AFRICA ENVIRONMENTAL REVIEW Journal

ISSN: 1727-8341

AER Journal Volume 4, Issue 1, pp. 1-9, December, 2020

RESEARCH ARTICLE

Available Online at *http://www.aer-journal.info*

Bioefficay of *Chrysanthemum cinerariifolium* and *Allium sativum* Oil Extracts against *Sitophilus zeamais*

S. Lutta[1*], L. Kituyi[1], S. Barasa[1] and M. Macharia[2]

[1]*Department of Chemistry and Biochemistry, University of Eldoret, P.O. Box 1125-3100 Eldoret, Kenya*

[2]*KALRO – Njoro (Food Crops Research Centre), Private Bag – 20107, Njoro, Kenya*

[*]*Corresponding Author's Email: mukolwesam@gmail.com*

Abstract

Post harvest grain loss due to insect pests remains a major challenge to farmers globally. The application of synthetic chemicals in pest control has been limited by toxicity, evolution of insect resistance and environmental degradation. This study was done to evaluate the ability of garlic oil extract to synergize pyrethrin against Sitophilus zeamais a significant cereals pest. The phytochemical analysis on pyrethrin with garlic oil confirmed the presences of secondary metabolites that possess insecticidal activities. The experimental design used for bioassay was completely randomized design and tests were done in triplicates. The parameters applied to evaluate efficacy were mortality rates, treatment concentrations and insect's exposure time. The treatment solutions used for bioassay were varying concentration mixtures of pyrethrin with garlic oil prepared in a ratio of 1:10. The mortality rates were evaluated after 24, 48 and 72 hours. The result showed piperonyl butoxide as the most effective pyrethrin synergist with mean mortality of 90%. The non-synergized pyrethrum containing 20 mg/ml pyrethrin had less mortality (-%) compared to treatments with lower concentration of pyrethrin that were mixed with garlic oil. The findings revealed that garlic oil can enhance the potency of pyrethrin against Sitophilus zeamias.

Keywords: *Sitophilus zeamias*, Phytochemicals, Treatments, Synergist, Mortality

INTRODUCTION

Maize is major staple food in Kenya and also one of the leading cereal crops that are cultivated worldwide (Awika, 2011). A large proportion of maize produce is utilized for human consumption apart from United State of America where one third is used in production of biofuel (Ranum & Garcia-Casal, 2014). Natural pyrethrin is preferred in pesticides formulations due to its broad-spectrum effect on pests, low toxicity to non target organisms and low effective dose (Shawkat & Majeed, 2011). The garlic (*Allium sativum)* essential oil and powder have been applied in pest management in some countries across the world (Yang *et al.*, 2009).

The damages due to maize weevils (*Sitophilus zeamais*) have been found to have devastating effects on both the quality and quantity of harvested grains (Mutungi *et al.*, 2019). Researchers have also shown that metabolic products of *Sitophilus zeamais* increase moisture content on the surface of grains which facilitates growth of *Aspergillus flavus* and production of aflatoxin mould that is a cancer agent (Bhusal & Khanal, 2019).

The limitations associated with the use of synthetic insecticides are high production cost, toxicity to non-target organism and environmental pollution due to their non-biodegradable nature (Donia & Alqasoumi, 2012). Botanical plants insecticidal bioactivity has been studied with findings indicating they can provide a solution to the challenges associated with current conventional pesticides (Mkindi *et al.*, 2017). Phytochemicals contain secondary metabolites, which form the biomolecular basis for natural plants bioefficacy poteintial against pests (Abbas *et al.*, 2012).

Piperonyl butoxide (PBO) is commonly used as a synergist in pyrethrin to enhance its insect's potency and to reduce the amount of pyrethrin in the formulation of insecticide making it to be cost effective (Gleave *et al.*, 2018). Piperonyl butoxide has been found to be harmful as it impedes molecular signals and retards the development of animal cell (Wang *et al*., 2012). Garlic extract in aqueous mixture of humic acids has been positively assayed for insect repellency - a practice applied in pest management (Knueppel *et al.,* 2015). This study was carried out to investigate the bioefficacy of pyrethrin and garlic oils as green pesticides against *Sitophilus zeamias.*

MATERIALS AND METHODS

Rearing of Maize Weevils

The research was conducted at University of Eldoret laboratories located at altitude of 2090m above sea level, latitude of 00° 55` North and longitude of 34° 50`East. The mean maximum and minimum temperatures of the laboratory were 29±4 °C and 16±2 °C, respectively during the periods of the experiment. Mass rearing for both insects was done as described by (Hategekimana *et al.,* 2017) with some few modifications. The culture for mass rearing of weevil was obtained from infested hybrid maize grain breed from local farmers and had no history of being treated with pesticide prior to the study. The non infested seeds were sorted, disinfested in oven for 1 hour at 60°C and cooled in desiccators before being used for mass rearing of the insects.

The rearing was done in Biological Science Department incubation room using clean disinfected 2 litres 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 on the surface with tanglefoot oil to prevent mites and foreign insects from entering the culture. The non infested grains were sorted, disinfested in oven for 1 hour at 60 °C and cooled in a desiccator before being used for mass rearing of the insects.

The average moisture content of maize grain used for rearing of insects was 13%. After two weeks of oviposition the adult weevils were sieved out, the maize grains containing eggs were reintroduced into a clean disinfected 2l rearing jar and left for 30 days. The adult weevils from the culture were removed; 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 the range of 25-29 °C, 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

Chemical reagents used for this experiment were analytical grade obtained from Department of Chemistry and Biochemistry at University of Eldoret. Garlic bulbs were sourced from the main local market in Uasin Gishu County. The pyrethrum, *Chrysanthemum cinerariaefolium* oil 0.2% was obtained from Pyrethrum board of Kenya, Nakuru. The outer dry layer of the garlic bulbs was peeled off, the remaining part was cut into tiny pieces then placed in a well-ventilated area in the laboratory and left to dry for two weeks. They were then milled to 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 concentrated in rotor evaporator at 50°C to extract the garlic oil. The refractive index of garlic oil was determined at 25°C using refactometer to assay the purity of the extract. Preliminary test for qualitative analysis of phytochemicals was done for the test solutions to confirm the presence of secondary metabolites.

Phytochemical Test

Phytochemical screening of the test solution was done using standard procedure as explained by (Manouze *et al.,* 2017) and (Patil *et al.*,2016). The tests were done in parallel with a test tube containing phytochemical test reagents alone as a blank for comparative analysis. Tannins were determined by measuring 2 ml of extracts stock test solution and stirring in 3 ml of distilled water. This was followed by addition of drops of 5% $FeCl_3$ solution. The formation of a green precipitate indicated the presence of tannins. Saponins were assayed by measuring 5 ml of extracts test solution, shaking in 5 ml of distilled water in a test tube and warming, formation of stable foam was taken as a positive test for the saponins.

Test for flavonoid was performed by measuring 2 ml of extracts test solution and adding 1 ml of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonoids. Anthraquinones were analysed by measuring 2 ml of test solution and boiling with 10% hydrochloric acid for 10 minutes on water bath, filtered and cooled. Same volume of chloroform 2 ml was added to the filtrate followed by 3 ml of 10% ammonia. The mixture was then heated and non appearance of pink-rose colour was taken as negative test for anthraquinones.

Test for terpenoids was conducted by measuring 2 ml of extracts test solution and dissolving in 2 ml of chloroform followed by evaporation to dryness. A 2 ml of concentrated sulphuric acid was then added and heated for about 2 minutes, greyish colour indicated the presence of terpenoids. The steroids test was done by measuring 2 ml of extracts test solutions, dissolving 2 ml of chloroform and 2 ml concentrated sulphuric acid. Red colour produced on lower layer indicated the existence of steroids.

Alkaloids test was performed by measuring 3 ml of extract test solution and stirring with 3 ml of 1% HCl on a steam bath. This was followed by addition of Mayer's reagent (a solution of mercuric chloride and potassium iodide) and Wagner's reagents (solution of iodine in potassium iodide) formation of turbidity by precipitate was taken as positive test for alkaloids.Testing of glycosides was done by measuring 2 ml of extracts test solutions, dissolving 2 ml of chloroform and 2 ml ethanoic acid, formation of blue greenish colour was taken as a confirmatory test for glycosides.

Bioassay Tests

This was done as illustrated by (Mulungu *et al.,* 2011) and (Suleiman & Majeed, 2012). The experimental design used for the bioassay test was Complete Randomized Design (CBD) and each test was performed in triplicate. Ten pairs of unsexed *Sitophilus zeamais* were introduced into the 250 ml plastic jars of diameter 7.5 cm and height 15 cm with lids that had four small holes diameter of 1 cm that were covered with muslin cloth for sufficient ventilation of air. The plastic jars each contained 20 gm of maize grains that acted as a substrate that were treated with solutions of different concentrations. The treatments contained pyrethrin and garlic oil extract in ratio of 1:10 prepared using acetone as solvent. The concentration ratios applied varied from 14 mg/ml: 140 mg/ml to 20 mg/ml: 200 mg/ml.

The standard conventional insecticide contained 20 mg/ml actellic dust and standard pyrethrum for the study was a mixture of 20 mg/ml pyrethrin and 200 mg/ml piperonyl butoxide. The maize grains used in control experiments were not subjected to any treatment. The mortalities were assayed after 24, 48 and 72 hours time intervals. The parameters used to determine the efficacy of the treatments were concentrations, exposure time and mortality rate.

Data Collection

Mortality rates were evaluated after the time intervals by touching the insects using fine brush and considered dead if no movements were observed under magnifying lens. The dead insects were removed from the jars once counted. The software for data analysis was R CRAN using Duncan test. One way analysis of variance (ANOVA) and linear regression statistical analysis were used to determine 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 garlic oil extract was yellow-greenish in colour insoluble in water with characteristic pungent smell and the refractive index was 1.450 at 25°C. The phytochemical screening results confirmed the presence of tannins, saponins, flavonoids, alkaloids, steroids, terpennoids, glycosides and glycosides. The test for anthraquinone was negative as shown below.

Table 1: Phytochemical Test

Phytochemicals	Results
Tannins	+
Saponins	+
Flavoniods	+
Anthraquinones	-
Terpenoids	+
Alkaloids	+
Steroids	+
Glycosides	+

Key: + Present - Absent

The bioassay test results showed there was significant (P<0.05) increase in mortality rates with concentration of treatments solutions and exposure times (Table 2). These differences are further illustatrated in Figure 1. The treatments containing 16 mg/ml pyrethrin with 160 mg/ml garlic oil extract had a mortality rate of 27% after 24 hours while 14 mg/ml pyrethrin with 140 mg/ml had 23%. These values were more than non synergized pyrethrum that had 20 mg/ml oil pyrethrin only which had mortality rate of 20% during same interval. The standard conventional 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 80% and 70% respectively after 24 hours. Both acetone which served as blank and the untreated which was control for the tests recorded no mortality at the same time interval.

The percentage mortality rate of 18 mg/ml pyrethrin with 180 mg/ml garlic oil extracts was 50% after 48 hour. The treatment solutions containing concentration ratio of pyrethrin: garlic oil extracts of 14:140 and 16:160 recorded mortality 33% while 20 mg/ml non-synergized pyrethrin oil alone had 27% mortality within the same interval. The standard convectional insecticide 20 mg/ml actellic and pyrethrum containing 20 mg/ml pyrethrin with 200 mg/ml PPO had highest mortality rate of over 95% within the same interval.There were no mortalities observed in acetone (blank) and untreated with the same period.

The highest mortality rate after 72 hours was 100% observed in both the standard actellic and 20 mg/ml pyrethrin with 200 mg/ml PPO. The treatment solutions containing concentration ratio of pyrethrin: garlic oil extracts of 14:140 and 16:160 recorded mortality 37%. The non-synergized pyrethrum containing 20 mg/ml pyrethrin oil had 40% mortality rate which was less than 18 mg/ml pyrethrin with 180 mg/ml garlic oil extracts that had 53%

within the same interval. Acetone had mortality rate of 3% within the same exposure time while the untreated sample which served as control recorded zero mortality through out the period the bioassay test was done.

The treatments with the highest mean percentage mortality after 72 hours was 20 mg/ml actellic dust with 93%. The treatment with lowest was 20 mg/ml non-synergized pyrethrin 29%. The standard pyrethrum (20 mg/ml with 200 mg/ml piperonyl butoxide) had mean mortality of 90%. The treatment solution with 18 mg/ml pyrethrin and 180 mg/ml garlic oil had a mean percentage mortality of 49% while 16 mg/ml pyrethrin and 160 mg/ml garlic oil had 32%. The treatment solution containing 14mg/ml pyrethrin and 140 mg/ml had 31% while acetone which was the blank and solution used to prepare treatment solutions had percentage mean mortality of 1%. The table below shows the variation of percentage mortality rate of *Sitophilus zeamia*s with varying concentration of tests solutions and exposure time. This further explained in Figure 2.

Table 2: Mortality Rates of *Sitophilus zeamais* at Different Concentrations of Treatment with Time

Treatment (concentration in mg/ml)	Variation in Mean % mortality with time				
	24 hrs	48 hrs	72 hrs	Mean	Groups
20 mg/ml actellic	83.33	96.67	100.00	93.33	a
20 mg/ml + piperonyl butoxide 200 mg/ml	73.33	96.67	100.00	90.00	a
Pyrethrin 20 mg/ml+ Garlic oil 200 mg/ml	43.33	50.00	56.67	50.00	b
Pyrethrin 18 mg/ml + Garlic oil 180 mg/ml	43.33	50.00	53.33	48.89	b
Pyrethrin 16 mg/ml + Garlic oil 160 mg//ml	26.67	33.33	36.67	32.22	c
Pyrethrin 14 mg/ml+ Garlic oil 140 mg/ml	23.33	33.33	36.67	31.11	c
Pyrethrin 20 mg/ml	20.00	26.67	40.00	28.89	c
Acetone (blank)	0.00	0.00	3.33	1.11	d
Untreated	00.00	00.00	00.00	00.00	d

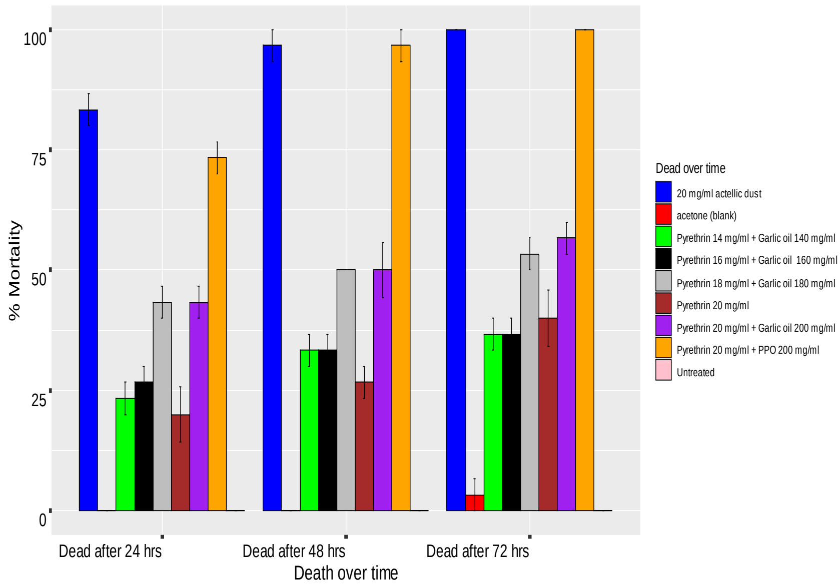

Figure 1: Mortality Rates of *Sitophilus zeamais* at Different Concentrations of Treatment and Time.

There was a significant variation on the mean percentage mortality of *Sitophilus zeamais* with treatments at different exposure times (Table 3 and Figure 3) with highest mean of 47% for 72 hours and lowest of 34% for 24 hours. The mean value for percentage mortality after 48 hours was 43% as shown below.

Table 3: Mortallity Rates of *Sitophilus zeamais* at Different Times

Time	% Mortality	groups
Dead after 24 hrs	34.81	a
Dead after 48 hrs	42.96	b
Dead after 72 hrs	47.41	c

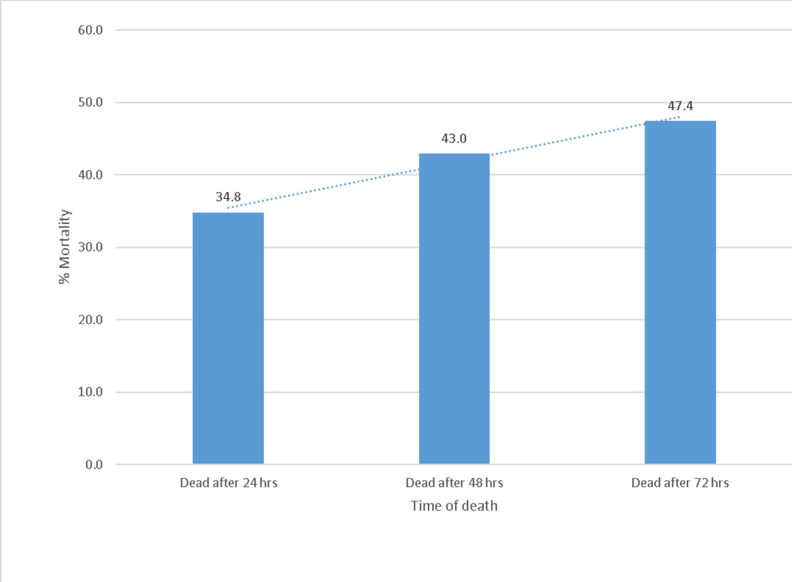

Figure 2: Mortallity Rates of *Sitophilus zeamais* at Different *Times.*

DISCUSSION

The physical properties of garlic oil extract had close similarities to the findings by Rafe (2014) making it suitable for the study. Insecticidal activity of the test solutions may be associated with essential oils found in plants that have been ascertained to repel, distract desire for food and interfere with reproduction in insects (Brari & Kumar, 2019). The phytochemical screening results confirmed the presence of secondary metabolites that included tannins, saponins, steroids, terpenes, glycosides and alkaloids while anthraquinone was not detected showing some close similarities to some study done on whole garlic plant extract (Singh & Kumar, 2017).

A study conducted on flavan glycoside a secondary metabolite produced by plant for defense mechanism shows it inhibits digestive enzymes and discourage feeding leading to retarded growth in insects (War *et al.*, 2012). Saponins have been found to interact with cholesterol resulting in down regulation on biosynthesis of steroids in insects which is essential for growth and moulting (Ikbal, 2010).

Acetone which was the solvent for test solution and served as blank for bioassay test had mean mortality of 1.1% which might have been due to natural factors (Ngwej *et al.*, 2019). Since the mortality rate due to acetone was not significantly different ($p<0.05$) from untreated (Table 1) with a mean of less than 5% in all the tests conducted, consequently the values were taken as non-substantial with reference to the experimental data Denlinger *et al.*, 2015). Abbott corrected mortality formular was not applied since control had zero mortality (WHO, 2013).

The increase in mortality rates of combined pyrethrin with garlic extracts can be attributed to enhanced penetration of insecticides through the insects cuticles by essential oils found in plants which act as surfactants (Tak & Isman, 2017). This may also be due to aromatic compounds that are found in secondary metabolites like flavonoids present in the garlic oil that have been confirmed to have synergistic and antagonistic effects (Pavela, 2014). Despite actellic dust and piperonyl butoxide showing higher efficacy, they are limited by their non-biodegradable nature and toxicity towards non-target organisms (Oso, 2015).

Garlic plant has been used as food spice with current studies indicating it is safe and contains medicinal values that have multiple benefits to human cells (Sethi *et al*., 2014). The concentrations of garlic oil extract used for the study are safe based to a study by Yamato *et al.* (2018) that found administering garlic extract dose of 90 mg/kg daily for three months did not manifest any detrimental effects on the body tissues of *Canis* species.

CONCLUSION

The results from phytochemicals analysis established pyrethrin combined with garlic oil contain plant secondary metabolites which possess biopesticides activities. The findings from this study scientifically confirmed that garlic oil has the ability to enhance the efficacy of pyrethrin oil against *Sitophilus zeamais.* The efficacy of actellic dust, which is a standard convectional insecticide was superior compared to both pyrethrin combined with garlic oil and the pyrethrum standard. The efficacy of standard pyrethrum synergized with piperonyl butoxide (PBO) was high compared to pyrethrin combined with garlic oil. Thus, despite toxicity and non-biodegradable nature PBO, it still remains the most effective pyrethrum synergist.

References

Abbas, M., Shahid, M., Rehman, H. M., Sharif, S., Muhammad, R., Khan, A., and Munawar, I. (2012). Screening of Selected Medicinal Plants for Secondary Metabolites. *Abstract Accepted for Poster Presentation In11 International and 23 National Chemistry Conference Held at NCEPC, University of Peshawar* , **8**(3), 119.

Awika, J. M. (2011). Major cereal grains production and use around the world. In *American Chemical society Symposium Series,* **1089**, 1–13.

Bhusal, K., and Khanal, D. (2019). Role of Maize Weevil, Sitophilus zeamais Motsch. on Spread of Aspergillus section flavi in Different Nepalese Maize Varieties. *Advances in Agriculture*, **1**,1–5.

Brari, J. and Kumar, V., (2019). Antifeedant activity of four plant essential oil against major stored product insects .*International journal of pure and applied zoology*,**7**(3), 41-45.

Denlinger, D. S., Lozano-Fuentes, S., Lawyer, P. G., Black, W. C., and Bernhardt, S. A. (2015). Assessing Insecticide Susceptibility of Laboratory Lutzomyia longipalpis and Phlebotomus papatasi Sand Flies (Diptera: Psychodidae: Phlebotominae). *Journal of Medical Entomology*, **52**(5), 1003–1012.

Donia, A., and Alqasoumi, S. (2012). Phytochemical screening and insecticidal activity of three plants from Chenopodiaceae family. *Journal of Medicinal Plants Research*, **6**(48), 5863–5867.

Gleave, K., Lissenden, N., Richardson, M., and Ranson, H. (2018). Piperonyl butoxide (PBO) combined with pyrethroids in long lasting insecticidal nets (LLINs) to prevent malaria in Africa. *Cohrane Database of Systematic Reviews.* 2017. **10**.1002/14651858.cd012776.

Hategekimana, A., Night, G., Rutikanga, A., Uzamugura, J. M. V. and Erler, F. (2017). Insecticidal and grain-protecting properties of a pyrethrum-based product against stored maize weevil, Sitophilus zeamais (Coleoptera: Curculionidae). *Fresenius Environmental Bulletin*,**26**(8),5136-5141.

Ikbal, C.,(2010). Saponins as insectiides: A Review. *Tunisian Journal Plant Protection*,**1**,39.

Knueppel, D. I., Yap, M. C., Sullenberger, M. T., Hunter, R., Olson, M. B. and Wessels, F. J. (2015). Pesticidal compositions and related methods. Retrieved from https://patents.google.com/patent/US8883229B2/en.

Manouze, H., Bouchatta, O., Gadhi, A. C., Bennis, M., Sokar, Z. and Ba-M'hamed, S. (2017). Anti-inflammatory, antinociceptive, and antioxidant activities of methanol and aqueous extracts of Anacyclus pyrethrum roots. *Frontiers in Pharmacology*, **8**(SEP).

Mkindi, A., Mpumi, N., Tembo, Y., Stevenson, P., Ndakidemi. P., Mtei, K ., Machunda, R. and Belmain, S. (2017). Invasive weeds with pesticidal properties as potential new crops. *Industrial Crops and Products*, **110** (December 30), 113-122.

Mulungu, L.S., Ndilahomba, B., Nyange, C. J., Mwatawala, M. W., Mwalilino, J. K., Joseph, C. C. and Mgina, C. A. (2011). Efficacy of *Chrysanthemum cinerariaefolium*, *Neorautanenia mitis* and *Gnidia kraussiana* against Larger Grain Borer (*Prostephanus truncatus* Horn) and Maize Weevil (*Sitophilus zeamays Motschulsky*) on Maize (*Zea mays L.*) Grain Seeds (*Sitophilus zeamays Motschu. Journal of Entomology*, **8**(1), 81–87.

Mutungi, C. M., Muthoni, F., Bekunda, M., Gaspar, A., Kabula, E. and Abass, A. (2019). Physical quality of maize grain harvested and stored by smallholder farmers in the Northern highlands of Tanzania: Effects of harvesting and pre-storage handling practices in two marginally contrasting agro-locations. *Journal of Stored Products Research*, **84**,12.

Ngwej, L. M., Hattingh, I., Mlambo, G., Mashat, E. M., Kashala, J. C. K., Malonga, F. K. and Bangs, M. J. (2019). Indoor residual spray bio-efficacy and residual activity of a clothianidin-based formulation (SumiShield® 50WG) provides long persistence on various wall surfaces for malaria control in the Democratic Republic of Congo. *Malaria Journal*, **18**(1), 72.

Oso, J. (2015). Pesticide residues and potential health risk through the consumption of fish from Ibadan, Oyo state, Nigeria.Master's thesis, College of Science Kwame Nkrumah University of Science and Technology.

Patil, U. S. and Deshmukh, O. S. (2016). Preliminary phytochemical screening of six medicinal plants used as traditional medicine. International Journal of Pharma and Bio Sciences, **7**(1),77-81.

Pavela, R. (2014). Acute synergistic and antagonistic effects of some aromatic compounds on the *Spodopter littoralis Boisd* (Lep, Noctuidae) larvae .*Industrial crops and products* , **60**, 247–251.

Rafe, A. (2014). Physicochemical Characteristics of Garlic (Allium sativum L.) Oil: Effect of Extraction Procedure. *International Journal of Nutrition and Food Sciences*, **3**(6), 1.

Ranum, P., Peña-Rosas, J. P. and Garcia-Casal, M. N. (2014). Global maize production, utilization, and consumption. *Annals of the New York Academy of Sciences*, **1312**(1), 105–112.

Sethi, N., Kaura, S., Dilbaghi, N., Parle, M. and Pal, M. (2014). Garlic: a Pungent Wonder From Nature. *International Research Journal of Pharmacy*, **5**(7), 523–529.

Shawkat, M. S., Khazaal, A. Q. and Majeed, M. R. (2011). Extraction of Pyrethrin from Chrysanthemum cinerariaefolium petals and study its activity against beetle flour Tribolium castanum**.** *Iraqi Journal of Science*, **52**(4), 456–463.

Singh, V. and Kumar, R. (2017). Study of Phytochemical Analysis and Antioxidant Activity of Allium sativum of Bundelkhand Region. *International Journal of Life-Sciences Scientific Research*, **3**(6), 1451–1458.

Suleiman, M., D. Ibrahim, N. and Majeed, Q. (2012). Control of Sitophilus zeamais (Motsch) [Coleoptera: Curculionidae] on Sorghum Using Some Plant Powders. *International Journal of Agriculture and Forestry*, **2**(1), 53–57.

Tak, J. H. and Isman, M. B. (2017). Enhanced cuticular penetration as the mechanism of synergy for the major constituents of thyme essential oil in the cabbage looper, Trichoplusia ni. *Industrial Crops and Products*, **101**, 29–35.

Wang, J., Lu, J., Mook, R. A., Zhang, M., Zhao, S., Barak, L. S. and Chen, W. (2012). The insecticide synergist piperonyl butoxide inhibits hedgehog signaling: Assessing chemical risks. *Toxicological Sciences*, **128**(2), 517–523.

War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S. and Sharma, H. C. (2012). Mechanisms of plant defense against insect herbivores. *Plant Signaling and Behavior,* **7**(10), 1306–1320.

WHO (2013). Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. In *WHO Library Cataloguing-in-Publication Data*.

Yamato, O., Tsuneyoshi, T., Ushijima, M., Jikihara, H. and Yabuki, A. (2018). Safety and efficacy of aged garlic extract in dogs: Upregulation of the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway and Nrf2-regulated phase II antioxidant enzymes. *BMC Veterinary Research*, **14**(1), 373.