

Insecticidal Activity of *Chrysanthemum Cinerariifolium* & *Allium Sativa* Natural Oils against *Prostephanus truncatus*

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Abstract

The larger grain borer (Prostephanus truncatus) is one of the most destructive post-harvest pest that affects maize and other crops like cassava. Synergists are substances added to pyrethrin increase efficacy and also to reduce the quantity of insecticides in formulation. The application of chemicals in pest control has been limited by toxicity, insect resistance and environmental degradation. A research was conducted to determine the ability of natural garlic oil to enhance the efficacy of pyrethrin against Prostephanus truncatus. The treatment solutions used were mixtures containing garlic bulb extract and pyrethrin oil. The experimental design used for bioassay was completely randomized design while the tests were done in triplicate. The test solutions used for bioassay tests were prepared by combining pyrethrin with garlic oils in ratio of 1:10. The concentrations ratio of pyrethrin to garlic oil used were between 20 mg/ml: 200 mg/ml to 14 mg/ml: 140 mg/ml while mortality rates were assessed after 24, 48 and 72 hours. Analysis of garlic oil extract using Fourier transform infrared spectroscopy showed the presence of several functional groups linked with insecticidal activity. There was significant variation in percentage mortality rates of P truncatus with change in concentration of treatment solutions and exposure time. The pyrethrin synergized with garlic oil recorded over 50% mortality rate during the test period. The non-synergized pyrethrin containing 20 mg/ml pyrethrin oil alone had a mean mortality rate of 29%. This was same as 14 mg/ml pyrethrin with 140 mg/ml garlic oil but less than 16 mg/ml pyrethrin with 160 mg/ml garlic oil that had 46%. The parameters used to estimate the efficacy during bioassay test were mortality rates which were compared with the concentrations and insect's exposure time. The findings from this study revealed that garlic oil can augment the efficacy of pyrethrin against P truncatus.

Keywords: *Prostephanus truncatus*, Garlic, Pyrethrin, Mortality, Efficacy.

INTRODUCTION

Maize is the major source of energy food for people worldwide (Slavin & Carlson, 2014). The impact of post-harvest grain pests is devastating affecting both the quantity and quality of stored grains (Bakoye *et al.*, 2017). Garlic bulb biochemical ingredients have been investigated and found to have a wide range of pharmaceutical benefits with minimum side effects (Mikail, 2010). Pyrethrin is preferred as an insecticide due to its broad spectrum effect on pests, minimum toxicity to non-target insects and low effective dose (Shawkat *et al.*, 2011).

The larger grain borer (*Prostephanus truncatus*) is one of the most destructive pests that affects maize and other crop in the tropical region (Ognakossan *et al.*, 2013). *P. truncatus* originated from American continent and was introduced to Africa through imported maize that came from Mexico to Tanzania in 1970s. This later spread to other parts of the continent causing serious post-harvest damages to maize and some non-cereal crops like cassava (Gueye *et al.*, 2008).

Biochemical components found in plant extracts have shown the potential of producing affordable natural insecticides with low toxic levels towards non target animals (Abreu-Villaça & Levin, 2017). Despite the effectiveness of natural pyrethrin in vector control they are photosensitive which reduces their insecticidal activity, thus they are preferably used together with triglycerides from vegetable oil that contains plant secondary metabolites like tannins to stabilize them (Wanyika *et al.*, 2009).

The use of chemically synthesized pesticides has been associated with the rapid development of resistant strains in pest control and environmental degradation due bioaccumulation leaving the use of botanical insecticide as an alternative to these effects (Mkenda *et al.*, 2015). Another study revealed that there is association between declines in population of earthworm which improves soil fertility with consistent use of chemically synthesized insecticides (Tiwari & Pandey, 2019). Plant essential oils have exhibited insecticidal activity with various modes of actions that have high efficacy on pests and low toxicity towards non target organisms (Pavela & Benelli, 2016).

Pyrethrum (*Chrysanthemum cinerariifolium*) is the most frequently used natural insecticide that has a potent knock down effects on target pests (Barnes, 2010). Pyrethrin is oil extracted from *C. cinerariifolium* mainly from flowers and is the main active ingredient in its insecticidal activity (Freemont *et al.*, 2016). Plant oils as a natural synergist have been found to enhance the efficacy of pyrethrin against *P. truncatus* (Kaguchia *et al.*, 2018). The investigation done on bioactivity of garlic oil against mealworm beetle (*Tenebrio molitor*) at high dose showed it interferes with mobility of the insects due to induced paralysis (Plata-Rueda *et al.*, 2017).

Pyrethrin exerts its toxicity by penetrating the cuticle and binding to sodium channels leading death of the target pest as a result of shutting down of the central nervous system (Duke *et al.*, 2010). Synergists like piperonyl butoxide (PBO) are added to pyrethrin increase potency by inhibiting the activities of detoxification enzymes which reduces efficacy of insecticides (Joffe *et al.*, 2015). Therefore, a study was done to assess the potential of garlic oil to enhance insecticidal activity of pyrethrin against *P. truncatus*.

METHODOLOGY

Rearing of maize weevils

This study was carried out at University of Eldoret laboratories situated in a region with an altitude of 2090 m above sea level, latitude of 00° 55' North and longitude of 34° 50' East. The mean maximum and minimum temperature of the laboratory were 29±4°C and 16±2°C, respectively during the period of the experiment. Mass rearing for both insects was done as described (Tefera *et al.*, 2010) with some slight modifications. The culture for mass rearing of *P. truncatus* was obtained from infested hybrid breed maize grain from a local farmer and had no history of being

treated with insecticide before the research was conducted. The non-infested seeds were sorted, disinfested in oven for 1 hour at 60 °C and cooled in desiccators before been used for mass rearing of the insects.

The mass rearing of insects was done using clean and disinfected 2 liter plastic jar of diameter 15 cm and height 25 cm with lids containing four small holes of diameter 2 cm and muslin cloth to ensure sufficient circulation of air. The rearing jars were placed on top of glass containers treated with tangle foot oil to prevent mites and foreign insects from entering the culture. The non-infested seeds were sorted, disinfested in oven for 1 hour at 60 °C and cooled in desiccators before been used for mass rearing of the insects.

The average moisture content of maize grains used for rearing of insects was 13 %. After two weeks of ovipositional the adult weevils were sieved out, the maize grains containing eggs were returned in a clean disinfected rearing jar and left for 30 days. The adult weevils from the culture were sieved using 1mm sieve mesh and those retained were taken as of the same age. The rearing of insects was done at day temperature within a range of 25-29 °C and relative humidity of 55-62% and predominantly 12:12 light: dark photo period interval. The F1 generation from the culture was used for bioassay test.

Sampling and preparation of materials

The chemical used for this experiment were analytical grade reagents obtained from department of Chemistry and Biochemistry University of Eldoret. The pyrethrum, *C cinerariaefolium* oil 0.2% stock solution was obtained from Pyrethrum Board of Kenya Nakuru. The garlic bulb was purchased from main local market in Uasin Gishu County as a bulb.

Extraction of garlic oil

The garlic oil was extracted from the bulb by removing the outer layer, cutting into small pieces and transferring in a well ventilated room where they were left for two weeks to dry. They were then grind into fine powders using an electrical blender and sieved. The fine powder was soaked in *n*-hexane for three days, filtered using whatman filter paper and the solution was transferred into a flask and concentrated in a rotor evaporator at 50 °C.

The hexane was evaporated and the extracted oil remained in the rotor flask. The extracted oil was weighed and stored in a refrigerator. The refractive index of garlic oil was determined using refractometer at 25 °C to assay the purity of the extract.

Fourier transforms infrared spectroscopy (FTIR) analysis of garlic oil

The extracted garlic oil was further assayed for biochemical activities by determining the presence of functional groups linked to plants secondary metabolites using Fourier transform infrared spectroscopy (FTIR). The analysis was performed as previously described by Kannan, (2014) and Raju *et al.*, (2016) with few modifications where 1 ml of garlic oil was transferred in a transparent glass sample cell and placed in sample holder for analysis in a FTIR spectrophotometer (Shimadzu, Japan) that had a scanning range from 400 to 4800 cm^{-1} with a resolution of cm^{-1} .

Bioassay tests: Bioassay tests were done as illustrated by Mulungu *et al.*, (2011). The experimental design used for the bioassay test was Complete Randomized

Design and each test was carried out in triplicates. Ten pairs of unsexed *P truncatus* were introduced into the 250 ml plastic jars of diameter 7.5 cm and height 15 cm with lid that had four small holes of 1 cm diameter that were covered with muslin cloth for sufficient ventilation of air. The plastic jars used contained 20 gm grain maize as substrate that was treated with solutions of different concentrations. The treatments contained pyrethrin and garlic oil extract in ratio of 1:10 prepared in acetone as solvent. The concentration ratios of pyrethrin to garlic oil extract varied from 14mg/ml: 140 mg/ml to 20 mg/ml: 200 mg/ml. The standard conventional insecticide contained 20 mg/ml actellic dust and standard pyrethrum designed for the study contained 20 mg/ml pyrethrin and 200 mg/ml piperonyl butoxide. The maize grains used in control experiment were not exposed to any form of treatment. The mortality rates were determined after 24, 48 and 72 hours' time intervals. The parameters used to determine the efficacy of treatments solutions were concentrations, exposure time and mortality rate.

Data collection

Insect's mortality was determined by touching them with fine brush and they were considered dead if no movements were observed under magnifying lens. Dead insects were removed from the jars once identified and counted. The software applied for data analysis was R CRAN using Duncan test. One Way Analysis of Variance (ANOVA) and linear regression statistical analysis were used to evaluate the relationship between mortalities rate, concentrations of treatment solutions and exposure time. The value $p \leq 0.05$ was considered to be statistically significant during data analysis.

RESULTS

The extracted garlic oil extract of was yellow-greenish in colour, insoluble in water and had characteristic pungent smell. The refractive index of the oil was 1.450 at 25°C. The interpreted results for FT-IR spectrum showed the presence of methylene, amides, strong aromatics, sulphonates and other hydrocarbons functional groups associated with lipids and essential oils.

Table 1: Wavenumbers for FT-IR analysis spectrum for garlic oil

Peak no	Wavenumber in cm^{-1}	Functional group	Comment
1	2916.42	C-H stretching Alkane	predominant for lipids
2	2848.91	C-H stretching Alkane	predominant for lipids
3	1735.96	C=O stretching Amide	mainly amide bond for protein
4	1462.07	C-H bending Alkane	CH_2 bending for lipids
5	1172.74	S=O stretching Sulfonate	presence of sulfur organic compound
6	719.46	C=C bending/aromatics Alkenes	Presence of lipids

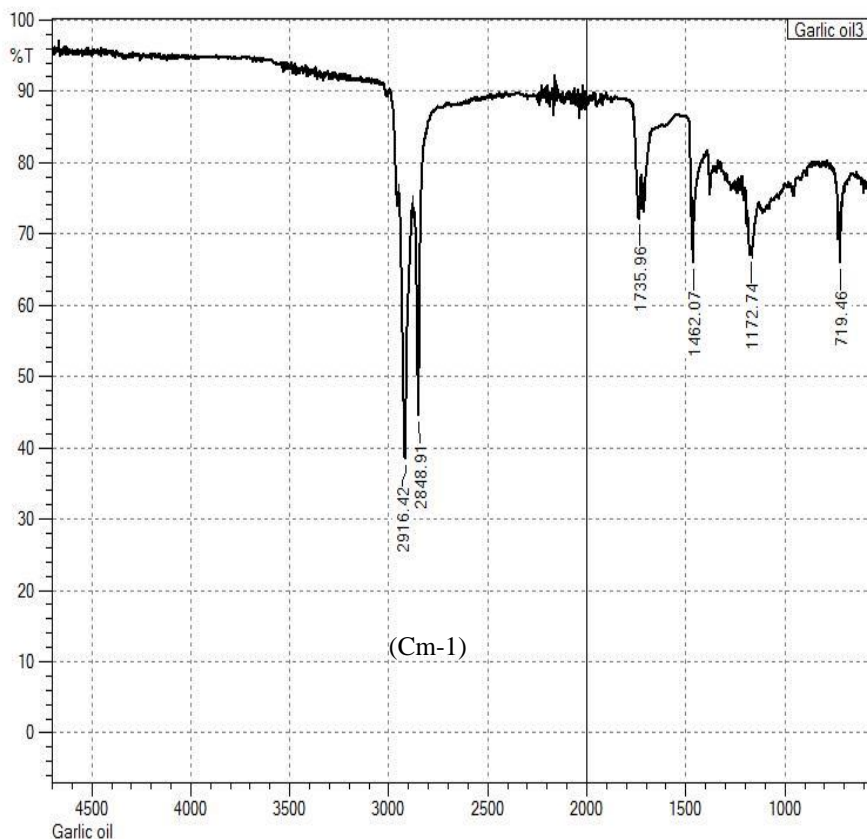


Figure 1: FT-IR analysis spectrum for garlic oil extract of hexane

The bioassay test results showed there were significant ($P < 0.05$) increase in mortality rates with concentration of treatment solutions and exposure times. Treatment solutions containing 16 mg/ml pyrethrin with 160 mg/ml garlic oil extract had a mortality rate of 37% after 24 hours while 14 mg/ml pyrethrin with 140 mg/ml had 33%. These values were more than for non-synergized pyrethrum that had 20 mg/ml oil pyrethrin only with mortality rate of 27% during the same interval. The standard convectional insecticide which contained 20 mg/ml actellic dust and standard pyrethrum (20 mg/ml with 200 mg/ml piperonyl butoxide) recorded the highest mortality rate of over 100 % and 93 %, respectively. The blank (acetone) and untreated (control) samples recorded no mortality during the 24-hour interval.

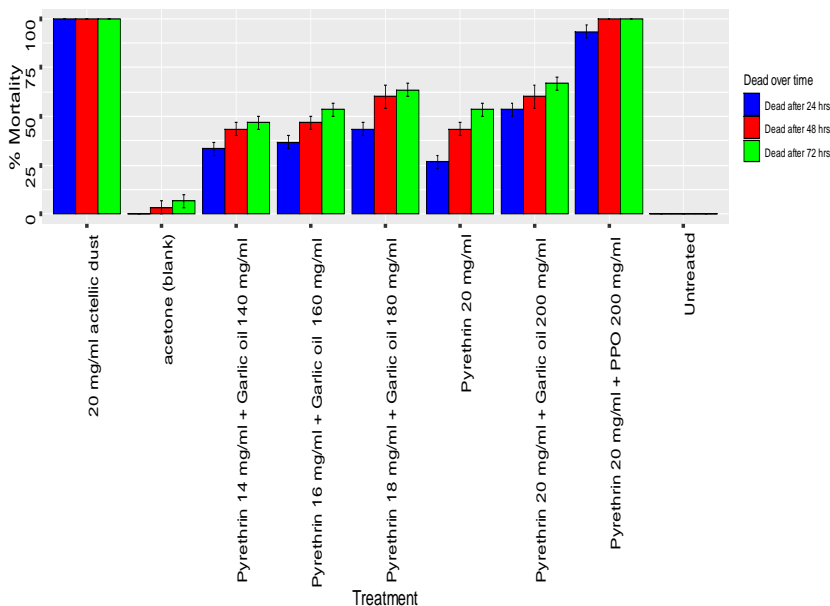


Figure 2: % mean mortality of *Prostephanus truncatus* at varying concentrations of treatment with time

There was a significant difference on percentage mortality of *Prostephanus truncatus* with treatments at varied exposure times with highest mean of 54% for 72 hours and lowest of 43% for 24 hours. The mean percentage mortality for 48-hour time was 43%. Acetone the blank for the tests had mean percentage mortality of 1.1 %.

Table 3: Mean of % mortality of *Prostephanus truncatus* at varying time of death

	% Mortality	Groups
Dead after 72 hrs	54.44	a
Dead after 48 hrs	50.74	B
Dead after 24 hrs	42.96	c

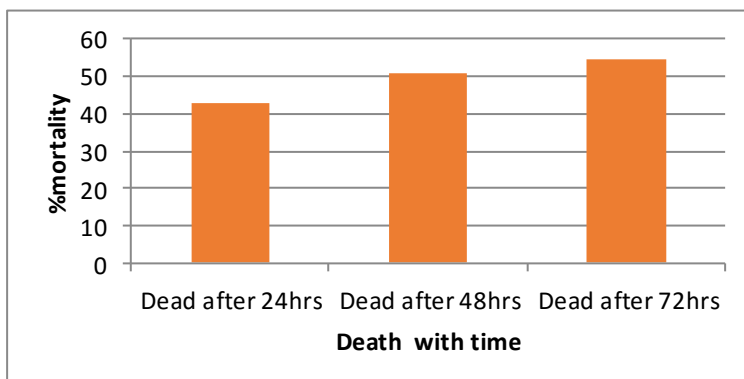


Figure 3: Mean of % mortality of *Prostephanus truncatus* at varying time of death

DISCUSSION

The physical properties of the garlic oil extract are consistent to earlier findings by (Rafe, 2014) making the characteristics of the garlic oil extracted appropriate for the bioassay test. The hydrocarbons in the FTIR spectrum are related to essential oils which contain terpenes that have been found to impede bioactivities in insects (Beg, 2017). The functional groups that were established to be present in the IR spectrum of garlic oil have been verified to exhibit pharmaceutical properties (Chowański *et al.*, 2016). Sulphur compounds which are vital for bioactivity in garlic oil are represented by S=O stretch in the FTIR spectrum (Satyal *et al.*, 2017).

The increase in efficacy of pyrethrin garlic oil extract can in addition be attributed to terpenes which when studied with *Sitophilus oryzae* were found to hinder the actions of acetylcholinesterase and adenosine triphosphatases (Mona & Samir, 2018). The essential oils have also been found to control insects as repellants, distracting desire for food and interfering with reproduction in insects (Brari & Kumar, 2019). The study results show relations to investigations done on essential oils derived from *Lippia javanica spreng* which were found to be effective in control of *Sitophilus zeamais* (Kamanula, 2017). The bioassay test results can be correlated to research done on efficacy of garlic oil combined with mint oils that establish they were effective in management of the black cutworm *Agrotis ipsilon* at all stages of the insect life (Sharaby & El-nujiban, 2015).

The increase in mortality rates with increase in concentration of treatment can as well be associated altering cellular physiological processes, cell development and behavioural modification by the essential oils (Sarwar, 2015). The bioassay test results showed there was significant ($p < 0.05$) increase in mortality rates with concentration of treatment solutions and exposure times (Vedovatto *et al.*, 2015). Acetone which served as the blank for the tests had mean mortality of 1.1 % which may have been as a result of natural factors (Ngwej *et al.*, 2019). The mean percentage mortality rate for acetone which was the solvent for test solutions and served as the blank was less than 5 % and not significantly different ($p < 0.05$) from the untreated hence the values were taken as non-substantial with reference to the bioassay results (Denlinger *et al.*, 2015). Abbott corrected mortality formular was not applied since there was no mortality observed on the control experiments (WHO, 2013). The increase in mortality rates of can be attributed to enhanced penetration of pyrethrin through the insect's cuticles by essential oils present in garlic which act as surfactants (Tak & Isman, 2017). This may also be due to aromatic compounds C-H "oops" strong bonds that occur at frequency of 719 cm^{-1} that were present in garlic oil FT-IR spectrum that has been found to with antagonistic and synergistic effects (Pavela, 2014).

Mammals possess complex multifaceted detoxification mechanism compared with arthropods thus cannot be affected by pyrethrin toxic dose designed for insect pests (Ensley, 2007). This makes application of pyrethrin with garlic oil concentrations used in this study safe and still with allowance to be optimized further. Though the capability of piperonyl butoxide as pyrethrin synergist was higher than garlic oil its application has been limited due its non-biodegradable nature and toxicity (Jansen & Warnier, 2010).

CONCLUSION

The FT-IR analysis showed hexane extract of garlic contains functional groups connected to plant essential oils that possess biopesticides activities. The results from this study established that garlic oil has the potential to enhance insecticidal activity of pyrethrin against *Prostephanus truncatus* hence can be applied as a natural biodegradable synergist. The efficacy of acetellic was superior to pyrethrin synergized by piperonyl butoxide and garlic oil. Piperonyl butoxide was found to be a more potent pyrethrin synergist compared to garlic oil.

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