

Toxicity Effect of Slow-Release Pelletized Edible Essential Oils on Cowpea Seed Bruchid, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) Infesting some Legume Grains in Lafia, Nasarawa State

Ismaila Jibrin¹, Folorunso A. Ajayi^{1,2*}, Isaac M. Ogara¹, Anayo C. Etonihu³, Olobayo O. Kunle⁴, Shehu A. Rahman², Eunice A. Adgiz¹, Kafayat O. Ajelara⁵, Samson O. Okunade⁶, Akindele O. Ogunfunmilayo⁷, James Y. Oyeniyi⁸, and Shehu A. Dattijo⁹

¹Faculty of Agriculture, Nasarawa State University, Keffi, P.M.B. 135, Lafia, Nasarawa State, Nigeria.

²Faculty of Agriculture, Federal University of Lafia, P.M.B. 146, Lafia, Nasarawa State, Nigeria.

³Faculty of Natural and Applied Sciences, Department of Chemistry, Nasarawa State University, Keffi, Nasarawa State, Nigeria.

⁴National Institute for Pharmaceutical Research Development, Abuja, FCT, Nigeria.

⁵Department of Zoology and Environmental Biology, Lagos State University, Lagos, Nigeria.

⁶Nigeria Stored Product Research Institute, Ilorin, Kwara State, Nigeria.

⁷Nigeria Agricultural Quarantine Service, Ibadan Office, Ibadan, Nigeria.

⁸Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

⁹Department of Pest Management Technology, Audu Bako College of Agriculture, Dambatta, Kano State

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CORRESPONDING AUTHOR

Folorunso A. Ajayi

Faculty of Agriculture, Federal University of Lafia, Nasarawa State, Nigeria

faajayi@agric.fulafia.edu.ng, and

faajayi@nsuk.edu.ng

+234-803-648-2672

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Abstract

*The evaluation of acute toxicity of three slow-release pelletised edible essential oils (SRPEEOs) extracted from clove (*Syzygium aromaticum* (L. Merr. and L. M. Perry), West African black pepper (WABP), (*Piper guineense* Schumach. and Thonn.) and Ginger (*Zingiber officinale* Roscoe) on the bean bruchid, *Callosobruchus maculatus* (F.) infesting cowpea, pigeon pea, Bambara groundnut, Lima and Soya beans. The research was conducted under laboratory conditions at the Faculty of Agriculture, Nasarawa State University, Keffi, Nigeria. Evaluation of the SRPEEOs on susceptibility and lethal concentration (LC₅₀) was conducted using 0.25, 0.5, 0.75, and 1.0 g/5 g legumes. The treatments were replicated three (3) times, and the experiments were laid out in a complete randomised block design. Data analyses were conducted using Statistix 10, an analytical software package, in a two-way factorial analysis. All data were transformed before analysis. The results of the LC₅₀ showed that WABP SRPEEO caused the highest mortality of bruchids (LC₅₀: 1 g/5 g) compared to 0.25, 0.5, and 0.75 g/5 g for clove and ginger, respectively. The three SRPEEOs were very effective in conferring protection on the legume varieties against infestation by the bruchid and can also serve as an alternative to synthetic pesticides. However, SRPEEOs from WABP proved to be more effective and can thus repel colonisation of legumes by the cowpea seed bruchid.*

Keywords:

Cowpea seed bruchid, Pelletised edible essential oils, Slow-release, Toxicity, Legumes.

Introduction

The word “legume” originates from the Latin word “legumen,” which translates to “seeds harvested in pods” (Vasconcelos et al., 2020). Legumes, also known as pulses, have been a staple of farmers' diets since the Neolithic Revolution, dating back to the very beginning of farming practices by humans, when they were domesticated alongside grasses as early as 10,000 years ago (Hancock, 2012; Huebbe & Rimbach, 2020). Among the earliest domesticated legume crops were chickpea (*Cicer arietinum* L.), garden pea (*Pisum sativum* L.), and lentil (*Lens culinaris* Medik.) (Sprent, 2009; Hancock, 2012; Smýkal et al., 2015). The domestication of other important legumes followed later on in different regions of the world, for example, soybean (*Glycine max* (L.) Merr.) in East Asia (Sedivy et al., 2017), Azuki bean (*Vigna angularis* (Willd.) Ohwi & Ohashi) in West Asia (Lee, 2012), or common bean (*Phaseolus vulgaris* L.) in Mesoamerica (Lopez et al., 2013).

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In agriculture, the legume family (Fabaceae) is second in importance only to the cereals (Poaceae) (Maphosa & Jideani, 2017) based on an area of harvested land and total world production, with more than 650 million tons of grain legumes produced on 240 million ha as at 2011 (FAOSTAT, 2016). Grain legumes provide one-third of the plant protein and a similar proportion of the vegetable oil used for human consumption (Graham & Vance, 2003). The amino acid composition of legumes complements that of cereals and root crops (Wang et al., 2003), which may explain why the two groups were domesticated together (Gepts, 2004). Legumes are also essential forage crops in temperate and tropical regions (Singh et al., 2019), providing vital minerals to consumers (Grusak, 2002). The growth of agriculture-based economies worldwide depends on the sustained supply of high-quality seeds (World Bank, 2023). Among the pulses, cowpea, soybean, Bambara nut, and pigeon pea are the most common and important legume crops worldwide (Mahdi & Rahman, 2008). Legumes have been widely recognised as important sources for fortifying wheat-based traditional bakery products, such as biscuits (Tiwari et al., 2011). Legumes, when combined with cereals or grains, form a complete protein food (Boye et al., 2010). In developing countries, research attention is being paid to the better utilisation of legumes in addressing protein malnutrition and food security issues. Lima beans have been recognised as a potential supplement or even a substitute for the expensive soy meal and groundnut meal, which constitute the major portion of conventional protein sources (Kathirvel & Kumudha, 2011; Adebayo & Okoli, 2017). Legumes have been reported to be rich in secondary metabolites, such as polyphenols, alkaloids, and saponins, which are important defence compounds that protect the plant against herbivores and pathogens, and act as signalling molecules between the plant and its biotic environment (Ndakidemi & Dakora, 2003; Ku et al., 2020). These secondary metabolites have been suggested to protect the human consumer against certain cancer-related causal organisms (Madar & Stark, 2002) and offer benefits in the treatment of diabetes (Jenkins et al., 2003). The consumption of grain legumes has been shown to reduce blood cholesterol and exhibit a hypoglycemic effect. Other secondary compounds include antinutritional factors, such as trypsin inhibitors (Samtiya et al., 2020) and allergens (Spergel & Fiedler, 2001). Botanical insecticides, including powders, extracts, and essential oils, have been used for crop protection

for many years (Isman, 2006). The use of botanicals or plant-derived insecticides played a significant role in traditional storage methods in many parts of Africa (Bekele & Hassanali, 2001). Plants possess compounds such as terpenoids, alkaloids, and phenols that have been demonstrated to have various effects against insect pests, including toxicity, antifeedant, repellency, growth inhibition, and feeding deterrence (Divekar et al., 2022). Numerous studies have examined the insecticidal activity of powders, extracts, and essential oils from the families Apiaceae and Rosaceae (Kim & Ahn, 2001; Al-Jabr, 2006; Ebadollahi, 2011; Abdulhay, 2012; Lucca et al., 2015; Mohamed & Helaly, 2018). Various essential oils have been reported to play significant roles in protecting stored grains against insect infestations (Bakkali et al., 2008; Pérez et al., 2010). Products from edible spices, particularly oils, have been screened for their efficacy in suppressing stored product pests. They do not constitute a nuisance to farmers and the environment, as they do not add to the weight of the produce, unlike the use of powders, ash, or leaves (Ajayi and Lale, 2000). Monoterpenoids found in essential oils are known to be neurotoxins, and most of them are volatile, thus acting as fumigants (Tisserand & Young, 2013; Sattayakhom et al., 2023; Babarinde et al., 2025). The use of formulated essential oils (FEOs) as pellets for slow release is scantily reported in the literature (Etonihu et al., 2008). However, there is a need to explore the possibility of determining the efficacy of slow-release pelletised EEOs in forming slow-release pelletised edible essential oils (SRPEEOs) when used as a protectant for grains and seeds in storage. A pesticide formulation typically consists of an active ingredient and several inactive materials, known as adjuvants or additives. The primary purpose of additives is to increase the effectiveness of the active ingredient. Some common additives include spreaders, stickers, wetting agents, compatibility agents, and foaming agents. The formulated essential oils in the form of slow-release pelletised edible essential oil (SRPEEOs) were evaluated on their toxicity effect on the cowpea seed bruchid, *Callosobruchus maculatus* (F.) infesting five legume grains, cowpea (*Vigna unguiculata*, Lima beans (*Phaseolus lunatus*), soya beans (*Glycine max*), Bambara groundnut (*Vigna subterranea*), and pigeon pea (*Cajanus cajan*) under laboratory conditions in Lafia, Nasarawa State, Nigeria.

Materials and Methods

Experimental site

The study was carried out in the laboratory of the Department of Agronomy, Faculty of Agriculture (Shabu-Lafia Campus), Nasarawa State University, Keffi. Lafia, located at 08.330N and 08.320E, in North Central Nigeria (Lyam, 2000).

Insect culture

Infested cowpea seeds (var. Kananade) were used to raise the initial culture of the cowpea seed bruchid, *Callosobruchus maculatus*, in the laboratory. Infested cowpea seeds were raised inside a 500 mL transparent Kilner jar, covered with muslin cloth held in place with a rubber band, until the emergence of adults. The infested cowpea seeds were left for a period of 10 days, after which all living and dead insects were removed. The culture was then left to develop until the emergence of adults. Gravid adult female *C. maculatus* as described by Singh and Pandey (2001) that emerged from the initial stock were collected with the aid of a pooter and used to infest pristine cowpea seeds for new emergence. This was carried out for five consecutive generations until the insects had acclimatised and there were enough insect populations to commence the trials.

Source of legume grains and treatment

The legumes, which comprise cowpea (*Vigna unguiculata*), Lima bean (*Phaseolus lunatus*), soya bean (*Glycine max*), Bambara nut (*Vigna subterranea*), and pigeon pea (*Cajanus cajan*), were purchased from Lafia main market, Lafia, Nasarawa State. The legume seeds were carefully sorted, and only pristine seeds were collected from the lots. The legume seeds were transferred into a double-layered polythene bag, and the opening was sealed with a tight rubber band to prevent water seepage in the freezer and infestation by cowpea seed bruchid. The polythene bag containing the legume seeds was kept in a freezer for five days. This process was carried out to disinfest the legume seeds of any eggs or larvae that may have been harboured. After five days of cold treatment, the seeds were placed and spread on a polythene sheet on a laboratory bench, covered with screen gauze, and held in place at the edges with heavy stones, allowing them to equilibrate to atmospheric laboratory conditions for three days. Thereafter, the legume seeds were packed into disinfested 0.5-litre Kilner jars and kept on the laboratory bench, following the method of Lale & Ajayi (1999) and Ajayi & Lale (2000-2001), until ready for use.

Source of Slow Release Pelletized Edible Essential Oils (SRPEEOs) used for the study

The slow-release, pelletised edible essential oils from clove (*Syzygium aromaticum*), ginger (*Zingiber officinale*), and West African black pepper (*Piper guineense*) were obtained from already formulated pellets in the Pharmaceutical Technology and Raw Materials Department of the National Institute for Pharmaceutical Research, Abuja.

Experimental procedure and design

The ambient average temperature and relative humidity of the experimental laboratory were 32 °C and 65% relative humidity, respectively, throughout the trial period.

Determination of lethal concentration (LC₅₀) of the slow-release pelletised edible essential oils on adult *C. maculatus* infesting legumes

The determination of the Probit analysis for the slow-release pelletised edible essential oils was carried out using 3-day-old adult *C. maculatus* in a 100 mL Kilner jar and treated with 0.0 (control without pellets), 0.25, 0.50, 0.75, and 1.0 g each of the slow-release pelletised edible essential oils on 5 g legume grains. Adult mortality was recorded 24 hours after infestation, using the method described by Ajayi & Lale (2000-2001) and Ajelara et al. (2018). Any adult *C. maculatus* was considered dead if unresponsive to a probe by gently touching it with a fine thistle brush (Owolabi et al., 2014). The experiment was laid out in a completely randomised design, and each treatment was replicated three times.

Statistical analysis

All percentage data obtained were arc-sine transformed before being subjected to two-way ANOVA. The differences between treatment means were separated using a Probit test to determine the LC₅₀ at a $P \leq 0.05$ probability level, as analysed with STATISTIX 10 Vision (2018) analytical package.

Results and Discussion

Toxicity effects of three slow-release pelletised edible essential oils against *Callosobruchus maculatus* infesting Bambara nut

The toxic effects of clove, West African black pepper (WABP), and ginger slow-release pelletised edible essential oils (SRPEEOs) against the adult seed bruchid, *C. maculatus*, are presented in Figures 1, 2, and 3. The results showed that the essential pellets from ginger, with an LC₅₀ of 0.35 g/5 g, were the most toxic, followed by WABP pellets at an LC₅₀ of 0.36 g/5

g. In contrast, the lowest mortality of the adult *C. maculatus* was recorded from clove pellets, with an LC₅₀ of 0.41 g/5 g, which had significantly lesser contact toxicity.

The Fiducial confidence level limits between the three SRPEEOs were 0.349-0.422, 0.400-0.492, and 0.442-0.609, respectively, for clove, WABP, and ginger slow-release pelletised edible essential oils. The regression analysis in Figures 1, 2, and 3 inveterate that there is an increase in toxicity effect with an increase in concentration among the three SRPEEOs and the r² regression analysis showed that the interaction between the concentrations and the use of the SRPEEOs as contact toxicity on adult *C. maculatus* were significant for clove, 0.929; WABP, 0.953 and ginger, 0.727, respectively.

Toxicity effects of three slow-release pelletised edible essential oils against *Callosobruchus maculatus* infesting Soya bean.

The contact toxic effect of ginger, clove, and WABP slow-release pelletised edible essential oils

(SRPEEOs) against the adult seed bruchid, *C. maculatus*, is presented in Figures 4, 5, and 6. The results showed that the essential pellets from WABP were the most toxic, with an LC₅₀ of 0.27 g/5 g seed, followed by clove pellets at an LC₅₀ of 0.29 g/5 g. In contrast, the lowest mortality of the adult *C. maculatus* was recorded with ginger pellets, which had an LC₅₀ of 0.34 g/5 g, indicating less contact toxicity.

The Fiducial confidence level limits between the three essential SRPEEOs were 0.414-0.522, 0.432-0.697, and 0.412-0.687, respectively, for ginger, clove, and WABP slow-release pelletised edible essential oils. The regression analysis in Figures 4, 5, and 6 indicates that there is an increase in toxicity effect with increasing concentration among the three SRPEEOs. The r² regression analysis showed that the interaction between concentrations and the use of SRPEEOs as contact toxicity on adult *C. maculatus* was significant for ginger (0.932), clove (0.904), and WABP (0.896), respectively.

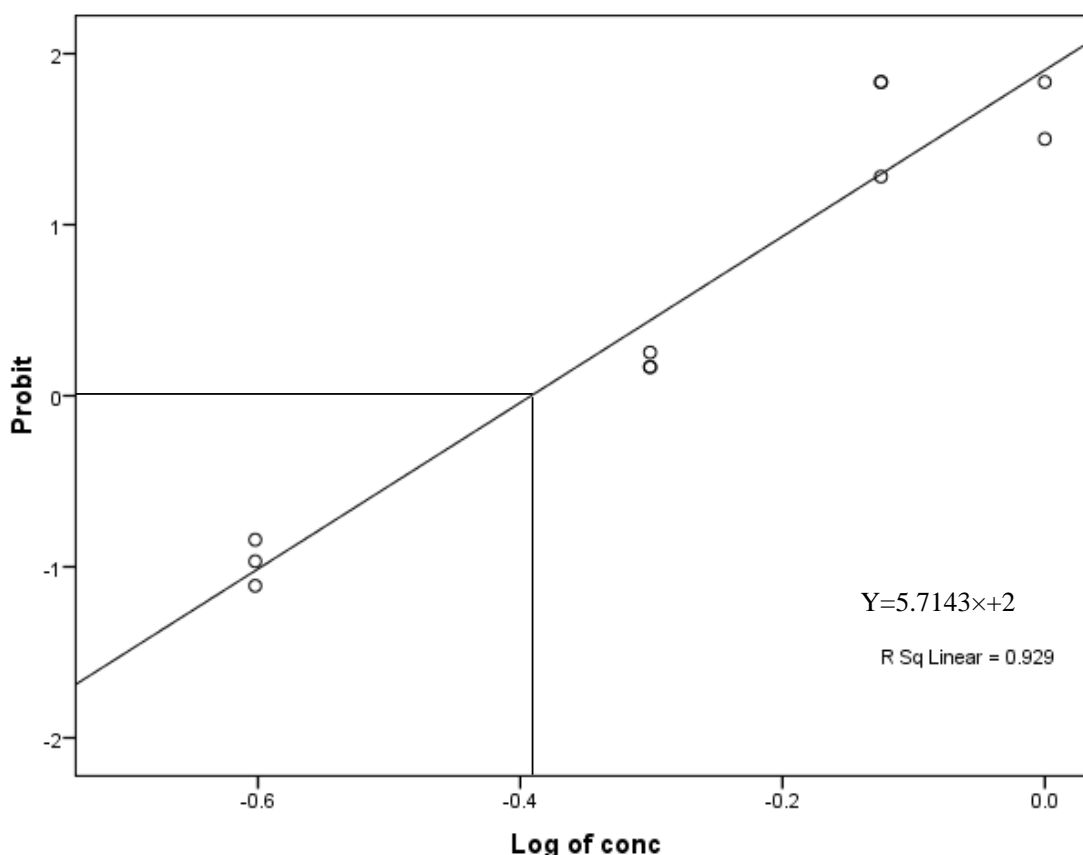


Figure 1: Toxicity of Clove Slow-Release Pelletised Edible Essential Oils on Bambara nut [(Log. Conc.-.383, LC₅₀=0.41); Fiducial Limits (Confidential Interval)]: 0.349 - 0.422 g/5 g seed.

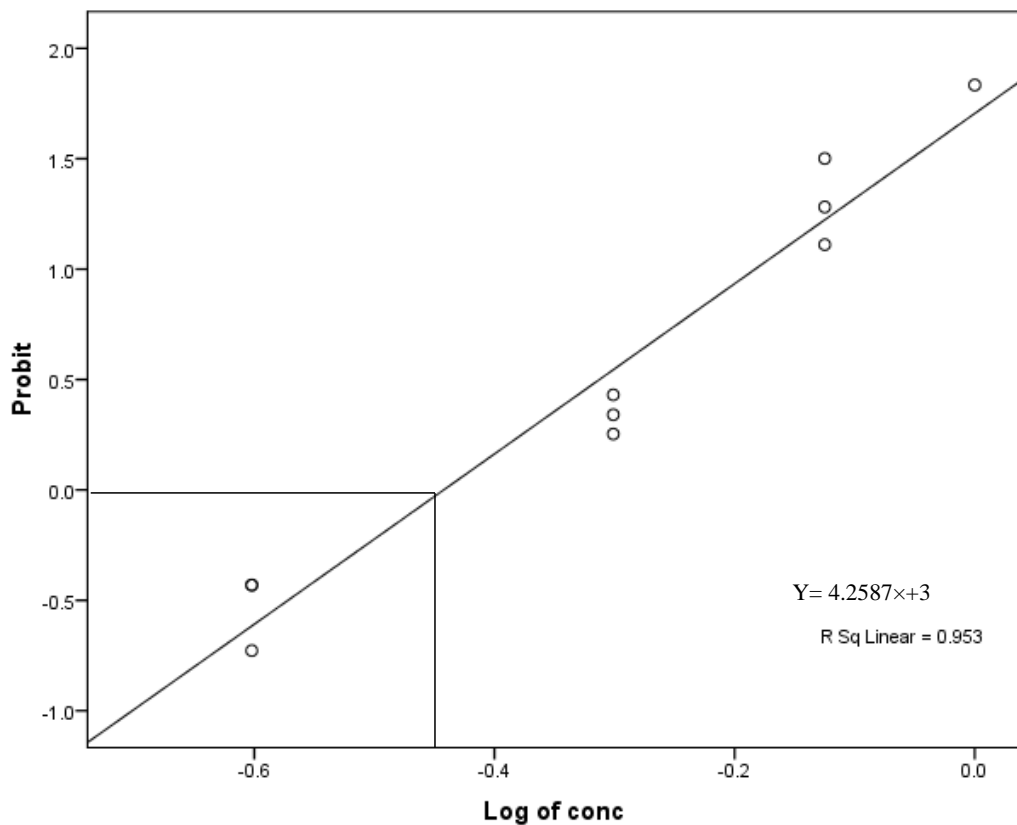


Figure 2: Toxicity of WABP Slow-Release Pelletised Edible Essential oil on Bambaranut (Log. Conc.-.442, LC₅₀=0.36); Fiducial Limits (Confidential Interval): 0.400-0.492 g/5g seed.

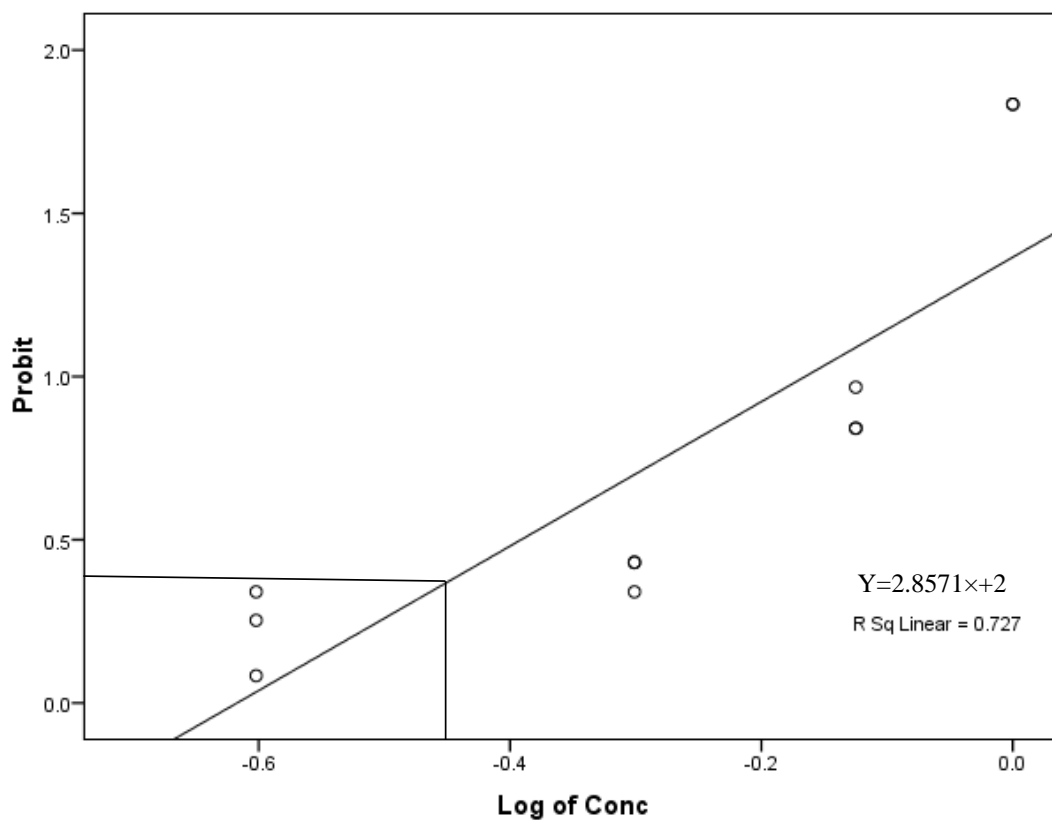


Figure 3: Toxicity of Ginger Slow-Release Pelletised Edible Essential oil on Bambara nut (Log.Conc.-.450, LC₅₀ = 0.35); Fiducial Limits (Confidential Interval): 0.442-0.609 g/5 g seed

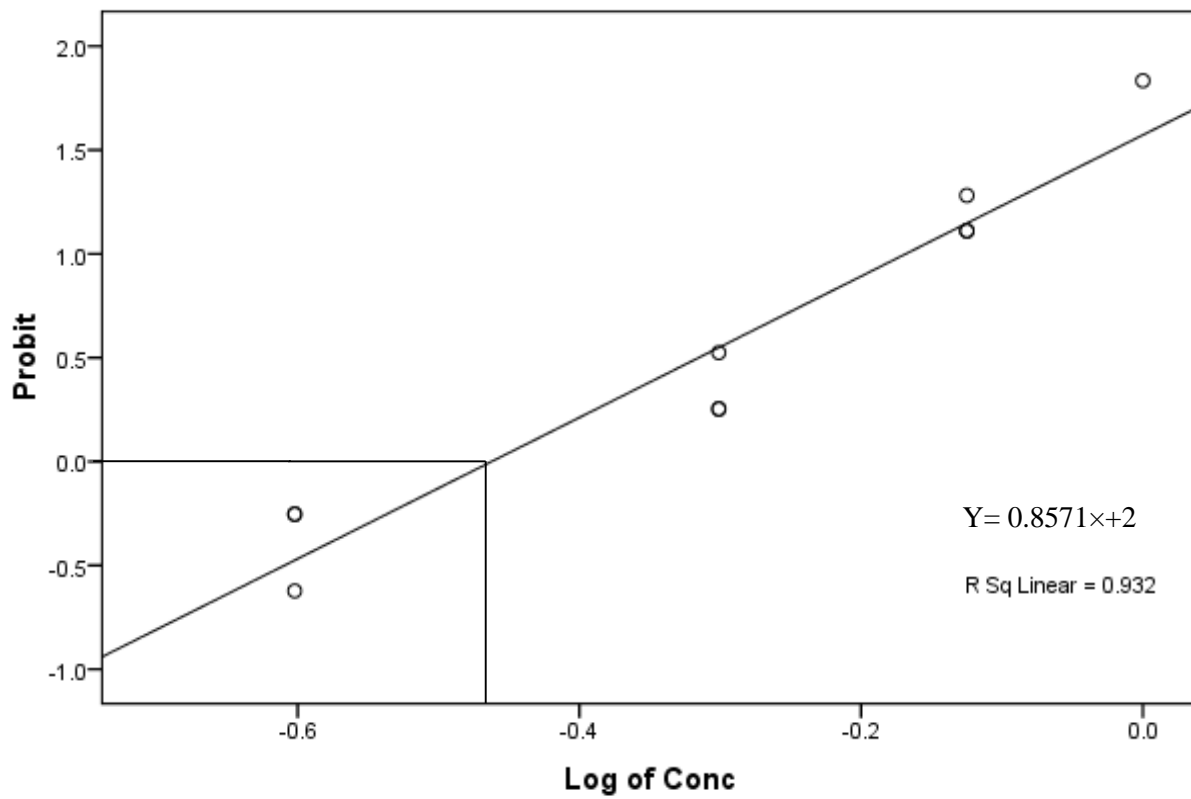


Figure 4. Toxicity of Ginger Slow-Release Pelletised Edible Essential Oil on Soya bean (Log. Conc.-.463, $LC_{50}=0.34$); Fiducial Limits (Confidential Interval): 0.416-0.522 g/5 g seed.

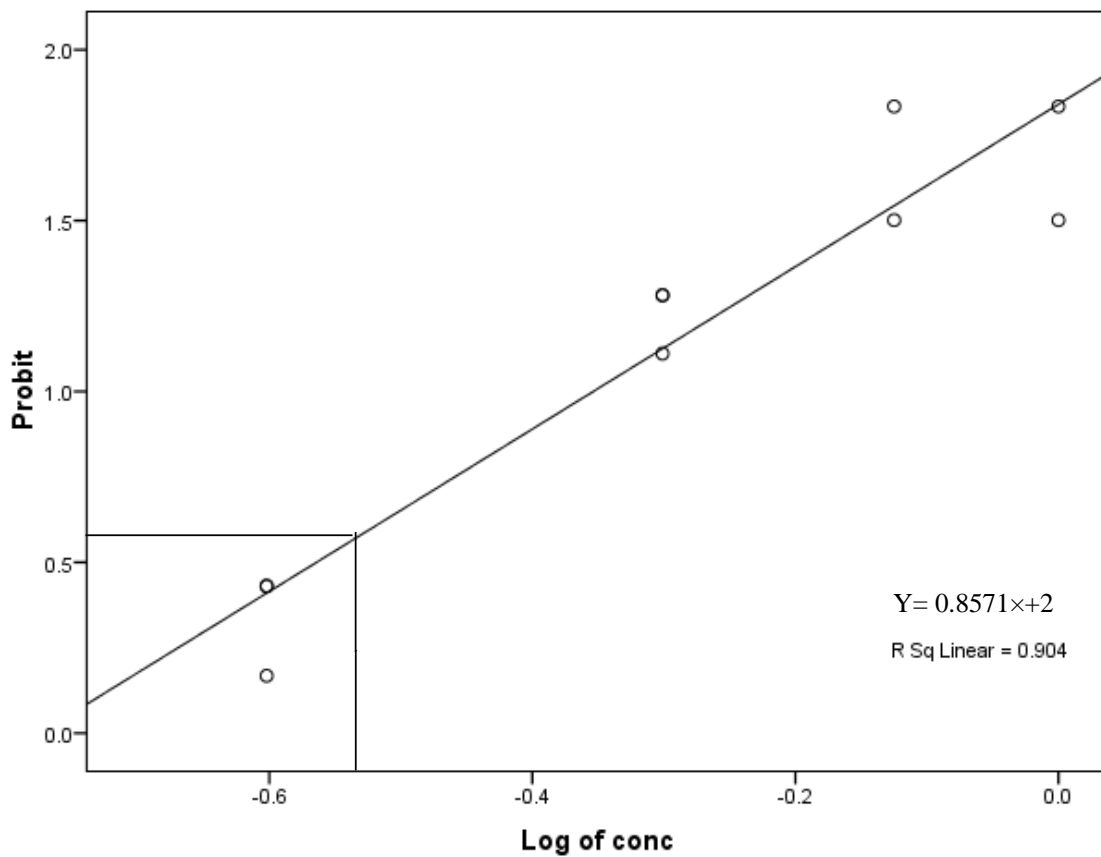


Figure 5: Toxicity of Clove Slow-Release Pelletised Edible Essential Oil on Soya bean (Log. Conc.-.532, $LC_{50}=0.29$); Fiducial Limits (Confidential Interval): 0.697-0.432 g/5 g seed.

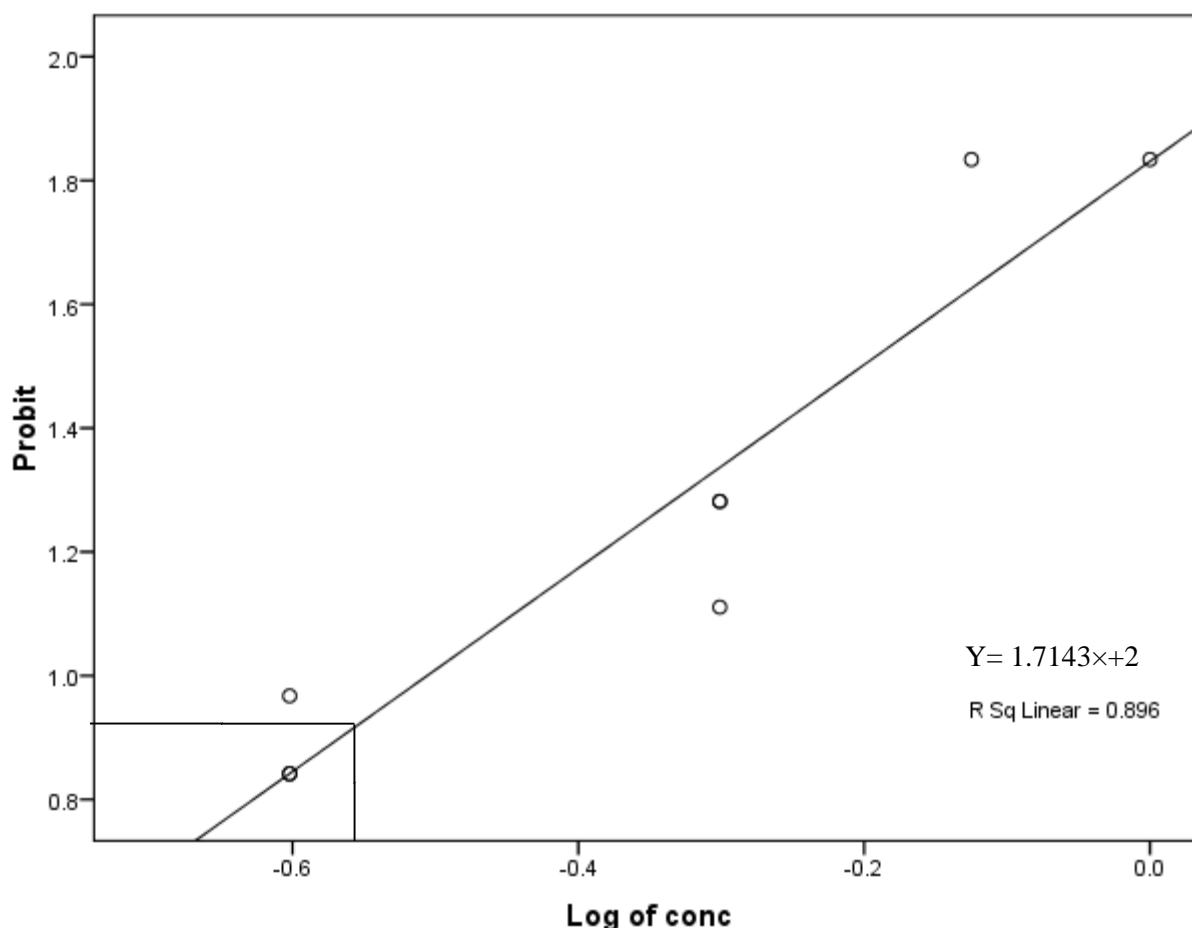


Figure 6: Toxicity of WABP Slow-Release Pelletised Edible Essential Oil on Soya bean (Log. Conc.-.560, LC₅₀=0.27); Fiducial Limits (Confidential Interval): 0.877- 0.477 g/5 g seed.

Toxicity effects of three slow-release pelletised edible essential oils against *Callosobruchus maculatus* infesting Pigeon pea.

The contact toxicity effect of clove, ginger, and WABP slow-release pelletised edible essential oils (SRPEEOs) against the adult seed bruchid, *C. maculatus*, is presented in Figures 7, 8, and 9. The results showed that the essential pellets from WABP were the most toxic, with an LC₅₀ of 0.25 g/5 g seed, followed by ginger pellets at an LC₅₀ of 0.27 g/5 g. Meanwhile, the lowest mortality of the adult *C. maculatus* was recorded with clove pellets, which had an LC₅₀ of 0.34, indicating less contact toxicity.

The Fiducial confidence level limits between the three essential pellets were 0.416-0.522, 0.481-0.675, and 0.587-0.748, respectively, for clove, ginger, and WABP. The regression analysis is presented in Figures 7, 8, and 9. Indicates that there was an increase in toxicity effect with an increase in concentration among the three SRPEEOs, and the r² regression analysis showed that the interaction between the concentrations and the use of the SRPEEOs as contact toxicity on adult *C. maculatus* was significant for clove, 0.932; ginger, 0.8; and WABP, 0.818, respectively.

Toxicity effects of three slow-release pelletised edible essential oils against *Callosobruchus maculatus* infesting Zebra bean.

The contact toxicity effect of WABP, ginger, and clove slow-release pelletised edible essential oils (SRPEEOs) against the adult seed bruchid, *C. maculatus*, is presented in Figures 10, 11, and 12. The results showed that the essential pellets from clove were the most toxic, given an LC₅₀ of 0.30 g/5 g seed, followed by ginger pellets at an LC₅₀ of 0.31 g/5 g; while the lowest mortality of the adult *C. maculatus* was recorded with clove WABP pellets, recording an LC₅₀ of 0.35 g/5 g, signifying less contact toxicity.

The Fiducial confidence level limits between the three essential SRPEEOs were 0.446-0.548, 0.434-0.609, and 0.492-0.677, respectively, for WABP, ginger, and clove. The regression analysis in Figures 10, 11, and 12 inveterate that there is an increase in toxicity effect with an increase in concentration among the three SRPEEOs and the r² regression analysis showed that the interaction between the concentrations and the use of the SRPEEOs as contact toxicity on adult *C. maculatus* were significant for WABP, 0.916; ginger, 0.892 and clove, 0.806, respectively.

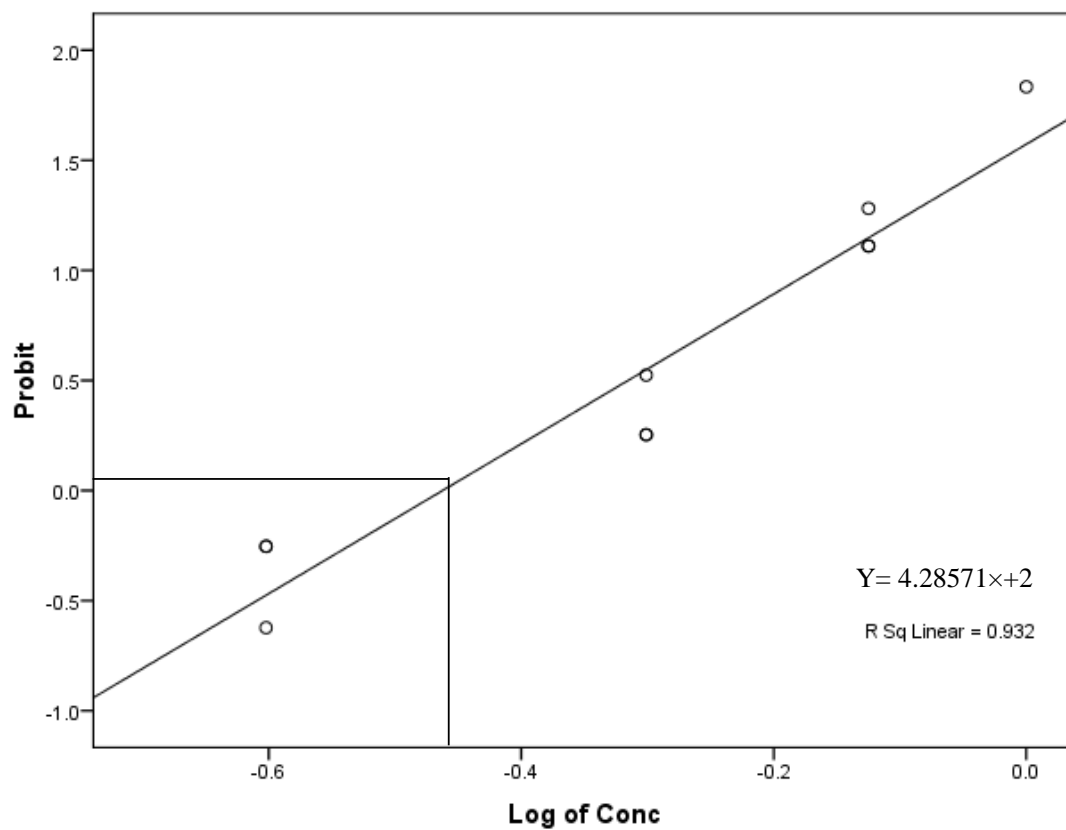


Figure 7: Toxicity of Clove Slow-Release Pelletised Edible Essential Oil on Pigeon pea (Log. Conc. -.463, $LC_{50} = 0.34$); Fiducial Limits (Confidential Interval): 0.416-0.522 g/5 g seed

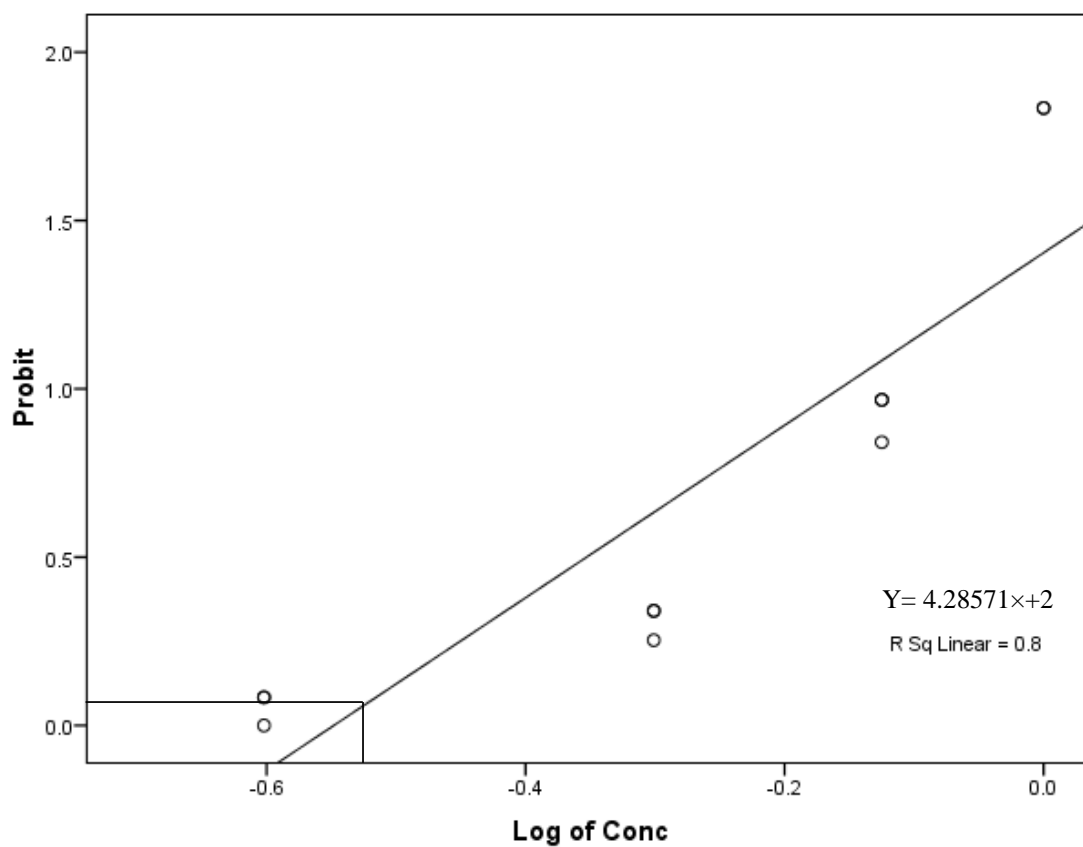


Figure 8: Toxicity of Ginger Slow-Release Pelletised Edible Essential Oil on Pigeon pea (Log. Conc. -.558, $LC_{50} = 0.27$); Fiducial Limits (Confidential Interval): 0.481-0.675 g/5 g seed

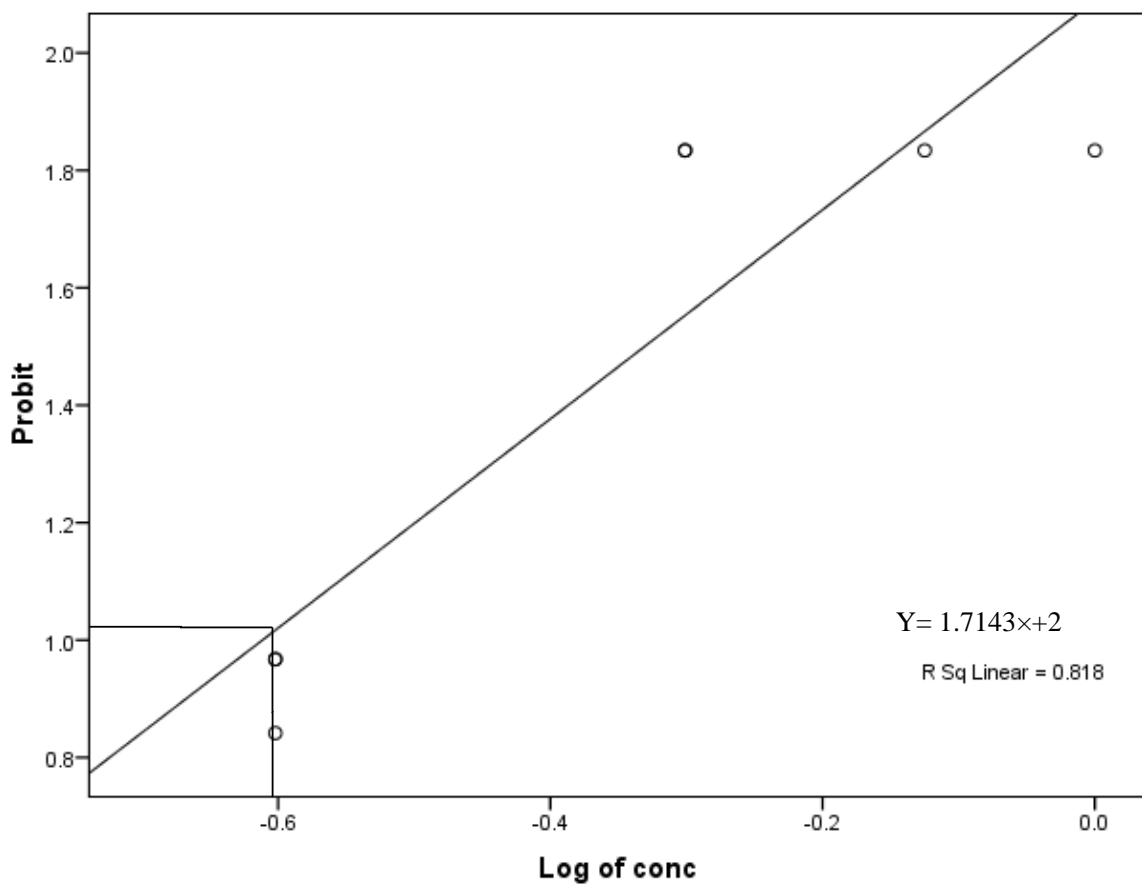


Figure 9: Toxicity of WABP Slow-Release Edible Essential Pellet Oil on Pigeon pea (Log. Conc.-.601, $LC_{50} = 0.25$); Fiducial Limits (Confidential Interval): 0.587-0.748 g/5 g seed.

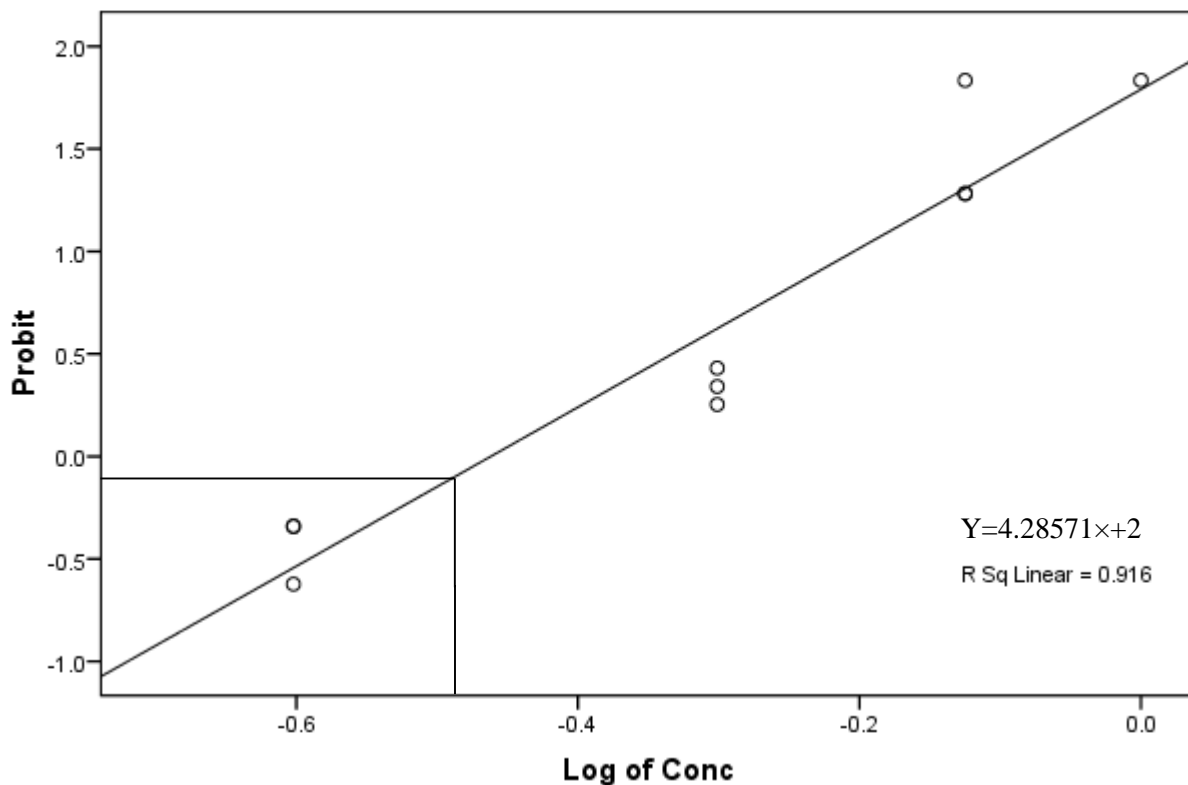


Figure 10: Toxicity of WABP Slow-Release Edible Essential Pellet Oil on Zebra bean (Log.Conc.-.491, $LC_{50} = 0.35$); Fiducial Limits (Confidential Interval): 0.446-0.548g/5 g seed

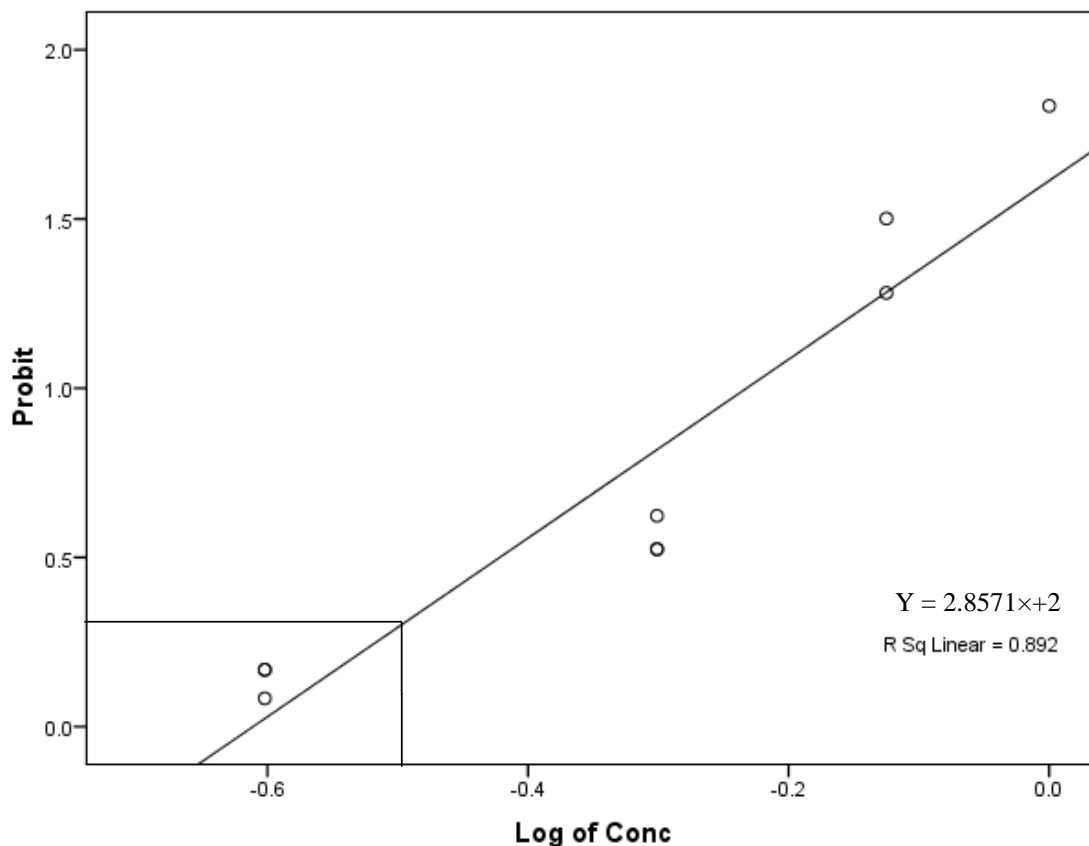


Figure 11: Toxicity of Ginger Slow-Release Pelletised Edible Essential Oil on Zebra bean (Log. Conc. -.499, $LC_{50} = 0.32$); Fiducial Limits (Confidential Interval): 0.434-0.609 g/5 g seed.

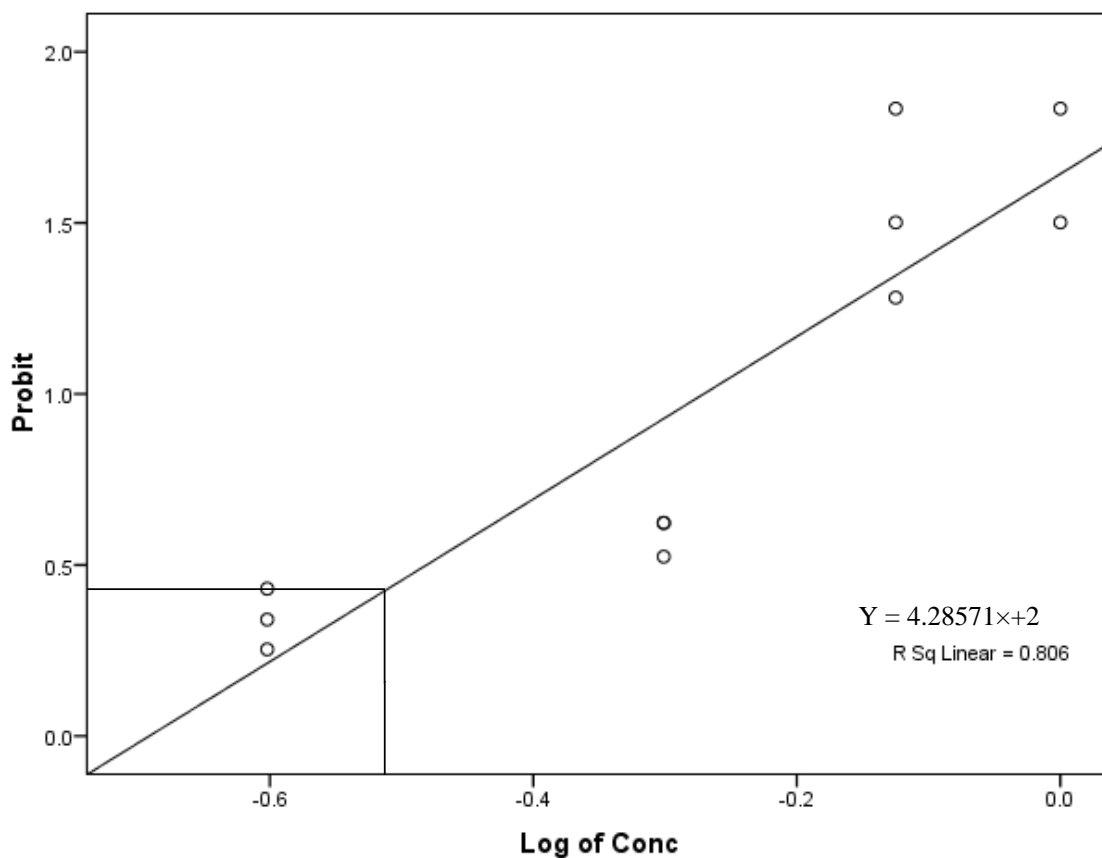


Figure 12: Toxicity of Clove Slow-Release Pelletised Edible Essential Oil on Zebra bean (Log. Conc. -.5.10, $LC_{50}=0.30$); Fiducial Limits (Confidential Interval): 0.492-0.677 g/5 g seed.

Toxicity effects of three slow-release pelletised edible essential oils against *Callosobruchus maculatus* infesting Lima bean.

The contact toxicity effect of ginger, WABP, and clove slow-release pelletised edible essential oils (SRPEEOs) against the adult seed bruchid, *C. maculatus*, is presented in Figures 13, 14, and 15. The results showed that the essential pellets from clove were the most toxic, given an LC₅₀ of 0.29 g/5 g seed, followed by WABP pellets at an LC₅₀ of 0.33; while the lowest mortality of the adult *C. maculatus* was recorded with ginger pellets, recording an LC₅₀ of 0.34 g/5 g, signifying less contact toxicity

The Fiducial confidence level limits between the three essential oils were 0.438-0.561, 0.423-0.558, and 0.427-0.632, respectively, for ginger, WABP, and clove. The regression analysis in Figures 13, 14, and 15 inveterate that there is an increase in toxicity effect with an increase in concentration among the three SRPEEOs and the r² regression analysis showed that the interaction between the concentrations and the use of the SRPEEOs as contact toxicity on adult *C. maculatus* were significant for ginger, 0.836 WABP, 0.795; and clove, 0.658, respectively.

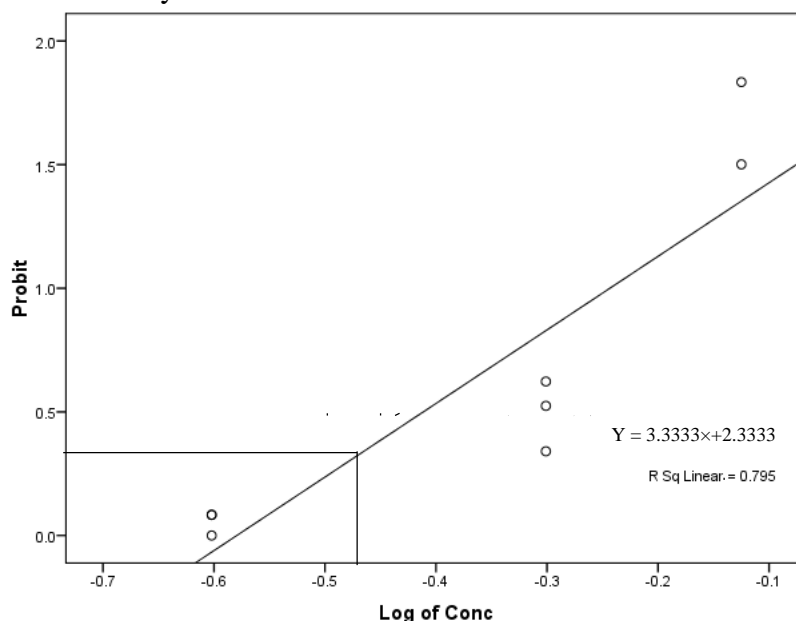


Figure 13: Toxicity of Ginger Slow-Release Pelletised Edible Essential Oil on Lima bean (Log. Conc. -.481, LC₅₀ = 0.33); Fiducial Limits (Confidential Interval): 0.423-0.558 g/5 g seed.

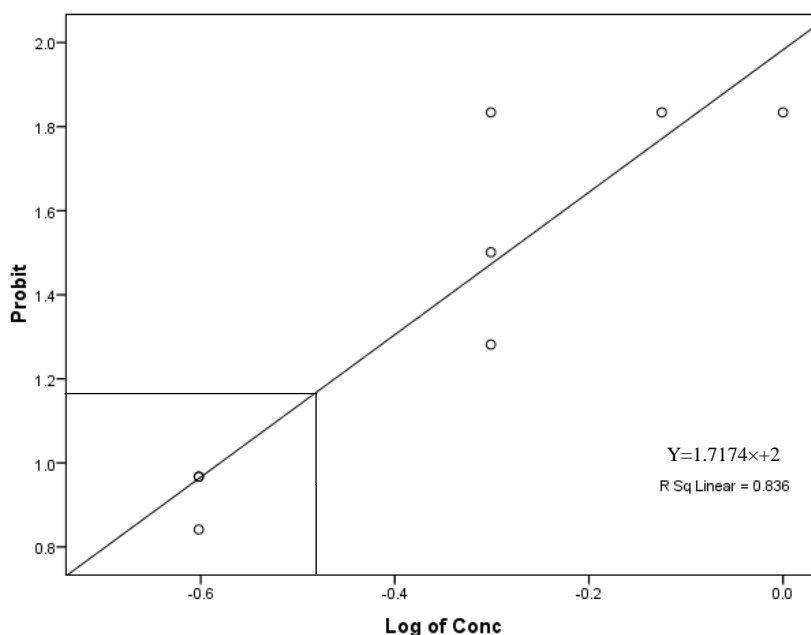


Figure 14: Toxicity of WABP Slow-Release Pelletised Edible Essential Oil on Lima bean (Log. Conc. -.471, LC₅₀ = 0.34); Fiducial Limits (Confidential Interval): 0.438-0.561 g/5 g seed

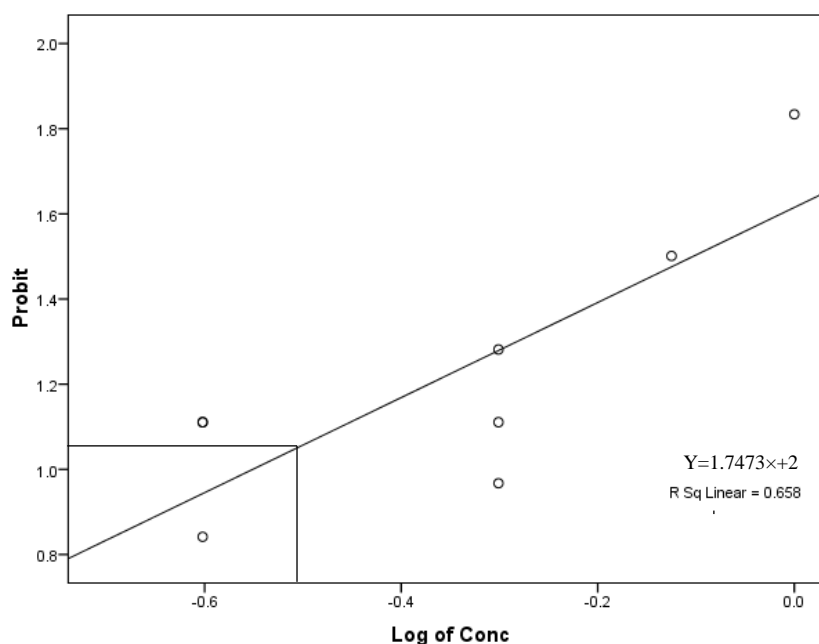


Figure 15: Toxicity of Clove Slow-Release Pelletised Edible Essential Oil on Lima bean (Log. Conc. -.532, $LC_{50} = 0.29$); Fiducial Limits (Confidential Interval): 0.427-0.632 g/5 g seed.

In toxicity evaluations involving products derived from other plant sources, it was reported that insect mortality was attributed to the biologically active components of the plant products. The results in Figures 1 to 12 indicated that the SRPEEOs from clove, WABP, and ginger were able to cause mortality of adult *C. maculatus* at very low concentrations (LC_{50}). The results obtained from this study are in concordance with those of Ajayi & Lale (2000-2001), who reported the toxicity of clove, WABP, and ginger oils on *C. maculatus* at very low LC_{50} values. The propensity of the SRPEEOs to cause mortality in the adult *C. maculatus* could be due to the constituents in the plant product pellets (Raja et al., 2001). The results obtained from this study are in general agreement with the reports of some workers, such as Oparaëke and Dike (2005), Adedire et al. (2011), and Mukanga et al. (2010), who observed that certain botanicals are effectively toxic against storage pests, including *C. maculatus*. The resultant mortality rate of *C. maculatus* in this research could be attributed to the harmful effects of the chemicals in the tested plant products. Although all botanical plants showed promise as insecticides, their toxicity against *C. maculatus* varied, likely due to differences in their phytochemical content.

Conclusion

The findings from this study indicate that the use of botanical extracts for protecting stored produce is highly significant in reducing insect pest damage, which small-scale farmers commonly experience. The

present study demonstrated that four legume varieties were susceptible to varying degrees of damage by cowpea seed bruchid *C. maculatus*. Cowpea, Zebra bean, Bambara nut, and Pigeon pea were susceptible. The soybean and lima bean showed resistance and high resistance, respectively. In the toxicity test of SRPEEO from WABP, it was more effective, as it recorded the highest mortality compared to clove and ginger.

Recommendation

It is recommended that SRPEEOs be applied at a rate of 1 g/5 g (100 g/50 kg bag) to confer protection on legumes.

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