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In Vitro Effects of Some Insecticides on Rumen Fluid Fermentation

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ABSTRACT

A study to investigate the effects of different levels (0,5,10 and 15ppm) of three insecticides (Endosulfan, Phosalone and Chlorpyrifos) on rumen fluid fermentation characteristics (rumen pH, rumen ammonia، rumen V FA and *in vitro* dry matter disappearance) was carried out in the laboratory of biology in the University of Gezira, Wad Medani, Sudan in1998 . The study indicated that all the levels of each insecticide significantly ($P<0.05$) inhibited the in vitro dry matter disappearance (IVDMD), the rumen fluid volatile fatty acids and the rumen fluid total protozoa, and elevated the rumen fluid ammonia compared to the control. However, the rumen fluid pH was not affected. Also, the study revealed that endosulfan significantly ($P<0.05$) inhibited the rumen protozoa and the IVDMD percent than both phosalpne and chlorpyrifos.

INTRODUCTION

Many factors such as type of feed (Zervas *et al*, 1999; Ivan. *et al*،. 2000), antibiotics (Chalupa, 1988) and insecticides (Kutches *et al.*, 1969) the rumen microbes and hence their food fermentation affect characteristics. Kutches *et al*. (1969) reported that toxaphene was the most inhibitory of the insecticides tested and resulted in greater decreases of the in vitro dry matter disappearance (IVDMD), volatile fatty acids (VFA) and rumen protozoa. However, Abdalla (1993) reported an increase in serum urea and activity of aspartate transferase in goats which received Endosulfan at 5 mg/kg body weight.

Most of the studies on the effects of insecticides on protozoa dealt with their effects on water protozoa (*Tetrahymena pyriformis*). Inhibitory effects of organophosphates such as Phosphomidon, Chlorpyrifos and phosalone on *Tetrahymena spp* were reported by Hoskin *et al* (1984) . Landis et al and Agarwal and Saxena (1990) Hoskin *et al* (1984) and Landis *et al*. (1987) found that the homogenate of T. *thennophila* can hydrolyse the potent acetyl choline-esterase inhibitor 0,0 di-isoporpyl phosfluoridaze and 0, tetra methyl-methyl propyl fluoridate (enzyme responsible for detoxification). The presence of such enzymatic system was also suggested by Surender *et al.* (1989).

The effects of organochlorine insecticides on *T. pyriformis* were studied by Alchalabi *et al.* (1987) who found that all the tested concentrations of Lindane inhibited the synthesis of DNA, RNA, protein and reduced carbohydrate contents of the microbes.

In irrigated schemes such as Gezira and Rahad, ruminant animals graze on residues of insecticide treated-cotton crop. Intake from such grazable substances represents an important route of rumen contamination with insecticides (El Obied, 2003). This stimulates the present study with the objective of detecting the most inhibitory insecticide (in common use) to rumen fluid protozoa, pH, ammonia, VFA and the IVDMD

MATERIALS AND METHODS

Studies were conducted to investigate the effects of different levels (0,5,10 and 15ppm) of different insecticides (Endosulfan, Phosalone and Chlorpyrifos) on rumen fluid fermentation characteristics (rumen pH، rumen ammonia, rumen V FA and *in vitro* dry matter disappearance) in 1998 in the laboratory of biology in the University of Gezira, Wad Medani, Sudan. A completely randomized design, in a factorial arrangement (3x4) with two replications was used. Rumen fluid for the *in vitro* fermentation was obtained by stomach tubing of sheep (52kg body weight) maintained on *Cynodon dactylon* (Nageela) forage with no history of insecticide treatment. The fermentation studies were conducted according to Kutches *et al.* (1969). One hundred ml beakers fitted with no. 10 one hole rubber stopper containing a gas release valve were used Stock solutions of 100 mg/ml of each Endosulfan, Phosalone and Chlorpyrifos were used to prepare different concentrations (5,10 and 15ppm). A control containing no insecticide was included. Each concentration from each of the three insecticides (dissolved in acetone) was poured on lg of grass (*Cynodon dactylon*) (in duplicate) in each vessel. The solution (acetone) was evaporated. Then 10 ml of inoculum and 35ml of buffer (sodium hydrogen orthophosphate, Na2 C03, KCL and Mg CL_2) and 35 mg from each urea and glucose were added. The vessels were swept with $C0_2$ and the inoculum was allowed to proceed 24 hr at 39^0C. Then the microbial activity was stopped by addition of Iml of 2N H_2S0_4.

The VFA was determined according to Erwin *et al*. (1961). However، the rumen fluid ammonia (RF-NH_3) was obtained according to the procedure described by Fenner (1965). The rumen fluid pH (RF-pH) was immediately read (during rumen fluid withdrawal) by the use of electrode pH-meter (Mini pH ATC Meter). The IVDMD was obtained by filtering he fermentation contents through 50 ml sintered glass Gooch crucible and dried at 105^0C in an oven for 24hrs.

RESULTS AND DISCUSSION

Table (1) shows no significant differences between the RF-pH treated with different types of insecticides and even between the different levels of insecticides. In both organophosphates (Phosalone and Chlorpyrifos) treated RF, a dose-dependant decrease in pH (though was not significant) was found at both levels of 5 and 10 ppm. While this trend was not noticed in Endosulfan treated RF.

Table 1. Interaction effects of insecticides and their concentrations on the pH of sheep rumen fluid.

Insecticide level (ppm)	Endosulfan	Phosalone	Chlorpyrifos	Mean
O(control)	6.48	6.48	6.48	6.48
5	6.50	6.23	6.22	6.32
10	6.56	6.38	6.36	6.44
15	6. 44	6.51	6.42	4.46
Mean	6. 49	6.40	6.37	4.43

SE± for insecticides = 0.01, level = 0.013, interaction = 0.015.

LSD for insecticide = 0.23, level = 0.25, interaction = 0.27.

The investigation indicated that Endosulfan (organochlorine) significantly ($P<0.05$) elevated RF-NH_3 compared to both Phosalone and Chlorpyrifos (Table 2). However, among the two organophosphates، Chlorpyrifos was significantly ($P<0.05$) potent in elevating the RF-NH_3 than phosalone. Also, the RF-NH_3 increased significantly ($P<0.05$) as the level of insecticide increased (Table 2). With the exception of Phosalone at 5ppm level, the other concentrations of the insecticides increased the RF-NH_3 significantly ($P<0.05$) in a dose dependent pattern.

Table 2. Interaction effects of the insecticides and their concentrations on ammonia (mg/ 100ml) of sheep rumen fluid.

Insecticide level (ppm)	Endosulfan	Phosalone	Chlorpyrifos	Mean
O(control)	21.00	21.00	21.00	21.00
5	28.77	17.88	23.88	23.51
10	33.30	27.51	30.92	30.58
15	36.69	34.95	35.90	35.85
Mean	29.90	25.36	27.95	27.74

SE± for insecticides =0. 61, level = 0 .75, interaction = 0.87.
LSD for insecticide = 1.72 level = 1.91, interaction = 2.04

Generally, at 5 and 10 ppm levels, Endosulfan was more potent than Phosalone and Chlorpyrifos in elevating the RF-NH_3, while at 15 ppm all of the three insecticides elevated the RF-NH_3 without significant differences between them.

The mechanism by which the RF-NH_3 was elevated was not clear, but it might be due to some selective effects of these insecticides on some of the rumen microorganisms and hence the rumen metabolism, These results were in line with Alchalabi *et al*. (1988) and Aganwal and Saxena (1990).

It was obvious from Table (3) that the three insecticides significantly ($P<0.05$) decreased the total VFA, propionate and acetate compared to the control. The acetate to propionate ratio (A/P) was significantly ($P<0.05$) increased. This increase in A/P was mainly due to the greater decrease in the propionate levels. As shown in Fig. la and lb, the acetate followed the same trend of the total VFA. Only Endosulfan decreased both the acetate and total VFA at 5 ppm while both Phosalone and Chlorpyrifos increased them. This might indicate that both Phosalone and Chlorpyrifos decreased the acetate and the total VFA at levels higher than 5 ppm. While Endosulfan exerted its decreasing effects on acetate and total V FA at levels lower than that of Phosalone and Chlorpyrifos. The propionate level was reduced at 5 ppm by all insecticides used but Endosulfan was more potent at this level than both organophosphates. Both organophosphates were more effective in reducing the propionate production at 10 ppm than Endosulfan. It was well indicated in Fig. Ic that the level of 15ppm of the three insecticides was more inhibitory to propionate production and caused a wide range of A/P than the other levels. Generally, the production of VFA and their composition ratio were affected by the three insecticides used in this study.

Table 3. Main effects of insecticides on rumen fluid VFA (μ mol/100ml)

Insecticide VFA	Control	Endosulfan	Phosalone	Chlorpyrifos	Mean
Acetate	44.05	31.66	29.26	31.34	44.05
Propionate	14.80	6.11	7.22	6.01	14.80
Butyrate	1.94	2.12	1.29	2.62	I .94
Total VFA	60.70	39.89	37.77	40.47	60.70
A/P	2.97	4.78	3.17	5.22	2.97

SE(±) Acetate = ‹0.03 =Propionate = 0.01, Butyrate = 0.028, Total VFA= 0.03‹ A/P=0.005.

LSD for Acetate= 0.36, Prop.= 0.25. Buty. = 0.29, total VFA= 0.40, A/P= 0. 15.

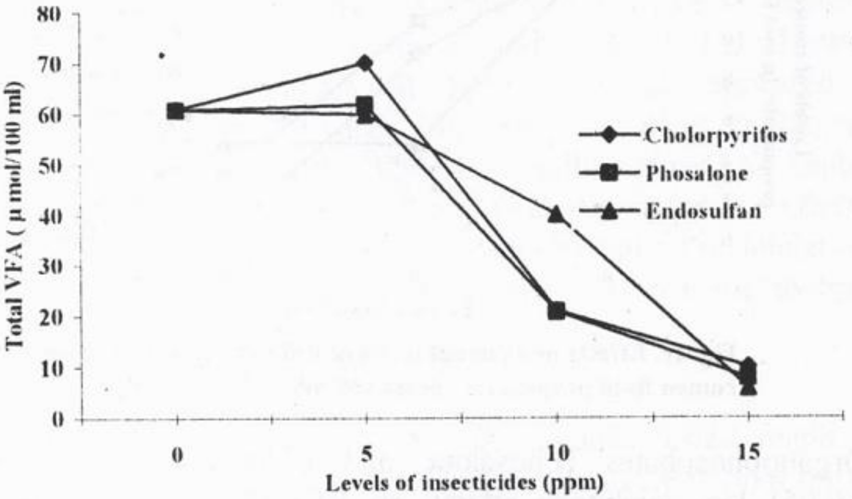

Fig. 1a. Effect of different levels of insecticides on total volatile fatty acids (µ mol/100 ml).

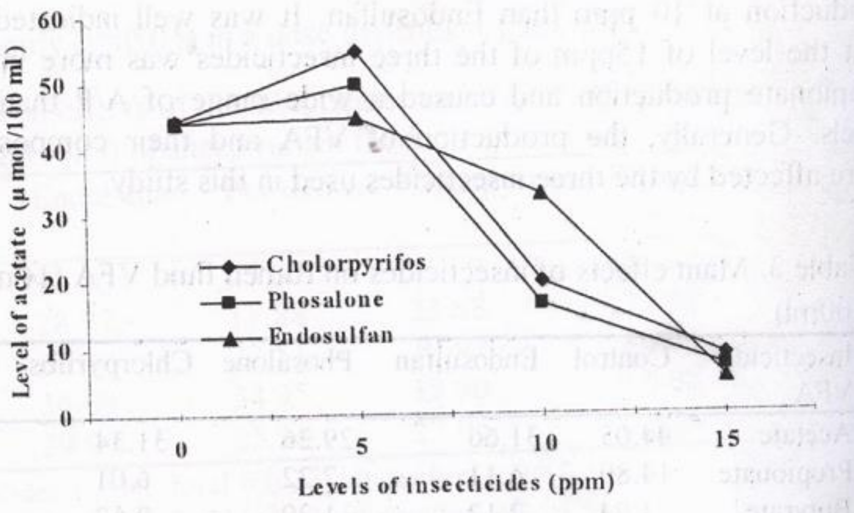

Fig. 1b. Effects of different levels of different insecticides on rumen fluid acetate (µmol/100 ml).

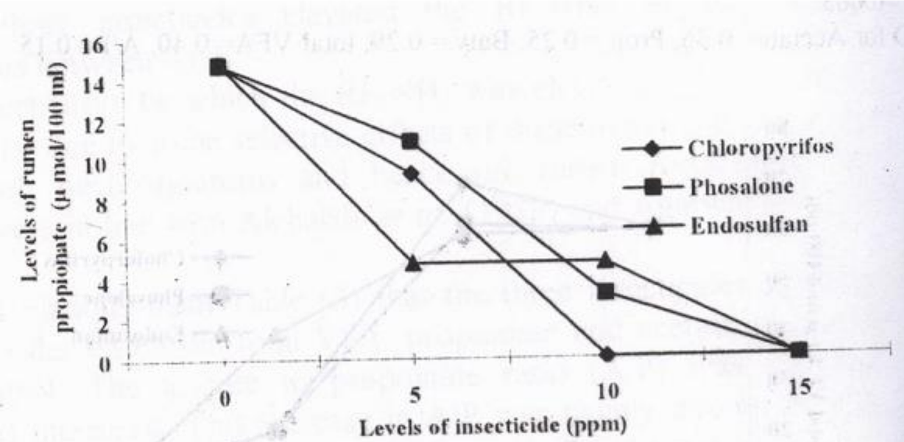

Fig. 1c. Effects of different levels of different insecticides on rumen fluid propionate (µmol/100 ml).

Organophosphates (Phosalone and Chlorpyrifos) had significantly ($P<0.05$) less inhibitory effects on RF-TPC than the organochlorine Endosulfan (Table 4). However, the RF-TPC decreased linearly with an increasing level of the insecticide. This finding was consistent with Kutches *et al*. (1969) who used insecticides other than those used in this study. Agarwal and Saxena (1990) reported that in some instances the protozoal number increased at concentrations of insecticide between 0. I to 10 ppm. With respect to this study, the RF-TPC decreased as the concentration of insecticide increased. Although they were in the range reported by Agarwal and Saxena (1990) for phosphamidin, but their study involved different insecticides and different types of protozoa. The inhibition of RF-TPC with increasing dose of insecticides (Table 4) might well explain the inhibition of IVDMD, RF-VFA production and RF-NH_3 elevation.

Table 4. Interaction effects of insecticides and their concentrations on rumen fluid protozoal count (TPCxl0^5/ml)

Insecticide level (ppm)	Endosulfan	Phosalone	Chloipyrifos	Mean
O(control)	0.63	0.63	0.63	0.63
5	0.56	0.62	0. 61	0.60
10	0.37	0.61	0.62	0.53
15	0.25	0.52	0.57	0.45

SE ± for insecticide = 00. level = 00. interaction = 00.
LSD for insecticide = 0.04, level = 0.04, interaction = 0.04.

As seen in Fig. 2, Endosulfan (organochlorine) was much inhibitory to IVDMD compared to both Phosalone and Chlorpyrifos. However، Phosalone was much inhibitory at 5ppm level. It appeared that organophosphates (Phosalone and Chlotpyrifos) were inhibitory to IVDMD at levels below 15ppm. While Endosulfan caused an inhibition of IVDMD in a dose-dependent fashion. The reason for the effects of organophosphate on IVDMD was not clear. Perhaps the inhibition of IVDMD (degradation of grass) was reflected on rumen metabolism of. VFA and NH_3

CONCLUSION

It could be concluded that the three insecticides tested might have exerted some inhibitory effects at concentrations above 5 ppm on some rumen microbial activity. These inhibit01Y effects were well reflected on increased rumen fluid ammonia and decreased V FA and percent of IVDMD

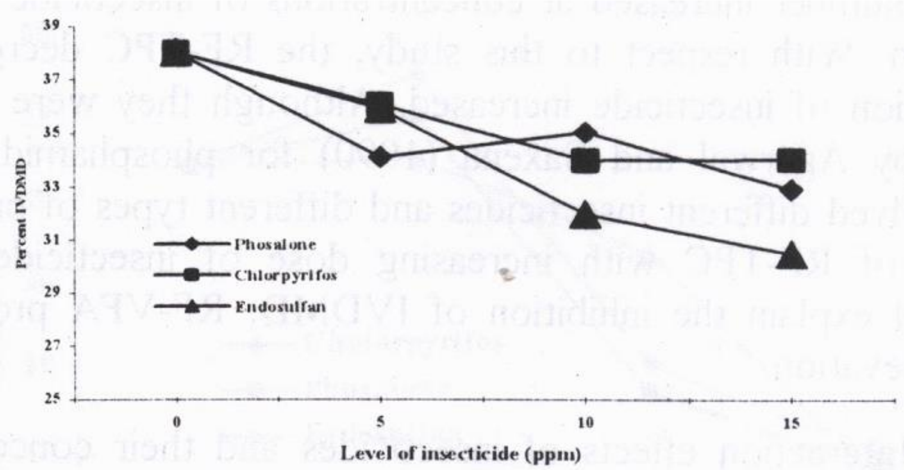

Fig. 2. Effect of different levels of insecticides on *in vitro* dry matter disappearance percent.

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