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Original Research Article

Screening of Plant Extracts Possessing Methylenedioxyphenyl (MDP) Group as Potential Synergists in Insecticide Formulations using the Maize Weevil, *Sitophilus Zea-mais* (Motsch.)

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Abstract

Synergists are compounds that are either negligibly toxic or non-toxic to insects when applied on their own, but enhance the efficacy of an insecticide. Many insects are able to detoxify insecticide molecules and survive their application. Early studies found that most methylene-dioxyphenyl (MDP) agents themselves possess relatively low intrinsic toxicity, but strongly influence the actions of other xenobiotic in mammals and insects. The current commercial synergist, Piperonyl butoxide (PBO), though effective as a synergist, is not classified as an organic product in many countries. 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. Searches for effective synergists have not yet yielded many compounds that have the viability equivalent to that of PBO. This paper screened plant extracts possessing MDP rings as potential synergists in insecticides formulations using the maize weevil, *Sitophilus zea-mais.* Dose-mortality experiments were carried out on *S. zea-mais* at four concentrations of synergists. Topical application of synergist on *S. zea-mais* was done in triplicate in a CRD. Experiments were conducted under controlled laboratory conditions of 27 ± 2^{O}C and 60 ± 5%RH with normal daylight hours. Analysis of Variance (ANOVA) was used to obtain the mean mortality differences of *S. zea-mais* at P ≤ 0.05 while Duncan's Multiple Range Test (DMRT) was used to rank significant concentration means within a synergist. Black pepper seed hexane extract (BPSHE) and PBO after 48 h exposure were statistically significant (P ≤ 0.05) with the average percentage mortalities of 10% and 20% at 10,000 ppm and 20,000 ppm respectively, while PBO and Coriander leaves hexane extract (CLHE) were significant P ≤ 0.05 after 72 h. PBO was the most toxic synergist (36.67 ± 3.33%) followed by CLHE (26.67 ± 3.43%) at 20,000 ppm. It can be concluded that the plant extracts tested and statistically significant at a particular exposure time can be used to replace the standard PBO in insecticide formulations since their inherent toxicity is low. At a higher concentration (20,000 ppm) PBO was the most toxic synergist which could be contributing to the toxicity of insecticide when used in formulations.

Keywords: Methylenedioxyphenyl (MDP) ring, Plant extracts, Synergist, Toxicity

INTRODUCTION

Synergists are compounds that are either negligibly toxic or non-toxic to insects when applied on their own, but enhance the efficacy of an insecticide (Joffe, 2012). Synergists can be used in combination with insecticides possessing metabolic resistant mechanisms and in susceptible insect strains since they inhibit the metabolic pathway involved in detoxification of an insecticide (Casida, 1970; Metcalf, 1967). The use of a synergist enhances efficacy, allowing more cost-effective formulations.

Synergism has particularly been associated with the use of pyrethroids and pyrethrins formulations because of their moderate toxicity to insects on their own (Norries *et al*., 2019). Many insects are able to detoxify these molecules and survive their application (Casida and Quistad, 1995; Scott, 1990). For this reason and due to the high cost of pyrethrum, unsynergised insecticides formulations are rarely applied. Among the first compounds to be tested for synergism was sesame oil that was found to synergise pyrethrum, having no insecticidal activity of its own. The active compounds were shown to be sesamin and the more potent sesamolin (Beroza, 1954; Haller *et al*., 1942a,). The synergistic effects were attributed to its methylenedioxyphenyl (MDP) ring, and the constituents on the benzene ring (Haller *et al*., 1942b).

The MDP substituent is a structural feature present in many plant chemicals that deter foraging by predatory insects and herbivores. Exposure of insects to MDP containing synergists in the environment, in the absence of co-administered pesticides may also enhance xenobiotic detoxification (Murray, 2012). Early studies found that most MDP agents themselves possess relatively low intrinsic toxicity, but strongly influence the actions of other xenobiotics in mammals and insects by modulating cytochrome P-450 (CYP)-dependent biotransformation (Murray, 2012). Such MDP - containing natural products are frequently associated with prolonged inhibition of CYP450 activities because they undergo biotransformation to reactive intermediates that generate tight-binding complexes with the cytochrome Thus, after exposure to MDP chemicals, an initial phase of CYP inhibition is followed by sustained phase of CYP induction. In insects CYP inhibition by MDP agents underlies their use as pesticide synergists (Murray, 2012)

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 an organic product in many countries; it is expensive, toxic and also in short supply (Lang'at *et al*., 2008). Important qualities of a good synergist would include low mammalian toxicity, effectiveness against a variety of insects, rapid rate of absorption into the insect's body, stability in storage, good solubility characteristics, availability and a low cost of production or extraction (Casida, 1970; Beroza and Barthel, 1957). Searches for effective synergists have not yet yielded many compounds that have the viability equivalent to that of PBO. This paper seeks to screen plant extracts possessing (MDP) rings as potential synergists in insecticides formulations using the maize weevil, *Sitophilus zea-mais.*

MATERIALS AND METHODS

The experiments were carried out at Chuka University research laboratory, Kenya. All the bioassays were maintained throughout under controlled storage experimental growth chamber conditions of 27 ± 2 ^{0}C and 60 ± 5% RH with normal daylight hours. Completely Randomised Design (CRD) was used throughout the experiments. The treatments were assigned completely at random with each experiment unit having the same chance of receiving any one treatment. The experiments compared the values of dependent variables (response as percentage mortalities of *S. zea-mais*) based on different levels of independent variables (concentrations).

Experimental maize

A freshly harvested shelled and susceptible maize variety H512 (5x90kg bags) was procured from Kenya Agriculture and Livestock Research Organization – Katumani Research Institute, Kenya. Dust and debris of shelled maize grains were removed by sieving using a 6.0 mm sieve. The cleaned maize was then dried in the sun until the moisture content was less than 13% then transferred into a storage chamber free from insecticides and storage pests.

Rearing of insects

Maize weevil parent stock was obtained from National Agriculture Research Laboratory (NARL), Nairobi, Kenya. Cultures of the insects were established to supply similar aged weevils for the experiments. Maize weevils were cultured on the clean disinfected maize grains in 14 jars, each jar with 1.5L capacity, 500gms maize grains were put into the jars. 50 Unsexed adult insects 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

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

Table 2. Mass of each extract obtained after extraction of plant parts

Plant part	Mass of powder before extraction (g)	Mass of crude extract (g)
BPSHE	210.62	15.21
BPSME	170.20	13.20.
CLHE	93.49	10.71
CLME	82.89	12.24
CRHE	10.11	3.79
NMHE	72.30	9.78
PBO	N/A	N/A

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 daylight hours.

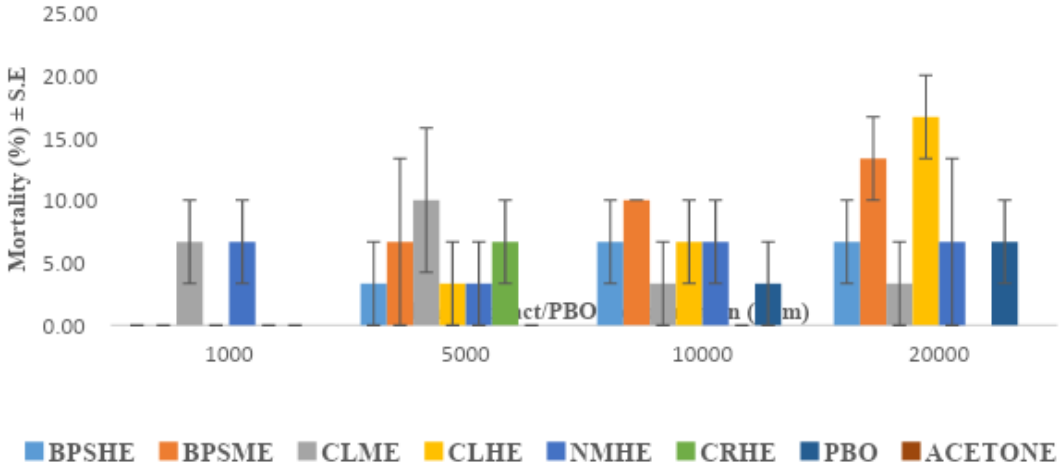
Synergists

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 . Table 1

Collection of plant parts and extraction

Seeds of *Piper nigrum* and Myristica 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 quantities of extracts/oils obtained are given in Table 2. The concentrated extracts/oils were then kept in vials at 4^{o}C until ready for use. The plant extracts/ oils used as synergists were given codes for easy identification (Table 2). Likewise, the formulation containing either of the plant extracts and insecticides was given a code similar to that of the plant extract.

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, 1,0000 ppm and 20,000 ppm respectively.



PBO and the plant extracts BPSHE, CLME, NMHE and CRHE were not statistically significant (P > 0.05).

Figure 1. Mean percentage mortality (± S.E) of *S. zea-mais* adults at 24 h after topical application of each plant extract at four concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm), PBO and acetone as the controls.

Data Collection

Dose-mortality bioassays were conducted to determine the toxicity of plant extracts to *S. zea-mais*. The bioassays followed procedures previously described by Viteri Jumbo *et al*, (2014). Four concentrations of each plant extract (1,000ppm, 5,000ppm, 10,000ppm and 20,000ppm) and the standard synergist, PBO were used in the bioassays. Acetone was included in the bioassay since it was used as the solvent in the dilutions. Here, batches of 10 newly emerged 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 synergist 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.

Data analysis

ANOVA was used to test the significant difference of the mean mortality of *S. zea-mais* P ≤ 0.05 taking control mortality into account Abott (1925). Significant means were ranked using DMRT to ascertain any differences between the concentrations. Data analysis were done via Statistical Package for Social Scientist (SPSS) and Microsoft Excel and the report was done on Microsoft Word.

RESULTS

It was found that after 24 h exposure (Figure 1), CLHE had higher average mortality 16.67 ± 3.33% mortality compared with BPSME with an average mortality rate of 13.33 ± 3.33% at synergist concentration of 20,000 ppm. The mean difference of percentage deaths of BPSME and CLHE were both statistically significant at P ≤ 0.05 (P = 0.015 and P = 0.017 of BPSME and CLHE respectively). As the concentration of the extracts increased, higher percentage mean mortality in both BPSME and CLHE also increased. BPSME at 1,000 ppm is shown to be statistically different from 20,000 ppm while 5,000 ppm and 10,000 pm perform the same way according to DMRT. In CLHE, concentration at 20,000 ppm ranked differently from the lower concentrations tested (1,000 ppm, 5,000 ppm and 10,000 ppm) which were not statistically different.

Figure 2 shows the synergists efficacies when exposure duration was increased to 48 h. PBO, BPSHE and CLHE showed that the mean difference of

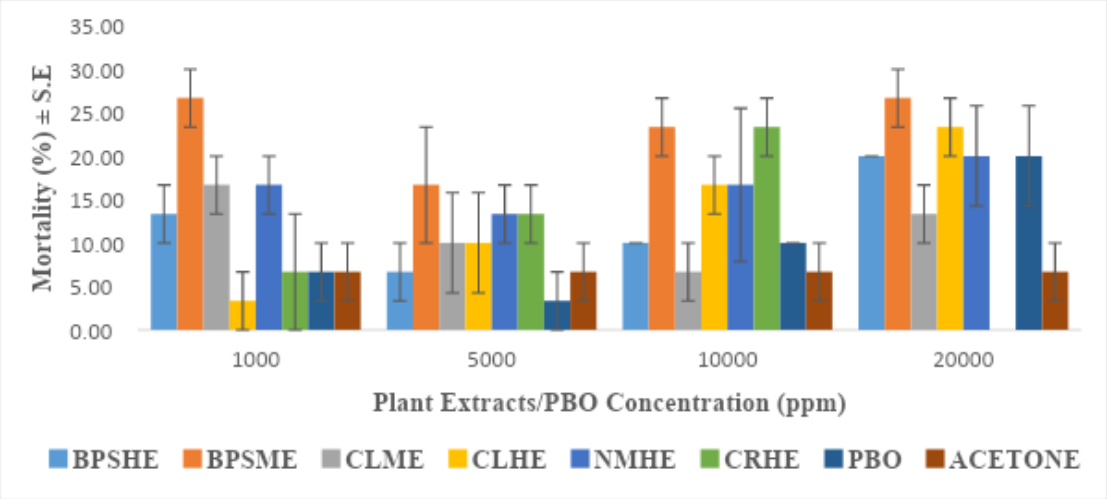


Figure 2. Mean percentage mortality (± S.E) of *S. zea-mais* adults at 48h after topical application of each plant extract at four concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm), PBO and acetone as the controls.

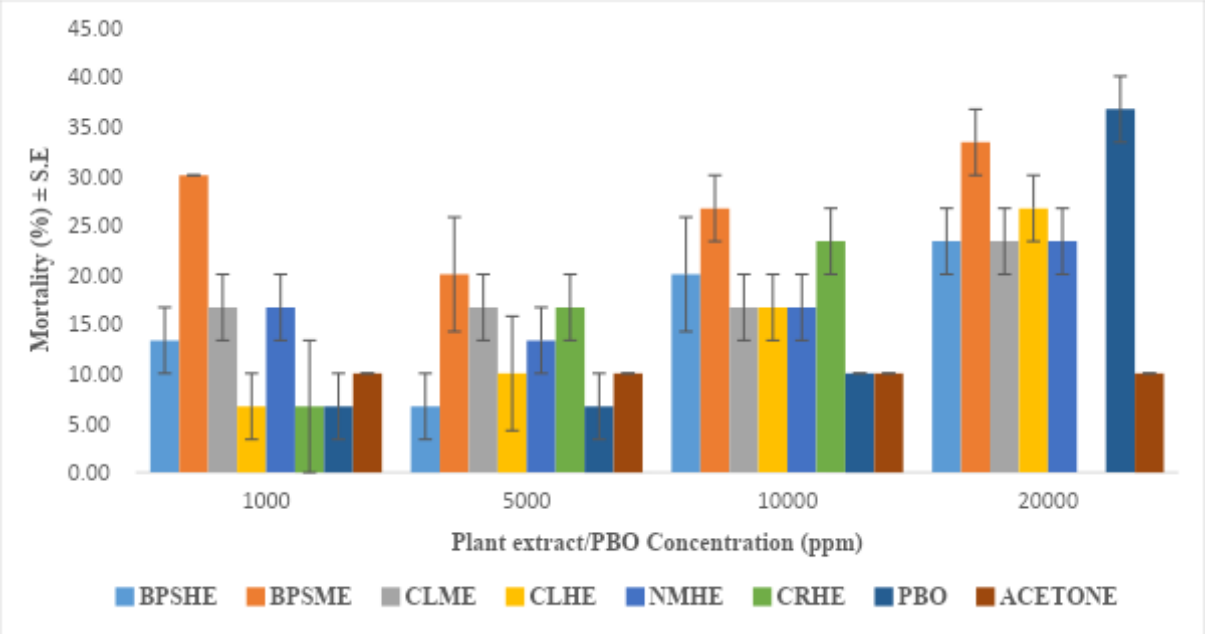


Figure 3. Mean percentage mortality (± S.E) of *Sitophilus zea-mais* adults at 72 h after topical application of each plant extract at four concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm), PBO and acetone as the controls.

percentage deaths was statistically significant $P \leq 0.05$ ($P = 0.04$, $P = 0.021$ and $P = 0.041$ of PBO BPSHE and CLHE respectively). CLHE had higher average mortality at 10,000 ppm and 20,000 ppm of 16.67 ± 3.33% and 23.33 ± 4.33% respectively compared with PBO and BPSHE each with 10 ± 0% and 20 ± 5.77% mortalities respectively. At 48 h exposure, PBO and BPSHE performed more or less the same at higher synergist concentrations tested. Also, the mean percentage deaths in CLHE increased consistently with increase in concentrations being higher than PBO and BPSHE at 5,000 ppm, 10,000 ppm and 20,000 ppm (10.00 ± 5.77%), 16.67 ± 3.33% and 23.33 ± 4.33% respectively).

However, plant extracts BPSME, CLME, NMHE and CRHE were not significant $P > 0.05$. The increase in average percentage mortality of *S. zea-mais* after 48 h of exposure suggests that plant extracts and PBO were slow in acting on the insects at first or their toxicity might have been contributed by the solvent, acetone which recorded an average percentage mortality of 6.67% mortality

since it was used in dissolving the synergists.

It was found that after 72 h exposure (Figure 3), the mean difference of percentage deaths of PBO and CLHE were both statistically significant P ≤ 0.05 (P =0.001 and P=0.036 of PBO and CLHE respectively). PBO had the higher average mortality 36.67 ± 3.33% mortality compared with the CLHE with an average mortality rate of 26.67 ± 3.43% at synergist concentration of 20,000 ppm both with percentage mortality of 6.67 ± 3.33% at synergist concentration of 1,000 ppm. A similarity was observed between CLHE and the standard synergist PBO the percentage mortalities recorded at the synergist concentrations tested. However, PBO was shown to be more toxic (36.67 ± 3.33%) to the insects than CLHE (26.67 ± 3.43%) when tested at 20,000 ppm concentration.

BPSHE, BPSME, CLME, NMHE and CRHE were not statistically significant at 5% significance level with no difference in mortality means when the concentration of the extracts was increased. For instance, in CLME at 1,000 ppm, 5,000 ppm and 10,000 ppm the average percentage mortality was 16.67% meaning the synergist concentration did not affect percentage mortality of the test insects. This consistency shows that CLME could be utilized as a synergist since it showed minimal variations in percentage mortality of the test insects. Figure 3

DISCUSSION

Generally, at lower concentrations (1,000 and 5,000 ppm), toxicity of all synergists tested was low. Toxicity of a plant extract is used to form a basis to determine whether it can be a synergist or an additive in an insecticide formulation (Joffe, 2012) and therefore the percentage mortalities recorded are important when selecting a synergist. If high mortalities have been recorded, it would imply that compounds contribute to the overall mortalities whenever they are used in formulations of insecticides. At 24 h exposure time, CLHE and BPSME recorded 16.67% and 13.33% mortalities at 20,000ppm concentration respectively. With increase of exposure time to 48 h and 72 h, CLHE percentage mortality of the maize weevils increased to 23.33% and 26.67% respectively. This consistency of increase in CLHE could indicate the presence of a particular chemical component in coriander leaves which gets activated with time and could be responsible for the results obtained. Characterization of these compounds may explain which component would be responsible for the observed results.

BPSHE and PBO after 48 h exposure were statistically significant at 5% significance level with the average percentage mortalities of 10% and 20% at 10,000 ppm and 20,000 ppm respectively while after 72 h, PBO and CLHE were significant. These results imply that the plant extracts tested and statistically significant at a particular exposure time could be potential synergists since their toxicity to the maize weevil is generally low. CRHE, CLME and NMHE were not statistically significant at all concentrations tested, that is, increasing the concentrations of the plant extract did not correspond to increased percentage morality of the maize weevils.

A synergist by definition is a negligibly toxic compound that enhances the efficacy of an insecticide when used in combination with insecticide (Casida 1970, Metcalf 1967). Early studies found that most MDP agents themselves possess relatively low intrinsic toxicity but strongly influence the action of other xenobiotics in mammals and insects by modulating cytochrome P-450 dependent biotransformation thus in insects CYP inhibition by MDP agents underlies their use as pesticide synergists (Murray, 2012). The low average mortalities of the plant extracts tested and statistically significant in this study are in harmony with earlier studies and that indeed they could be potential synergists in insecticide formulations.

CONCLUSION

It can be concluded that the plant extracts tested and statistically significant at a particular exposure time can be used to replace the standard PBO in insecticide formulations since their inherent toxicity is low. At a higher concentration (20,000 ppm) PBO was the most toxic synergist which could be contributing to the toxicity of insecticide when used in formulations.

RECOMMENDATIONS

The plant extracts possessing MDP ring structure can be synergists at a particular exposure time and concentration since their inherent toxicities are generally low.

Contribution to the Body of Knowledge

Addition to the list of inexpensive compounds functioning as effective synergists which will benefit both the insecticide industry, as it expands the use of botanical active ingredients by making them more viable and affordable for pest management.

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REFERENCES

Beroza M (1954). Pyrethrum synergists in sesame oil. Sesamolin, a potent synergist. *J. Ame. Oil Chemists' Society31*, 302-305.

Beroza M, Barthel WF (1957). Insecticide synergists: Chemical structure and activity of pyrethrin and allethrin synergists for control of the housefly. *J. Agric. Food Chem. 5*, 855-859.

Casida JE (1970). Mixed-function oxidase involvement in the biochemistry of insecticide synergists. *J. Agric. Food Chem. 18*, 753-772.

Casida JE, Quistad GB (1995). *Metabolism and synergism of pyrethrins. In: Pyrethrum flowers: Production, Chemistry, toxicology, and uses*. Casida, J.E. & Quistad, G.B. (Eds). Oxford University Press, New York. Pp 258-276.

Haller HL, LaForge FB, Sullivan WN (1942a). Some compounds related to sesamin: their structure and their synergistic effect with pyrethrum insecticides. *J. Organic Chem.* 7:185-188

Haller HL, McGovran ER, Goodhue LD, Sullivan WN (1942b). The synergistic action of sesamin with pyrethrum insecticides. *J. Organic Chem.* 7:183-184

Joffe T (2012). Evaluation of potential pyrethrum synergists on agriculturally significant insect pests. *Industry and investment NSW, Tamworth Agricultural Institute, Australia.*

Lang'at MK, Cheplogoi PK, Deng LR, Michura CG (2008). Effects of plant extracts on potency of pyrethrins against the housefly, *Musca domestica* Linne. (Diptera: Muscidae) *J. Kenya's Chem. Soc. 5*, 1811-5934.

Metcalf RL (1967). Mode of action of insecticide synergists. *Annual Review of Entomology* 12: 229-256.

Methylenedioxyphenyl- Substituted Chemicals in Mammals and Insects. *J. Toxicol. Environ. Health Part B* 15(6): 365-395

Murray M (2012). Toxicological Actions of Plant Derived and Anthropogenic

Norries EJ, Gross AD, Bartholomay LC, Coats JR (2019). Plant essential oils synergize various pyrethroid insecticides and antagonize malathion in *Aedes aegypti*. *Medical and Veterinary Entomology*, (33)453-466

Viteri Jumbo LO, Faroni LR, Oliveira EE, Pimentel MA, Silva GN (2014). Potential use of clove and cinnamon essential oils to control the bean weevil, *Acanthoscelides obtectus* Say, in small storage units. *Industrial Crops and Products*, 56, 27-34