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Determination of LC_{50} of malathion, dicofol and α-cypermethrin on semi-engorged females of the brown dog tick, *Rhipicephalus sanguineus* (Acari: Ixodidae)

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Semi-engorged females of the brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) were dipped in different concentrations of three acaricides (malathion, dicofol and α-cypermethrin) for three minutes. Probit analysis has been done using a proper software and the results were presented in terms of lethal concentrations of acaricides affording 50% mortality (LC_{50}). As per probit analysis, dicofol was the most effective acaricide with LC_{50} 88.6 ppm (79.419–97.429 ppm fiducial limits) followed by malathion (LC_{50} = 1299.8 ppm; 1223.7–1375.5 ppm fiducial limits). This work can be utilized as a convention with different chemicals, to determinate the LC_{50}, basic steps for investigations of control, resistance and conduct of ticks treated with acaricides, especially the brown dog tick, *Rhipicephalus sanguineus*. Additionally, the knowledge of the LC_{50} gives an idea on the strength of chemicals, the affectability of arthropods to them and even gauges on nuisance control.

Ticks are obligatory blood-sucking arachnid arthropods of medical and veterinary concern which act as vectors of several pathogens (e.g. protozoa, bacteria, viruses and nematodes) that cause tick-borne diseases (TBDs) in domestic animals and humans (Jongejan and Uilenberg 2004). Considered internationally, ticks are second only to mosquitoes regarding the vectorial importance to humans (Goddard 2007).

The genus *Rhipicephalus* comprises around 79 species. The brown dog tick or the kennel tick *Rhipicephalus sanguineus* is the most widespread tick species in the world, particularly within the altitudes of 35° S and 50° N (Dantas-Torres 2008). Chemical acaricides particularly synthetic pyrethroids and organophosphates are currently the most used method of tick control but the indiscriminate and incessant use with improper concentrations for an extended period of time has probably contributed to the development of resistance to these acaricides (FAO 2004), so that many studies are now in progress to determine the most effective control strategy that would reduce the damage caused by these ectoparasites. Fipronil, amitraz, carbaryl and pyrethroids (deltamethrin, permethrin and cypermethrin), are amongst the most commonly used acaricides for controlling *R. sanguineus* (Otranto *et al*. 2005; Oliveira *et al*. 2011), Nevertheless, malathion, dicofol and specifically α-cypermethrin were not studied well in control of ticks. Thus, due to the widespread use of the chemical compounds in the control of ticks, this study shows the detailed procedure of laboratorial determination of LC_{50} for three different acaricides, malathion, dicofol and α-cypermethrin on semi-engorged females of *R. sanguineus* adult using dipping test since a few descriptions are so old.

In the current study, a total of 450 semi-engorged females (ranging in weight from 20–30 mg) of *R. sanguineus* that never been treated with any kind of pesticides were used during this experiment. The ticks culture was obtained from the Department of Applied Entomology, Faculty of Agriculture, Alexandria University, Egypt which maintains the colonies under laboratory conditions (27 ± 3°C, 75 ± 5% RH) as parasite on New Zealand albino rabbit, *Oryctolagus cuniculus*. The feeding procedure on the rabbits was performed using the direct infection method to the host described by Londt and Van der Bijl (1977) and Troughton and Levin (2007) as well. The chemicals used are: [malathion 57% EC (diethyl (dimethoxyphosphinothioyl-thio) succinate)], [dicofol 24.5% EC (2,2,2-trichloro-1,1-bis(4-chlorophenyl) ethanol), [α-cypermethrin 10% EC (racemate comprising (R)-α-cyano-3-phenoxybenzyl (1S,3S)-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate and (S)-α-cyano-3-pheno-xybenzyl (1R,3R)-3-(2,2 dichloro-vinyl)-2,2-dimethylcyclopropanecarboxylate]. All che-mical compounds used in this experiment were provided by Shoura Chemicals (Kilo 28, Cairo-Alexandria desert road, Egypt).

To evaluate the mortality percentages of ticks, four concentrations of each of the previously selected compounds were prepared beside the control group. Concentrations considered for malathion were 1800, 1500, 1200 and 1000 ppm (based on formulation) prepared in distilled water. Similarly, for dicofol the concentrations were 180, 150, 100 and 50 ppm. Finally, for α-cypermethrin were 3500, 3000, 2000 and 1000 ppm. Formulation of the used acaricides were used to prepare the stock solution and different concentrations were prepared in distilled water from the stock solution to use against the ticks. Large scale of concentrations were experimented firstly and probit analysis was done, then narrowing the concentrations was done to the most suitable and effective ones.

Each concentration of the above chemical was tested on thirty adult females in three replicates of 10 adults each. The ticks were dipped for three minutes in a flask filled with test concentrations, then dried on filter paper to remove excess liquid. The volume of each concentration of every acaricide was 30 ml. The control group received the same volume of distilled water (30 ml) and during the experiment period, they were not allowed to feed. After treatments, the semi-engorged females were held at 27 ± 3°C and 75 ± 5% RH in clean glass vials covered with a piece of cloth and secured with rubber bands. The mortality rates were taken after 24h, 48h and 72h. Death confirmed by fixing the treated ticks with two-sided adhesive tape in Petri dishes on their dorsal sides and only ticks capable of movement were considered alive. In this study, the results obtained from bioassay were subjected to probit analysis using LDP-line software (Ehab Soft) to calculate LC_{50}, Slope and fiducial limits according to Finney (1971).

The results of the bioassay experiments showed 0% response of *R. sanguineus* females of the control groups. The application of malathion at 1000 ppm showed a minor response of the females with 30 ± 0.1684% mortality response, these responses increased during the application of 1200, 1500 and 1800 ppm gradually with 50 ± 0.1406%, 60 ± 0.1751% and 80 ± 0.1711% respectively. These responses were along with the observation of behavior changing such as, activity increasing, repeated movement, spreading out the legs, paralysis followed by death. LC_{50} has been determined after 24, 48 and 72 hours.

The application of dicofol showed also a gradual response during the concentration increasing starting from 50 ppm through the series of 100, 150 and 180 ppm with a mortality of 20 ± 0.1710%, 60 ± 0.2150%, 70 ± 0.2670% and 90 ± 0.1640% respectively along with a similar behavior of the treated *R. sanguineus* females. Whereas, the application of α-cypermethrin showed its activity by the series of 1000, 2000, 3000 and 3500 ppm with a mortality percentage 30 ± 0.1857%, 60 ± 0.1960%, 70 ± 0.2496% and

80 ± 0.1689% respectively with same observed dying behavior. LC_{50} for both compounds also have been calculated after 24, 48 and 72 hours.

The analysis of data obtained in the present study showed that, the dicofol compound which belongs to organochlorine (LC_{50} = 88.6 ppm) was the most toxic compound against *R. sanguineus* and was 50 times more toxic than malathion which belongs to organophosphates (LC_{50} = 1299.8 ppm), followed by α-cypermethrin which belongs to synthetic pyrethroids (LC_{50} = 1642.8 ppm) as judged by the 24h LC_{50} values. The highest efficacy of treatment was obtained on the second and third day after treatment by the dicofol as judged by the LC_{50} value (LC_{50} = 77.0 ppm) (Table 1). It should be considered in that treatment of the three acaricides, the activity of these acaricides increased from 24h to 48 and 72h post-treatment and the same results were between both 48h and 72h. The results also in this study revealed that the increase in the concentration of the three used acaricides caused a clear increase in the mortality of *R. sanguineus* adult females.

Table 1. Toxicity of malathion, dicofol and α-cypermethrin on the brown dog tick, *Rhipicephalus sanguineus* under laboratory conditions (27 ± 3ºC and 75 ± 5% RH)

Compound	LC_{50} (ppm)	LC_{99} (ppm)	Slope	Chi-square	Fiducial limits (LC_{50})		Fiducial limits (LC_{99})	
					Lower	Upper	Lower	Upper
Malathion								
24 hours	1299.8	3576.3	5.2923	1.4362	1223.7	1375.5	2901.5	5081.4
48 hours	1112.6	2995.2	5.4087	2.9804	1022.8	1182.9	2495.3	4079.4
Dicofol								
24 hours	88.6	435.6	3.363	5.9909	79.419	97.429	339.7	626.7
48 hours	77.0	439.1	3.077	1.5730	67.336	85.872	334.7	660.2
α-Cypermethrin								
24 hours	1642.8	15713	2.3721	0.8997	1391.1	1870.6	10177.3	32735.4
48 hours	1408.2	25175	1.8576	1.0649	1076.8	1676.6	13386.2	86404.9

Medical, veterinary and economic implications of tick infestation are well documented. The brown dog tick, *R. sanguineus* produces debilitating effects due to blood losses, skin lesions and secondary infections, such as myiasis and abscess in affected animals (Balashov 1972; Wall and Shearer 1997) besides transmitting many pathogens. Numerous studies are currently underway to find an effective control strategy that would minimize the damage caused by these parasites. A new tick control approach is an immunological one, consisting in the identification, isolation and synthesis of proteins that protect tissues and organs of the tick, mainly those of the reproductive system, with the aim of developing a vaccine (Tellam *et al.* 1992; Willandsen 1997). Biological control agents are in principle highly desirable but their efficacy, mass production, application and stability present serious challenges. However, nowadays the most efficient method to control tick populations is by using chemical compounds, the toxicity of an acaricide is defined as extent or degree to which a chemical substance to kill or injure the target pest. In this way, the toxicity of a drug is determined by running laboratorial tests on ticks and it is expressed as LD_{50} (Lethal dose fifty) and LC_{50} (Lethal concentration fifty) values and are the amount or concentration, respectively, of the pesticide's active ingredient that is required to kill 50% of the tested animals under standardized tests conditions (Garcia-Garcia *et al.* 2005). The concentration and conditions of exposure are the main factors determining the toxic effect of given compound (Ottoboni *et al.* 1990).

Acaricidal activity of three compounds belong to three different group of chemicals were tested by direct contact application in various concentrations against adults of the brown dog tick, *R. sanguineus* considering their safety to the host animal especially dogs. According to recent reports, malathion is classified as a GUP (General Use Pesticide) and is slightly toxic category III and it is not carcinogenic, whereas, dicofol is classified by the World Health Organisation as a class II 'moderately hazardous' but with less toxicity to vertebrates and as a general rule, dogs, livestock (cattle, sheep, goats, swine), horses, and poultry tolerate cypermethrin and most synthetic pyrethroids very well. Chemical statistical analysis using the method of Finney (1971), proved both the insignificant heterogeneity of the results and goodness of fit of the drawn LCP lines.

Different chemical agents (organochlorines, organophosphates and pyrethroids) have predominantly been used to control ticks on domestic animals worldwide (Enayati *et al*. 2010). *Rhipicephalus sanguineus* females subjected to permethrin concentrations of 384000, 38400 and 25600 ppm showed morphological, physiological and behavioral changes, as a result of the toxic effect of these compounds. According to Hervé (1983) and Sfara *et al.* (2008), these changes are caused by pyrethroids mainly absorbed by the tick's integument, which produces a series of uncoordinated nerve impulses as a result of changes in the permeability of membranes to sodium-repetitive effect. Consequently, sensory organs and nerve terminations are especially sensitive, triggering a state of excitation followed by paralysis and death of parasites (Mencke *et al.* 2003). Oliveira *et al.* (2011) mentioned that the LC_{50} of fipronil of semi-engorged *R. sanguineus* females was extremely low (LC_{50} = 9.6 ppm), demonstrating its high potential for pest control. Thus, of the three pesticides tested, dicofol compound is the most promising for possible use against *R. sanguineus* due to the low doses required to produce a high mortality in the adult females. However, dicofol, like other organochlorine which is used in public health could have a harmful effect on non-targeted organisms and environment. In order to obtain one integrated, environmental safe but adequate method for tick control, it is necessary to integrate theoretical and practical data.

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