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Biodegradation of Applied Plant Extracts of *C. cinerariifolium*, *E. camaldulensis* and *N. tabaccum* in the Habitats of Third Instar Larvae of the Malaria Vector *Anopheles gambiae s.s.* Giles (Diptera: Culicidae) Larvae is a Function of Time

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Abstract: ***Experiments for determination of extracts persistence were carried out in accordance with the methods used by Attia et al. (2015). One highest extract of each plant was placed in 300 ml disposable bowls separately in their relevant concentrations: DCM C. cinerariifolium (164.86 ppm), DCM E. camaldulensis (168.65 ppm) and ethanol extract of N. tabaccum (189.58 ppm). Distilled water was added into each bowl to make 300 ml of water and concentration in the form of pools. By use of a mouth aspirator 25 3rd instar larvae were collected and dipped into the bowl of extract and distilled water solutions. Observation for larval mortality was not a requirement for extract persistence but larvae were included as it could be done under the normal mosquito control programme in the field. Then samples of the solution were taken daily, hourly and in minutes to observe how the extracts were reducing in time to the point of zero. Sample analysis was done using Gas Chromatography- Mass Spectrometry (GC-MS) technique. The results of the three plants indicated that under light regime C. cinerariifolium took 5 hours and 30 minutes to completely decompose under light regime and 28 days to decompose under darkness. E. camaldulensis decomposition under dark regime was 35 days and in light it took 12 days to decompose. The dark-light degradation periods for N. tabaccum struck a balance in the two regimes taking 18 days of light and 28 days of darkness. Decomposition of all the plant extracts were generally impressive as the periods were very short in comparison with the decomposition periods of chemical insecticides.***

Keywords: Degradation, Time, Dark, Light, Analysis, *C. cinerariifolium, E. camaldulensis, N. tabaccum,* Gass Chromatography- Mass Spectrometry (GC-MS).

1. Introduction

Unlike *C. cinerariifolium* the degradation of *Eucalyptus camaldulensis* and *Nicotiana tabaccum* is not documented. Therefore, in this section environmental degradation of pyrethrins, the toxic products of *C. cinerariifolium* will be cited.

Pyrethrins were declared an effective mosquito control agent for reducing malaria in 1942. However, there was a reduction in pyrethrins use after advances in synthetically manufactured insecticides led to the discovery of dichlorodiphenyltrichloroethane (DDT) in 1945 and later to the synthetic version of pyrethrins and pyrethroids (Gunasekara, 2005).

Although pyrethrins are one of the oldest natural pesticides currently in use, there are limited environmental fate data available. Therefore, many of environmental fate parameters have been estimated using chemical property estimation methods as opposed to determined in laboratory or field studies (Antonious *et al.* (2004). Pyrethrins as vapour phase compounds spray in air, are susceptible to rapid degradation via direct photolysis and by reaction with hydroxyl radicals, ozone and nitrate radicals (Todd *et al.* 2003). The estimated octanol/water (K_{ow} partition coefficients suggest that group I pyrethrins are more lipophilic than the group II pyrethrins that also have higher estimated water solubilities. (Antonious *et al.* 2004). Timated (2001) similarly found octanol/water K_{ow} for pyrethrin I (416,869) to be higher than the more soluble pyrethrin II (3631).

In soils and microbial interactions compost amended soil, having two times greater organic matter content than native soil, was found to absorb more pyrethrins and their mobility was reduced by humic acids, a major component of organic matter (Antonious *et al.* (2004). It was also determined by Antonious *et al.* (2004) that increasing the humic acid concentration significantly reduced the mobility of pyrethrins. Further, Antonious *et al.* (2001) found that compost with high organic matter content absorbed more of pyrethrin I (0.056 µg/g) than non-mulch (0.026 µg/g) and fescue strip soils (0.002 µg/g) in potato field trials. The study indicated that pyr ethrin I bound strongly to soils ($K_{ow\ rly}$ = 26915) while pyrethrin II did not (K_{ow} = 2042). These experimental data are similar to the estimates of Crosby (1995) who reported higher K_{ow}s for the pyrethrins of group I as compared to group II. Crosby (1995) has predicted the microbial degradation of pyrethrins occur via oxidative metabolism. These oxidative processes are expected to occur at unsaturated side-chains, reactive methylene groups, and secondary alcohol groups of pyrethrins. The estimated octanol/water (K_{ow}) partition coefficient suggest that group I are more lipophilic than group II pyrethrins that also have higher estimated water solubilities (Antonious *et al.* 2001). However Crosby (1995) has shown that the estimated values are very similar to the experimentally observed K_{ow} values by USA Environmental Pollution Agency (EPA) for pyrethrin I and II. Photodegradation of pyrethrins is rapid in the presence of oxygen and sunlight. Casida and Chen (1969) determined a photodecomposition period of 5 hours and 30 hours light-dark periods respectively for

pyrethrin 1, allethrin, phthalthrin and dimethrin. Casida and Chen (1969) also determined that oxidation of pyrethrin I was stable for 24 hours under nitrogen and oxygen conditions in the dark but highly unstable in the presence of oxygen and light. In dilute solution in an organic solvent, but in the virtual absence of atmospheric oxygen, pyrethrins decompose and the principal reaction is isomirizationof the pyrethrolone side-chain, from a cis-(Z-) to a trans-(E-) configuration (Kawano *et al.*, 1980; Ramirez, 2013; Bullivant & Pattenden, 1976). Under aerobic conditions, photooxidation predominates. Other than pyrethrin 1 all of the other pyrethrins (Bioallethrin, Cinerin I, Jasmolin I, pyrethrin II, Cinerin II, and Jasmolin II) also undergo photodegradation. Pyrethrin I and cinerin I are degraded more rapidly than pyrethrin II and cinerin II (Brown *et al*., 1957). The rapid and extensive decomposition of the pyrethrins very likely is due primarily to UV-energized autoxidation (direct reaction with atmosphere triplet oxygen). Pyrethrins are stable in the dark (Chen and Casida, 1969; Dickinson, 1982) indicated 5 hours and 30 hours light-dark periods respectively for pyrethrin 1, allethrin, phthalthrin and dimethrin.

1.1 Methods and materials

The highest crude flower and leaf extracts (ppm) in each plant were selected for use in persistence experiments and these were methanol extract of *C. cinerariifolium* (164.86ppm), DCM extract of *E. camaldulensis* (168.65 ppm) and ethanol extract of *N. tabaccum* (189.58ppm). Experiments for determination of extracts persistence was carried out in accordance with the methods used by Attia *et al*. (2015). One highest extract of each plant was placed in 300 ml disposable bowls seperately in their relevant concentrations: DCM *C. cinerariifolium* (164.86 ppm), DCM *E. camaldulensis* (168.65 ppm) and ethanol extract of *N. tabaccum* (189.58 ppm). These were all set separately in 300 ml beakers. Distilled water was added into each bowl to make 300 ml of water and concentration.

By use of a mouth aspirator 25 3rd instar larvae were collected and dipped into each of the three solutions. Observation for larval mortality was not a requirement for extract persistence. To monitor the decline of extracts in the solution with time was the essence of the experiment. The larvae were an inclusion in this experiment so as to allow some extract solution taken by the larvae as expected under normal mosquito control programme. The samples were then placed in a shed outside the laboratory to monitored under external environmental conditions (temperature, humidity, etc.) excluding exposure to strong sunshine to avoid solutions evaporation. This was a requirement for the compliance of what would normally occur in mosquito breeding places such as natural water pools. The concentrations were then monitored daily, hourly and in minutes on how they reduced under these natural conditions to reach zero ppm with time. Monitoring was accomplished by taking 1ml of sample from each solution of *C. cinerariifolium, E.camaldulensis and N. tabaccum* for laboratory analysis. However, extract which showed higher rate of degradation than others was sampled more at closer interval of time such as *C. cinerariifolium*. This was an up-down procedure observing how a concentration reduced from its initial high concentration to zero. One extract was done at a time to avoid accumulation of samples in the laboratory.

Samples were labelled to indicate species of plant, time sample taken, date of sampling, name and address of sampler, and indication of what was to be analysed. They were stored at 4^0 C awaiting to be taken to the laboratory for analysis.

For transportation to the laboratory the samples were well packed in a cold box to avoid exposure to the sun. Analysis was carried out using Gass Chromatography-Mass Spectrometry (GC-MS)-(Pelkin Elmer SQ 8 GC/MS) analysis (Hashmi *et al.* 2013). Each sample analysis results at the expiry of each extract concentration from the solution indicated the zero ppm point. The period of time taken for complete degradation to zero point of the extract was calculated based on day and time sampling started to time when zero point was reached. Extract degradation periods results were recorded in hours and days.

2. Results

The results of the three plants extracts degradation indicated that under light regime *C. Cinerariifolium* took 5 hours and 30 minutes to completely decompose and 28 days to decompose under darkness. *E. camaldulensis* decomposition under dark regime was 35 days and that of light regime was 12 days. The dark-light degradation periods for *N. tabaccum* were 18 days of light and 28 days of darkness. These results for *C. cinnariifolium* concur with those of Chen and Casida (1969) who determined a photodecomposition period of 5 hours and 30 hours light-dark respectively. Table 1 indicated the decomposition modes of the three extracts.

Table 1: Percent recovered oils in the degradation of pyrethrum, E.

Hrs	Pyr	Euc	N.t	Pyr	Euc	N.t	Pyr	Euc	N.t
0	164.6	144.45		151.50-182.70	132.4-157.2	132.4-155.3		y=10.64x+35.50	Y=13.88x+9.35
30	124.6			113.25-132.55				y=9.40x+17.80	
1	100.00			62.40-97.35				y=8.75x+20.75	
1.30	95.00			29.50-53.70				y=11.40x+12.15	
2.00	90.00	4.00	12.50	11.80-33.55				y=12.70x+21.70	
2.30	50.00	4.50	6.60	0-00					
3.0	25.00	5.00	1.50						
3.30	20.50	5.30	0.00						
4.00									
4.30									
5.00									
Days									
2		75.00	117.30		62.45-89.70	98.5-129.3		y=13.25x+9.70	y=7.85x+16.20
3		62.50	112.45		51.70-80.50	82.6-127.4		y=8.25x+9.40	y=6.35x+10.25
4		50.00	100.00		37.65-61.70	90.40-114.0		y=16.7x+9.25	y=10.70x+13.15
5		46.70	98.70		30.45-58.20	81.7-113.7		y=7.45x+8.00	y=9.00x+11.55
6		25.45	96.40		16.28-34.70	79.8-111.5		y=11.85x+9.40	y=4.78x+8.15
7		25.45	85.25		13.60-32.80	69.6-100.4		y=6.40x+12.80	y=5.55x+10.20
8		25.00	76.80		13.45-32.50	60.2-88.7		y=7.70x+14.80	y=3.87x+9.10
9		25.00	75.00		13.45-32.50	69.87.9		y=8.80x+9.40	y=7.00x+10.85
10		12.50	62.50		3.55-19.70	51.3-76.5		y=16.25x+10.50	y=9.25x+3.98
11		6.25	54.00		1.12-4.80	36.7-63.8		y=5.20x+41.60	y=17.20x+10.16
12		1.00	52.00			32.8-60.7		y=11.70x+8.85	y=2.58x+6.25
13		0.00	50.00			31.7-59.2		y=27.50x+11.50	y=3.95x+4.80
14			48.00			37.5-56.8		y=21.50x+9.95	y=6.15x+7.15
15			46.30			35.3-54.6		y=10.75x+6.25	y=8.15x+5.55
16			43.40			33.8-52.9		y=9.85x+7.95	y=3.95x+5.25
17			25.00			12.7-32.6		y=17.80x+12.70	y=9.50x+16.50
18			24.00			12.3-32.1		y=14.75x+13.55	y=5.15x+21.85
19			19.40			10.5-28.7		y=18.25x+12.85	y=4.90x+8.65
20			10.27			1.75-17.5		y=5.45x+15.99	y=8.15x+13.25
21			0.00			0.00			
	Control		0.00						

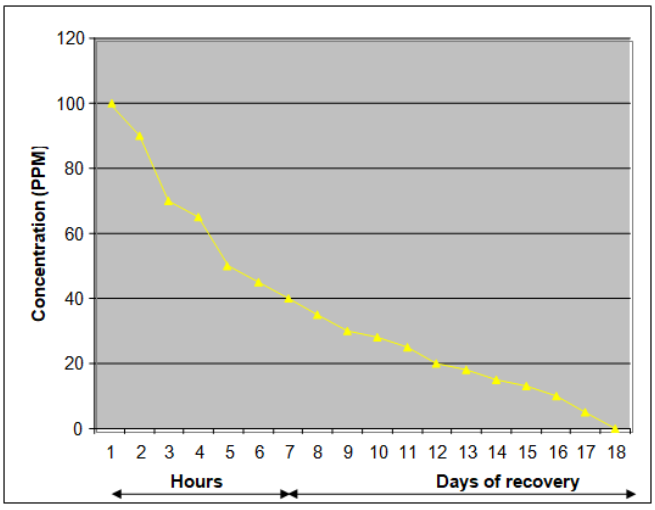

Figure 1: Degradation of *N. Tabaccum* essential oil under light regimes

Degradation of *N. Tabaccum* under light indicated resistance from day 1 to day 5. From day 6 up to 18th the extract seemed not to experience any resistance but decomposed steadily and was more of a 45^0 angle slope downward to day 18 when decomposition got completed without any resistance.

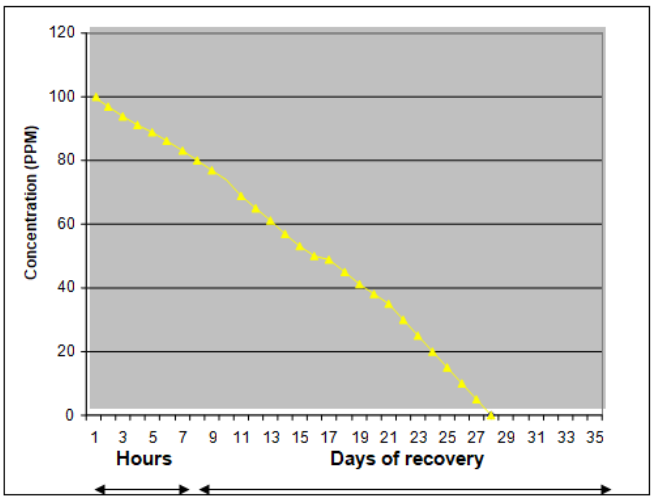

Figure 2: Persistance of Nicotiana *tabaccum* essential oil under dark regimes

Nicotiana tabaccum showed a long period of biodegration (28 days) under darkness. The extract did not show any major resistance to biodegradation over the period of 28 days, and its degradation was steady and exhaustive as indicated by its straight but slight downward curving slope. No doubt that the oil components of *Nicotiana tabaccum* have high resistance and therefore may direct the user to the knowledge of its application for mosquito control.

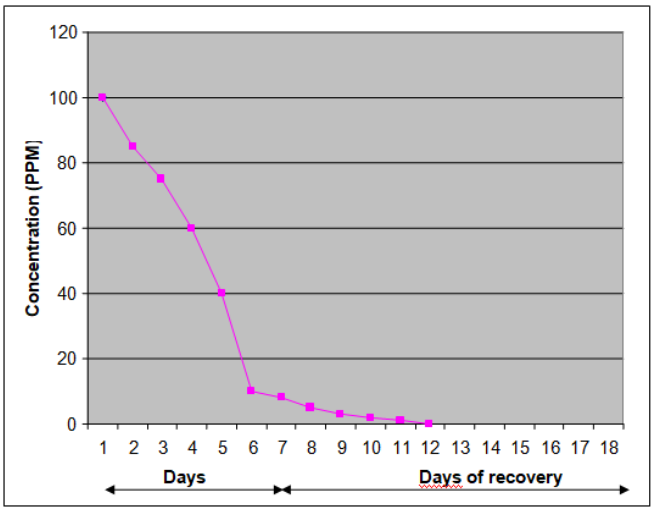

Figure 3: Degradation of Eucalyptus *camaldulensis* essential oil under light regimes

Decomposition of *Eucalyptus camaldulensis* showed a quick and sharp drop from day of extract input up to the 6th day, showing a dropping weakness for the decomposition at the first 6 days.

After 6th day, the extract inclined at a slightly straight and gentle slope ending quickly at the twelve day when the decomposition of the extract was finally completed.

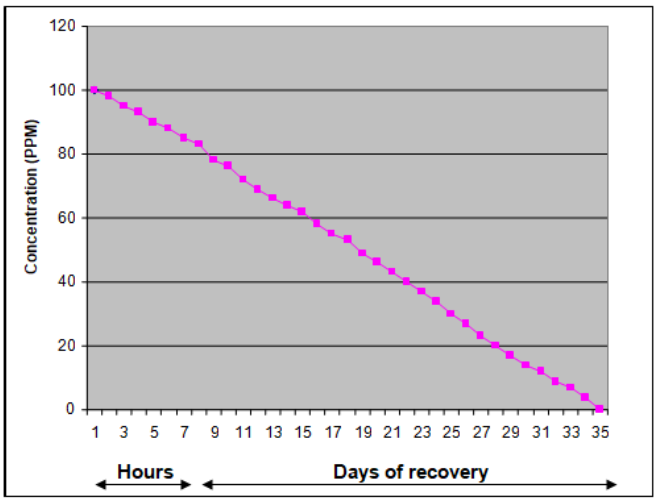

Figure 4: Persistance of Eucalyptus *camaldulensis* essential oil under dark regimes

Eucalyptus camaldulensis degradation was the longest but indicated constant and unobstructed progress from time of extract input until the extract expired at the end of 35 days. The biodegration of this extract is the longest amongst *Nicotiana tabaccum and Chrysanthemum cinareriifolium.* Its moderately steep slope was an indication of a steady-state of decomposition given conducive environmental conditions. Under mosquito control programmes, it would be necessary to make a decision on which extract between *Nicotiana tabaccum and Eucalyptus camaldulensis* to apply because of their long period of persistence.

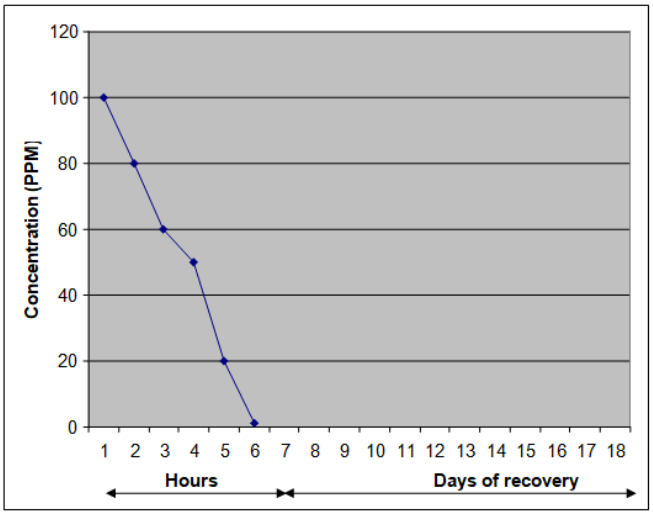

Figure 5: Degradation of *Chrysanthemum cinareriifolium* essential oil under light regimes

Chrysanthemum cinareriifolium was the weakest extract amongst the three. The extract indicated a very sharp drop from its input to its end of decomposition within a period of 5 hours and 30 minutes. However, for unknown reasons, the extract was observed to have developed resistance between 3rd and 4th hour. Because of the shortest time it took to decompose, makes *Cc* the best extract to apply under mosquito control programmes; in addition to the fact that its short period of degradation will enhance the protection of untargeted organisms.

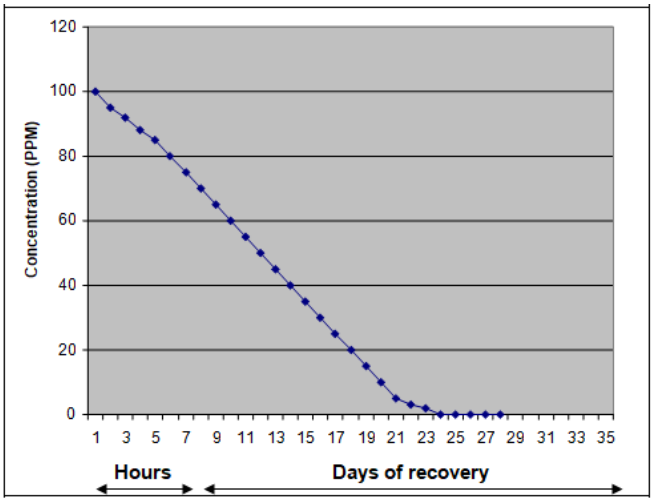

Figure 6: Persistence of *Chrysanthemum cinerariifolium* essential oil under dark regimes

Chrysanthemum cinerariifolium under dark regime indicated a reasonably stable decomposition from day 1 to 21st day because its curve appeared unobstructed for a period of 21 days. However, on day 21 to day 28 the extract indicated little resistance each day for 6 days, but the extract got its complete biodegradation on the 7th day. In total the extract took 28 days to clear off.

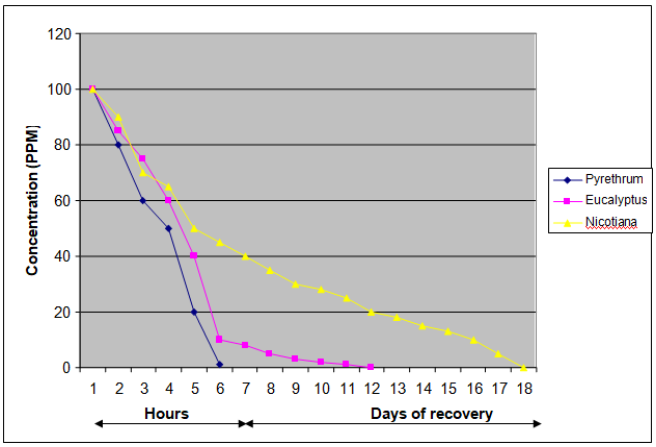

Figure 7: Comparison of the degradation periods of *C. cinerariifolium*, *E. camaldulensis*, and *N. tabaccum* essential oils under light regimes

Figure 7. Shows degradation periods of the three plants extract as *Cc* 5.5 hrs, *Ec* 12 days and *Nt* 21 days under light regimes. These periods of degradation expressed in a ratio of hours gave 5.5 :720:1260 against total hours of 1985.5. Hence the ratio was further reduced against base 1986 and *Cc* taken as common factor (with least degradation period) arrived at ratio 1:15:9. This ratio was then translated to mean that the decomposition of *Cc* was 15 times faster than *Ec* and 9 times faster than *Nt* under light regimes.

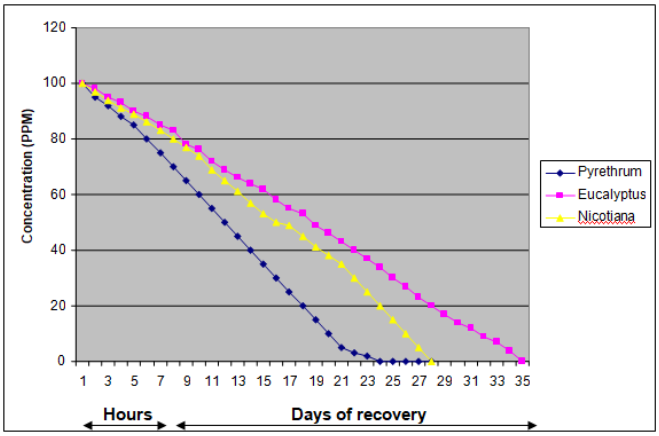

Figure 8: Comparison of the degradation periods of *C. cinerariifolium*, *E. camaldulensis*, and *N. tabaccum* essential oils under dark regimes

Nicotiana tabaccum showed a long period of biodegration (28 days) under darkness. The extract did not show any major resistance to biodegradation over this period. Its degradation was steady and exhaustive as indicated by its straight but slightly downward curving slope. No doubt that the oil components of *Nicotiana tabaccum* have high resistance and therefore may direct the user to the knowledge of its application for mosquito control.

3. Discussion

The photodecomposition results for *C. cinerariifolium* concur with those of Chen and Casida (1969) whose their results of study indicated 5 hours and 30 hours light-dark periods respectively for pyrethrin 1, allethrin, phthalthrin and dimethrin. Other researchers including Gunasekera (2005) and Crosby (1995) also attempted tests on environmental fate of pyrethrins.

The breakdown (deactivation) of natural pyrethrins when exposed to daylight is perhaps their most prominent and best-recognized chemical characteristic (Chen and Casida, 1969). Pyrethrin photodegradation is rapid. In dilute solution in an organic solvent, but in the virtual absence of atmospheric oxygen, the principal reaction is isomirizationof the pyrethrolone side-chain, from a cis-(Z-) to a trans-(E-) configuration (Kawano *et al.*, 1980; Ramirez, 2013; Bullivant & Pattenden, 1976). Under aerobic conditions, photooxidation predominates. Other than pyrethrin 1 all of the other pyrethrins (Bioallethrin, Cinerin I, Jasmolin I, pyrethrin II, Cinerin II, and Jasmolin II) also undergo photodegradation. Pyrethrin I and Cinerin I are degraded more rapidly than pyrethrin II and Cinerin II (Brown *et al.*, 1957). The rapid and extensive decomposition of the pyrethrins very likely is due primarily to UV-energized autoxidation (direct reaction with atmosphere triplet oxygen). Pyrethrins are stable in the dark (Chen and Casida, 1969; Dickinson, 1982).

These differences in degradation could have possibly been as a result of the chemical composition of the plants' oils. This is so because some compounds in an extract may resist decomposition while others may decompose in a very short time depending on the type of chemical in the extract. Components of *N. tabaccum* and

*E.camaldulensis*oils may require further investigation to confirm which components of the oils have high resistance to decomposition. Since the trend of insecticides application for mosquito control in Kenya takes place during the day it is therefore important to take into account of the period of the extracts biodegradation under light (solar energy) regimes. The persistence of *E. camaldulensis* and *N. tabaccum* are not documented and hence no record available for comparison to this study.

However, it must be realized that application of insecticides for mosquito control in Kenya takes place during the day. For this reason, it could be considered whether it would be appropriate to apply insecticides for mosquito control at dusk other than applying insecticides in the early hours of the day.

Consequently, *C. cinarirariifolium* flower extract was superior to the leaf extracts of *E. camaldulensis* and *N. tabaccum.* These differences in degradation could have possibly been as a result of the chemical composition of the plants' oils. This is so because some compounds may resist decomposition while others may decompose in a very short time. Components of *N. tabaccum* and *E.camaldulensis* oils may require further investigation to confirm which components of the oils have high resistance to decomposition. Since the trend of insecticides application for mosquito control in Kenya takes place during the day it is therefore important to take into account of the period of the extracts biodegradation under light (solar energy) regimes.

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