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SYNERGISTIC BIO-PESTICIDE COMBINATION OF PYRETHRINS AND ROTENOIDS FOR THE CONTROL OF THE COCKROACH *AMERICANA PERIPLANETA*

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ABSTRACT

Pyrethrins and Rotenoids extracts from *Chrysanthemum cinerariaefolium* and *Tephrosia vogelii* plants respectively were combined at various ratio mixtures and tested against adult cockroach *Americana periplaneta* for their efficacy. A mortality rate of 86% in 200 minutes with a synergistic ratio mixture of 28.5:1 w/w was established. The mixture was found to have an observed LC_{50} of 0.23mg/g and a theoretical LC_{50} of 0.92mg/g calculated from the individual Pyrethrins and Rotenoids extracts. The synergism expressed as co-toxicity coefficient CC, was found to be 4. The photodegradation of Rotenoids in the combination was found to have a half life of $t_{1/2}$ of 6.1hrs compared to that of Rotenone and Pyrethrins of 3hrs respectively.

KEYWORDS: Synergism, Pyrethrins, Rotenoids, *Americana periplaneta*, Photodegradation

INTRODUCTION

Bio-pesticides, based on plant extracts such as rotenone, nicotine and pyrethrum have been commonly used in pest management during the earlier half of this century. After the Second World War, cheap synthetic organic chemicals were produced in large quantities and rapidly substituted most other pesticides. The legume *Tephrosia vogelii,* a shrubby plant indigenous to Africa but distributed to many parts in the tropics is used as shelter, cover crop, fish poison and as a pesticide. It was introduced to the United States of America in the sixties for the purpose of commercial production of rotenone and is regarded as a more promising plant than *Derris* and *Lonchocarpus spp* which thereto have been the main sources of the pesticide (Abiy *et al*, 2004, Barnes and Freyre, 1966). The principle active ingredient is rotenone but there are other Rotenoids such as Tephrosin and Deguilin which are toxic to fish and mammals. The insecticidal effects have been shown on several different insect species (Atkinson *et al*, 2004,).

Rotenone acts by inhibiting the conversion of nutrients into energy at the cellular level (cellular respiration). Insects quickly stop feeding and death occurs several hours to a few days after exposure. Rotenone is a broad-spectrum contact and stomach poison that is effective against leaf-feeding insects, such as aphids, certain beetles (asparagus beetle, bean leaf beetle, Colorado potato beetle, cucumber beetle, flea beetle, strawberry leaf beetle, and others) and caterpillars, as well as fleas and lice on animals (Abiy *et al*, 20043). Rotenone degrades rapidly when exposed to air and sunlight. It is not phytotoxic and moderately toxic to mammals by inhalation than by ingestion. Skin irritation and inflammation of mucous membranes may result from skin contact (Njiru, 2006).

Pyrethrins on the other hand are highly concentrated active compounds, which are extracted from the daisy-like flower of *Chrysanthemum cinerariaefolium*, commercially grown in Kenya, Tanzania, Rwanda in East Africa as well as Tasmania in Australia. Most insects are highly susceptible to low concentrations of pyrethrins. The toxins cause immediate knockdown or paralysis on contact, but insects often metabolize them and recover. They act specifically by disrupting the

sodium and potassium ion exchange process in insect nerve fibers and interrupting the normal transmission of nerve impulses (Cassida and Quistad, 1995). Pyrethrins break down quickly on exposure, have a short residual, and low mammalian toxicity, making them among the safest insecticides in use. However, people may have allergic skin reactions and cats are highly susceptible to poisoning (e.g., flea powder) (Crosby, 1995). Pyrethrins may be used against a broad range of pests including ants, aphids, roaches, fleas, flies, and ticks (Darwazeh *et al*, 1981).

Although Rotenoids and Pyrethrins have different chemical structures and mode of action, they do have similar general traits. These include; fast breakdown effect, fast action, low mammalian toxicity, selectivity, low or no phytotoxicity and are found to exhibit individual synergism (Njiru, 2006). Pyrethrins are often mixed with synergists such as sesame oil, Piperonyl butoxide (PBO), MGK 264, rotenone, or rynia to increase their effectiveness. PBO has been implicated as a carcinogen, and may not be used in some organic certification programs (Casida and Quistad, 1995). Mixtures of pesticides have been found to be more effective, allow broader spectrum application, reduce the rate of application as well as delay the development of resistance of pests to pesticides (Langat *et al*, 2011).

In toxicology, synergism refers to the effect caused when exposure to two or more chemicals at the same time results in health effects that are greater than the sum of the effects of the individual chemicals. When chemicals are synergistic, the potential hazards of the chemicals should be re-evaluated, taking their synergistic properties into consideration (Kariuki *et al*, 2003).

MATERIALS AND METHODS

Pyrethrum plant extracts containing 50% pyrethrins were obtained from Pyrethrum Board of Kenya (PBK), Nakuru Kenya. PBK extracts pyrethrins from the *Chrysanthemum cinerariaefolium* flowers using hexane 99% followed by 95% methanol refining. The flowers are grown in the Kenyan highland counties, including Nakuru, Nyandarua, Kisii, West Pokot and delivered to the Nakuru factory (Casida and Quistad, 1995).

Plant extracts containing 8.3% Rotenoids were obtained from leaves of *Tephrosia vogelii* through extraction using chloroform. The leaves were harvested from Mr. Kibugi's farm in Kikuyu, 16km from Nairobi, identified at the School of Biological Sciences, University of Nairobi, Hebarium. The leaves were dried in the Department of Chemistry, University of Nairobi, Laboratory and then ground into powder using a blender. 250g of plant material was extracted using soxhlet extraction method and extract evaporated to near dryness on a rotary evaporator at reduced pressure and temperature of 40^{O}C to give a dark oily chloroform crude residue sample. The sample was then stored under refrigeration at a constant temperature of 4^{O}C.

UV/Vis absorption wavelengths for the detection was established by dissolving one gram of Pyrethrins, Rotenoids extracts and Rotenone standard each separately in $100cm^3$ of acetone.

Table 1: Optimum Absorption Wavelengths Ultra Violet Visible Spectrometer

Sample	Wavelengths (λ nm)
Pyrethrins (50%)	329
Rotenoids	340, 570, 695, 665, 666
Rotenone (95%)	340

Table 1 above shows the optimum absorption wavelengths responding to the extracts. The absorption wavelengths were used as the detection wavelengths with the HPLC UV detector analysis for identification during separation and extraction.

Evaluation of Pyrethrins and Tephrosins Extract Combinations

Stock solution of Rotenoids chloroform extract was prepared by weighing one gram and dissolving in 100cm^3 of ethanol. Several mixture combinations of Rotenoids and Pyrethrins extracts were made by mixing appropriate amounts of the extracts in the ratios *Tv*: *P*, 1:3, 2:3, 4:3, 2:1, 1:6, 1:24, 1:30, 1:36 respectively. They were then transferred into amber sample bottles prior to bioassay tests. Table 2 shows the various combination mixture concentrations and sample coding.

Table 2: Combination Ratios of Rotenoids and Pyrethrins Extracts

Sample Code	Concentration Ratio Rotenoids: Pyrethrins (%)	Volume Ratio Rotenoids: Pyrethrins (v/v)
A	1 : 454	1:3
B	1 : 227	2:3
C	1 : 113.5	4:3
D	1 : 28.5	2:1
E	0 : 0.4645g/cm^3	0:1
E2	0.00306735g/cm^3 : 0	1:0
F	1: 908	1:6
G	1: 2270	1:24
H	1: 2724	1:30
I	1: 3178	1:36

Bioassay Tests against Adult Americana Periplaneta

20 adult *American periplaneta* insects were transferred into each insect holding jar. A dose of 1.0cm^3 from each mixture ratio was transferred into the insect holding jars. The control was maintained at the same ethanol content of 1.0cm^3. The mortality in each set of experiment was recorded after a specified period of time of exposure. The experiment set of each bioassay was replicated five times. These experiments were set according to the standard World Health Organization's protocol (WHO, 1975).

Photodegradation of Rotenoids

1 gm of each was dissolved in 100cm^3 of acetone. 1cm^3 of the stock solution of Rotenoids was withdrawn in four replicas and each portion transferred into a clear bottle and stored in the dark. Another set of 1cm^3 was withdrawn and put into clear bottles marked 3, 6,9,12, 15 and 18hrs and exposed in natural sunlight. This procedure was repeated for the mixture combination (1:28.5), the Rotenone and Pyrethrins standard samples. The samples were stored in the dark and the solvent allowed to evaporate at room temperature. The bottles were then exposed to direct sunlight and removed at prefixed time intervals and then stored in the dark at room temperature for analysis. The half-life of each was determined by investigating the decay rate in natural sunlight.

The solid residue in the bottle was dissolved in 5cm^3 of solvent and injected for analysis to determine the concentration of Rotenoids in the solid residues. The decay rate was then calculated as a first-order kinetics reaction, where half-life $t_{1/2}$=ln2/r.

RESULTS AND DISCUSSIONS

Synergism against *Americana periplaneta*

The combination mixture of Rotenoids and Pyrethrins resulted to synergism at varying degrees for each individual mixture combination. Figure 1 below shows mortality efficacy of various combinations on *Americana periplaneta*.

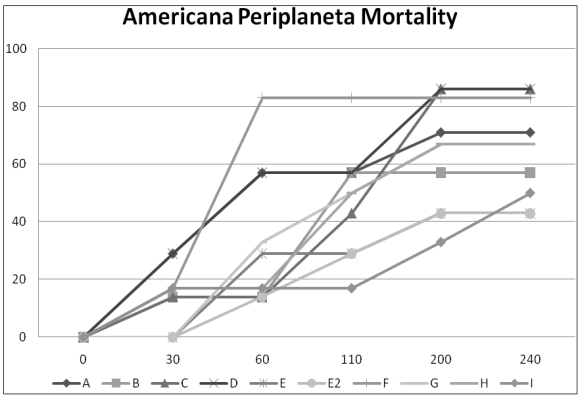

Figure 1: Mortality Rate of Various Combinations of Rotenoids and Pyrethrins Extracts on Adult Americana Periplaneta

The mortality rate of the individual extracts, Rotenoids and Pyrethrins, Sample Code E and E2 recorded similar lower mortality rate of 43% in 240 minutes. This implies that Rotenoids extract was theoretically 152 times more toxic than Pyrethrins.

The highest mortality rate observed was 86% in 200 minutes by Sample Codes C and D, however, Sample Code F had recorded mortality rate of 83% as early as 60 minutes. Sample Codes A and D also recorded a high early mortality rate of 29% in 30 minutes while Sample C retained a lower mortality rate of 17% in the first 60 minutes. Sample Code E and E2 recorded 0% mortality rate in the first 30 minutes and in 60 minutes, E had risen to 29% while E2 to 14%. Thus Pyrethrins have an earlier mortality compared to Rotenoids extracts. Sample Code G recorded an early high in 60 minutes of 33% rising to 50% in 110 minutes and tapering off at 200 minutes with 67% mortality rate. Sample Code I recorded the lowest mortality rate of 50% in 240 minutes of all the combinations. This shows that the insects were more susceptible to all the combinations with high early and final mortality rates than either the Pyrethrins or Rotenoids extracts alone. The insects were found to be most susceptible to Sample Code D with the highest early and final mortality rates of 29% in 30minutes and 86% in 200 minutes respectively. It was then investigated for the observed LC_{50E}.

Co-Toxicity Coefficient

This was established by investigating the LC_{50} of each ratio mixture of the Pyrethrins and Rotenoids extracts and comparing it with the individual extracts before combination. The mixtures were then assessed for synergism, additive, and antagonism by calculating the co-toxicity coefficient (CC). CC is calculated by taking the theoretical LC_{50} (LC_{50T}) and dividing by the observed LC_{50E} of the combination [CC= LC_{50T}÷ LC_{50E}]. The theoretical LC_{50T} is calculated as LC_{50T} = (LC_{50P}X %P) + (LC_{50TV}X %TV). A CC value of <<2 indicates antagonism of the combination, that of CC=2 indicates additive, while that of much greater than CC>>2 shows synergism (Kariuki *et al*, 2003).

The observed LC_{50E} for the combination was 0.23mg/cm^3 Pyrethrins 0.94mg/cm^3 while that of the Tephrosins at 0.47 mg/cm^3. The LC_{50T} was established = (0.94X28.5/29.5) + (0.47X1/29.5) = 0.9240722mg/ cm^3. CC was established as 4 which is significantly larger than 1 thereby showing great synergism in the combination mixture of the two bio-pesticides.

Photodegradation of Rotenoids and Rotenones

Photodegradation of the Rotenoids or Rotenone explains natural environmental exposure degradability hence the environmental sustainability of the combination mixture. The concentration of Rotenone decreased from initial concentration of 0.5mg/cm^3 as indicated in Table 3 below.

Table 3: Photodegradation of Rotenone Standard Sample

Time (Hours)	Conc. mg/cm^3	Log C_t	Log Co-LogC	r
0	0.50	-0.30	-	-
1	0.36	-0.44	0.14	0.3224
3	0.23	-0.64	0.34	0.2610
6	0.15	-0.82	0.52	0.1996
9	0.11	-0.96	0.66	0.1682
12	0.08	-1.10	0.80	0.1535
15	0.05	-1.30	1.0	0.1535
18	0.01	-2.0	1.7	0.2125

C=Concentration after time t
Co-Initial Concentration
r=decay rate

In 18hrs only 2% of Rotenone was remaining in the sample residue with a degradation rate r=0.21 and a $t_{1/2}$ =3.2hrs.

The concentration of Rotenoids decreased progressively with time from an initial concentration of 0.59mg/cm^3 as shown in Table 4 below.

Table 4: Photodegradation of Rotenoids in *Tephrosia Vogelii* Extract

Time (Hours)	Conc. mg/cm^3	Log C_t	Log Co-LogC	r
0	0.59	-0.23	-	-
1	0.50	-0.31	0.08	0.184
3	0.42	-0.38	0.15	0.115
6	0.30	-0.52	0.29	0.111
9	0.25	-0.60	0.37	0.0951
12	0.19	-0.72	0.49	0.094
15	0.14	-0.85	0.62	0.095
18	0.19	-1.04	0.81	0.104

C=Concentration after time t
Co-Initial Concentration
r=decay rate

The photodegradation of Rotenoids in the extract was slower than that of Rotenone in the standard sample. The Rotenoids progressively decreased with time and by 18 hrs only 15% was recoverable with a mean decay rate r=0.104. This corresponded to a half-life of 6.1hrs compared to that of Rotenone standard sample of 3hrs.

CONCLUSIONS

On evaluation it was found that in Sample Code D, Pyrethrins and Rotenoids have a synergistic effect against *Americana periplaneta*. On applying the LC_{50} co-toxicity coefficient formulae, a coefficient of 4 was obtained indicating that there is great synergism between Rotenoids and Pyrethrins (1: 28.5). Comparison of the observed and expected mortalities confirmed this synergism against *Americana periplaneta*. The synergistic effect is presumably due to the combination of the two natural insecticides that individually comprises of several different molecules, 6 in Pyrethrins and 4 in Rotenoids, thereby overwhelming the various insect detoxification defense mechanisms by reinforcing their combined impact through different mode(s) of action.

The photodegradation of the molecules in the extract was established at $t_{1/2}$=6.1hrs implying that the mixture is environmentally friendly and would thereby allowing for a Post Harvest Infestation of at least 7hrs. Synergism has been studied and applied on commercial products for more than 60 years and has significantly contributed to improved efficacy

of insecticides as well as solving resistance problems that arise from over use of single synthetic molecule insecticides. The quantities used in synergistic combinations are minimal compared to use of individual insecticides and in the case of naturally occurring insecticides, the preservation of our environment is upheld.

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