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Dissipation Studies of Amitraz in Cattle Dips in Bureti, Kericho County- Kenya

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ABSTRACT

Dissipation study of amitraz acaricide was conducted in two cattle dips in Bureti, Kericho County. Farmers in the region experience frequent reoccurrence of ticks resulting into huge management losses due to tick borne diseases. Whereas amitraz is widely used for ticks control, little success has been achieved against management of ticks in Bureti. The objective of this study was to establish whether the application rates used in the dips met the recommended guidelines, and whether dissipation affected efficacy of the pesticide in the dip solutions. We collected cattle dip solutions after 30 minutes of application, then 3, 24, 48, 72, 96, 120 and 144 hours. We extracted amitraz from aqueous solution following liquid-liquid extraction technique using dichloromethane. Amitraz content in the samples was analysed by Gas Chromatograph equipped with Mass Selective Detector, GC-MS. Both dip solutions contained amitraz levels below the recommended dosing rate 1.49 ± 0.11 mg/L and 1.46 ± 0.28 mg/L on day 0 in dips 1 and 2 respectively. The half-life of amitraz was found to be 17 and 18 hours for dips 1 and 2 respectively. The fast dissipation rate of amitraz in the dips contributed to fast declining levels of the pesticide residues in the dip solutions leading to poor control of ticks. The results suggest the need to replenish the dip solutions with adequate amounts of amitraz to effective control ticks in Bureti.

Keywords : Amitraz Dissipation, Cattle Dips Efficiency, Pesticide Half-Life, Bureti Kericho County Kenya.

I. INTRODUCTION

The use of acaricides remains the most popular approach to economic management of ticks.[1] Whereas various nonchemical methods of controlling ticks have been explored including tick-resistant cattle breeding,[2] anti-tick pastures,[3] manual removal of ticks and rotational grazing[4] among others. These methods have not fully succeed in tick control on their own. However, the greatest challenge to application of acaricides in ticks control remains the ability of ticks to develop resistance. This has frustrated tick control efforts and spark continuous search for new acaricides. The discovery of amitraz acaricides brought renewed trust in acaricides as effective tick control measures. Amitraz is a non-systemic formamidine insecticide and acaricide with contact and respiratory action.[5] According to Hollingworth,[6] formamidines acaricide-insecticides are novel both in their range of biological activities and in their mode of action. The group is generally of low hazard for non-target species with the significant exception of predaceous mites. Combined with the effectiveness of amitraz against strains of ticks resistant to other chemical classes of ixodicide[7] hence more popular tick control agent. Amitraz has a different mode of action from carbamates or organophosphorus and making it an acaricide of choice where others failed.[8] Figure 1 below shows the chemical structure of amitraz.[1]

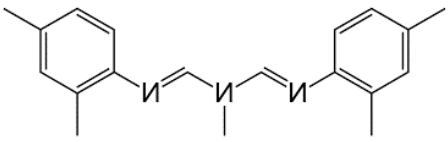

Figure 1. Chemical structure of amitraz [1, 5 di-(2, 4-dimethylphenyl)-3-methyl-1, 3, 5-tri-azapenta- 1,4 diene).

Livestock farmers in Bureti Sub-County depend on the use of acaricides for economic management of ticks. However, there is increasing concern about the effectiveness of cattle dipping in the sub-County due to frequent reoccurrence of ticks after dipping. Whereas it is known that for efficient tick control, it is important to maintain the concentration of the acaricides at effective levels, the ability to determine and maintain the effective concentration of acaricides in the dips solutions is low. Therefore it is difficulty to assure immediate effect of the treatment as well as reduced chances of developing tick resistance.[9] Resistance of ticks to acaricides causes livestock to suffer from tick-transmitted diseases like East coast fever and anaplasmosis which are easily controlled using acaricides.[10] Resistance is accompanied by adverse economic consequences to farmers who depend on cattle rearing for beef and dairy products for their economic development. In their attempt to counter tick resistance to acaricides, some farmers often switch from one acaricide to the other. Others have reduced their cattle dipping intervals. This always attracts huge loses and costs that compromise the economic stability of these farmers.[11] In addition, pesticides contamination pose environmental hazards and adverse health effects to non-target organisms including human. Furthermore, there is no data on the extent of environmental contaminations from cattle dips in the Bureti sub-county. However, previous studies conducted elsewhere in and out of the country have revealed that pesticides used in agricultural activities can contaminate surface waterways and groundwater, as well as soils.[12,13] In addition, cattle dip effluents are discharged into open wetlands, which have high potential of contaminating the water sources, soil and crops through various fate and transport processes.[13] Further contamination by runoff from the wetlands can cause negative effects on the environment. The main objective of this study was to determine dissipation rates of acaricides used in cattle dip wash in Bureti sub-county, Kericho County, Kenya.

II. MATERIALS AND METHODS

A. Percent Recovery Analysis:

500 ml of distilled water was transferred into a 1 L separating funnel. The pH was adjusted to 10 by adding drops either 1N NaOH or 1N HCl because amitraz is stable in basic media.[14&15] The mixture was spiked with 500 µL of the 100 ppm working standard. The mixture was extracted thrice with 30 ml analytical grade DCM and the extracts combined in a round bottom flask. Extraction was done in triplicate per sample. The extract was concentrated using a rotary evaporator to about 1 mL and transferred into Agilent vials using Pasteur pipettes. The volume was reduced further to 500 µL under a stream of white spot nitrogen gas. 1 µL was injected into the GC-MS for quantification of amitraz residues (Figure 2).

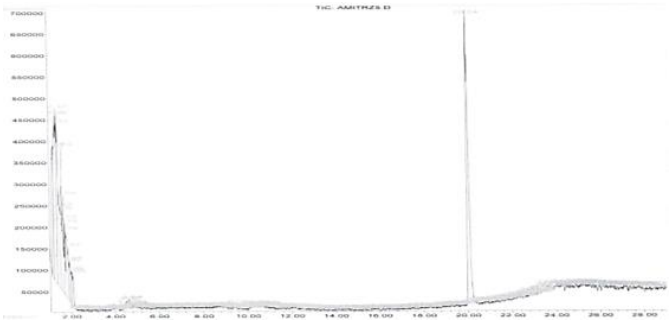

Figure 2. GC-MS ion chromatogram of amitraz

The recovery ranged from 90 to 110% which was considered acceptable.

$$\% \text{ recovery} = \frac{(\text{Spiked sample} - \text{plain sample})\ \text{mg/L} * 100.}{\text{Amount spiked (mg/L)}}$$

B. Analysis of the dip wash:

100 ml of the dip solution was transferred into a 250 ml separating funnel and extracted thrice with 30 ml analytical grade dichloromethane (DCM). For each sample, 2.0 g of analytical sodium chloride was introduced to enhance phase separation. The organic layer was collected in a pre-cleaned dry 250 ml conical flask and extraction repeated twice. The extracts were combined and eluted a 25 cm long x 1.5 cm id packed with anhydrous sodium sulphate to remove any traces of water and solids. The eluent was collected in a round bottom flask. 2 ml of iso-octane was added as a keeper. The mixture was reduced to 1 ml using a rotary evaporator.

The concentrates were carefully transferred into Agilent vials using Pasteur pipettes and reduced further to 0.5 ml under a fine stream of nitrogen gas. The reduced extracts were kept in a fridge at -4 ºC awaiting analysis via GC-MS.

C. GC-MS Analysis and Quantification of the Extract Samples:

Analysis of the acaricide concentration was carried out using Agilent 6890 GC equipped with 5772A MSD at the University of Nairobi, pesticide research laboratory. Helium gas of 99.99% purity was used as the carrier gas at a flow rate of 1.0 ml/min. Oven temperature was maintained initially at 70 °C for 1 min, increased at 15 ° C/min to 175 ° C, then at 2 °C/min to 215 ° C, then 10 °C/min to 265 ° C and finally at 20 °C/min to 290 °C and held for 8 min. Injection volume was 1µL, in split less mode, while the injection temperature was maintained at 250 °C. The data obtained was analyzed using Microsoft Excel software half-life of amitraz was calculated using Langmuir - Hinshelwood first order reaction rate equation

III. RESULTS AND DISCUSSION

A. Calibration curve and recovery:

Calibration was made using multiple point standardization with concentrations ranging from 5 mg/L to 100 mg/L. The calibration curve was linear with the R2 value of 0.99 suggesting a high correlation between instrument response and analyte concentration (Figure 3). Percent recovery of 103.5 ± 1.51 % was obtained which was within the acceptable range of 70 to 110%, hence no correction was made to the results.

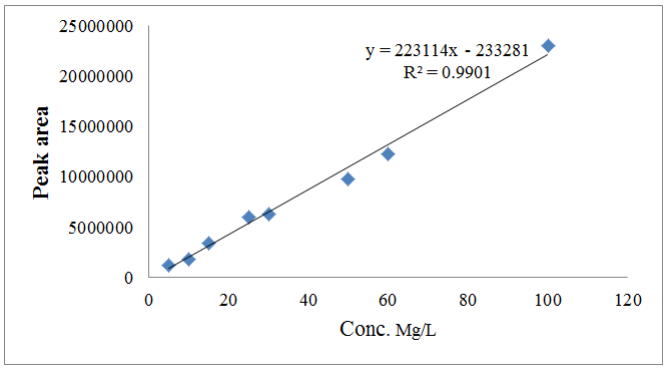

Figure 3. Calibration curve of Amitraz

1) B. Dissipation of amitraz in the dip solutions:

Amitraz levels in dip 1 decreased rapidly from the time of application to below detection limit by the 6th day as shown in Figure 4.

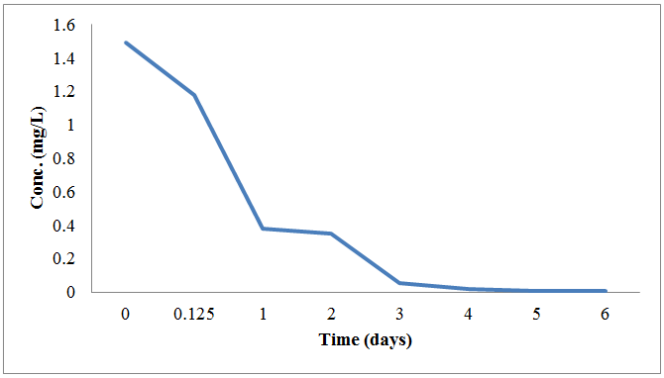

Figure 4. Dissipation trend of amitraz in dip 1

Over the same study period, a similar dissipation trend was observed in cattle dip 2. However, unlike in dip1, amitraz was undetected at day 5. The discrepancy may be attributed to such factors as differences in: day 0 concentrations, prevailing environmental conditions and number of livestock dipped among others. The dissipation trend observed in dip 2 is presented in Figure 5.

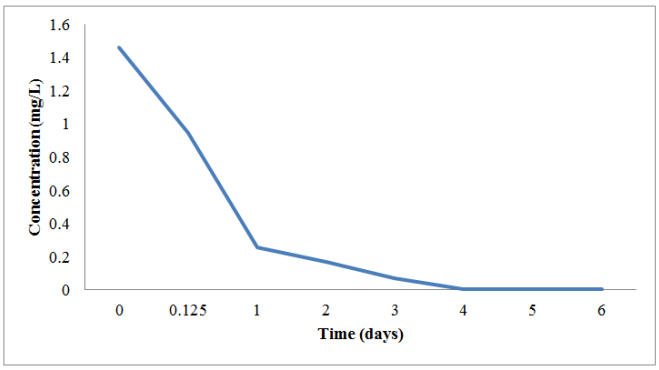

Figure 5. Dissipation trend of amitraz in dip 2

Dissipation of the acaricide followed a first-order kinetic model according to the equation:

$$k = -\ln(C/C0)/t \quad \text{---------- (1)}$$

Where, k is the degradation rate constant expressed in day^{-1}, C and C0, the mean acaricide concentrations ($mg.L^{-1}$) of the samples taken at day t and day 0 respectively. The degradation constant was estimated by linear regression analysis. The persistence of amitraz was estimated by the half-life ($t_{1/2}$) equation:

$t_{1/2} = \ln 2/k$ ----------(2).

C. Linear regression curves:

Based on first order kinetic, a plot of negative ln concentration of amitraz residues versus time t (days) was made. Figures 6 and 7 show the regression curves based on linear equations: y = 0.953x - 0.924 and y = 0.95x - 0.924 observed in dips 1 and 2, respectively.

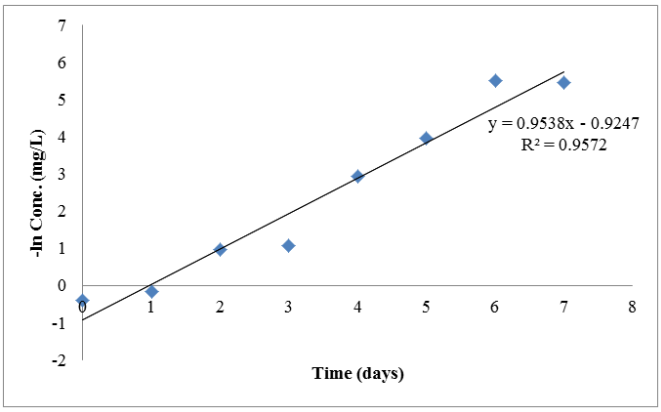

Figure 6. Regression curve for disappearance of amitraz in dip 1

The gradients 0.954 and 0.950 are equivalent to the degradation constant K. Using equation (2), the half-life of amitraz in the dip wash was found to be 17 hours in dip 1 and 18 hours in dip 2. The observed dissipation pattern followed the Langmuir-Hinshelwood kinetic model.[17,18] On the other hand, the regression curve for the disappearance of amitraz in dip 2 is presented in Figure 7 below.

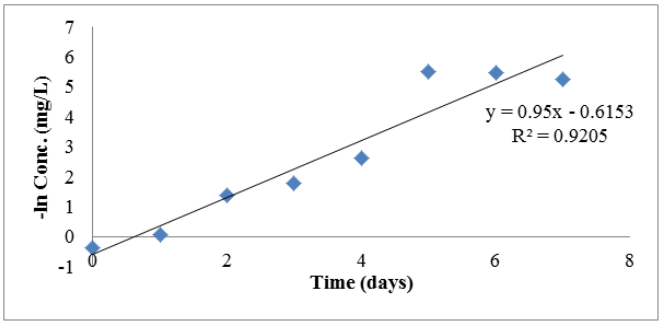

Figure 7. Regression curve for disappearance of amitraz in dip 2

The dip-wash test results indicated that amitraz degrades fast in dips confirming reports from previous studies.[1,14] As such, with each dipping, it necessary to use freshly re-constituted acaricides so as to ensure efficacy in the dips. The fast degradation of amitraz observed gives an implication that by the time of emptying the dip, the amount of acaricide residue in the contents likely to pollute the environment would be minimal. However, since the number of dips studied were few, there is a need to conduct a similar study covering many dips so as to confirm the phenomenon with greater certainty.

Despite its fast degradation potential, previous studies have demonstrated amitraz as a superior tick control acaricide.[1,6] Moreover, amitraz is said to be economical when a large number of livestock are to be dipped.[1] As such, amitraz remains an acaricide of choice in cattle plunge dips for a long time to come. However, there is need to control environmental contamination protect environment and human health due to associated toxicity of the compound[19] against non-target organism.

V. CONCLUSION

All acaricides employed in cattle dips in the sub-county contained amitraz as the main active ingredient. There was a significant loss of amitraz dip strength during the cattle dipping process. The concentration of the acaricide in dip 1 was 1.49 ± 0.11 mg/L on day 0 and 0.0043 ± 0.0002 mg/L after the dipping on day 6. Similar observation was made at dip 2 which had initial concentration of the acaricide at 1.46 ± 0.28 mg/L on day 0 and 0.0052 ± 0.0006 mg/L on day 6. The significant drop in acaricide concentration could be attributed to the decrease in efficacy observed in acaricides employed in the dips. The results further revealed that amitraz dissipated very fast under the prevailing environmental conditions in Bureti cattle dips contributing to the loss of efficacy against ticks. The half-lives of 17 and 18 hours were obtained for dips 1 and 2, respectively. The concentration of amitraz was negligible by the 5th day suggesting the need to replenish the dips with adequate amount of amitraz on weekly basis. Further research covering more cattle dips should be conducted to widen the scope of the study to the entire county level.

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