International Journal of Plant & Soil Science

33(23): 226-245, 2021; Article no.IJPSS.73108
ISSN: 2320-7035

Assessing the Toxic Effects of Insecticides on Honey Bees in the West Gonja District of the Savannah Region of Ghana

Joseph Lambon [a*] and Abdul-Rahaman Issahaku [b]

[a] Bagabaga College of Education, P .O. Box 35, Tamale, Ghana.
[b] Department of Environment, Water and Waste Engineering, University for Development Studies, P. O. Box 1350, Tamale, Ghana.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2021/v33i2330737
Editor(s):
(1) Dr. Ahmed Medhat Mohamed Al-Naggar, Cairo University, Egypt.
Reviewers:
(1) Habiba Usman Aliyu, Federal University of Kashere, Nigeria.
(2) Terd Disayathanoowat, Chiang Mai university, Thailand.
(3) Bamphitlhi Tiroesele, Botswana University of Agriculture and Natural, Botswana.
Complete Peer review History, details of the editor(s), Reviewers and additional Reviewers are available in this link: https://www.sdiarticle5.com/review-history/73108

Original Research Article

Received 04 September 2021
Accepted 09 November 2021
Published 22 November 2021

ABSTRACT

Aims: To examine the toxic effects of insecticides on bees in farming communities in the Savannah Region of Ghana.
Study Design: The study employed five different doses of insecticides to 3 groups of 10 honey bees in each group using 3 types of insecticides. The number of dead bees were registered and used for the estimation of LC_{50} of each insecticide.
Place and Duration of Study: The experiment was conducted at Damongo Agricultural Training College, Ghana, between August 2019 and September 2019.
Methodology: We collected bees from farms in the West Gonja District of the Savannah Region of Ghana. Controller Super 2.5 EC, Pyrinex 48 EC and Golan SL were insecticides used for the experiment. Live adult bees were randomly obtained from beehives at 2:00 am from the farms when the bees were not aggressive. The bees were collected by hand and placed into a perforated plastic container and transported from the site of collection to the experimental site. They were allowed to acclimatize to the experimental conditions for a period of three hours under room temperature of 24 °C and a relative humidity of 49 percent throughout the study.

**Corresponding author: E-mail: lanbonjoseph@yahoo.com, lambonjoseph7@gmail.com;*

Results: Mortalities were recorded 10 minutes after administering the concentrations and thereafter at every 10 minutes continuously till 60 minutes. The LC_{50} was calculated using $\frac{(\log LC_{84} - \log LC_{16})}{\sqrt{2N}}$ Where N is the number of honey bees in each group

Controller Supper 2.5 EC at a concentration of 6.7 ml/L gave the highest mean mortality (10 bees) at the 50th minute while the concentration of 1.0 ml/L gave the lowest mean mortality (0.0 bees) in the same 50th minute.

Conclusion: The LC_{50} for the three insecticides used were within the recommended concentrations provided by the Environmental Protection Agency of Ghana. The overall mortalities occurred when honey bees were exposed to different concentrations of all the three insecticides.

Keywords: Insecticide; mortality; concentration; pollinators; lethal dose.

1. INTRODUCTION

There are several uses of insecticides in modern times. Insecticides are used during flowering and or preservation of crops for consumption. This is important to increase agriculture production and income of farmers [1]. Insecticides are important in controlling pest in crop production. Insecticides are used directly in crop production and are deposited on the plant parts. The deposits get in contact with honey bees when they visit the plants for juice [2]. This makes insecticides harmful to bees and affects human lives [3, 4]. Literature search indicates a link between insecticide presence in food stuffs and illnesses such as immune deficiency, cancers and mental disorders. Some insecticides contain active ingredients which can affect the endocrine system in humans leading to nervous systems disorders and asthma. --. Insecticides also affect the skin, conjunctiva, gastrointestinal tract and the lungs. They also affect non target species as well as cause reduction in the shells of eggs of birds. The negative effects of insecticides on honey bees have received global attention. The food and agricultural organization (FAO) of the United Nations has recommended that effective use of insecticides and good management of pollinators was inevitable to increase crop production. They therefore advocated the use of integrated pest management (IPM) in the control of pest and diseases [5].

The differential use of insecticides depends on their toxicity level, registration by Ghana's Environmental Protection Agency (EPA) and the availability of the pesticide in the market. For this reason, all agrochemicals including insecticides undergo evaluation by the EPA of Ghana before being used in the field by the farmers. Evaluation is very important to ensure the acceptability of the active substance to honey bees. The free trade policy in Ghana however has opened up the boarders of Ghana for insecticides to be imported and used by farmers without approval from the EPA of Ghana.

[6] observed that honey bees play a crucial role in pollination and food production in the form of honey as well as providing many other important products such as bee wax and proppolis which has many crucial benefits to humans. In modern times, honey bees' population is reducing due to the use of pesticides. [7].

1.1 Problem Statement

The desire to increase crop production encouraged the quest to control pest and diseases using pesticides by farmers [8] in the West Gonja District. Commercial farmers in particular have relied on pesticides to increase acreage of their farms. Yet, the use of pesticides impact negatively on honey bees' population directly when ingested. Pesticides weaken the immune system of bees and leads to death [9]. This is because, some of these pesticides are systemic or contact based. If they are consumed or get into contact with the insecticides, they penetrate into their immune system thereby resulting in their death.

1.2 Justification for the Study

The abundance and diversity of bees is a determining factor in environmental degradation. Bees dictate the diversity and abundance of plant species in an ecosystem. Excessive use of agro-chemicals such as, insecticides endangers the survival of honey bees [10, 11]. Measuring the toxicity level response of honey bees to insecticides serves as the basis for accessing its impact on crop production [12]. The toxicity of an

insecticide can be determined by finding the acute contact toxicity value (LD_{50}). This is the contact level that can cause a mortality of half of the target population [13]. Compared to other insect species, honey bees lack effective defense system against toxins [4]. This difference of honey bees to other insects make them more vulnerable to insecticides compared to other insect species. For this reason, the study sought to evaluate the impact of insecticide use on honey bees in the West Gonja Municipality in the Savanna Region of Ghana.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out at Damongo Agricultural Training College in the West Gonja District of the Savannah Region of Ghana. The District shares boundaries with Wa East District in the North West, Central Gonja District in the south, Sawla-Tuna-Kalba Districts in the West, North Gonja District in the East and Mamprugu Mogduli District of the North East Region in the North (Fig. 1).

The West Gonja District lies between latitudes 8º32'N and 10 º2'N, and longitudes 1º5'W and 2º58'W. Damongo town lies within latitudes 9° 5' 0" N and longitudes 1° 49' 0" W whilst the Larabanga town lies latitudes 9° 13' 0" N and longitudes 1° 51' 0" W.

The district covers a land area of about 8,352 square. km representing about 12 percent of the total land area of the Northern Region. The district has two forest reserves and these are the Mole National Park and Kenikeni Forest Reserve both having a rich array of flora and fauna .The Mole National Park which is located about 30 km west of Damongo, is the largest in the country and occupy a land area of 3800 km.^{2} It serves as home for various wildlife species including honeybees. It has an altitude of between 150 – 200 meters above sea level and a generally undulating terrain with the Damongo Escarpment as the only high land.

The area generally records high temperatures with the mean monthly temperature being 27°C. Humidity is very low with the average being 50 percent [14].The natural vegetation is Guinea Savanna with scattered trees except in most valleys where isolated woodland or forest are found. Most trees are deciduous shedding their leaves during the dry season to conserve water [14]. The area is suitable for cultivation of crops such as millet, sorghum, maize, groundnuts, vegetables and root crops.

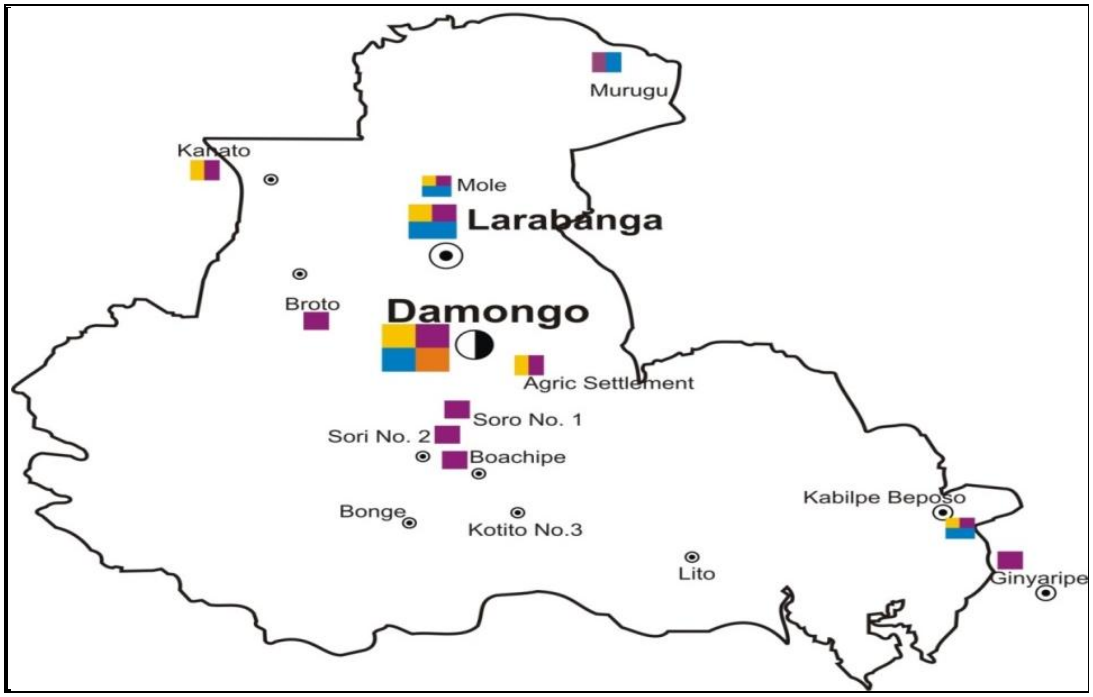

Fig. 1. Map of the West Gonja District showing Damongo and Larabanga

2.2 Sampling

Live adult bees were obtained from beehives by wearing a mask at 2:00 am (in the morning of the experiment) when the bees were outside the hive and not aggressive. The bees were obtained from the Damongo Agricultural Training College in the West Gonja District at night when they are dormant. A perforated plastic container with a lid was used to collect the bees at random by fetching the bees with the hand into the container. Hand gloves were worn to avoid stinking. Also wellington boots, coveralls and goggles were worn for protection. The bees were collected and used for the study from August to September, 2019.

2.3 Materials

Materials used for the study were 0.20 cm nylon mesh, 3.81 cm nails, 1.27 cm plywood, and 11.43 cm by 11.43 cm square test cages, wellington boots, overall coat, mask, gloves, torchlight, pipe-borne water and distilled water. A clinical syringe and one litre calibrated spraying bottle were obtained from the Veterinary Department at the Damongo Agricultural Training College. Three different brands of insecticides namely Controller Super 2.5 EC, Pyrinex 48 EC and Golan SL were purchased from Wumpini Agrochemical Limited in Tamale that gets its supply from K. Badu Agrochemical Company Limited in Kumasi.

2.4 Construction of Test Cages

Sixteen wooden cages were constructed to carry out the study, but only twelve of them were used for the study. Cages were constructed using 3.81 cm nails with the assistance of a professional carpenter from the Damongo Agricultural Training College. Each cage had six sides and measured 11.43 cm by 11.43 cm square. The four sides of the cages were covered with 1.27 cm thick plywood and the top covered with 0.2 cm nylon mesh to provide enough ventilation for the bees and proper vision while the bottom was left opened.

The bees were collected by hand and placed into a perforated plastic container and were immediately transported from the site of collection to the experimental site. The twelve wooden cages were put into four groups with each group containing three cages. The open end of each cage was placed on a flat floor and twelve to fifteen bees were released from the perforated plastic container into each cage through the open side by gently lifting it. This was done with the aid of torchlight. They were left to acclimatize to the experimental conditions for a period of three hours. They were maintained under standard room conditions (natural darkness) at a room temperature of 24 °C and a relative humidity of 49 percent throughout the study. In the morning of the experiment, the bees were reduced to ten in each cage after the dead and moribund ones were removed from each cage and where necessary, replacements were made.

Handling procedures including preparation of concentrations, administration, observations and recording were conducted under day light. All collections and experimentations were done in August and September to coincide with the right environmental conditions for field applications.

2.5 Preparation of Concentrations and Administration

The method used for the calculations and preparation of the various concentrations of the insecticides and application was according to the recommended application rates on the labels on the various plastic bottles by the manufacturers of the various insecticides for field application. A clinical syringe was used to measure the calculated concentrations of each insecticide into a one litre calibrated spraying bottle containing 200 ml of water as a carrier. The solution in the litre calibrated spraying bottle was topped up to one litre mark. The same procedure was used to prepare all the other concentrations (Table 1).

Table 1. Concentrations of insecticides used for the study

Insecticide Type	Active ingredient	Concentration ml/L				
Controller Super 2.5 EC	Fenitrothion 50% (W/V)	1.0	1.6	3.3	5.0	6.7
Pyrinex 48 EC	Chrorpyrifos Ethyl (EC)	0.5	1.0	1.5	2.0	2.5
Golan SL	Acetamiprid 200g (Soluble liquid)	0.5	1.0	1.5	2.0	2.5

Distilled water was used as the control..

2.6 Application of Doses

Each formulated insecticide was gently sprayed on top of each test cage containing ten bees. To ensure uniformity, 10ml was sprayed on each test cage for five seconds. This was repeated in the other two cages to cover the set of three cages for each insecticide. It The spraying bottle was then immediately rinsed several times with clean water after which the process was repeated for the other two insecticide brands. One litre of distilled water was then poured into a well washed and rinsed spraying bottle and used to spray a set of three other test cages containing ten bees each to serve as control. This was repeated for the remaining concentrations on different days [15].

2.7 Test duration and Observations

The test lasted for 90 minutes for each concentration administered with their respective controls but recordings ended at the 60th minute since it was observed that the control mortality started occurring after the sixtieth minute onwards [16].

2.8 Determination of Recommended Formulated Values

Using the data, the recommended application rates of the various insecticides were determined using Microsoft Excel. Pearson's correlation analysis was conducted to investigate relationships between different concentrations levels used.

2.9 Validity of the Test

For the test to be valid the following conditions were observed:

- Only healthy adult live bees were used for the test. Bees were collected and kept under field conditions for three hours before application of the various doses.
- Preparations of all doses were done using the prescribed application rates for the application of the various insecticides to specific crops in the field.
- The average mortality for the total number of controls did not exceed 10 per cent at the end of each test session.
- The LC_{50} of the toxic standard met the specified range.
- All instruments used for the test were always washed with a detergent and hot water, rinsed with tap water and finally with distilled water before use. After using them for a particular dosage, the same was repeated before using them for the next one.

2.10 Percentage Mortalities and Calculation of LC_{50}

The bees were observed at ten minutes interval for ninety 90 minutes for any toxic signs. The number of dead bees in each cage was counted and the percentage of mortality was calculated [15]. The percentage of bees that died at each dose was then transformed to probit using Finney's method. The probit values obtained were plotted against log-concentration and the concentration corresponding to probit 5 (A type of regression where the dependent variable can take only two values), i.e., 50% was found. The graph obtained gave the probit versus log-concentration Curve. The S.E of LC_{50} was calculated using the formula of [15].

$$\text{Approx S.E of } LC_{50} = \frac{\left(\log LC_{84} - \log LC_{16}\right)}{\sqrt{2}N}$$

2.11 Estimation of Acute Toxicity of the Insecticides When Applied to Honey Bees

The standard method to evaluate the toxicity of the insecticides that could potentially be in contact with the honey bees consisted of the calculation of an acute toxicity data. The acute toxicity of an insecticide was determined by the calculation of median lethal concentration (LC_{50}), that is, the concentration that will kill 50 percent of animals of a particular species.

The corrected % Formula for 0% mortality and 100% were calculated using the formula of [15].

For 0% dead = 100 * (0.25/n)
For 100% dead = 100 - (100*0.25/n).

Where n is number of honey bees in each group [17].

2.12 Median Lethal Concentration (LC_{50}) of Controller Super 2.5 EC

Three groups of adult bees of 10 bees in each group were placed in wooden boxes. Five different concentrations of 1.0 ml/l, 1.7 ml/l, 3.3

ml/l, 5.0 ml/l and 6.7 ml/l in the case of Controller Super 2.5 EC were applied. The number of bees with behavioral modifications or dead during 10, 20, 30, 40, 50 and 60 minutes were recorded.

The percentage of honey bees that had died at each concentration level was then transformed to probit. A control group experiments were performed using distilled water without any agrochemical. The probit values were plotted against log-concentrations (Fig. 1); the concentration corresponding to probit 5, that is, 50 percent was found.

2.13 Median lethal concentration (LC_{50}) of Pyrinex 48 EC

In the case of Pyrinex 48 EC, five different concentrations (0.5 ml/l, 1.0 ml/l, 1.5 ml/l, 2.0 ml/l and 2.5 ml/l) were applied to the three groups of adult bees (10 bees in each group). The number of bees with behavioral modifications or dead during 10, 20, 30, 40, 50 and 60 minutes was recorded. The percentage of bees that had died at each concentration level was then transformed to probits. The probit values were plotted against log-concentrations (Fig. 2); the concentration corresponding to probit 5, that is, 50 percent was found [16].

2.14 Median Lethal Concentration (LC_{50}) of Golan SL

Five different concentrations (0.5 ml/l, 1.0 ml/l, 1.5 ml/l, 2.0 ml/l and 2.5 ml/l) of Golan SL were applied to adult live bees in three cages (of 10 bees in each group). The number of bees with behavioral modifications or dead during 10, 20, 30, 40, 50 and 60 minutes was reported. The percentage of bees that had died at each dose level was then transformed to probits in a probits table. The results obtained were used to plot a probit versus log-concentration Curve for Golan SL and the concentration that would kill 50% of the bee population determined [16].

2.15 Calculation of Standard Error (S.E)

The S.E of the LC_{50} of the insecticides was calculated from the following formula of [15].

$$\text{Approx S.E of } LC_{50} = \frac{(\log LC_{84} - \log LC_{16})}{\sqrt{2N}}$$

Where N is the number of honey bees in each group

2.16 Statistical Analysis

The data collected were presented in tabular form, showing for each treatment group, as well as control group, the number of bees used and mortality at each observation time. The tables are found at the appendices. All observations (mortality data) were analysed using Microsoft Excel to generate the various curves with statistical equations where appropriate for data analysis. Specifically, the data were analysed by tabulations and descriptive statistics of the Microsoft Excel output. Statistical Package for Social Sciences (SPSS) was also used to establish relationships between different concentrations. All statistical tests were estimated at 95% confidence level. Control mortality was made using Abbott's correction [18].

3. RESULTS AND DISCUSSION

3.1 Commonly used Insecticides in the two Communities

From the interview conducted, the commonly sold insecticides in the area are Controller Super 2.5 EC, Pyrinex 48 EC, Goland SL, Pyrinex Quick 256 SC, Insector T. 45 and Sunhalothrin 2.5% EC. Controller Super 2.5 EC, Pyrinex 48 EC, and Goland SL were the most commonly used insecticides in the two communities studied. All the 40 farmers interviewed stated that they sprayed their crops with the insecticides twice before harvesting in every planting season. The insecticides are used on crops such as maize, millet, rice, groundnuts, yams, cowpea and vegetables. Spraying was always done during flowering and fruiting. The targeted insects are usually caterpillars, beetles, aphids, moths, whiteflies, grasshoppers, crickets and locusts which feed on plants.

The oral interview conducted in the study area showed that the commonly used insecticides were Controller Super 2.5 EC, Pyrinex 48 EC and Golan SL. The people explained that the insecticides were effective in controlling pests, were less expensive compared to other types of insecticides, were less toxic to humans and other animals and were always available and accessible to farmers. The variation of the toxicity of these insecticides to honey bees may be due to the active ingredients they contain and the concentrations administered. The likelihood of exposure of honey bees to the insecticides could occur when honey bees living near

agricultural fields go foraging on food crops sprayed with the insecticides.

3.2 Observations Recorded during the Study

In the morning of the experiment, bees were found resting on the nylon mesh covering the top part of the cages. When the insecticides were sprayed on them, they became aggressive (They were violently flying within the test cages) and started flying all over inside the cages. This occurred one to three minutes after spraying depending on the insecticide type and the concentration. All mortalities occurred after the bees fell from the nylon mesh and were crawling on the floor. Mortalities were recorded 10 minutes after administering the concentrations and thereafter at every 10 minutes continuously till 60 minutes [16]. Observations continued to the ninetieth minute but no recordings were made between 60 to 90 minutes since the control mortalities started occurring within that period. In some of the insecticide types and concentrations, all the bees were dead (They were motionless) by the sixtieth minute.

3.3 LC_{50} of Controller Super 2.5 EC

The plot of probits versus log - concentration for calculation of LC_{50} for Controller Supper 2.5 is presented in Fig. 2.

Log LC_{50} was found to be 0.29 and LC_{50} was 1.95 ml/L. The standard error of Controller Super 2.5 EC was calculated to be 0.53 using probit of 6 and a log concentration of 84 and probit of 4 and a log concentration of 16 from the plot of probits versus log - concentration for calculation of LC_{50}. The LC_{50} of the insecticide Controller Super 2.5 EC applied was 1.95 ± 0.53 with 95% confidence interval of 2.48 ml/L – 1.42 ml/L.

3.4 LC_{50} of Pyrinex 48 EC

The plot of probits versus log - concentration for the calculation of LC_{50} for Pyrinex 48 EC is given in Fig. 3.

In the case of Pyrinex 48 EC, Log LC_{50} was 0.04 and LC_{50} was 1.1 ml/L. The standard error of Pyrinex 48 EC was calculated to be 0.37 using the probits of 6 and log concentration of 84 and probit of 4 and log concentration of 16 from the plot of probits versus log - concentration for the calculation of LC_{50}. LC_{50} of the insecticide Pyrinex 48 EC when applied was 1.1 ± 0.37, with 95% confidence interval of 1.47 - 0.73.

3.5 LC_{50} of Golan SL

The plot of probits versus log - concentration for the calculation of LC_{50} for Golan SL is shown in Fig. 4.

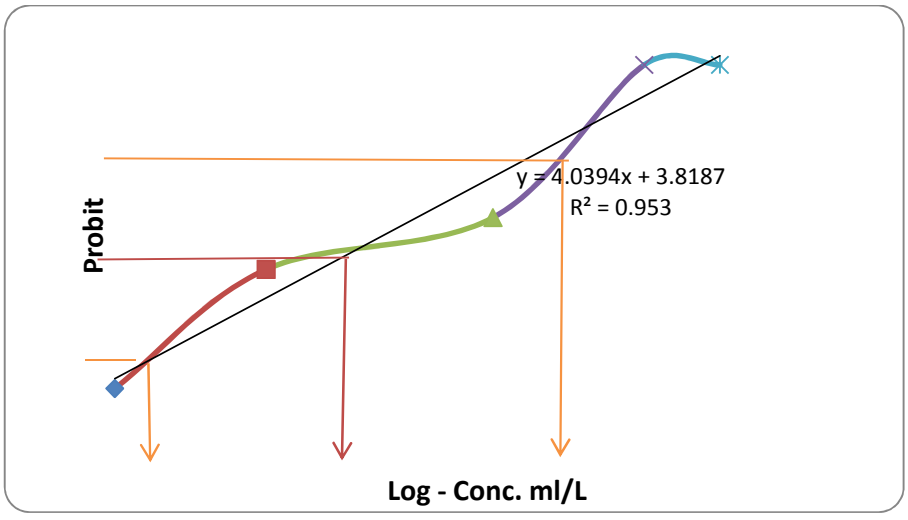

Fig. 2. Plot of probits versus log - concentration for calculation of LC_{50} for Controller Supper 2.5

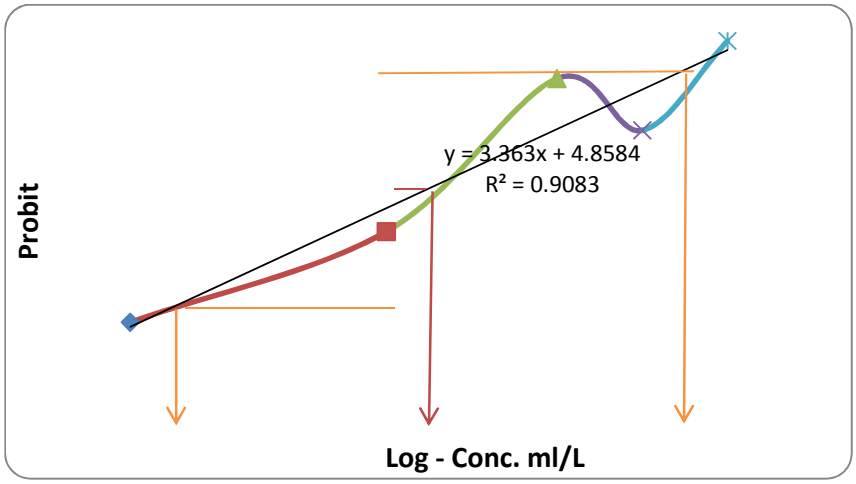

Fig. 3. Plot of probits versus log - concentration for calculation of LC_{50} for Pyrinex 48 EC

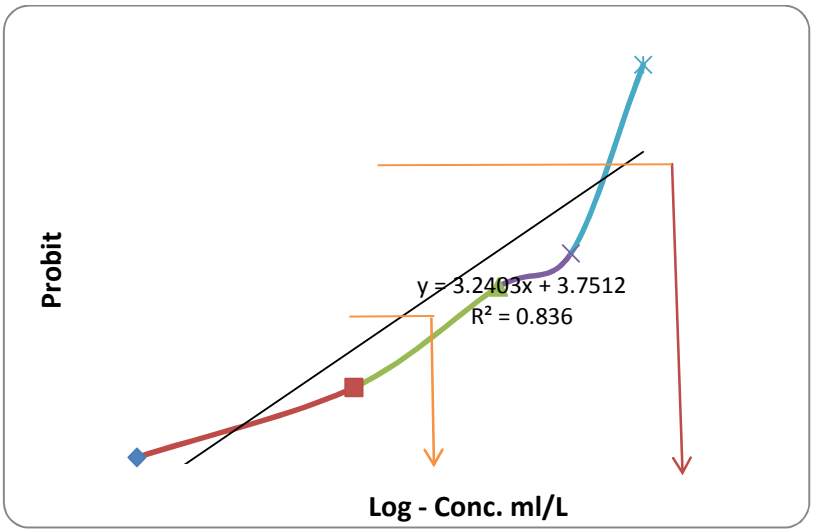

Fig. 4. Plot of probits versus log-concentration for calculation of LC_{50} for Golan SL

Log LC_{50} of Golan SL was 0.39 and LC_{50} was 2.45 ml/L**.** The Standard Error was calculated to be 0.83 using the probit of 6 and log concentration of 84 and probit 4 and log concentration of 16 from the probits versus log-concentration curve. The LC_{50} of the insecticide Golan SL when applied was calculated to be 2.45 ± 0.83, with 95% confidence interval of 3.28 ml/L -1.62 ml/L.

The calculated median lethal concentrations (LC_{50}) values of the Controller Super 2.5 EC, Pyrinex 48 EC and Golan SL were 1.95, 1.10 and 2.45 respectively. The result obtained was similar to the findings of [19], that the smaller the LC_{50} / LD_{50} value, the more toxic the chemical is. The opposite is also true: the larger the LC_{50} / LD_{50} value the lower the toxicity. Pyrinex 48 EC was the most toxic with an LC_{50} of 1.1 ± 0.37 ml/L, with 95% confidence interval of 1.47 to 0.73. Controller Super 2.5 EC demonstrated a similar level of toxicity to the Pyrinex 48 EC with an LC_{50} value of 1.95 ± 0.53 ml/L at 95% confidence interval of 2.48 – 1.42 ml/L. Golan SL was the least toxic among the three insecticides under consideration with LC_{50} value of 2.45 ± 0.83 ml/L, at 95% confidence interval of 3.28 - 1.62 ml/L. According to [20], the differences in the LC_{50} values of the insecticides were due to the type of active ingredients they contained and the concentrations administered. Controller Super 2.5 EC contained Lambda-cyhalothrin;

Pyrinex 48 EC contained Chlorpyrifos 480 GR/LT (O, O-Diethyl O-3, 5-6-trichloro-2-pyridyl phosphorothioate) and Golan SL contained acetamiprid. These active ingredients might be toxic to insects but may vary in toxicity. In the present case, Chlorpyrifos 480 GR/LT (O, O-Diethyl O-3, 5-6-trichloro-2-pyridyl phosphorothioate) in Pyrinex 48 EC might be the most toxic and acetamiprid in Golan SL the least toxic. The concentrations administered are also important in determining the toxicity (LC_{50}) since the results showed that there was significant difference between each concentration and the subsequent one as in the case of Controller Super 2.5 EC as follows: 1.0 ml/L and 1.7 ml/L (r = 0.82; p = 0.040); 1.7 ml/L and 3.3 ml/L (r = 0.99; p = 0.00) as well as 3.3 ml/L and 5 ml/L (r = 0.82; p = 0.040). However, there was no significant difference between the lowest and highest concentration levels of 1.0 ml/L and 6.7 ml/L (p = 0.440).

Pyrinex 48 EC showed significant difference between all concentration levels as follows: at 0.5 ml/L and 1.0 ml/L (r = 0.90; p = 0.010), 1.0 ml/L and 1.5 ml/L (r = 0.99; p = 0.000), 1.5 ml/L and 2.0 ml/L (r = 0.99; p = 0.000), 2.0 ml/L and 2.5 ml/L (r = 0.99; p = 0.000) as well as the lowest and highest concentrations of 0.5 ml/L and 2.5 ml/L (r = 0.94; p = 0.000).

Goland SL showed positive correlation such that there was significant difference between concentrations 1.5 ml/L and 2.0 ml/L (r=0.84; p=0.030); 2.0 ml/L and 2.5 ml/L (r = 0.95; p=0.000). Besides, there was no significant difference between concentrations 1.0 ml/L and 1.5 ml/L (r = 0.72; p = 0.110). However, 0.5 ml/L could not be used to establish relationships because the concentration level was zero at all the time recorded [16].

3.6 Mortality of Honey Bees after Exposure to the Different Concentrations of Controller Super 2.5 EC

The mean mortality of the honey bees after exposure to the different concentrations of Controller Super 2.5 EC for 60 minutes is given in Fig. 5.

Controller Supper 2.5 EC at a concentration of 6.7 ml/L gave the highest mean mortality (10 bees) at the 50th minute while the concentration of 1.0 ml/L gave the lowest mean mortality (0.0 bees) in the same 50th minute. The concentrations of 1.7 ml/L, 3.3 ml/L and 5.0 ml/l gave mean mortalities of 2.3, 4.3 and 8.3 respectively at the 50th minute (Fig. 5).

Results obtained from Pearson's correlation analysis conducted confirmed there was close relationships between concentrations at different levels as was observed by [18]. The concentration at 1.0 ml/L had significant correlations with the concentration at 1.7 ml/L (r = 0.82; p = 0.040). Also, there was a significant relationship between concentration levels

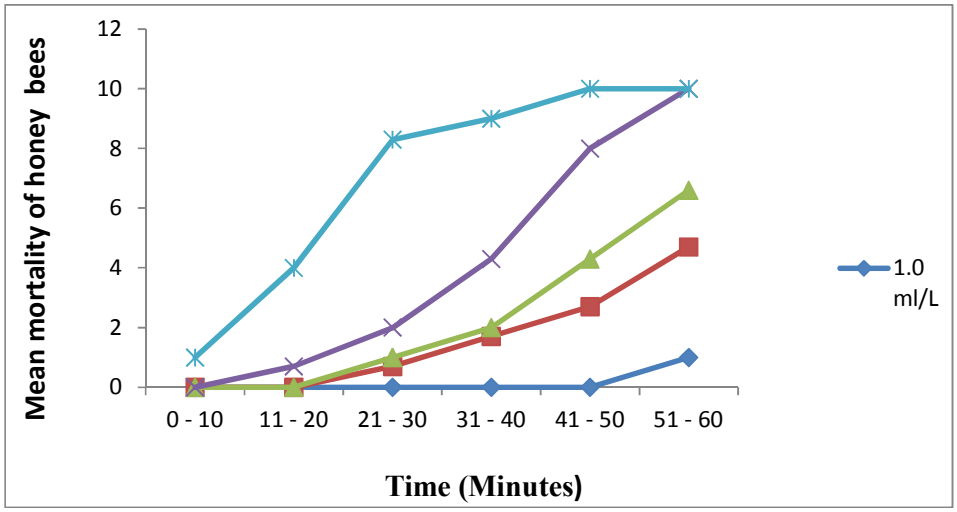

Fig. 5. Mean mortality of Honey Bees after Exposure to the Different Concentrations of Controller Supper 2.5 EC for 60 minutes

1.7 ml/L and 3.3 ml/L (r = 0.99; p = 0.000) as well as 3.3 ml/L and 5.0 ml/L (r = 0.82; p = 0.040). However, there was no significant difference between the lowest and highest concentrations of 1.0 ml/L and 6.7 ml/L (p = 0.440).

3.7 Mortality of Honey Bees after Exposure to the Different Concentrations of Pyrinex 48 EC

The mean mortality of the honey bees after exposure to the different concentrations of Pyrinex 48 EC are given in Fig. 6.

Pyrinex 48 EC at a concentration of 2.5 ml/L gave the highest mean mortality (9 bees) at the 60th minute while the concentration of 0.5 ml/L gave the lowest mean mortality (1.3 bees) in the 60th minute. The concentrations of 1.0 ml/L, 1.5 ml/L and 2.0 ml/L gave mortalities of 3.7, 8.3 and 7.0 respectively at the 60th minute.

Results from Pearson's correlation analysis demonstrated that there was a positive relationship (significant difference) between all the concentrations at different levels. Specifically, there was a significant difference between the concentration levels at 0.5 ml/L and 1.0 ml/L (r = 0.90; p = 0.010), 1.0 ml/L and 1.5 ml/L (r = 0.99; p = 0.000), 1.5 ml/L and 2.0 ml/L (r = 0.99; p = 0.000), 2.0 ml/L and 2.5 ml/L (r = 0.99; p = 0.000) as well as the lowest and highest concentrations of 0.5 ml/L and 2.5 ml/L (r = 0.94; p = 0.000). This observation is akin to Gerkin et al., (2001) that the potency of insecticide are at different levels of concentrations.

3.8 Mortality of Honey Bees after Exposure to the Different Concentrations of Golan SL

The mean mortality of the honey bees after exposure to the different concentrations of Golan SL are indicated in Fig. 7.

Golan SL at a concentration of 2.5 gave the highest mean mortality (7.3 bees) at the 60th minute while the concentration of 0.5 gave the lowest mean mortality (0.0 bees) at the 60th minute. Mean mortalities of 0.7, 1.7 and 2.7 were recorded at the concentrations of 1.0 ml/L, 1.5 ml/L and 2.5 ml/L respectively in the 60th minute. The study further established an association between the different concentrations levels recorded. Results demonstrated that concentration level at 0.5 ml/L could not be used to establish relationships because the concentration level showed zero mortality at all the time recorded. However, the concentration level at 1.5 ml/L and 2.0 ml/L showed significant difference (r = 0.84; p = 0.030). Also, there was a significant difference between the concentration levels 2.0 ml/L and 2.5 ml/L (r = 0.95; p = 0.00). Besides, there was no significant correlation between the concentration levels 1.0 ml/L and 1.5 ml/L (r = 0.72; p = 0.110).

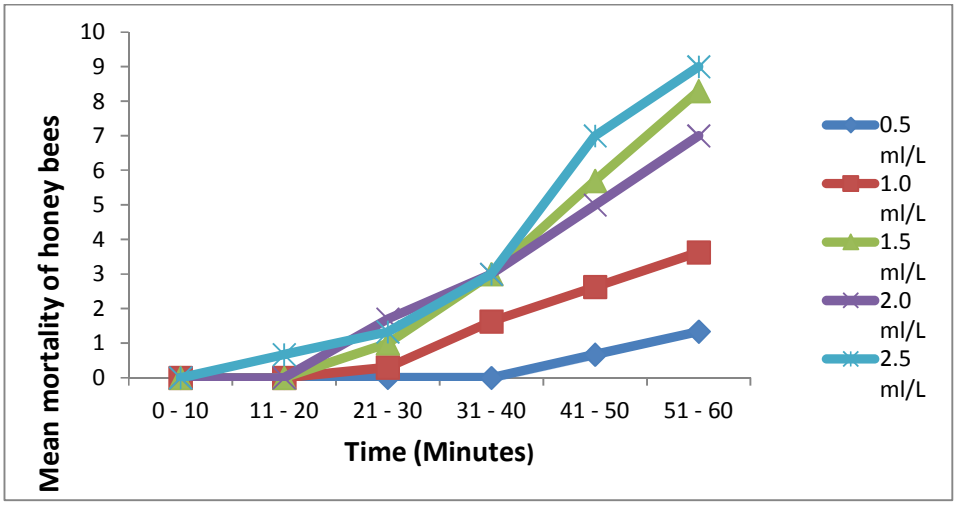

Fig. 6. Mean mortality of Honey Bees after Exposure to the Different Concentrations of Pyrinex 48 EC for 60 minutes

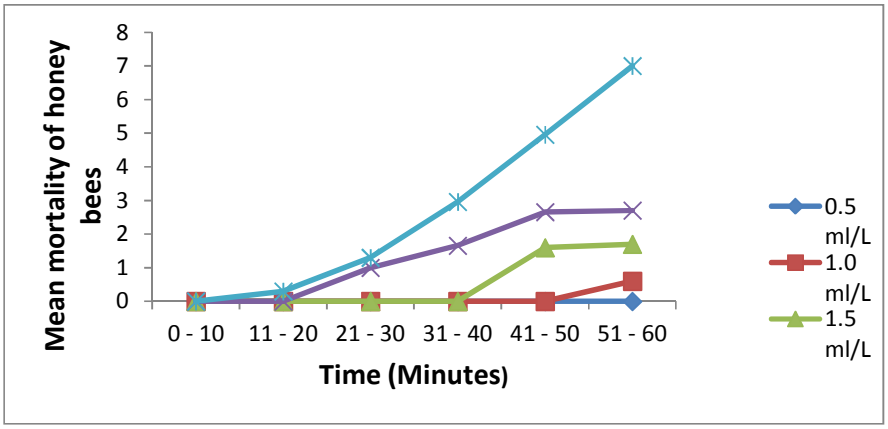

Fig. 7. Mean mortality of Honey Bees after Exposure to the Different Concentrations of Golan SL for 60 minutes

Mortality of bees due to exposure to the different concentrations of all the three insecticides (Controller Super 2.5 EC, Pyrinex 48 EC and Golan SL) was higher than the control. Generally as the concentration of the three insecticides increased, there was a corresponding increase in mortality of the bees. The highest concentrations of 6.7, 2.5 and 2.5 milliliters per liter of water for Controller Super 2.5 EC, Pyrinex 48 EC and Golan SL respectively used resulted in mean mortalities of 10.0, 9.0 and 7.3 respectively, after 60 minutes of exposure. However, the lowest concentrations of 1.0 ml/L, 0.5 ml/L, and 0.5 ml/L resulted in the mean mortalities of 1.0, 1.3 and 0.0 respectively in the 60th minute.

At the recommended formulation of 1.0 ml/L for Controller Super 2.5 EC, no mortality was recorded at the 50th minute while at 6.7 ml/l all the ten bees died at the 50th minute (Fig. 5). The finding is similar to that of [21] in their study of pollinator decline. However the recommended formulation of 1.0 ml/L produced some mortality (mean mortality of 1.3 bees) at the 60th minute. This might be due to the fact that the bees had to take in greater quantity of the insecticide before they could show any toxic signs. The concentration of 1.7 ml/L showed mean mortality of 0.7 bees in the 30th minute which increased steadily to 4.7 bees in the 60th minute. There was significant difference ($p = 0.040$) between 1.0 ml/L and 1.7 ml/L. Concentration of 3.3 ml/L gave the same mortality pattern as the 1.7 ml/L except that it showed a greater mean mortality (6.3 bees) in the 60th minute while the 1.7 ml/L gave mean mortality of 4.7 bees in the 60th minute. There was significant difference ($p = 0.000$) between 1.7 ml/L and 3.3 ml/L.

Concentration 5.0 ml/L also showed some mortality (0.3 bees) in the 20th minute which increased steadily to 10 at the 60th minute where all the bees died. There was significant difference ($p = 0.040$) between 3.3 ml/L and 5.0ml/L. The 6.7 ml/L concentration showed some mortality of 0.7 bees in the 10th minute which increased to 8.3 bees in the 30th minute. This might be that the active ingredients at that concentration exhibited greater toxicity after few minutes of exposure leading to death of the bees. All 10 bees were dead by the 50th minute. There was significant difference ($p = 0.05$) between 5.0 ml/L and 6.7 ml/L. The overall mean mortality recorded for the concentrations 1.0 ml/L, 1.7 ml/L, 3.3 ml/L, 5.0 ml/L and 6.7 ml/L were 1.3, 4.7, 6.7, 10 and 10 respectively at the 60th minute.

There was positive correlations among the various concentrations which gave the following r-values; 1.0 ml/L and 1.7 ml/L ($r = 0.82$; $p = 0.040$); 1.7 ml/L and 3.3 ml/L ($r = 0.99$; $p = 0.000$); 3.3 ml/L and 5 /L ($r = 0.82$; $p = 0.040$); 5.0 ml/L and 6.7 ml /L ($r = 0.82$; $p = 0.05$). This result is similar to the findings of [17] in Scotland that when colonies of bumble bees were exposed to recommended formulations of different concentrations of imidacloprid, it caused some mortality. It showed that bumble bees, which are wild pollinators, were suffering similar impacts of pesticide exposure to "managed" honey bees [18].

The recommended formulations of 0.5 ml/L for Pyrinex 48 EC showed mortality (mean mortality of 0.7 bees) at the 50th minute which increased steadily to the 60th minute (Fig. 5). Similarly, the

recommended formulation of 1.0 ml/L produced some mortality (mean mortality of 0.3 bees) at the 30th minute and steadily increased to the 60th minute. There was significant difference (p = 0.010) between 0.5 ml/L and 1.0 ml/L. Concentration 1.5 ml/L also showed some mortality (mean mortality of 8.3 bees) at the 60th minute while concentration 2.0 ml/L produced mean mortality of 7.0 bees at the 60th minute. This might be that the toxic chemicals at the concentration 1.5ml/L exhibited greater toxicity to the bees than the 2.0 ml/L during the exposure leading to more mortality of the bees (Fig. 6). Other possible reasons for the bees mortality includes stress since the bees were caught from their hives and transported to different location where they were caged for the study. Variations in environmental conditions such as temperature, relative humidity and light at the study site which could differ from what existed in the hive could also contribute to bees' mortality. The time and period of capture and exposure to the various insecticides could also affect the health of the bees leading to their mortality.

There was significant difference (p = 0.000) between 1.5 ml/L and 2.0 ml/L. The 2.5 ml/L produced some mortality of 0.7 bees in the 10th minute which showed a slow increase to the 40th minute (mean mortality of 3.0 bees) but increased steadily to the 60th minute. There was significant difference (p = 0.000) between 2.0 ml/L and 2.5 ml/L. The overall mean mortality recorded for the concentrations; 0.5 ml/L, 1.0 ml/L, 1.5 ml/L, 2.0 ml/L and 2.5 ml/L were 1.3, 3.7, 8.3, 7.0 and 9.0 respectively at the 60th minute (Fig. 6).

The study also showed positive correlations among the various concentrations which gave the following r-values: 0.5 ml/L and 1.0 ml/L (r = 0.90; p = 0.010), 1.0 ml/L and 1.5 ml/L (r= 0.99; p = 0.000), 1.5 ml/L and 2.0 ml/L (r = 0.99; p = 0.000), 2.0 ml/L and 2.5 ml/L (r = 0.99; p = 0.000), 0.5 ml/L and 2.5 ml/L (r = 0.94; p = 0.000).

At the recommended formulation of 0.5 ml/L for Golan SL, no mortality (mean mortality of 0.0 bees) was recorded at the 60th minute while at 2.5 ml/L seven bees died at the 60th minute (Fig. 7). This was be due to the fact that the active ingredients in the 0.5 ml/L concentration were too low to cause any mortality in the bees even after longer period of exposure. The concentrations of 1.0 ml/L, 1.5 ml/L and 2.0 ml/L showed similar mortality pattern of 0.7, 1.7 and 2.7 bees in the 60th minute. The mortality generally increased with increasing concentrations. There was significant difference (p = 0.030) between 1.5 ml/L and 2.0 ml/L. However, there was no significant difference (p = 0.110) between 1.0 ml/L and 1.5 ml/L. The 2.5 ml/L concentration showed some mortality of 0.3 bees in the 10th minute which increased steadily to 7.0 bees in the 60th minute. Also, there was a significant difference (p = 0.000) between 2.0 ml/L and 2.5 ml/L. The overall mean mortality recorded for the concentrations 0.5 ml/L, 1.0 ml/L, 1.5 ml/L, 2.0 ml/L and 2.5 ml/L were 0.0, 0.7 , 1.7, 2.7 and 7.0 respectively at the 60th minute (Fig. 7).

The study further indicated correlations between the different concentrations. Results demonstrated that concentration of 0.5 ml/L could not be used to show relationships because the concentration level showed zero mortality at all the time recorded. However, the following concentration levels gave positive correlations. At 1.5 ml/L and 2.0 ml/L (r = 0.84; p = 0.030); 2.0 ml/L and 2.5 ml/L (r = 0.95; p = 0.000) as well as 1.0 ml/L and 1.5 ml/L (r = 0.72; p = 0.110).

3.9 Determination of Recommended Levels of Insecticides Applied to Crops

Honey bees are vulnerable to many of the insecticides used to control pests on plants, fruits, vegetables, nuts, and seeds [20]. In the quest for crop farmers to maximize yield, they depend on honey bees for the pollination of their crops. One of the objectives of this study was to determine if insecticides applied at recommended levels to crops is capable of causing reduction in honey bee population.

This was based on estimating mortalities of honey bees due to exposure [22] to the insecticides (Controller Super 2.5 EC, Pyrinex 48 EC and Golan SL) and subsequently comparing the results with the calculated $LC_{50,}$ and error bars from the respective toxicity graphs.

3.10 Toxicity of Controller Super 2.5 EC

The number of mortalities of honey bees after one (1) hour exposure to Controller Super 2.5 EC to Honey bees produced a toxicity curve of Fig. 8 with the equation:

y = 1.5254x + 1.0987. Where 'Y' is the number of mortalities in bees and 'X' is the concentration of Controller Super 2.5 EC per one (1) liter of water.

If the 'X' value kills many bees, it means the concentration is more toxic.

The toxicity curve (y = 1.5254x + 1.0987) presented in Fig. 8 showed the recommended level of Controller Super 2.5 EC.

3.11 Toxicity of Pyrinex 48 EC

The number of mortalities of honey bees after one (1) hour exposure to Pyrinex 48 EC produced a toxicity curve of Fig. 9 with the equation y = 3.734x + 0.265.

Where 'Y' is the number of mortalities in bees and 'X' is the concentration of Pyrinex 28 EC. If the 'X' value kills so many bees, it means the concentration is more toxic.

The toxicity curve (y = 3.734x+ 0.265) presented in Fig. 9 showed the recommended level of Pyrinex 48 EC.

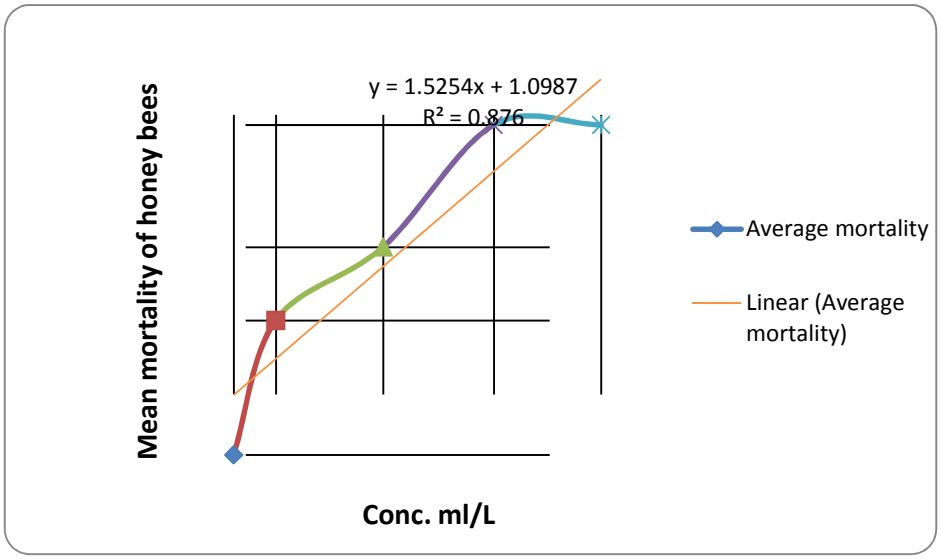

Fig. 8. Toxicity of Controller Super 2.5 EC due to one hour exposure to honey bees

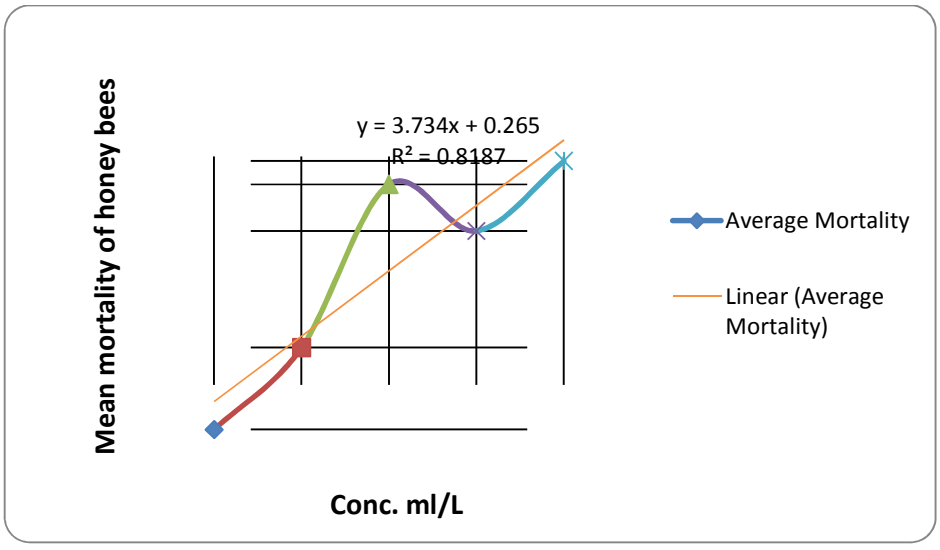

Fig. 9. Toxicity of Pyrinex 48 EC due to one hour exposure to honey bees

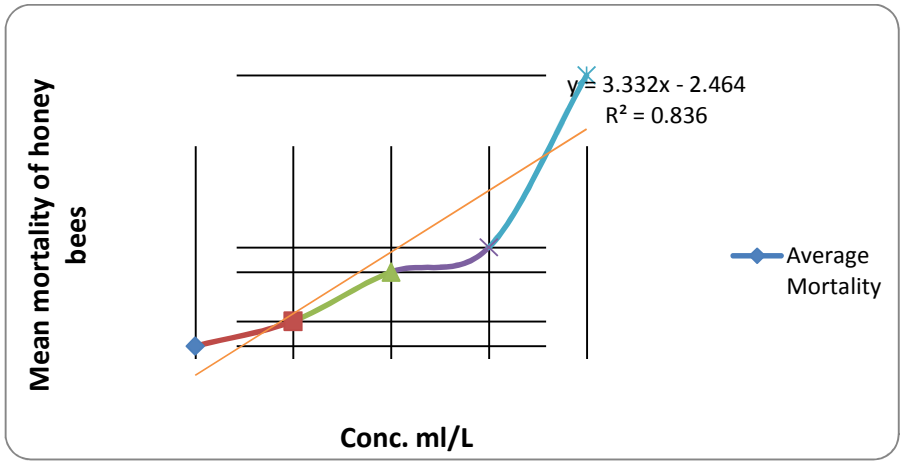

Fig. 10. Toxicity of Golan SL due to one hour exposure to honey bees

3.12 Toxicity of Golan SL

The number of mortalities of honey bees after one (1) hour exposure to Golan SL produced a toxicity curve of Fig. 10 with the equation: y = 3.332x - 2.464. Where 'Y' is the number of mortalities in bees and 'X' is the concentration of Golan SL. If the 'Y' value is large, the concentration is more toxic.

The toxicity curve (y = 3.332x - 2.464) presented in Fig. 10 showed the recommended level of Golan SL.

The recommended levels for application of Pyrinex 48 EC and Controller Super 2.5 EC on maize are 100 ml per 100 Liters of water and 100 ml per 15 Liters of water respectively. Honey bees which would be inadvertently exposed to the maize for 1 hour during or after the application of Pyrinex 48 EC will cause mean mortality of 4 bees out of every 10 bees (Fig. 9). However, when the Controller Super 2.5 EC is applied at the recommended concentration (100mls per 15 litres of water) on maize, it will cause total mortality within 1 hour [23, 24]. The recommended formulation concentration for Golan SL is 30 ml per 100 Liters of water on vegetables. This would show no mortality when honey bees are inadvertently exposed to it within 1 hour of application (Fig. 10).

Given the toxicity curves of Pyrinex 48 EC and Golan SL (as y = 3.734x + 0.265 and y = 3.332x - 2.464 respectively) (Figs. 9 and 10 respectively), after one (1) hour of exposure, the Pyrinex 48 EC will cause mean mortality of 2.1 out of every ten honey bees while Golan SL will show no mortalities.

The findings generally agreed with that of [25, 26] that pesticide use reduces biodiversity, contributes to pollinator decline, destroys habitat and threatens endangered species. The use of agricultural chemicals can have damaging effects on honey bees. This has been stated by [27] that crop farmers who depend on honey bees for the pollination of their crop (s) must constantly maintain a delicate balance between protecting their crops from pests and pathogens, and protecting the insects that are necessary to pollinate these crops.

4. CONCLUSION

The LC_{50} for the three insecticides used were within the recommended concentrations provided by the Environmental Protection Agency of Ghana. The overall result of the present study demonstrated that mortalities occurred when honey bees were exposed to different concentrations of all the three insecticides. However, higher mortalities occurred at higher levels of concentrations and longer period of exposure. It can therefore be concluded that the use of these insecticides continue to kill bees because of excessive exposure. It is recommended that: The Ministry of Food and Agriculture must ensure that the use of systemic insecticides by farmers on bee-pollinated crops as well as agro-chemicals (insecticides) use prior or during crop flowering should be abolished since these practices can injure pollinators; Also, the Environmental Protection Agency (EPA) of Ghana should compile a list of insecticides that are safe for use in combinations and make it

available and accessible to all local farmers and agriculture extension officers since insecticides stacking can lead to synergistic reactions in pollinators. All other combinations should be disallowed; In addition, research institutions such as the Centre for Scientific and Industrial Research should conduct continuous studies to determine the effects of long term low level exposure to multiple insecticides on the health and functioning of honeybee colonies foraging in agricultural environments; Furthermore, farmers should consider the use of alternative methods to pesticides use such as biological pest control (eg. pheromones and microbial pesticides); as well as safer methods of cultivation such as polyculture, crop rotation, timing planting according to when pests will be least problematic; genetic engineering; methods of interfering with insect breeding; the use of trap crops and application of compost yard waste should be encouraged instead of traditional chemical pesticides.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cooper J, Hans D. The benefits of pesticides to mankind and the environment. Crop Protection. 2007;26:1337-1348.
2. Odoux J, Henry M, Maxime B, Fabrice RR, Orianne D, Axel G. A common pesticide decreases foraging success and survival in honey bees. Science. 2012;20:348–350.
3. Hackett KJ. Bee benefits to agriculture. Forum Journal, ARS National Program, Agricultural Research, Biological ControlBeltsville, Maryland. 2004;4:23-35.
4. Desneux N, Decourtye A, Delpuech JM. The sublethal effects of pesticides on beneficial arthropods. Annu Rev Entomol. 2007;52:81–106.
5. FAO. Agricultural and consumer protection corporate document repository value added products from beekeeping: *United Nations.* 2014; 24:36 -38.
6. Alaux C, Brunet JL, Dussaubat C, Mondet F, Tchamitchan S, Le Conte Y. Interactions between *Nosema microspores* and a neonicotinoid weaken honeybees (*Apis mellifera*). Environmental Microbiology. 2010;12:774-782.
7. Pettis JS, van Engelsdorp D, Johnson J, Dively G. Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema naturwissenschaften*. 2012;99:153±158.
8. Kwapong P. Benefits of taxonomy: African Pollinator Initiative (API). Taxonomy for Ghana's development and conservation – assessing the needs, Ghana-UK project 2006-7, Workshop Presentation; 2006.
9. James RR, Xu J. Mechanisms by which pesticides affect insect immunity. J. Invertebr. Pathol. 2012;109:175-182.
10. OEPP/EPPO. EPPO Standards PP1/170 (4). Efficacy evaluation of plant protection products, Side-effects on honey bees. OEPP/EPPO Bulletin. 2010;40:313-319.
11. Morse RA, Calderone NW. The value of honey bees as pollinators of U.S. crops in 2000, Bee Culture. 2000;128:1-15.
12. Wu J, Anelli C, Sheppard W. Sub-lethal effects of pesticide residues in Brood comb on worker honey bee (*Apis mellifera*) development and longevity. PLoS ONE. 2011;6: e14720:58, 78-86.
13. Hunt GJ, Krukpe CH, Eitzer BD, Andino G, Given K. Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS One. 2012;7:e29268.
14. West Gonja District Assembly. Medium Term Development Plan 2010-2013; 2010. Available:http://westgonja.ghanadistricts.gov.gh/?arrow=dnf&_=89&r=6&rlv=climate (July 15th, 2019)
15. Gerken A, Suglo JV, Braun M. Crop protection policy in Ghana. Pokuase Accra:

Integrated Crop Protection Project, PPRSD/GTZ; 2001.

16. Miller LC, Tainter ML. Estimation of LD_{50} and its error by means of log-pribit graph paper. Proc. Soc. Exp. Bid Med. 1944;57:261.
17. Ghosh MN. Toxicity studies. In: Ghosh M. N. (ed). Fundamentals of Experimental Pharmacology, Scientific Book Agency, Calcutta, India. 1984;153-158.
18. Abbott WS. A method for computing the effectiveness of an insecticide. Jour. Econ. Entomol. 1925;18:265-267. DOI:https://doi.org/10.1093/jee/18.2.265a18
Penelope PS, Connor O, Wackers FL, Goulson D. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science. 2012;20: 351–352.
19. Colin ME, Bonmatin JM, et al. Quantitative analysis of the foraging activity of honey bees: relevance to the sub-lethal effects induced by systemic insecticides. Arch. Environm. Contam. Toxicol. 2004;47:387-395.
20. Krupke CH, Greg MJ, Hunt BD, Eitzer G, Andino KG. Multiple routes of pesticide exposure for honey bees living near agricultural fields. DOI:10.1371/ journal. pone.0029268,2012;7-14.
21. Potts SG, Kremen JC, Neumann P, Schweiger O, Kunin WE. Global pollinator declines: trends, impacts and drivers. Trends in Ecology and Evolution. 2010;25: 345–353.
22. Rortais AG, Arnold MP, Halm F, Touffet B. Modes of honeybees exposure to systemic insecticides, estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie. 2005;36:71-83.
23. Hodgson A. The high cost of pesticide poisoning in northern Ghana. Pestic. News. 2003;62(3): 4–8.
24. Kunin WE. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science. 2006;313:351–354
25. Penelope PS, Connor O, Wackers FL, Goulson D. neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science. 2012;20:351–352.
26. Palmer WE, Bromley PT, Brandenburg RL. Wildlife and pesticides on Peanuts. North Carolina Cooperative Extension Service. 2007;21-28. Retrieved on 2013-10-11.
27. Feldman H. Protecting Pollinators: Stopping the Demise of Bees". Pesticides and You, Beyond Pesticides; 2011.

APPENDIX

Appendix 1:. Mortality Observation of honey bees when exposed to the Control Experiment (1L of distilled Water)

TIME (Minutes)	Mortality observation of control experiment			Mean mortality
	Box 1	Box 2	Box 3	
1 to 10 min	0.0	0.0	0.0	**0.0**
11 to 20 min	0.0	0.0	0.0	**0.0**
21 to 30 min	0.0	0.0	0.0	**0.0**
31 to 40 min	0.0	0.0	0.0	**0.0**
41 to 50 min	0.0	0.0	0.0	**0.0**
51 to 60 min	0.0	0.0	0.0	**0.0**
61 to 70 min	1.0	0.0	0.0	**0.3**
71 to 80 min	1.0	0.0	1.0	**0.7**
81 to 90 min	0.0	1.0	1.0	**0.7**
91 to 100 min	1.0	1.0	1.0	**1.0**

Appendix 2. Mortality evolution of honey bees when exposed to different doses of Controller Super 2.5 EC

Concentration	Time	1 to 10 min	11 to 20 min	21 to 30 min	31 to 40 min	41 to 50 min	51 to 60 min
1.0 ml/L	Box 1	0.0	0.0	0.0	0.0	0.0	2.0
	Box 2	0.0	0.0	0.0	0.0	0.0	1.0
	Box 3	0.0	0.0	0.0	0.0	0.0	0.0
	Average	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**1.0**
1.7 ml/L	Box 1	0.0	0.0	1.0	3.0	0.0	5.0
	Box 2	0.0	0.0	1.0	0.0	2.0	4.0
	Box 3	0.0	0.0	0.0	1.0	3.0	5.0
	Average	**0.0**	**0.0**	**0.2**	**1.0**	**1.7**	**4.6**
3.3 ml/L	Box 1	0.0	0.0	1.0	2.0	5.0	7.0
	Box 2	0.0	0.0	1.0	3.0	5.0	7.0
	Box 3	0.0	0.0	1.0	0.0	3.0	6.0
	Average	**0.0**	**0.0**	**1.0**	**1.7**	**4.3**	**6.7**
5.0 ml/L	Box 1	0.0	1.0	3.0	5.0	9.0	10.0
	Box 2	0.0	1.0	2.0	5.0	8.0	10.0
	Box 3	0.0	0.0	1.0	3.0	7.0	10.0
	Average	**0.0**	**0.2**	**2.0**	**4.3**	**8.0**	**10.0**
6.7 ml/L	Box 1	2.0	5.0	9.0	9.0	10.0	10.0
	Box 2	1.0	4.0	9.0	10.0	10.0	10.0
	Box 3	0.0	3.0	7.0	8.0	10.0	10.0
	Average	**1.0**	**4.0**	**8.3**	**9.0**	**10.0**	**10.0**

Appendix 3. Mortality evolution of honey bees when exposed to different doses of Pyrinex 48 EC

Concentration	Time	1 to 10 min	11 to 20 min	21 to 30 min	31 to 40 min	41 to 50 min	51 to 60 min
0.5 ml/L	Box 1	0.0	0.0	0.0	0.0	1.0	2.0
	Box 2	0.0	0.0	0.0	0.0	1.0	1.0
	Box 3	0.0	0.0	0.0	0.0	0.0	1.0
	Average	**0.0**	**0.0**	**0.0**	**0.0**	**0.7**	**1.3**
1.0 ml/L	Box 1	0.0	0.0	1.0	2.0	3.0	3.0
	Box 2	0.0	0.0	0.0	2.0	3.0	5.0
	Box 3	0.0	0.0	0.0	1.0	2.0	3.0

Concentration	Time	1 to 10 min	11 to 20 min	21 to 30 min	31 to 40 min	41 to 50 min	51 to 60 min
	Average	**0.0**	**0.0**	**0.3**	**1.6**	**2.7**	**3.7**
1.5 ml/L	Box 1	0.0	0.0	2.0	4.0	7.0	9.0
	Box 2	0.0	0.0	1.0	3.0	5.0	8.0
	Box 3	0.0	0.0	0.0	2.0	5.0	8.0
	Average	**0.0**	**0.0**	**1.0**	**3.0**	**5.7**	**8.3**
2.0 ml/L	Box 1	0.0	0.0	2.0	4.0	6.0	9.0
	Box 2	0.0	0.0	2.0	3.0	5.0	7.0
	Box 3	0.0	0.0	1.0	2.0	4.0	5.0
	Average	**0.0**	**0.0**	**1.7**	**3.0**	**5.0**	**7.0**
2.5 ml/L	Box 1	0.0	1.0	2.0	3.0	8.0	10.0
	Box 2	0.0	1.0	1.0	3.0	7.0	9.0
	Box 3	0.0	0.0	1.0	3.0	6.0	8.0
	Average	**0.0**	**0.7**	**1.3**	**3.0**	**7.0**	**9.0**

Appendix 4. Mortality evolution of honey bees when exposed to different doses of Golan SL

Concentration	Time	1 to 10 min	11 to 20 min	21 to 30 min	31 to 40 min	41 to 50 min	51 to 60 min
0.5 ml/L	Box 1	0.0	0.0	0.0	0.0	0.0	0.0
	Box 2	0.0	0.0	0.0	0.0	0.0	0.0
	Box 3	0.0	0.0	0.0	0.0	0.0	0.0
	Average	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
1.0 ml/L	Box 1	0.0	0.0	0.0	0.0	0.0	1.0
	Box 2	0.0	0.0	0.0	0.0	0.0	0.0
	Box 3	0.0	0.0	0.0	0.0	0.0	1.0
	Average	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.7**
1.5 ml/L	Box 1	0.0	0.0	0.0	0.0	2.0	2.0
	Box 2	0.0	0.0	0.0	0.0	1.0	1.0
	Box 3	0.0	0.0	0.0	0.0	2.0	3.0
	Average	**0.0**	**0.0**	**0.0**	**0.0**	**1.7**	**2.0**
2.0 ml/L	Box 1	0.0	0.0	2.0	2.0	3.0	3.0
	Box 2	0.0	0.0	1.0	2.0	3.0	3.0
	Box 3	0.0	0.0	0.0	1.0	2.0	2.0
	Average	**0.0**	**0.0**	**1.0**	**1.7**	**2.7**	**2.7**
2.5 ml/L	Box 1	0.0	1.0	2.0	4.0	6.0	8.0
	Box 2	0.0	0.0	1.0	2.0	4.0	7.0
	Box 3	0.0	0.0	1.0	3.0	5.0	7.0
	Average	**0.0**	**0.3**	**1.3**	**3.0**	**5.0**	**7.3**

Appendix 5. Concentration - Response Values for Controller Super 2.5 EC

Conc. group	Concentration Ml/l	Log - concentration	mean mortality	Percentage mortality	Corrected percent	Probit
1.	1.0	0.0	1.0	10.0	10.0	3.72
2.	1.7	0.2	4.7	46.7	46.7	4.91
3.	3.3	0.5	6.7	66.7	66.7	5.43
4.	5.0	0.7	10.0	100.0	97.5	6.96
5.	6.7	0.8	10.0	100.0	97.5	6.96

Appendix 6. Concentration - Response Values for Pyrinex 28 EC

Conc. Group	Concentration Ml/l	Log - concentration	Mean mortality	Percentage mortality	Corrected percentage	Probit
1.	0.5	-0.3	1.3	13.3	13.3	3.89
2.	1.0	0.0	3.7	36.7	36.7	4.66
3.	1.5	0.2	8.3	83.3	83.3	5.96
4.	2.0	0.3	7.0	70.0	70.0	5.52
5.	2.5	0.4	9.0	90.0	90.0	6.28

Appendix 7. Concentration - Response Values for Golan SL

Conc. group	Concetration Ml/l	Log - concentration	Mean mortality	Percentage mortality	Corrected percentage	Probit
1.	0.5	-0.3	0.0	0.0	2.5	3.04
2.	1.0	0.0	0.67	6.7	6.7	3.50
3.	1.5	0.2	2.0	20.0	20.0	4.16
4.	2.0	0.3	2.7	26.7	26.7	4.38
5.	2.5	0.4	7.3	73.3	73.3	5.62

Source: Authors construct from Laboratory Experiment 2019

Appendix 8. Pearson's Correlations between different concentration Levels (Controller Super 2.5 EC)

Concentration Level		1.0 ml/L	1.7 ml/L	3.3 ml/L	5.0 ml/L	6.7 ml/L
1.0 ml/L	Pearson Correlation	1	.821*	.795	.702	.390
	Sig. (2-tailed)		.045	.059	.120	.445
	N	6	6	6	6	6
1.7 ml/L	Pearson Correlation	**.821***	1	.994**	.979**	.767
	Sig. (2-tailed)	**.045**		.000	.001	.075
	N	**6**	6	6	6	6
3.3 ml/L	Pearson Correlation	.795	**.994****	1	.988**	.763
	Sig. (2-tailed)	.059	**.000**		.000	.078
	N	6	**6**	6	6	6
5.0 ml/L	Pearson Correlation	.702	.979**	**.988****	1	.820*
	Sig. (2-tailed)	.120	.001	**.000**		.046
	N	6	6	**6**	6	6
6.7 ml/L	Pearson Correlation	**.390**	.767	.763	**.820***	1
	Sig. (2-tailed)	**.445**	.075	.078	**.046**	
	N	**6**	6	6	**6**	6

**. Correlation is significant at the 0.05 level (2-tailed).*
***. Correlation is significant at the 0.01 level (2-tailed).*

Appendix 9. Pearson's Correlations between different concentration Levels (Pyrinex 48 EC)

Concentration Level		0.5 ml/L	1.0 ml/L	1.5 ml/L	2.0 ml/L	2.5 ml/L
0.5 ml/L	Pearson Correlation	1	.907*	.939**	.907*	.942**
	Sig. (2-tailed)		.013	.006	.013	.005
	N	6	6	6	6	6
1.0 ml/L	Pearson Correlation	**.907***	1	.995**	.987**	.986**
	Sig. (2-tailed)	**.013**		.000	.000	.000
	N	**6**	6	6	6	6
1.5 ml/L	Pearson Correlation	.939**	**.995****	1	.993**	.994**
	Sig. (2-tailed)	.006	**.000**		.000	.000
	N	6	**6**	6	6	6
2.0 ml/L	Pearson Correlation	.907*	.987**	**.993****	1	.984**
	Sig. (2-tailed)	.013	.000	**.000**		.000

Concentration Level		0.5 ml/L	1.0 ml/L	1.5 ml/L	2.0 ml/L	2.5 ml/L
	N	6	6	**6**	6	6
2.5 ml/L	Pearson Correlation	**.942****	.986**	.994**	**.984****	1
	Sig. (2-tailed)	**.005**	.000	.000	**.000**	
	N	**6**	6	6	**6**	6

**. Correlation is significant at the 0.05 level (2-tailed).*
***. Correlation is significant at the 0.01 level (2-tailed).*

Appendix 10. Pearson's Correlations between different concentration Levels (Pyrinex 48 EC)

		0.5 ml/L	1.0 ml/L	1.5 ml/L	2.0 ml/L	2.5 ml/L
0.5 ml/L	Pearson Correlation	.^{a}	.^{a}	.^{a}	.^{a}	.^{a}
	Sig. (2-tailed)		.	.	.	.
	N	6	6	6	6	6
1.0 ml/L	Pearson Correlation	.^{a}	1	.716	.539	.748
	Sig. (2-tailed)	.		.109	.269	.087
	N	6	6	6	6	6
1.5 ml/L	Pearson Correlation	.^{a}	**.716**	1	.847*	.919**
	Sig. (2-tailed)	.	**.109**		.033	.009
	N	6	**6**	6	6	6
2.0 ml/L	Pearson Correlation	.^{a}	.539	**.847***	1	.956**
	Sig. (2-tailed)	.	.269	**.033**		.003
	N	6	6	**6**	6	6
2.5 ml/L	Pearson Correlation	.^{a}	.748	.919**	**.956****	1
	Sig. (2-tailed)	.	.087	.009	**.003**	
	N	6	6	6	**6**	6

**. Correlation is significant at the 0.05 level (2-tailed).*
***. Correlation is significant at the 0.01 level (2-tailed).*
a. Cannot be computed because at least one of the variables is constant.

Appendix 11. Transformation of percentages to probits

%	0	1	2	3	4	5	6	7	8	9
0	----	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.20	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
---	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

Source; http://userwww.sfsu.edu/efc/classes/biol710/probit/ProbitAnalysis.pdf

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/73108