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Original Research Article

Synergistic Evaluation of Selected Plant Extracts on Potency of Pyrethrins against the Maize Weevil, *Sitophilus zea-mais* (MOTSCH.)

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Abstract

The interest in utilization of botanical insecticides, particularly essential oils have become increasingly relevant in the control of insect pests. Many plant products and their bioactive compounds with repellent, antifeeding or insecticidal activity against stored product insect pests have been reported however, their levels of control are still wanting. The major issue is that the oil yields obtained are low, unstable and high costs involved in production to use on a commercial scale. Use of these oils in combinations or in formulations with botanical actives as synergists or stabilizers, may make them economically viable insecticides. The present study focussed on evaluating plant extracts (Black pepper seeds, Nutmeg seeds, coriander leaves and roots) as potential synergists in formulations with pyrethrins for possible use in protection of stored grains against Sitophilus zea-mais. Full dose response and synergism experiments were carried out on *S. zea-mais* at four concentrations of synergists at ratio 1:1 (synergist: pyrethrins) Topical application of synergist/ formulation on *S. zea-mais* was done in triplicate using Factorial Design in a completely Randomized Design layout. Experiments were conducted under controlled laboratory conditions of 27 ± 2^{O}C and 60 ± 5%RH with normal day light hours. Probit analysis was used to determine the lethal concentration (LC) of pyrethrins and ANOVA was used to obtain the mean mortality differences of *S. zea-mais* at $P \leq 0.05$. LC_{20} value for pyrethrins was found to be 2,200 ppm. In formulations of synergist: pyrethrins, coriander leaves extract (CLHE), Nutmeg seed extract (NMHE) and Black pepper seed methanol extract (BPSME) were statistically significant ($P \leq 0.05$) 24 h after exposure. PBO registered higher percentage (83.33 ± 12.02%) mortality followed by CLHE (46.67 ± 3.33%), BPSME (43.33 ± 6.67%). BPSME and NMHE co-toxicity values were below 20 and -20 respectively while in PBO, BPSHE and CLHE values were above 20. Plant extracts BPSHE and CLHE and PBO synergized the toxicity of pyrethrins in all concentrations over 72 h exposure duration with co-toxicity factors above 100. BPSME was shown to be an additive in the formulation and only a synergist at 20,000ppm while NMHE was antagonistic to pyrethrins. Increasing the concentration of a plant extract did not correspond to increased efficacy of the formulation. Efficacy of the synergist-pyrethrins formulations at lower concentrations can be economically viable since average percentage mortalities will not be different from that of higher concentrations when time of interaction is prolonged. It is recommended that BPSHE and CLHE can be synergists to pyrethrins when formulations are exposed to insects for 72 h duration.

Keywords: Efficacy, Exposure time, Formulation, Plant extracts, Synergism

INTRODUCTION

The interest in utilization of botanical insecticides, particularly essential oils have become increasingly relevant in the control of insect pests (Isman and Grieneisen, 2014; Regnault-Roger *et al*., 2012. Many plant products and their bioactive compounds with repellent, antifeedant or insecticidal activity against stored product insect pests have been reported (Akhtar *et al*., 2008). Essential oil extracts from leaves of wormseed, *Chenopodium ambrosioides* Linn. (Chenopodiaceae) were found to be effective against six common species of grain beetles in western highlands of Cameroon. In laboratory experiments, Carvacrol, a compound from *Thujapois dolabrata*; linalool, a bioactive molecule from *Ocimum canum*; estragole and fenchone from *Foeniculum vulgare* have been found to be toxic against adults of *S. oryzae* and *R. dominica* (Akhtar *et al*., 2008; Kim et al., 2003). Also, bioactive compounds isolated from roots of *Decalepis hamiltonii* have shown to protect grains by suppressing the emergence of F_1 progeny of *S. oryzae, R. Dominica, T. casteneum* and *C. cinensis* in treated grain by contact bioassays for a period of upto three months (Rajashekar *et al*., 2010). Shaaya *et al.* (2016) tested four edible oils: pure and crude cotton seed oil, pure soya bean oil, crude rice bran oil and crude palm kernel oil as fumigants against insects in beans and wheat which were reported to be effective during the initial four months' storage period on average.

Although these essential oils have been tried for use as alternative to synthetic insecticides, their levels of control are still wanting. The major issue is that the oil yields obtained are low, unstable and high costs involved in production to use on a commercial scale (Kumar and Kalita, 2017). Use of these oils in combinations or in formulations with botanical actives as synergists or stabilizers, may make them economically viable insecticides. Synergism has the role of increasing the potency of insecticides and speeding their reaction time by preventing detoxification within the insect. Studies done by (Liu *et al*., 2015) on synergistic effects of various compounds to pyrethrins, documented an optimal biological ratio for different pest species and each individual synergist. Piperonyl butoxide (PBO) is the main synergist employed to boost efficacy of low levels of pyrethrins by binding onto cytochrome P-450 dependent microsomal oxidase, the defence mechanisms employed by the insects in counteracting pyrethrins (Hamilton, 1995). Synergists enable the use of an active ingredient in very small quantities by preventing its detoxification within the insect thus unsynergised formulations are rarely applied for the control of insect pests.

With the current focus on decreasing environmental contamination and increasing demand for organic products, a natural compound for use as a synergist would be ideal. PBO, although effective as a synergist, is not classified as organic product in many countries; it is expensive, toxic and also in short supply (Lang'at *et al*., 2008). Searches for effective synergists have not yet yielded many compounds that have the viability equivalent to that of PBO and thus alternative strategies have to be sought. The present study focussed on evaluating plant extracts possessing MPD ring similar to that of the commercial synergist, PBO as alternatives in formulations with pyrethrins for possible use in protection of stored grains against coleopteran pests.

MATERIALS AND METHODS

The experiments were conducted at Chuka University research laboratory, Kenya. All the bioassays were maintained throughout under controlled storage experimental conditions of 27 ± 2 ^{0}C and 60 ± 5% RH with normal day light hours.

Rearing of maize weevils

Maize weevil parent stock was obtained from National Agriculture Research Laboratory (NARL), Nairobi, Kenya. Cultures of the maize weevil were established to supply similar aged weevils for the experiments. Maize weevils were reared on the clean disinfected maize grains in 14 jars, each jar with 1.5L capacity, 500 gms maize grains were put into the jars. 50 Unsexed adult maize weevils were introduced into each of the seven (7) jars of grain. The jars were then covered with muslin cloth and fixed with a rubber band to allow for aeration and prevent escape of insects. After seven days (period allowed for oviposition), all parent insects were removed from each jar by sieving using a 6.0 mm sieve and placed on the other seven jars with grains and kept at the same conditions. Removal of parent insects and placement on a fresh maize medium was repeated until sufficient numbers of laboratory reared weevils were obtained. The jars were kept at the experimental growth chamber maintained at a constant temperature of 27 ± 2 ^{0}C and 60 ± 5% RH with normal day light hours.

Synergists

Potential synergists were chosen on the basis of possessing a MDP ring structure similar to that of the standard synergist, PBO. The plant extracts and oils were prepared for use in this study at the Chemistry Research Laboratory, Chuka University, Kenya. The plant extracts/oils used as "synergists" were given codes for

Table 1. Codes given to Standard Synergists and Plant Extracts/Oils Possessing MDP ring tested for Synergistic Potential

Codes of Plant extracts/oils (synergists)	Description of sample	Extract/oil
PBO	Piperonyl butoxide	standard synergist
BPSHE	Black pepper seeds	Hexane extract
BPSME	Black pepper seeds	Methanol extract
CLHE	Coriander leaves	Hexane extract
CLME	Coriander leaves	Methanol extract
CRHE	Coriander roots	Hexane extract
NMHE	Nutmeg seeds	Hexane extract

easy identification. Likewise, the formulation containing either of the plant extracts and insecticides was given a code similar to that of the plant extract. Table 1

Collection of plant parts and extraction

Seeds of *P. nigrum* and *M. fragrans* originally from Kerala, India were obtained from a commercial spice supplier in Nakuru, Kenya. Fresh leaves and roots of *C. sativum* were obtained from Farming Systems of Kenya, Nakuru branch. The plant materials (roots, leaves or seeds) were authenticated at the Botany Department of Chuka University, Kenya. The plant materials were air dried in a well-ventilated room temperature away from direct sunlight in order to avoid any decomposition of the compounds present by ultraviolet light. Drying was allowed until a constant weight was obtained so as to enhance maximum extraction of the compounds. The seeds of *P. nigrum* and *M. fragrans*, dried leaves and roots of *C. sativum* were milled into powder using a Binatone electric blender (BLG-400) fitted with a 2mm sieve. The powders were each, successively extracted using Soxhlet apparatus, initially using analytical grade *n*-hexane (1 L) and followed by extraction using analytical grade methanol (1 L) with each solvent for 24 h. The solvents were evaporated to dryness using a rotary vacuum evaporator (Resona type WB) under reduced pressure. *n*-hexane solvent was used to extract non-polar compounds whereas methanol solvent was used to extract semi-polar and polar compounds. Using these two solvents offers partitioning of compounds to two types of extracts with different polarities. The resulting extracts/oils were air-dried at room temperature to remove excess solvent. The concentrated extracts/oils were then kept in vials at 4^{o}C until ready for use.

Dilutions of the plant extracts were prepared to obtain different concentrations (1,000 ppm, 5,000 ppm, 1,0000 ppm, 20,000 ppm) of each synergist. 20mls of each concentration was prepared by weighing the required weight of the extract using a weighing balance and then transferred into 50mls vials. Approximately 20mls of acetone was measured using a measuring cylinder to dissolve plant extracts. The weights used were 0.002gm, 0.01gm, 0.02gm and 0.04gm which yielded concentrations of 1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm respectively.

Topical application of pyrethrins, and synergist-pyrethrins formulations

Batches of 10 newly hatched unsexed adult maize weevils aged between 7-14 days were selected for the treatments. The adult weevils were obtained by sieving from the maize cultures into a petri-dish, covered with a muslin cloth and placed in a freezer (-20^{o}C) for 10 minutes to immobilize them. The pyrethrins/synergist-pyrethrins formulation dosages were applied separately on the dorsal part of the thorax of each test insect using a hand operated 10-µL topical applicator to dose each insect with 1µL of synergist concentration. Dosages were done in a geometric progression from the lowest concentration to the highest. After each dose, test insects were transferred to 250 ml plastic containers with sufficient quantity of food (fresh maize) and covered with muslin cloth held in place by rubber bands to allow ventilation. The containers were then kept in a recovery growth chamber maintained at a constant temperature of 27 ± 2 ^{0}C and 60 ± 5% RH with normal daylight hours. Mortality was assessed after 24 h, 48 h and 72 h exposure period. Insects were considered dead if they were incapable of moving when probed with a fine forceps at the abdomen (two subsequent touches in one-minute interval) were counted as dead. Three replications were done for all concentrations.

Full dose response bioassay for Pyrethrins

Pyrethrins, of technical grade solutions of 50% (w/w) were sourced from Pyrethrum Processing Company of Kenya (PPCK) Ltd, Nakuru, Kenya. Dilutions of the pyrethrins were prepared to obtain different concentrations of pyrethrins, (100 ppm, 1,000 ppm, 2500 ppm, 5,000 ppm, 1,0000 ppm, 25,000 ppm, 5,0000 ppm,

1,00000 ppm and 20,0000 ppm diluted in acetone). A conversion of 0.0001% = 1 ppm was used for this study. To obtain these concentrations the formula $C_1V_1 = C_2V_2$ was used where C_1 = 50% (500,000ppm), V_1 = volume to be measured, C_2 = required concentration and V_2 = required volume. These were used to determine dose-response curve for pyrethrins using the maize weevils. Acetone was applied as the control. The data was analysed with a probit analysis (Finney, 1971) to determine the lethal concentration (LC) of pyrethrins to the maize weevils taking control mortality into account. LC_{20} was generally used for synergism bioassays as a fixed concentration of pyrethrins in combination with potential synergists, to compare the relative efficacy of the synergists to one another and to unsynergised pyrethrins on *S. zea-mais.*

Synergism Bioassay

These were done using discriminatory dose-mortality bioassays of *S. zea-mais* using mixtures of pyrethrins plus synergists. A concentration of 2200ppm pyrethrins were chosen as discriminating doses equivalent to approximately LC_{20} of pyrethrins. Low LC-value with pyrethrins alone was chosen so that the synergistic abilities of the plant extracts/oils (synergists) could be compared. PBO, the standard synergist was used as the control.Synergism bioassays followed a previously described method (Ribiero *et al*., 2003). Here, formulations were prepared to contain the synergists at the prepared four plant extracts/synergist concentrations and the discriminatory dose of pyrethrins (2200 ppm) at ratio of 1:1synergist: pyrethrins. Piperonyl butoxide was used throughout this study as a commercially used synergist.

Synergism Calculations

Synergy of plant extracts and pyrethrin formulations was obtained using the method of Mansour *et al*, (1966) where the co-toxicity factor was obtained using the equation:

$$\text{Co-toxicity factor} = \frac{(\text{Observed \% mortality} - (\text{Expected \% mortality}}{(\text{Expected \% mortality})} \times 100$$

Where expected mortality was calculated as the sum of the average percentage mortalities achieved by pyrethrins at LC_{20} and the plant extracts. The co-toxicity factor was used to assess whether a plant extract could be antagonistic, additive or synergistic within the formulations compared with pyrethrins alone. Values lower than -20 suggest an antagonistic relationship, values between -20 and 20 suggest an additive character and values greater than 20 suggest synergism.

Data Analysis

ANOVA was used to test the significant difference of the mean mortality of maize weevils at $P \leq 0.05$. Co-toxicity factors were used to determine whether a plant extract was a synergist, antagonist or an additive. Data analysis was done via Statistical Package for Social Scientist (SPSS) and Microsoft Excel and the report was done on Microsoft Word.

RESULTS

The results of formulations at the ratio of 1:1 synergist: pyrethrins are presented in Figures 1, 2, and 3. It was found that after 24 h exposure, formulations with PBO and the plant extracts CLHE, NMHE and BPSME were statistically significant at $P \leq 0.05$ (P = 0.0126, P=0.033, P = 0.013, and P = 0.009 of PBO, CLHE, NMHE and BPSME respectively). As the synergist concentration increased, higher percentage mean mortality resulted in the synergist formulations. However, PBO registered higher percentage mortalities than all the synergists concentrations tested. For example, at 20,000 ppm, PBO had 83.33 ± 12.02% mortality of *S. zea-mais,* followed by CLHE (46.67 ± 3.33%), BPSME (43.33 ± 6.67%) and NMHE (26.67 ± 3.33%). Ranking of the means revealed that concentration of 20,000 ppm was different from the other lower concentrations in the synergists that were significant ($P < 0.05$) except for NMHE where the highest percentage mortality was recorded at 10,000 ppm.

In addition, these compounds when formulated with pyrethrins at this ratio gave significantly higher mortality than unsynergised pyrethrins (20% mortality at 2200 ppm) except BPSME and NMHE at 1,000 ppm (16.67 ± 3.33% and 6.67 ± 3.33% mortality respectively). This could mean that there was an antagonistic effect of BPSME and NMHE with pyrethrins that brought the overall mortality below 20%. From these results, PBO and CLHE were shown to be the most effective synergists with significantly higher mortality when applied with pyrethrins than all the other treatments

in all the four concentrations tested. However, formulations with CLME, BPSME and CRHE were not significant ($P > 0.05$). These extracts did not show differences in average percentage mortality of *S. zea-mais* with regard to increasing concentrations. Figure 1

When formulated with pyrethrins, BPSME, CLHE and PBO had significantly higher mortalities than unsynergised pyrethrins (20% mortality) treatment at 5%

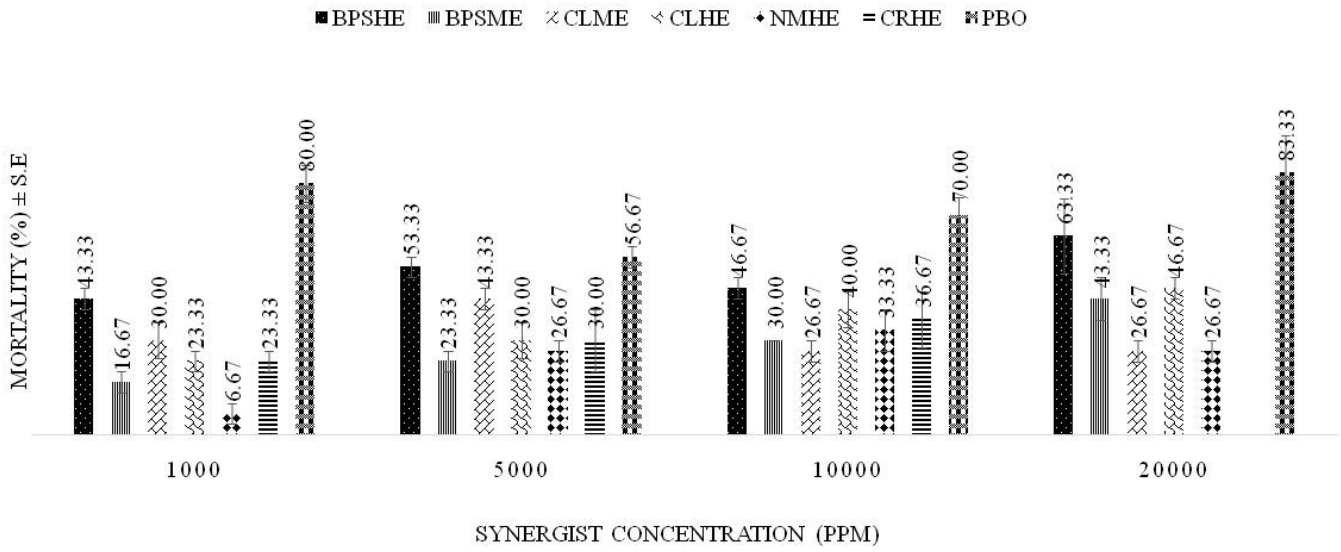

Figure 1. Mean percentage mortality (± S.E) in *S. zea-mais* adults at 24 h exposure after application of synergists at four concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) in combination with LC_{20} pyrethrins (2,200 ppm) at the ratio of 1:1 (synergist: pyrethrins).

significance level and that this plant extracts were negligible toxic on their own. The increase in mortality could therefore be ascribed to synergistic or additive effects. BPSME and NMHE with 16.67 ± 3.33% and 6.67 ± 3.33% respectively had mortalities below that of unsynergised pyrethrins of 20% mortality rate hence these plant extracts could have some antagonistic effects at 1,000 ppm level.

After 48 h exposure of synergised pyrethrins, the mean difference of percentage deaths of CLHE, BPSME, and NMHE were statistically significant at 5% significance level (P = 0.032, P = 0.026, and P = 0.001 of CLHE, BPSME, and NMHE respectively). However, formulations with BPSHE, CRHE, CLME and PBO were not significant (P ≥ 0.05).

The study showed that the plant extracts that were statistically significant at 24 h were also significant (P ≤ 0.05) at 48 h exposure time except PBO. Also, a higher average percentage mortality was recorded in the formulations (Figure 3). More time allowed the plant extracts to interact with pyrethrins yielding higher percentage mortality compared to 24 h duration. BPSME was the most effective pyrethrins synergist at 1,000 ppm (70.00 ± 5.77% mortality) followed by the CLHE (63.33 ± 3.33% mortality) and NMHE (16.67 ± 3.33%) of which mortalities recorded were higher than that of unsynergised pyrethrins (20% mortality) except for NMHE (16.67 ± 3.33%). BPSME and CLHE had 90 ± 5.77% average mortality at a synergist concentration of 20,000 ppm infering that BPSME and CLHE could have synergised pyrethrins at their respective concentrations since their toxicities to *S. zea-mais* were low (toxicity less than 20%).

Figure 2 shows the trends of synergist efficacy after 72 h exposure duration. Only BPSME and NMHE mortalities were statistically significant at 5% significance level (P = 0.012, and P = 0.0001 of BPSME and NMHE respectively). BPSME and NMHE showed significance (P < 0.05) over the entire exposure period of 72 h. NMHE at 1,000 ppm had 16.67% mortality which was below that of unsynergised pyrethrins (20% mortality) implying that NMHE could have an antagonistic effect to pyrethrins at 1,000 ppm compared to enhanced efficacy to 76.67% and 93.33% mortalities at 5,000 ppm and 10,000 ppm respectively. When the percentage mortality due to NMHE concentrations were ranked, 1,000 ppm was found to be significantly different from the higher concentrations (5,000 ppm, 10,000 ppm and 20,000 ppm) which in turn did not yield significantly different (P ≤ 0.05) percentage mortalities. This infers that it would be economical to use this plant extract at 5,000 ppm in formulations with pyrethrins as the percentage mortalities at higher concentrations (10,000 ppm and 20,000 ppm) would not be statistically different.

In general, the BPSME and NMHE recorded increased average percentage mortalities of *S. zea-mais* consistently over the exposure time of 24 h, 48 h and 72 h with higher percentage mortality observed after 72 h exposure for all the extracts.

The results of the joint action between the plant extracts (synergists) at the four concentrations (1,000 ppm, 5,000ppm, 10,000ppm and 20,000ppm) and pyrethrins at LC_{20} in the ratio of 1:1 (synergist: pyrethrins) determined according to the equation of co-toxicity factors are presented in Tables 1, 2 and 3. After 24 h exposure (Table 1), BPSHE and PBO co-toxicity values

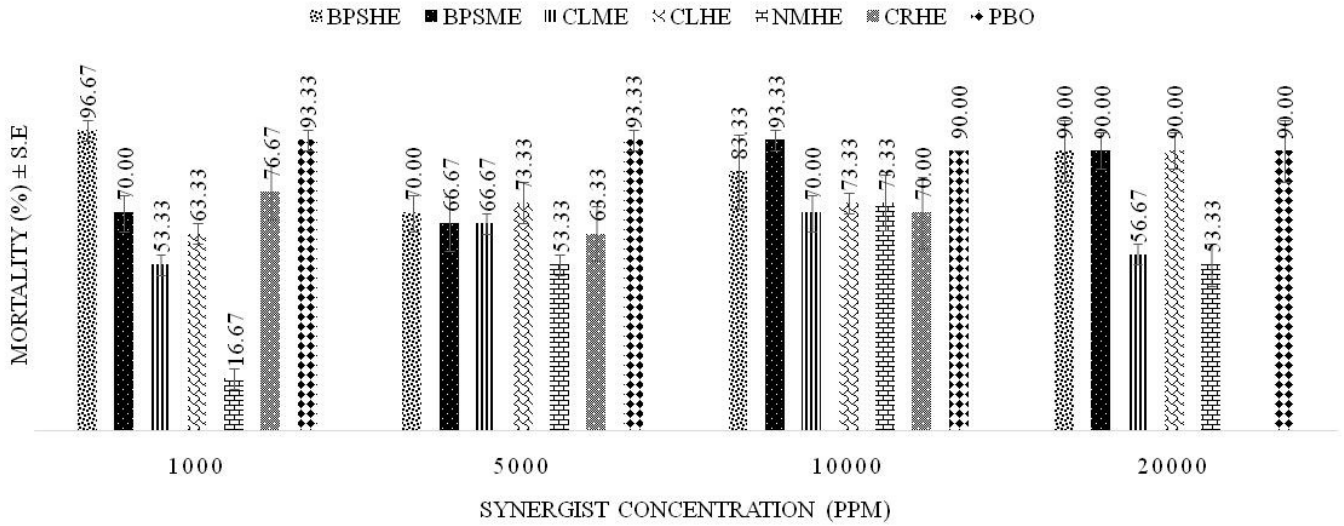

Figure 2. Mean percentage mortality (± S.E) in *S. zea-mais* adults at 48 h exposure after application of synergists at four concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) in combination with LC_{20} pyrethrins (2,200 ppm) at the ratio of 1:1 (synergist: pyrethrins).

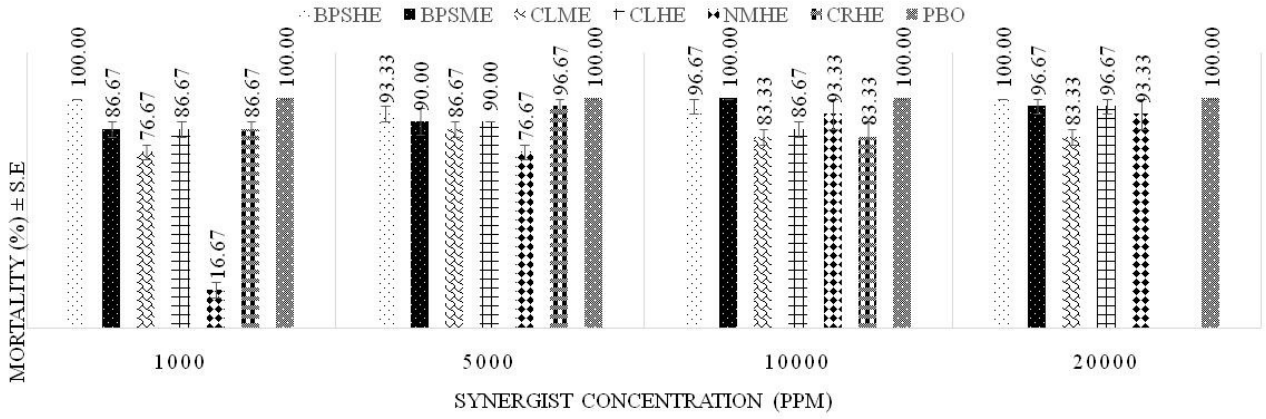

Figure 3. Mean percentage mortality (± S.E) in *S. zea-mais* adults at 72 h exposure after application of synergists at four concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) in combination with LC_{20} pyrethrins (2,200 ppm) at the ratio of 1:1 (synergist: pyrethrins).

obtained showed synergism (> 100 fold) in all the concentrations and ratios tested. BPSME with co-toxicity values of -16.7, -12.5 and 0 at concentration of 1,000 ppm, 5,000 ppm and 10,000 ppm respectively over 24 h period was an additive to the formulation and only a synergist at 20,000ppm. NMHE is antagonistic to pyrethrins at 1,000 ppm with co-toxicity value of -75 while at 5,000 ppm and 20,000 ppm it was an additive (14.3 and 0 values respectively). CLHE was only an additive at 1,000 ppm and a synergist at higher concentrations. Figure 3, Table 2

❖ The expected value is contributed by the constant 20% mortality of *S. zea-mais*due to pyrethrins (LC_{20}) and toxicity of the plant extract/synergist alone while observed mortality is due to the synergist-pyrethrins formulation Table 2 shows the co-toxicity values after 48 h exposure. NMHE was an antagonist at 1,000ppm (-54.5) when formulated with pyrethrins and a synergist at 5,000 ppm, 10,000 ppm and 20,000 with co-toxicity values of 90, 100 and 33 respectively. All the other plant extracts showed synergism. BPSHE and PBO had same synergism value (125 fold) followed by CLHE (107.7 fold) at 20,000 ppm while at 5,000 ppm, PBO was a better synergist (300 fold) followed by the plant extracts BPSHE (162.5 fold), CLHE (144.4 fold) and CLME (122.2 fold). Table 3

Table 2. Co-toxicity factors calculated on the basis of LC_{20} pyrethrins and synergists applied on *S. zea-mais* at ratio of 1:1 (synergist: pyrethrins) 24 h exposure. Values lowerthan -20 suggest antagonism, values between -20 and 20 suggest additive character and values greater than 20 suggest synergism

Plant extract/ synergist	Synergist - 1,000ppm			Synergist -5,000ppm			Synergist 10,000ppm			Synergist 20,000ppm		
	% Mortality			% Mortality			% Mortality			% Mortality		
	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor
BPSHE	20.0	43	117	23.3	53	129	26.7	47	75.0	26.7	63	138
BPSME	20.0	17	-16.7	26.7	23	-12.5	30.0	30	0.0	33.3	43	30.0
CLME	26.7	30	12.5	30.0	43	44.4	23.3	27	14.3	23.3	27	14.3
CLHE	20.0	23	16.7	23.3	30	28.6	26.7	40	50.0	36.7	47	27.3
NMHE	26.7	7	-75.0	23.3	27	14.3	26.7	33	25.0	26.7	27	0.0
CRHE	20.0	23	16.7	26.7	30	12.5	20.0	37	83.3			
PBO	20.0	80	300	20.0	57	183	23.3	70	200	26.7	83	213

Table 3. Co-toxicity factors calculated on the basis of LC_{20} pyrethrins and synergists applied on *S. zea-mais* at ratio 1:1 (synergist: pyrethrins) 48 h exposure. Values lower than -20 suggest antagonism, values between -20 and 20 suggest additive character and values greater than 20 suggest synergism

Plant extract/synergist	Synergist at 1,000ppm			Synergist at 5,000ppm			Synergist at 10,000ppm			Synergist at 20,000ppm		
	% Mortality			% Mortality			% Mortality			% Mortality		
	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor
BPSHE	33.3	97	190.0	26.7	70	162.5	30.0	83	177.8	40.0	90	125.0
BPSME	46.7	70	50.0	36.7	67	81.8	43.3	93	115.4	46.7	90	92.9
CLME	36.7	53	45.5	30.0	67	122.2	26.7	70	162.5	33.3	57	70.0
CLHE	23.3	63	171.4	30.0	73	144.4	36.7	73	100.0	43.3	90	107.7
NMHE	36.7	17	-54.5	33.3	53	60.0	36.7	73	100.0	40.0	53	33.3
CRHE	26.7	77	187.5	33.3	63	90.0	43.3	70	61.5			
PBO	26.7	93	250.0	23.3	93	300.0	30.0	90	200.0	40.0	90	125.0

❖ The expected value is contributed by the constant 20% mortality of *S. zea-mais*due to pyrethrins (LC_{20}) and toxicity of the plant extract/synergist alone while observed mortality is due to the synergist-pyrethrins formulation At 72 h exposure (Table 4), most plant extracts had higher synergism than the standard, PBO at a concentration of 20,000 ppm. For instance, BPSHE (130.8), NMHE (115.4), CLHE (107.1), CLME

Table 4. Co-toxicity factors calculated on the basis of LC_{20} pyrethrins and synergists applied on *S. zea-mais* at ratio 1:1 (synergist: pyrethrins) 72 h exposure. Values lower than -20 suggest antagonism, values between -20 and 20 suggest additive character and values greater than 20 suggest synergism

Plant extract/ synergist	Synergist at 1000ppm			Synergist at 5000ppm			Synergist at 10000ppm			Synergist at 20000ppm		
	% Mortality			% Mortality			% Mortality			% Mortality		
	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor
BPSHE	33.3	100	200.0	26.7	93	250.0	40.0	97	141.7	43.3	100	130.8
BPSME	50.0	87	73.3	40.0	90	125.0	46.7	100	114.3	53.3	97	81.3
CLME	36.7	77	109.1	36.7	87	136.4	36.7	83	127.3	43.3	83	92.3
CLHE	26.7	87	225.0	30.0	90	200.0	36.7	87	136.4	46.7	97	107.1
NMHE	36.7	17	-54.5	33.3	77	130.0	36.7	93	154.5	43.3	93	115.4
CRHE	26.7	87	225.0	36.7	97	163.6	43.3	83	92.3			
PBO	26.7	100	275.0	26.7	100	275.0	30.0	100	233.3	56.7	100	76.5

(92.3) and BPSME (81.3) while PBO had co-toxicity value of 76.5. These plant extracts could replace PBO when formulated at this ratio and concentration.

❖ The expected value is contributed by the constant 20% mortality of *S. zea-mais* due to pyrethrins (LC_{20}) and toxicity of the plant extract/synergist alone while observed mortality is due to the synergist-pyrethrins formulation.

DISCUSSION

In the discriminatory dose bioassays, each plant extract at four concentrations was mixed with pyrethrins (LC_{20}) at ratio 1:1synergist: pyrethrins and topically applied to the maize weevil. From the results obtained, PBO showed the highest efficacy as a pyrethrins synergist in all concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) tested, followed by CLHE at 24 h, 48 h and 72 h after treatment. NMHE, although statistically significant had low percentage mortalities below that of LC_{20} at 1,000ppm (6.67%, 16.67% and 16.67%) over 24 h, 48 h and 72 h respectively while BPSME had 16.67% mortality at 24 h suggesting that these plant extracts may have compounds that are either not sufficient enough to detoxify enzymes within the maize weevil or they are slow in penetrating the cuticle giving chance to the insect's enzymes to detoxify pyrethrins and render it ineffective. At higher concentrations of 5,000 ppm both BPSME and NMHE showed minimal increase in percentage mortalities but more than that of LC_{20}, the difference possibly being due to the toxicity of the plant extracts. Also, high synergist concentrations reducing synergism could mean that the mechanism of metabolism of an insecticide is not altered by the synergist but that the synergist could influence the reaction rate, or shift significant detoxification reactions to non-oxidative mechanisms (Casida, 1970). Oxidation or hydroxylation reactions form products of either reduced potency (detoxification) or enhanced potency (activation) thus a synergist might increase or decrease insecticide toxicity depending on the shift in balance of the competing activation or detoxification reactions.

Increasing the exposure period of the formulations to 48 h and 72 h also increased the overall percentage mortality of the maize weevils. For instance, NMHE at 10,000ppm achieved 33.33%, 73.33% and 93.33% while CLHE had 40.00%, 83.33% and 86.67% at 24 h, 48 h, and 72 h respectively. This showed that when the formulations were allowed time to interact with the maize weevils, higher mortalities were achieved. Though not tested in the present study, pre-treatment of insects with synergists have been found to increase the amount of synergism due to the time it takes for the synergist to maximally inhibit the enzymes within the insect (Bingham *et al*., 2007). In other studies, use of ethyl formate for control of stored grain pests were shown that varied dosages in an exposure period of 48-72 h controlled all stages of insects in stored grains and their products (Muthu *et al*., 2012) while methyl bromide gave complete disinfestations in 12-48 h (Anon, 2017).

Generally, it did not follow that increasing concentration of a synergist increased the percentage mortality of *S. zea-mais* geometrically except CLHE (24 h and 48 h) and PBO (24 h) where increase in concentration of synergist increased the percentage mortality of the maize weevil. In BPSME for instance, concentrations of 1,000 ppm, 5,000ppm, 10,000 ppm and 20,000 ppm yielded 70.00%, 67.67%, 93.33% and 90.00% percentage mortalities of *S. zea-mais* respectively while NMHE yielded 73.33% and 53.33% mortalities at 10,000 ppm and 20,000 ppm respectively. The findings suggest that penetration of the insect's cuticle may depend on the plant extract and that CLHE and PBO could be acting in a similar manner. These findings were contrary to a study by Joffe (2012) who found that an increase in the synergist concentration tended to correspond with a decrease in mortality of pollen beetles, *Meligethes aeneus* having to do with penetration of the cuticle of the insect. Possible explanation being increasing amount of synergist blocking the penetration of pyrethrins through the cuticle. Some synergists however, have been shown to reduce insecticide penetration through the cuticle, for example, piperonyl cyclonene, significantly reduced the absorption of topically applied pyrethroids in house flies *M. domestica* (Winteringham *et al*., 1955) and sesamex was shown to reduce the absorption of both labeled C^{14}-pyrethrin 1 and C^{14}-cinerin 1 by approximately half in houseflies, suggesting that sesame viscous oil possibly compete with the housefly epicuticle for any lipophilic compound.

To find out if the plant extracts synergized pyrethrins, co-toxicity factor was used as a means of assessing the synergistic potential of plant extracts when formulated with pyrethrins. From the results obtained BPSHE and PBO synergized the toxicity of pyrethrins in all the concentrations tested over the 72 h exposure time with co-toxicity factors above 100 with the highest being PBO at 1,000 ppm synergizing pyrethrins 300-fold which reduces as concentration is increased. BPSHE also consistently showed the highest co-toxicity factor of all the plant extracts tested. CLHE was only an additive at 1,000 ppm over 24 h exposure and a synergist in the rest of the concentrations and time. These observations indicate the potential of BPSHE to replace PBO in pyrethrins formulation at a ratio of 1:1 synergist: pyrethrins at all concentrations tested while CLHE can replace PBO when formulated with pyrethrins at 5,000 ppm, 10,000 ppm or 20,000 ppm.

BPSME with co-toxicity values of between -20 and 20 at concentration of 1,000 ppm, 5,000 ppm and 10,000 ppm respectively over 24 h period was an additive to the formulation and only a synergist at 20,000ppm while NMHE is antagonistic to pyrethrins at 1,000 ppm with co-toxicity values less than -20 over 24 h, 48 h and 72 h exposure period. NMHE showed significant synergism at concentrations of 1,000 ppm over the 72-h exposure period. These results suggest that some components in nutmeg plant extracts may have acted to inhibit oxidative processes important for the activation of pyrethrins. Faraone *et al*, (2015) found that some plant constituents like linalool from lavender (*Lavendula angustifolia*) and thymol from thyme (*Thymus vulgaris*) showed antagonistic action in imidacloprid against green peach aphid, *Myzus persicae*. This component in NMHE, though not isolated in this study may restrict the use of nutmeg oil as synergist at low concentrations. Studies by Gross *et al* 2017 suggested plant essential oils enhanced the toxicity of various type II pyrethroids and natural pyrethrins but the mechanism of action had not been fully explored. The plant extracts tested in this study were expected to portray the same synergistic activity as PBO because of the MDP ring structure thus the differences observed in their efficacy need further investigation. The data also suggest that some of these plant extracts may have higher levels of compounds that interfere with the insect's enzyme system than others which accounts for the variations in synergism observed. Further work need be done to specifically elucidate the structures of these plant extracts particularly that of CLHE whose efficacy compares well with that of PBO.

CONCLUSION

Plant extracts BPSHE and CLHE and PBO synergized the toxicity of pyrethrins in all concentrations at the ratio of 1:1 synergist: pyrethrins over 72 h exposure duration with co-toxicity factors above 100. BPSME was shown to be an additive in the formulation and only a synergist at 20,000ppm while NMHE was antagonistic to pyrethrins. Increasing the concentration of a plant extract did not correspond to increased efficacy in pyrethrins but rather depended more on the extract or possible interactions within the insect. Efficacy of the synergist- pyrethrins increased over the 72 h exposure period thus formulations at lower concentrations could be economically viable since average percentage mortalities will not be different from that of higher concentrations. BPSHE and CLHE could be used as synergists to pyrethrins when exposed to insects for 72 h duration.

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