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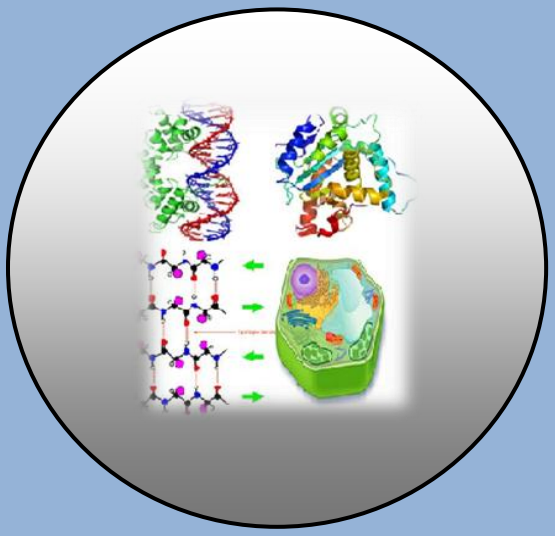

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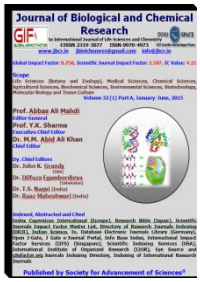

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Insecticidal activity of soluble concentrate formulation of *Khaya ivorensis* against pink bollworm, *Pectinophora gossypiella* (Saunders)

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ABSTRACT

The aim of the present study was to prepare a stable soluble concentrate formulation of ethanolic extract of Khaya ivorensis leaves. Stability studies were performed at different accelerated conditions to predict the stability of the prepared formulation. Different parameters, pH, viscosity, flash point, density, surface tension, refractive index, effect of centrifugation, persistent foam and dilution stability were determined. The prepared formulation exhibited good stability after preparation at different storage conditions. No separation was observed during the stability test. The effect of the prepared formulation on newly hatched larvae of pink bollworm Pectinophora gossypiella was evaluated under laboratory conditions. The results showed that the prepared formulation had insecticidal activity against newly hatched larvae of the Pectinophora gossypiella. The major components of ethanolic extract of Khaya ivorensis were determined by Gas Chromatography mass spectrometry analysis.
Keywords: formulation, Insecticidal activity, Khaya ivorensis, Pectinophora gossypiella. GC-MS analysis.

INTRODUCTION

Cotton (*Gossypium spp*.) is very important source of income for the local people and foreign exchange for the country (Ali et al. 2009). In many countries cotton is one of the most important fibre producing plants. Cotton crop not only provides fibre for the textile industry, but also plays a role in the feed and oil industries with its seed, rich in oil (18 – 24%) and protein (20 – 40%). An estimated 350 million people are engaged in cotton production either on-farm or in transportation, ginning, baling and storage. China consumes 40% of the world's raw cotton. Australia and Egypt produce the best quality cotton in the world. Insect-

pests are considered one of the important factors that influence cotton production and cause economical damage to the crop yield. Also, damage causing losses to fruit is frequently more destructive than that to leaves, stems and roots. Profitable cotton production in Egypt depends on successful and efficient pest management programs which reduce the disaster of crop losses particularly caused by insect-pests. In Egypt, about half million cotton feddan were cultivated in 2006 cotton growing season that represent about 6% of all cultivated area. Pests are such serious threat to cotton production and the cost of cotton pests control is about $12.5 million (Younis et al., 2007).

The pink bollworm *Pectinophora gossypiella* (Saunders) is one of the most injurious cotton pests in the world (Lykouressis et al. 2005; Al-kazafy et al., 2014; Ezzat et al., 2015). It is distributed in all most all cotton growing states of the country and has causes serious damage in cotton bolls resulting in high reduction in quantity and quality of cotton yield, so the success in controlling such insect is considered to be of great economical importance. It is a global pest that has many potential host plants, but feeds almost exclusively on cotton. Various control methods mainly the conventional chemical insecticides are used for pest control in agriculture. Synthetic pesticides are currently the most effective means of pest control. However, the increasing and indiscriminate uses of these substances have not only caused adverse effects on mammals' health, but have also affected on many other non-target organisms (Bughio and Wilkins, 2004). They are also responsible for the development of insecticide-resistance phenomenon (Lietti et al., 2005). In addition, over pollution of the environment, toxic residues, problems and carcinogenicity of these insecticides are well known and increased costs of insect control (Roy and Mukhopadhyay 2010).Therefore, using new approaches in pest management systems is highly encouraged. One solution for these problems is utilization of plants' bioactive molecules. Natural products based pesticides can sometimes be specific to the target species and have unique modes of action . Plant products have several uses in insect control. (Moreira et al., 2004; Jbilou et al., 2006; Moreno et al., 2011; Salari et al., 2012).

Khaya ivorensis A. Chev., producing plants from Meliaceae family, is one of the most popular traditional medicines in Africa, which occurs on the West Coast of Africa from Sierra Leone to Cabinda, is the best known African mahogany. It is a large malicious mahogany closely related to the African genus. It has insecticidal, antibacterial, antifungal, antimalarial, anticancer, antiviral and other clinical activities on humans (Abdelgaleil et al., 2005). The aim of the present study was to extract of *Khaya ivorensis* and formulated it in a suitable formulation; soluble concentrate formulation as a new alternative of conventional pesticide formulation for evaluating its efficacy against newly hatched larvae of the *P. gossypiella*.

MATERIAL AND METHODS

Plant Material

Fresh pieces from *Khaya ivorensis* were collected from Agricultural Research Center (ARC), Giza, Egypt. Leaves were air dried under natural laboratory conditions for one week.

Chemicals

Non-ionic surfactant, Polyethylene glycol dodecyl ether, Polyoxyethylene (23) lauryl ether (Brij 35) was purchased from Loba Chemie. India. Sodium sulphate was purchased from Oxford Laboratory.

Mumbai, India Propylene glycol, sodium hydroxide were purchased from ADWIC; El Nasr Pharmaceutical Chemical Co., Egypt, calcium carbonate was purchased from Sigma-Aldrich Chemie GmbH Steinheim, Germany, Magnesium oxide and methyl red were purchased from Qualikems Fine Chemicals. India. Ammonia solution was purchased from Prolabo, while the propionic acid was purchased from Chem-Lab, Belgium. Ethanol absolute was purchased from Fisher Scientific Company. Fair lawn, New Jersey, USA. Water used in all preparations obtained from Water distiller LABCONCO water PROT.M PS LABCONCO Corporation, Kansas City, Missouri 64132-USA.

Tested insect

The 1st instar larvae of pink bollworm, *Pectinophora gossypiella*, used in this study, were obtained from a standard laboratory colony, Bollworm Department, Plant Protection Research Institute; Agriculture Research Centre (ARC), Giza, Egypt, reared on an artificial diet at 27±1°C and 75±5% relative humidity for several generations away from any insecticidal contamination (Rashad and Ammar, 1985).

Preparation of ethanolic extract of *K. ivorensis*

Dried leaves of k*. ivorensis* were grounded using an electric mill sieved and kept for extraction. Plant extracts were prepared according to the method adopted by Freedman et al. (1979). One kilogram sample of plant material was soaked in 3 liters of ethanol and kept for 7 days in brown dark bottle with tight stoppers then the bottle was shaken for one hour by using a shaker. The extract was filtered over anhydrous sodium sulphate and the solvent was evaporated under reduced pressure using a Rotary Evaporator (Heidolph, LABOROTA A 4000-Germany) at 40-50°C to dryness. The resulting crude extract was weighted and kept in the deep freezer until evaluation.

Chromatography-Mass Spectrometry (GC-MS) analysis

Gas Chromatography-mass Spectrometry analysis of ethanolic extract of *khaya ivorensis*: The GC-MS analysis was performed with an Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column DB-5 (30 m x 320 µm x 0.25 µm film thickness). Helium was used as carrier gas at approximately 1.0 ml/min pulsed splitless mode. The solvent delay was 3 min, and the injection volume was 1 µl. The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 ev scanning from m/z 50 to 500. The ion source temperature was 230°C and the quadruple temperature 150°C. The electron multiplier voltage (EM voltage) was maintained 1050 v above auto tune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program was started at 60°C then elevated to 280°C at rate of 8°C /min, and 10 min hold at 280°C. The Detector and injector temperature were set at 280°C and 250°C, respectively.

Identification of components

Interpretation on mass spectrum of ethanol extract of *k. ivorensis* wee conducted using the database of National Institute of Standard and Technology (NIST) library and Willey (Chem Station data system).The spectrum of the compounds were compared with the spectrum of NIST library database. The name, molecular weight and structures of the compounds were ascertained.

Preparation of 40% soluble concentrate formulation of ethanolic extract of *k. ivorensis.*

Soluble concentrate formulation of *k. ivorensis* contains the amount of technical grade of ethanolic extract, antifreeze agents, preservative, and wetting agent.

The system was stirred (Magnetic stirrer with hot plate "Terrey Pines Scientific", USA) at high speed for 10 min until formation of the soluble concentrate formulation.

Physicochemical characterization

Visual Inspection

The soluble concentrated formulation was observed for homogeneity, dilution stability or separation during storage.

Dilution stability

Dilution stability is determined to ensure water soluble products dissolve readily and, when diluted, produce stable solutions without precipitation. The formulation, after the stability test at 54°C and following dilution with CIPAC Standard water D and standing at 30°C ± 2 for 18 h [CIPAC MT 18], will give a clear or opalescent solution, free from a trace of sediment and visible solid particles. Any visible sediment or particles produced pass through a 45 µm test sieve, using sieve shaker. [CIPAC MT 41].

Storage stability

After formulation, the accelerated storage tests were carried out according to CIPAC methods MT 39.3 and MT 46.3. The storage (stability) test (0°C) was performed during one week and the storage test (54°C) during two weeks.

Freeze -Thaw Cycles

Test tubes filled with the prepared formulation and hermitically closed were vertically stored for 12h in freezer at -20°C, and then for 12h at room temperature 25°C ±2. The formulation was observed for any change recorded. The formulation is considered "stable" if there is no substantial separation after four cycles.

Centrifugation test

Formulation was subjected to centrifugation at speeds up to 5400 rpm for 5 min by using a Laboratory Centrifuge REMI Centrifuge REMI Equipments Bombay-India- R32A.4000002.The formulation was centrifuged at 25°C.

Persistent foam

Specified amount of formulation was added to CIPAC standard waters A and D (95ml) in the measuring cylinder and made up to the mark. The cylinder is stoppered and inverted 30 times. Stand the cylinder on the bench and left undisturbed for the specified time. The volume of foam was noted. [CIPAC MT 47.2].

pH Measurement

pH value of (1%) prepared formulation was measured by using a pH Meter "Model: Jenway Instruments pH 3510pH meter. [CIPAC MT 75.3].

Surface Tension.

Surface tension of the prepared formulation was measured using "Sigma 700" by du Noüy Ring, a platinum/iridium ring. The instrument recalibrated before testing, the sample measured should be clean, homogenous and free from any bubbles and has a stable surface. Recording the surface tension of the prepared formulation.

Density measurement

Density of the prepared formulation was measured using digital density meter model DDM 2910 by touch screen. Rudolph Research Analytical, USA.

Refractive Index

Refractive index of the prepared formulation was measured by using ABBE Refractometer, ATAGO, Co., LTD, Japan. [ASTM, 2002].

Flash point

Flash point of the prepared formulation was carried out by tag open-cup method using Koehler instrument company, INC, USA. The flash point was recorded as the temperature at the thermometer when a flash appeared. [CIPAC MT12]**.**

Viscosity Measurement

Viscosity of the prepared formulation was measured at different shear rates, without dilution, using Brookfield DV II^+ PRO digital Viscometer. (Brookfield, USA). UL rotational adaptor. The temperature was kept at 25°C during the measurement by water bath TC-502. USA and each reading was taken after equilibrium of the sample. The flow curves of the prepared formulation were obtained by directly reading the viscosity (mPas) and shear rate (s^{-1}) from the viscometer. [ASTM, 2010].

Bioassay test

Thin film technique was used as a method of application in the present study. Newly hatched larvae of *P. gossypiella* were exposed to serial concentrations of the prepared formulation after dry the sprayed surface petri dishes (9 cm diam.). After an hour from exposure treated larvae were transferred individually on semi artificial diet into glass tubes (2x7.5 cm) covered with cotton piece and kept at 25±2°C and 70-85% R.H. according to Rofail et al., (1995). Each concentration was replicated three times. The control treatment was carried out by using distilled water. The number of dead larvae was counted after 24 hours of treatments. Mortality rate was recorded at time intervals after 1, 2, 3 and 5 days, respectively. The LC_{50} was used to investigate the effect of soluble concentrate formulation of ethanolic extract of *k. ivorensis* on the development of larval and pupal stages of *P. gossypiella*; duration larval and pupal weight. Reduction in their weight was estimated as follows:

$$\frac{\text{Weight of control} - \text{Weight of Treatment}}{\text{Weight of control}} \times 100$$

Statistical Analysis

The percentage of larvae mortality of the prepared soluble concentrate formulation LC_{50} values were determined by the linear regression (LPd line Computer Program) of the probit of the tested larval mortality vs. logs the concentrations (ppm) of the tested formulation. The obtained data were statistically analyzed using COSTAT Statistical program software and Duncan's multiple range tests at 5% probability level.

RESULTS AND DISCUSSION

GC-MS analysis of Ethanolic extract of *K. ivorensis*

GC-MS is one of the best techniques to identify the constituents of volatile matter, long and branched chain hydrocarbons, alcoholic acids, esters etc. Oladipupo et al., (2015). The results pertaining to GC-MS analysis leads to the identification of the number of compounds from the GC fractions of the ethanolic extract of *k. ivorensis* leaves and these compounds were identified through mass spectrometry attached with GC. Data in Table (1) and Fig (1) showed that, the most abundant components found in the ethanolic extract of *k. ivorensis* were (1); Hexadecanoic acid, methyl ester (23.64%) followed by (2); 8, 11- Octadecanoic acid, methyl ester (14.89%); (3) Phytol (12.40%) and (4); Hexadecanoic acid, ethyl ester (10.12%).

The mass spectrometer analyzes the compounds eluted at different retention times to identify the nature and structure of the compounds. The heights of the peak indicate that the relative concentrations of the components present in the plant extract. These mass spectra are fingerprint of that compound which can be identified from the data library. The chemical constituents of ethanolic extract of *k. ivorensis* leave using GC-MS. GC-MS analysis showed the existence of various compounds with different chemical structures. It is well known that the bioactivity of a plant extract could be due to the major compounds or a synergy between the major and minor constituents. It is evident that one or more compounds present in the extract may have been responsible for the observed insecticidal activity. Plant extract containing a large amount of Hexadecanoic acid, methyl ester; ethyl ester and phytol were known to exhibit insecticidal activity.(Anandan et al., 2012; Cruz-Estrada et al., 2013; Sha et al., 2013).

Table 1. List of Identified Phytocompunds of ethanolic extract of *k. ivorensis*

No.	Compounds	RT	Area %	MT.	MF.
1	Cyclohexanone,2-(2-nitro-2-propenyl)-)2-(2-Nitro-2-propenyl) cyclohexanone	183.09	3.03	15.83	$C_9H_{13}NO_3$
2	5-Benzimidazoline propionic acid, beta-methyl-2-oxo-	22.09	3.76	16.39	$C_{11}H_{12}N_2O_3$
3	2-Pentadecanone,6,10,14-trimethyl-	268.28	2.23	17.95	$C_{18}H_{36}O$
4	Hexadecanoic acid, methyl ester	270.26	23.64	18.95	$C_{17}H_{34}O_2$
5	Hexadecanoic acid, ethyl ester	284.27	10.12	19.57	$C_{18}H_{36}O_2$
6	8,11-Octadecanoic acid, methyl ester	294.26	14.89	20.98	$C_{19}H_{34}O_2$
7	Phytol	296.31	12.40	21.18	$C_{20}H_{40}O$
8	Linoliec acid ethyl ester	308.27	2.23	21.72	$C_{20}H_{36}O_2$
9	Cyclononasiloxane, octadecamthyl-	666.17	1.16	24.41	$C_{18}H_{54}O_9Si_9$
10	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl ester)	278.15	3.77	25.73	$C_{16}H_{22}O_4$
11	(+)-(P,1R,3S)-5-(4,5-dimethoxy-2-methyl-1-naphthyl)-6,8-dimethoxy-1,2,3-trimethyl-1,2,3,4-tetrahydroisoquinoline [(+)-O-Methylancistrocline	436.25	3.09	30.09	$C_{27}H_{34}NO_4$
12	Cyclodecsiloxane,eicosamethyl-	740.19	1.84	32.15	$C_{20}H_{60}O_{10}Si_{10}$

RT: Retention Time MW: Molecular Weight MF: Molecular Formula

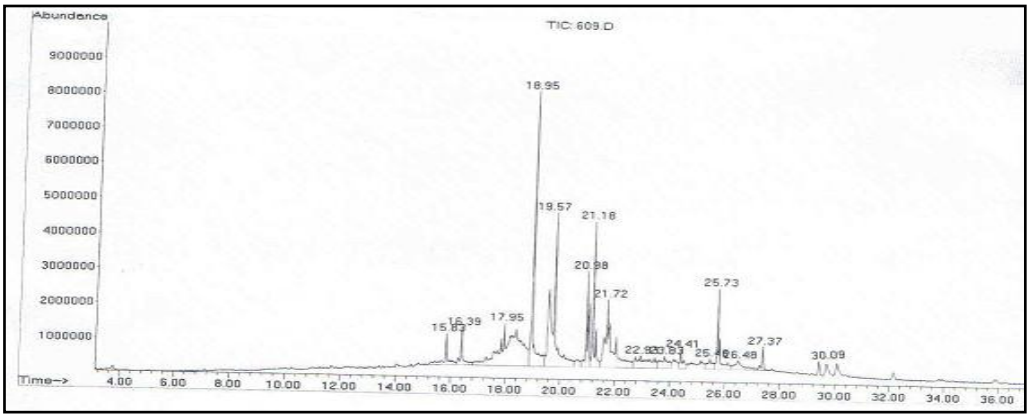

Figure 1. GC-MS Chromatogram of ethanolic extract of *k. ivorensis*

Formulation characteristics

The primary objectives of formulation technology are to optimize the biological activity of the pesticide, and to give a product which is safe and convenient for use. (Knowles, 2008).The simplest of all formulations to make is the solution concentrate; an aqueous solution of the active ingredient which merely requires dilution in the spray tank. (Knowles, 2008). The stability of soluble concentrate formulation can be predicted by measuring some physical parameters before and after accelerated tests. Storage at 0°C and 54°C has been used to control physical and chemical stability. It has been generally accepted that two weeks at 54°C represent 2 years in normal conditions. There is no evidence which indicate that a product has a satisfactory shelf life (of at least 2 years) in the different temperature zones. The test thus provides a useful guide for performance after storage in warm or continental temperature climates. However, it is not quite sure that the product which passes these tests will be satisfactory in field conditions. (Gašić et al., 2012).The soluble concentrate formulation of ethanolic extract of *k. ivorensis*, dissolved readily and, when diluted at a ratio of 5: 95 (v/v) soluble concentrate formulation: water with CIPAC standard waters A and D produced stable solution without precipitation and no change in color or appearance through the storage period. (7 days at 0°C ± 2 and 14 days at 54 ± 2°C). Such signs are good preliminary indication of physical stability.

Results of physical properties measurements are shown in Table (2). The pH values of the prepared formulation were in range (5.00-5.18), indicating that the prepared formulation in the different storage conditions having acidic character implying that it will have good biological activity. On the other hand, the prepared formulation having the surface tension ranges (28.99-29.62 mN/m). Lower surface tension is a desirable characteristic for most agricultural sprays because it 1) facilitates the spreading of droplets upon impaction on leaves or other target surfaces, to increase the surface active area, 2) improves penetration and uptake of the product into the plant, (Giardino et al., 2006) and 3) can facilitate retention of the material by the rain, ensuring improved rain fastness. Therefore, the pesticidal efficacy was increased. (Furmidge,1962).According to WHO specifications, the liquid formulations must have flash point not less than 22.8°C. The prepared formulation in the all storage conditions having high value of flash point more than 70°C and is quite safe. The variation of density was 0.9719-0.9885 g/cm^3. Results of persistence foam are given in Table 3. The volume of foam from the prepared formulation in CIPAC standard waters A and D is low and passed through the recommended rate of foam. Also, the prepared formulation of soluble concentrate formulation, showed shear-thickening behavior. Shear-thickening (ST) is a flow behavior marked by a shear viscosity increase with increasing applied shear rate or shear stress. (Ye et al., 2013).Fig. (2).

Table 2. Physicochemical properties of soluble concentrate formulation of *k. ivorensis* before and after storage.

Time	**Fresh formulation**	**After 7 days**	**After 14 days**	**Freeze-thaw**
Temperature	**room temp.**	**0°C**	**54°C**	**4 Cycles**
pH value (1%)	5.00	5.02	4.98	5.18
Refractive Index	29.62	28.99	29.27	29.59
Surface tension (mN/m)	1.3848	1.3853	1.3848	1.3859
density(g/cm^3)	0.9885	0.9719	0.9809	0.9852
Flash point (°C)	Over 70°C	Over 70°C	Over 70°C	Over 70°C

Table 3. Volume of persistence foam (cm^3) observed in soluble concentrate formulation of *K. ivorensis* before and after storage test.

Sample Code	CIPAC Water A			CIPAC Water D		
	1 min	5 min	12 min	1 min	5 min	12 min
Fresh formulation	2	3	4	3	4	35
After 7 days 0°C	3	4	5	2	3	4
After 14 days 54°C	4	4	5	3	3	4
Freeze-thaw cycles	3	3	4	4	3	4

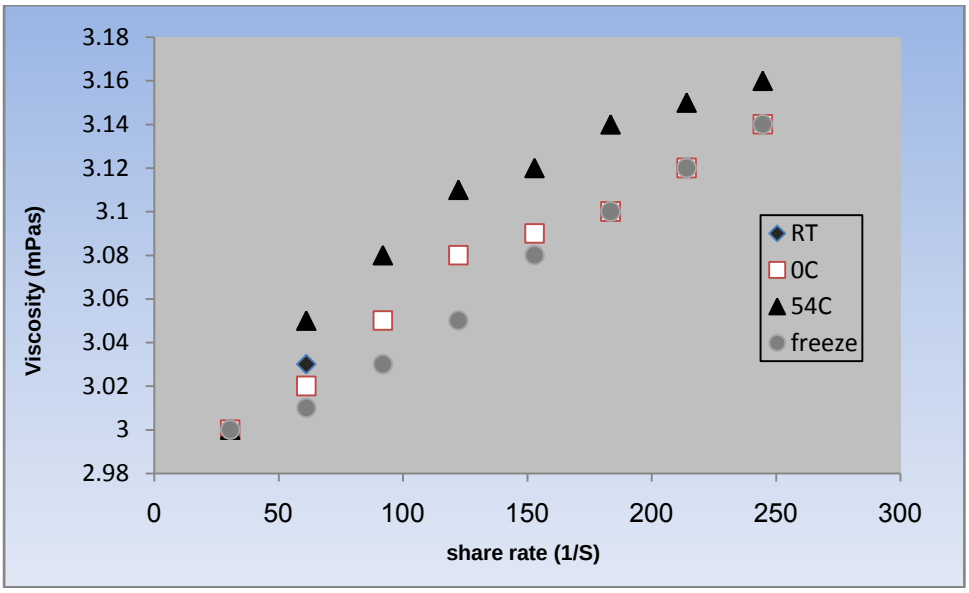

Figure 2. Rheological properties of soluble concentrate formulation of *K. ivorensis* before and after storage.

Table 4. Toxicity of soluble concentrate formulation of *Khaya ivorensis* on newly hatched larvae of *P. gossypiella*.

Conc.		% Mortality			
		24h	48h	72h	120h
12.5 ppm		24.99	25.65	32.52	49.06
25 ppm		28.08	33.08	39.53	54.47
50 ppm		35.63	42.05	46.90	59.79
100 ppm		46.28	51.46	54.38	64.94
200 ppm		60.79	60.79	61.70	69.83
400 ppm		69.53	69.53	66.09	74.42
800 ppm		77.28	77.28	77.95	78.55
LC_{50}		108.20	89.05	66.64	14.12
95 % Confidence limit	**Lower**	82.75	66.15	44.87	4.19
	Upper	142.21	118.78	94.11	27.26
Slope		0.84±0.08	0.77±0.0.04	0.63±0.08	0.45±0.08

Formulation activity

Toxicity of soluble concentrate formulation of *K. ivorensis* on newly hatched Larvae of *P. gossypiella*

Data presented in Table (4) showed that the prepared formulation had insecticidal activity against the newly hatched larvae of P*. gossypiella*. Also, the results revealed that the percentage of larval mortality fed on treated diet had a positive relationship with different concentrations of the prepared formulation comparing with untreated. i.e., the percentage mortality of *P.gossypiella* increases with increasing concentrations of the soluble concentrate formulation. The prepared formulation of soluble concentrate formulation could be successfully used for the control of pink bollworm *P. gossypiella* and may even replace the synthetic insecticide.

Biological activity of prepared formulation on larval and pupul stages

Data presented in Table (5) demonstrated that the prepared formulation caused significant increase in larval and pupal period which were 19.63 and14.5 days, respectively compared with untreated larvae which were 13.15 and 9.69 days, respectively. Also, the data showed that the prepared formulation caused decrease the newly hatched larval weight and the pupal weight when newly hatched larvae of *P. gossypiella* fed on treated diet compared with control. Fig (3). These results are in agreement with Hegab, (2008) who found that Z-seed oil of Zanzalacht extract (*Azadirachtin*) caused moderately decrease of the 4th instar larvae and severely decrease in pupal weight of Spiny boll worm.

Table 5. Biological activity of different stage of *P. gossypiella* after treated with soluble concentrate formulation of *K. ivorensis*.

Tested Compound	Larval duration			Pupal duration		
	Larval Period mean±S.E.	Weight mean±S.E.	R.E%	Pupal Period mean±S.E.	Weight mean±S.E.	R.E%
Formulation	19.63^{a}±2.13	0.023^{a}±0.008	20.69	14.5^{a}±1.70	0.022^{a}±0.002	42.11
Control	13.15^{b}±1.50	0.029^{a}±0.095	0.00	9.69^{b}±3.00	0.038^{a}±0.003	0.00

Figure 3. Morphological effects of *P. gossypiella* 4th instar larvae when treated with LC_{50} of soluble concentrate formulation of *Khaya ivorensis*. (C= Control).

The insecticidal, antifeedant and growth inhibitor action of plant extracts to several insect species may be due to the presence of substance such as sterols, terpnoids, flavonoids, alkaloids, Tannins, saponins,…….etc; fatty acid and fatty acid esters and phytol (Abdelgaleil and El-Aswad 2005; Anandan et al., 2012; Cruz-Estrada et al., 2013; Sha et al., 2013).Also, Simmonds, (2003) found that flavonoids affect the feeding behavior of a range of noctuidae larvae.

CONCLUSION

From the above mentioned data, characterization of the prepared formulation for its pH, viscosity, flash point, density, refractive index, surface tension, effect of centrifugation and dilution stability were determined during storage studies. It was noted that *k. ivorensis* could be successfully formulated in the form of a stable soluble concentrate formulation. Also, these studies demonstrate that the toxicity of this formulation as an insecticide due its repellency to *P.gossypiella* larvae. Further studies of using such formulation in the field or even greenhouse conditions to inspect the efficacy.

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