum concentrations of the individual acaricides were applied.

The effect of chlorfen vinphos on unfed nymph, unfed adult and fed adult stages of *A. variegatum* at 0.05 was significantly different ($p<0.05$). Conversely, there was no significant difference at 0.04 and 0.03 on the unfed and fed adults of the three host ticks ($p>0.05$) (Table 1). At 0.05 and 0.03 concentrations of diazinon, there was no significant difference ($p>0.05$) in the mortality rates of all the ticks stages after 24 hours of application. But at 0.04, the acaricidal effects differed significantly ($p>0.05$) (Table 2). For coumaphos, there was significant difference ($p>0.05$) between all the stages at 0.02 and 0.01 (Table 3). The study also recorded high mortality rates in the unfed stages of the ticks (nymphs and adults) as compared to the fed stage (adult) in all the pesticides evaluated.

When the mortality rates recorded in the unfed nymphs were compared after the use of the maximum recommended concentrations of the three pesticides, it was observed that there was no significant difference between chlorfenvinphos and coumaphos ($p>0.005$), but the mortality rate was higher in chlorfenvinphos (97.50±1.275%) than coumaphos (94.00±1.275%), however, a significantly ($p<0.05$) lowest mortality rate of 87.00±1.658% was observed in diazintol®. At the lowest concentrations, the mortality rates for all the pesticides were significantly different ($p<0.05$). The mortality rate decreased in the order, chlorfenvinphos, coumaphos and diazinon with values 95.00±1.768%, 79.50±1.275% and 61.00±1.696%, respectively (Table 4).

There was no significant difference ($p<0.05$) in the effects of chlorfenvinphos and coumaphos on unfed adults of *A. variegatum* when the maximum prescribed concentration for each product was used. chlorfenvinphos, coumaphos and diazinon produce the mortality rates of

Table 1: Acaricidal effects of different concentrations of chlorfenvinphos (Steladone®) on different stages of *Amblyomma variegatum* after 24 h of application

	Mortality rate (%)±SEM		
Treatment	Unfed nymphs	Unfed adults	Fed adults
0.05	[b]97.50±1.275	[b]89.50±2.550	91.50±1.275
0.04	89.50±2.550	*75.00±0.791	*77.00±3.984
0.03	[a]95.00±1.768	[a]*65.50±1.458	[a]*64.00±3.021

*: Data not significantly different at same treatment (p>0.05)

Table 2: Acaricidal effects of different concentrations of Diazinon (Diazintol®) on different stages of *Amblyomma variegatum* after 24 h of application

	Mortality rate (%)±SEM		
Treatment	Unfed nymphs	Unfed adults	Fed adults
0.05	*87.50±1.658	*83.50±1.275	*80.00±4.472
0.04	88.00±1.658	76.00±1.275	70.50±3.102
0.03	*61.00±1.696	*59.00±1.275	*59.50±2.151

*: Data not significantly different when same treatments are compared (p>0.05)

Table 3: Acaricidal effects of different concentrations of Coumaphos (Asuntol®) on different stages of *Amblyomma variegatum* after 24 h of application

Treatment	Mortality Rate (%)±SEM		
	Unfed nymphs	Unfed adults	Fed adults
0.02	94.00±1.275	89.00±1.275	59.00±1.458
0.01	83.50±2.031	68.00±1.837	48.00±3.571
0.008	79.00±1.275	*59.00±1.275*	59.73±2.463

*: Data not significantly different when same treatment is compared (P>0.05)

Table 4: Acaricidal effects of three organophosphates on unfed nymphs of *Amblyomma variegatum* after 24 h of applications at maximum and minimum recommended concentrations

	Mortality rate (%) ± SEM		
Treatment	Chlorfenvinphos	Diazinon	Coumaphos
Maximum Conc.	97.50±1.275	87.00±1.658	94.00±1.275
Minimum Conc.	95.00±1.768	61.00±1.696	79.5.00±1.275

Table 5: Acaricidal effects of three organophosphates on unfed adult of *Amblyomma variegatum* after 24 h of application when the maximum and minimum recommended concentrations for each are used

	Mortality rate (%) ± SED		
Treatment	Chlorfenvinphos	Diazinon	Coumaphos
Maximum Conc.	*89.50±2.550	83.50±1.275	*89.00±1.275
Minimum Conc.	65.50±1.458	*59.00±1.275	*59.00±1.275

*: Data not significantly different (p>0.05) when the pesticides are compared at the same concentrations

89.50±2.550%, 89.00±1.275% and 83.50±1.275% respectively. While the mortality rates of 65.50±1.458%, 59.00±1.275% and 59.00±1.275% were obtained when the minimum concentrations prescribed for chlorfenvinphos, coumaphos and diazinon, respectively were applied on unfed adult ticks (Table 5). On the fed

Table 6: Acaricidal effects of three organophosphates on fed adults of *Amblyomma variegatum* after 24 h of applications at maximum and minimum recommended concentrations

	Mortality Rate (%) ± SED		
Treatment	Chlorfenvinphos	Diazinon	Coumaphos
Maximum Conc.	91.50±1.275	80.00±4.472	59.00±1.458
Minimum Conc.	64.00±3.021*	59.50±2.151	*59.731±2.463

*: Data not significantly different (p>0.05)

adult ticks, the mortality rates of 91.50±1.275%, 80.73±4.472% and 59.00±1.458% were obtained when the highest prescribed concentrations of chlorfenvinphos, diazinon and coumaphos products were used. When the lowest concentrations recommended for each products were tested on the fed adult ticks, there was no significant difference (p>0.05) between diazinon and coumaphos treatments but significant and higher mortality rate was observed in those treated with chlorfenvinphos.

DISCUSSION

Generally, the result shows that all the stages of the *A. variegatum* under study were susceptible to chlorfenvinphos. The acaricidal effect decreases from unfed nymph, unfed adult and fed adult stages. This is in agreement with Natala (1997) that different stages of ticks are affected differently by different acaricides. The highest mortality rates in the unfed stages of the ticks (nymphs and adults) as compared to the fed stage (adults) in all the organophosphates evaluated is in agreement with Bittencourt *et al.* (1989) and Khurana *et al.* (1992) who observed that unfed stages of ticks are more susceptible to acaricides than the fed stages. The possible antagonistic interaction of blood and its constituents in the fed tick with the acaricides could be responsible to its reduced susceptibility to the pesticide.

The mortality rate of the ticks also decreased as the concentration of the acaricides decreased depicting that the acaricidal effects of these organophosphates is concentration-dependent as earlier reported (Ndumu *et al*., 1999).

At the highest and lowest recommended concentrations on all the ticks' stages, the mortality rates observed when chlorfenvinphos was used exceeded that of the other two acaricides. This shows that chlorfenvinphos has a higher acaricidal effect on all the stages of *A. variegatum* than coumaphos and diazinon. This agrees with Bafi-Yebo (1974) and Rawlins and Mansinga (1981) who compared chlorfenvinphos with lindane and dioxanthione on *Rhipicephalus* spp. respectively.

Even though all the organophosphates under study (chlorfenvinphos, coumaphos and diazinon) were efficacious on all the *A. variegatum* stages at the highest

recommended concentrations, chlorfenvinphos was superior to both coumaphos and diazinon. The highest acaricidal effect of chlorfenvinphos is in agreement with the efficacy study conducted earlier by Natala *et al.* (1998) on oviposition inhibition in *A. variegatum* and Silva-m-do *et al.* (2000) on *Boophilus microplus.*

Since the acaricidal effect of chlorfenvinphos is not significantly different from that of coumaphos at the highest recommended concentrations on the unfed nymphs and fed adults of *A. variegatum*, the two are equivalent in their acaricidal activity and are therefore interchangeable whereas diazinon is not equivalent with the above even though effective at higher concentrations. The *A. variegatum* mortality rates of chlorfenvinphos, coumaphos and diazinon are dependent on their concentrations and the stage of the ticks. In conclusion, chlorfenvinphos and coumaphos could be used interchangeably on the various stages of *Amblyoma variegatum* because of the equivalence in their acaricidal effects. For effective control of ticks on livestock, it is important to carry out efficacy study periodically, especially where resistant is suspected.

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