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Formulations of Chlorpyrifos-Ethyl in Non- Conventional Water-Based oil in water emulsion (EW) and micro emulsion (ME), and their Insecticidal Evaluation against Pink BollWorm (*P. gossypiella*), and migratory locust (*L. migratoria*)

Aly F. Aly [1]; Nasser A. Ibrahim [1]; Sameh S. Hafez [2]; Zakia K. Elkhiat [1]*
[1] *Pest. Form. Res. Dept., Central Agri. Pest. Lab., Agricultural Research Centre (ARC), Dokki, Giza, Egypt.*
[2] *Pest. Resist. Res. Dept. Central Agri. Pest. Lab., Agricultural Research Centre (ARC), Dokki, Giza, Egypt*

Abstract
With increasing the awareness of the effects of the conventional formulations of pesticides, there is a notable trend in the developed countries toward switching over from such formulations to more safe, less dosing eco-friendly water based formulations. In such non-conventional formulations, toxic and environmentally hazard organic solvents are replaced with water. Chlorpyrifos is well known and widely distributed organophosphorus insecticidein the form of EC formulation in Egypt and all over the world. In this study, two types of chlorpyrifos-ethyl as water based (24% EW and 24% ME) formulations were prepared and characterized. Physicochemical of the prepared formulations with their spray solutions were studied and compared with the standard formulation (Dursban H 48% EC). All studied parameters of the three formulations meet the specifications of FAO and WHO organizations. The insecticidal activity of the new formulations was evaluated against Pink bollworm (*P. gossypiella*) (Saunders) and 5th instar nymphs of the migratory locust (*L. migratoria*) and compared with the same standard insecticide. The insecticidal activity of the new formulations against Pink bollworm (*P. gossypiella*) (Saunders), showed promising activity where their LC_{50} values were 173.77, 179.062 and 311.12 ppm for EW, ME, and EC formulations respectively. While, field evaluation against migratory locust (*Locusta migratoria*) new formulations showed activity closed to the efficacy to that of the standard formulation. LC_{50} of the standard insecticide was 4.9 ppm and LT_{50} was 0.35 hrs. The LC_{50} values of the new formulations are
15.5 and 19.9 ppm for ME and EW formulations, and their average LT_{50} values are 4.71 and 7.28 hrs respectively.

Key words: Water-based EW and ME emulsion formulations, non-conventional formulation, physicochemical characteristics, chlorpyrifos, insecticidal activity, Pink bollworm (*Pectinophora gossypiella) and* migratory locust(*Locusta migratoria*).

1. Introduction

Organophosphorus (OP) insecticides which had been firstly used in 1960s are the most applied insecticides due to their high effectiveness potentiality and low environmental persistence [1]. Chlorpyrifos (O, O–diethyl–O–3, 5, 6–trichloro–2-pyridyl) phosphorothioate is one of the most world widely used organophosphate insecticides. It has played a key role in the pest management process around the world [2–4].

Conventional emulsifiable concentrate (EC) is the main commercially available formulation of chlorpyrifos in Egypt. In developing countries, pesticides mainly have been used in conventional or old technology formulations like dust, wettable powder, emulsifiable concentrate, solutions and others. Beside the undesirable characteristics of such formulations as dustiness and using volatile organic solvents in their preparation, they are applied in an increased dose rate or repeated applications to get the desired bio-efficacy. These properties maximize several problems related to environmental protection, pesticide residues in ecosystem and final fruit and vegetable products[5].Due to increasing the awareness of the toxic effects of conventional formulations and their related constituents, the developed world has progressed substantially in this regard to develop eco-friendly formulations which are safer to environment, users and with the targeted crops [6]. These formulations would not only replace toxic and non-degradable ingredients with safer ones, but also increase the efficacy of the final products through the

*Corresponding author e-mail: zakiaelkhiat@hotmail.com , Zakiaelkhait@arc.sci.eg (Zakia K. Elkhiat)
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proper choice and balance of all the components used in the formulation[7] .

Emulsion in Water (EW) is a liquid preparation of tiny oil droplets dispersed in water. the active ingredient is dissolved in a water-immiscible solvent, in the presence of surfactants, with vigorous stirring where, the oil phase dispersed as fine droplets in water. However, it still contains organic solvent but in smaller quantities than EC formulation [7–9] . An oil-in-water microemulsion (ME) is a thermodynamically stable, spontaneously formed, visually clear and optically transparent colloidal dispersion consisting of small spheroid particles [10,11]. Both EW and ME are non-conventional pesticide formulations where water is used instead of organic solvents [5,12] .In addition to having lower skin and eye toxicity as well as higher flash point, they also are safer when transport and storage as well as more compatible with other water based formulations as soluble concentrate (SC) formulations [5,13].

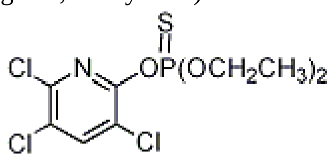
The objectives of the current study are to prepare safer and biologically more active water-based (EW and ME) emulsion formulations containing half amounts (240 g/l) of chlorpyrifos in the traditional formulations and to check their shelf-life stability under different storage conditions. Additionally, comparing their biological efficacy with Dursban H 48 % EC against the Pink bollworm (*P. gossypiella*) and migratory locust (*L. migratoria*).[14,15]. [16,17].

2- Experimental.

2.1- Materials

Insecticides

Dursban H 48% EC AGRIN SERVE local company, produced by Dow Agro Sciences LLC (Indianapolis, IN, USA). Chlorpyrifos [O, O-diethyl O-(3, 5, 6-trichloro-2-pyridinyl) Phosphorothioate] 97% from Egyptchem International for Agrochemicals (Technical grade, Purity 95%).



Surfactants and solvents

Polyoxyethylene sorbitan monooleate (Tween 80), Polyoxyethylene sorbitan esters (Tween-20), sorbitan fatty acid esters, (Span 80) were manufactured by Qualikems Fine Chem Pvt. Ltd. INDIA, Alkamuls RC (ethoxylated Castor oil) supplied by Rhodia-Home, Personal Care &Industrial Ingredients, Milano, Italy. Anionic Surfactants as Rodacal 60/BE (dodecyl benzene sulfonate, calcium salt) and (Geronol FF4) which is a mixture of ionic and non-ionic surfactant (calcium dodecyl benzene sulphonate in 2-methylpropan-1-ol and tristryphenol ethoxylates) by Rhodia. Xylene, toluene, solvesso 100, cyclohexanone, isopropyl alcohol, n-butanol, n-octanol as solvent and co-surfactant, was purchased from ADWIC, El Nasr Pharmaceutical Chemical Co., Egypt. Distilled water used in all preparation steps delivered by Water distillator LABCONCO water PROT. MPS LABCONCO Corporation, Kansas City, Missouri 64132-USA.

Equipment

Magnetic stirrer with hot plate “Terrey Pines Scientific” and 4 digit Sensitive balance, 220 g maximum load RADWAG, from USA used in the preparation of the two new chlopyrifos formulations. Melting point of technical material was measured by Stuart Automatic Melting Point, SMP40 according to [18] test method. Where small portion of substances inserted in capillary tube and placed inside instrument adjusted at ramp rate 5ºC/min. then record melting point of the substance at which it starts to convert from solid state to liquid state by heating.

Pink bollworm (*Pectinophora gossypiella*) (Saunders).

During the cotton season, a field population of green bolls Pink bollworm *(Pectinophora gossypiella)* were collected at the end of the cotton growth season was from the Sharkia government. The disposed larvae were released form the double infested seeds and reared under suitable temperature and humidity (25 ± 2ºC and 60 ± 5% R.H) according to rearing technique reported by [19,20] .

The migratory locust *(Locusta migratoria)*.

The culture of the migratory locust (*Locusta migratoria*) was provided by the Locust and Grasshoppers Research Division, Plant Protection Research Institute, ARC, Dokki, Giza. [21] described a rearing and handling approach for the insects. Daily food sources were sesbania and maize leaves. The cages of insects were kept at a constant room temperature of (32 ± 2ºC) and (30 – 50% RH) as relative humidity. The experimental nymphs were removed from the gregarious stock colony at the commencement of the first nymphal instar and kept in cages measuring 30 × 30 × 30 cm dimensions.

2.2- Methods

Preparation of emulsion in water (EW) formulation.

Emulsion oil in water 24% EW chlorpyrifos formulation was prepared by phase inversion method [9]. The oil phase containing the active ingredient is obtained by dissolving the calculated amount of technical material in aromatic hydrocarbon as xylene, toluene or cyclohexanone containing a blend of two non-ionic surfactants from tween 80, FF4, tween 20 and span 80, (with difference in HLB value 10-12). The aqueous phase was then slowly added to the oil phase at a constant rate of agitation to reach the inversion point at which the emulsion changes from water-in-oil to an oil-in-water emulsion. The process was carried out at 40ºC good stirring for 30-40 min to get chlorpyrifos 24% EW that is identified as CHEW.

Preparation of microemulsion (ME) formulation

Chlorpyrifos 24% ME microemulsion formulation was also prepared by the phase inversion method. The aqueous phase (mixture of water and isopropyl alcohol) was added slowly with continuous good stirring, at 30ºC, to oil phase containing chlopyrifos 24.72 wt. %, water immiscible solvent as cycloheaxanone, solvesso 100, or xylene, surfactants mixture two of FF4, span80, tween20 or tween80 to form chlopyrifos 24% ME that is identified as CHME.

Characterization of the studied EC, ME and EW formulations.

Particle size

The mean particle size and Poly dispersity index (PDI) of the new emulsions (ME and EW) were determined by light scattering method using Zeta sizer Ver.6.20 (Malvern Instruments Ltd., Worcestershire, England). Analysis process was carried out on freshly prepared samples. Each sample was diluted with deionized water (1:100), shaken vigorously before testing to avoid multiple scattering effects, and analysed in a clear disposable zeta cell. The best result of particle size was matched with % volume [22,23] .

Active ingredient content

The contents of the active ingredient in the studied formulations (EC, ME and EW) were detected by Gas Liquid Chromatography (Unicam pro GC) equipped with Electron Capture Detector (ECD) programmed for external standardization using peak area. Data obtained are illustrated in Table (1).

Formulation stability tests

These tests include emulsion stability, re-emulsification, stability at accelerated cold and hot storage conditions and persistent foam. Both accelerated cold and hot storage were carried out according to the test procedures [24] and [25] respectively. The first test was conducted by placing (about 100 ml formulation) in a glass bottle and placing the capped bottle and its contents in a refrigerator at 0 ± 2°C for 7 days. An accelerated hot storage procedure was executed by placing the sample (about 100 ml) in a bottle and placing the capped bottle and its contents in an oven at 54 ± 2°C for 14 days. The volume of any separated materials at the bottom of the bottles was observed. Many trails were conducted to get non separated materials. Emulsion stability test of each formulation was carried out visually according to [26]. Five ml of each formulation was added to a graduated cylinder (100 ml) with glass stopper filled with WHO standard hard water (95 ml) separately to produce 100 ml of aqueous emulsions. The cylinder was stoppered and inverted 10 times. Subsequently, the amount of free oil or creamy layer separated on the top or the bottom of emulsion was observed with various time intervals (initial time, 0.5h and 24h for re-emulsification test). Persistent foam was conducted to specify the volume of foam formed on 100 ml cylinder using the same dilution rate. According to [27], cylinder was filled with standard hard water (95 ml), 5 ml of formulation was added, cylinder was stoppered and inverted 30 times and lifted undisturbed. After 5 mins the volume of foam was measured and tabulated in Table (1).

Physicochemical properties of formulations and their spray tank solutions.

The following physicochemical properties for standard insecticide (Dursban H 48% EC), new formulations and their spray solutions were carried out to justify the formulation storage stability tests using the application rate dose of Drusban 48% EC (1L /feddan). Data obtained are illustrated in Tables (2 and 3).

Viscosity

It was measured for formulations and spray solutions using ″Brookfield DV II+ PRO″ digital viscometer (Brookfield, USA), according to the requirements of [28]. The temperature was kept at 25°C during all measurements using a water bath TC-502, USA. The principle of spindle rotation was used, in which the it was submerged in the sample to be analyzed, and the torsional force necessary to overcome the resistance of rotation was measured at rotational speed of max 200 rpm were specified for each sample.

Surface tension.

It was estimated for formulations and spray solutions using Force Tensiomate Sigma 700, USA by Whilmy plate method satisfying the test procedure [29]. This equipment was calibrated with distilled water. Water was used as the control in all assays used to determine the physicochemical characteristics of the spray mixtures.

Density and specific gravity.

Density and specific gravity of formulations and their spray solutions were measured with automatically with (Rudolph densitometer 2910, USA) fitted with autosampler, where up to 2 ml sample was drawn into instrument and it gave reading automatically according to test method [30].

Flash Point

The test was carried out according to [31] using the Koehler Flash Tester, USA. It was measured for formulations. In the method of sample under test was placed in the cup of the apparatus (2ml) and heated at a prescribed rate. A small test flame is directed into the cup at regular intervals, and the flash point was noted as the lowest temperature at which application of test flame causes the vapour above the sample to ignite with a distinct flash inside the cup.

Refractive Index:

The Refractive index is an optical measurement of a material's ability to bend a beam of light. Also, the refractive index may be used to determine the purity of the material; the refractive index of the EC samples was measured using a digital ABBE Refractometer, ATAGO, Co., LTD, Japan by placing one drop of the EC formulation on the slide at 25°C [32].

pH measurement

The test was carried out according to CIPAC specifications [33]. One g of the tested formulation was weighed and transferred to a measuring cylinder (100 ml) containing about 50 ml distilled water. The cylinder was made up to 100 ml and shook vigorously for 1 minute and then it was allowed to settle. The pH of the supernatant liquid was measured by Jenway pH meter 3510, UK supported by HANNA pH electrode.

Electrical Conductivity

The conductivity of spray solutions was measured by Conductivity and Salinity meter "Thermo Orion model 115A+, USA". The measurements were made at 25°C ± 2. Before the measurement according to [34] test procedure. One gram was weighed from sample into 100 ml distilled water in a beaker and shaken vigorously to mix completely, it was immersed into sample and left for 1-2 min during the measurement at a room temperature to allow the conductivity value to stabilize.

Insecticidal activity.

Insecticidal activity against Pink bollworm (*Pectinophora gossypiella*).

The experiment was conducted using the field application rate (1 L /feddan) to achieve 5 concentrations of 600, 480, 360, 240 and 120 ppm for reference EC formulation and 300, 240, 180, 120 and 60 ppm as concentration gradient for the prepared EW and ME formulations. Ten adult of Pink bollworm used for each replicate, three replicate for each treatment (concentration), glass chimney cages (6×9cm) were dipped in water of each formulation for 20 sec left to dray from exposure, the newly moth (zero day old) were exposed to residual test and kept at 26±1ºC and 70 - 85% R.H. according to [35, 36] Mortality was assessed after 24 hour exposure to thetested formulations. The LC_{50}, slope of toxicity line and toxicity index for each treatment were estimated and tabulated according to Ldp lines revealed the analogous response of the treated pests to the studied formulations in Table (4) [37,38] .

Insecticidal activity against grasshopper (*Locusta migratoria*).

Laboratory experiment

The leaf dipping technique was applied to deliver the chlorpyifos formulations to one-day old nymphs of the 5^{th} nymphal instars of *L. migratoria*. The nymphs were straved for 4-5 hrs before treatment. Castor leaves were dipped for two minutes in 12, 24, and 48 ppm spray solutions of the new (EW and ME) formulations as well as 24, 48, and 96 ppm of the reference EC formulation then supplied to the nymphs for feeding. Three replicates of ten nymphs each were given the treated leaves. The control was made by dipping the leaves in water only. All nymphs (treated and control) were given untreated fresh leaves a day after treatment. Every day for ten days, all mortalities of treated and control insects were recorded [39].

Field experiment

Field studies were conducted on September (2021) in a private field in El Baharia Oasis, Western Desert, Giza Governorate, during the 2021 season. The chosen area of 1L /feddan was divided into seven plots, one of which served as a control. Each plot covers approximately (35 x 15) = 525 m^2. A broad belt of 10 X 15 = 150 m^2 was used as a barrier zone between the plots to prevent pesticide drift and the migration of treated insects to the other plots and control. As general, the spraying process was carried out as the plots were located in the wind direction.

The motor spray, fitted with nozzle number 3 0.4 mm diameter, was utilized in all treatments and the spraying height was 0.5 m above the plants. The spraying speed was 40 meters per minute. The meteorological conditions at the applications were 3-5 m/sec wind and 30-40% humidity. The highest temperature was 28-30°C with a low of 18-20°C. The spraying took place between 8 and 10.5 a.m. Chlorpyrifos formulations were applied at half recommended dosage (500 ml/feddan) and recommended dosage (1000 ml/feddan) and sprayed on separate plots. Water was sprayed on the untreated plot as a control. Each treatment has three replicate cages measuring 0.5m×0.5m. Insects were gathered at random from the same treatment using a sweep-net and placed inside the cages immediately after application [39].

The cages were placed beneath the trees in the treatment area. Fifty nymphs were placed in each cage to feed on the treated cover. Ten days after application, mortality checks were performed. Before introducing the fresh food, routine tasks including removing the old uneaten food, dead nymphs, faeces, and counting the surviving insects were performed. The bioactivity of the tested formulations estimated by counting number of dead nymph and comparing it with that of control treatment in laboratory.

3- Result and Discussions

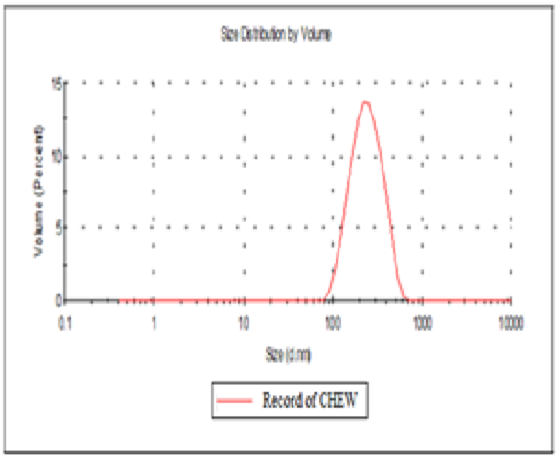
Characterization of the studied EC, ME and EW formulations.

Characterizations of the prepared ME and EW water-based formulations comparing with the standard EC one were measured in terms of their active ingredient contents, particle size (for new ME and EW formulations), creamy layer separation, emulsion stability, and persistent foam. Such parameters were studied for freshly prepared samples and after both accelerated cold and hot storage conditions. All obtained data are listed in Tables (1). Laboratory investigation showed no creamy layer separated or precipitated in all formulations and active ingredient contents (a.i) were found to be within the acceptable range with the tolerance of ± 5% for the standard 48% EC, and ± 6% for the new 24% WE and ME formulations [40]. Particle size is a key parameter that governed the stability and efficacy of pesticides. Usually finer droplets of pesticide formulation gave better long term stability and high field efficacy by enhancing the spread of formulation over the foliar surface [41]. Data also show that emulsion stability, and persistent foam tests meet the specifications of [42–44]

The presented data clarified that, particle size, PDI and % volume of EW formulation are 253.1 nm, 0.114 and 100% respectively. The results are in harmony with [22] who mentioned that, droplet size of the emulsion of oil in water formulation (EW) varied from 0.301 µm to 1.325 µm. In addition, the particle size, PDI and % volume of ME formulation, are 18.9 nm, 0.394 and 99.9% respectively, which in agreement with [45,46] They adopted that, particle size of microemulsions is in the range of 14.47 – 32.36 nm and PDI in the range of 0.19 – 0.74. [47] reported that, appearance of microemulsion as clear or translucent emulsion depends on the particle size that ranged from 10.0 – 300.0 nm. The importance of getting formulation with small particle size leads to enhancement the formulation stability, wettability, spray performance, and hence potential biological activity [48].

Table (1): Characterization of the new EW and ME emulsion and standard EC formulation.

Type of formulation	Standard 48% EC formulation			New 24% ME formulation			New 24 % EW formulation		
Types of sample	Fresh	After storage		Fresh	After storage		Fresh	After storage	
		Cold	Hot		Cold	Cold		Cold	Hot
A.I content (%)	48.0	47.5	47.1	22.6	23.3	25.7	23.4	23.0	25.3
Foam layer (ml)	1.0	1.0	1.0	1.0	0.5	0.5	1.5	1.0	1.0
Emulsion stability (ml)	2.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0
Re-emulsification (ml)	2.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0
Particle size (nm)	Not Detected			18.9			253.1		
PDI	Not Detected			0.394			0.114		
% volume	Not Detected			99.9			100.0		



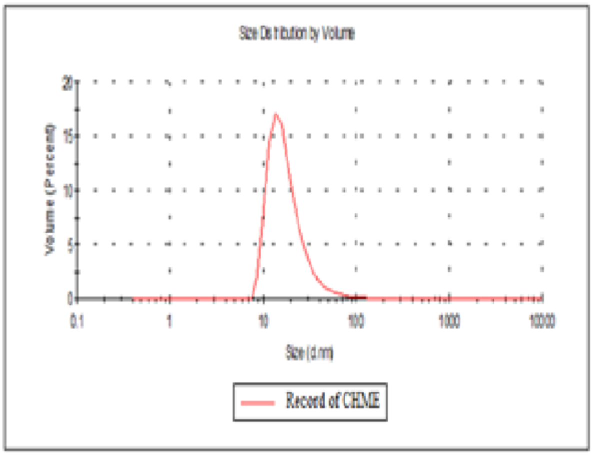


Figure (1): Particle size distribution of chlorpyrifos 24% EW (CHEW) and 24% ME (CHME) formulations.

Table (2): Physicochemical properties of the studied EC, EW and ME formulations.

Formulation /Conditions		Density[(a)] (g/cm^3)	Specific Gravity[(a)]	Refractive Index[(b)]	Surface Tension[(c)] (dyne/cm)	Viscosity[(d)] (cP)	pH[(e)]	Flash Point[(f)] (°C)
		Physicochemical characteristics						
EC	Pre storage	1.077	1.080	1.514	30.063	3.38	5.13	47
	Cold storage	1.078	1.081	1.514	30.146	3.34	5.11	47
	Hot storage	1.077	1.081	1.513	30.100	3.33	4.92	47
EW	Pre storage	1.062	1.065	ND	32.035	7.29	6.17	Over 70
	Cold storage	1.059	1.062	ND	32.316	7.18	4.93	Over 70
	Hot storage	1.053	1.057	ND	32.216	4.98	4.72	Over 70
ME	Pre storage	1.069	1.072	1.447	27.140	20.25	5.12	Over 70
	Cold storage	1.039	1.043	1.444	25.832	14.06	5.44	Over 70
	Hot storage	1.053	1.056	1.443	25.877	14.49	4.88	Over 70

(a)= value ±0.0009 **(b)** = value ±0.0461 **(c)** = value ±0.068 **(d)** = value ±0.115 **(e)** = value ±0.041 **(f)** = value ±0.51 these results values ± uncertainty

Physicochemical properties of the studied EC, ME and EW formulations.

Physicochemical parameters of the new EW and ME formulations were studied, and compared with that of the standard formulation (Dursban H 48% EC). Such parameters were studied according to the international specifications [42–44]. Table (2) showed the physicochemical properties of the three tested chloropyrifos formulations under different storage conditions. Data indicate that, values of both density and specific gravity are more than one (≥ 1) and so close to each other with no significant differences which have a high effect on the spontaneity of the formulation on dilution with water during preparation of the spray solutions. Refractive index increases with increasing the percentage of chloropyrifos in formulation. Its values ranged from 1.514 – 1.513 and 1.447 – 1.443 for both standard EC and new ME formulation respectively. These findings are in agreement with the published works [49–51]. They approved the direct relationship between the value of refractive index and the concentration of the solute in solution. Refractive index of EW is not applicable because it is opaque.

Surface tension is an important property of liquid formulations as it describes the force of interaction between its molecules, especially those found on its surface. Data in Table (2) proved that, the new prepared microemulsion (ME) recorded the least surface tension values (27.140, 25.832 and 25.877 dyne/cm) under the three tested conditions. This may be attributed to the solubilisation phenomenon which is a spontaneous dissolving of the substance by the reversible interaction with the micelles of surfactant in the solvent to form thermodynamically stable isotropic ME formulation [52]. A perusal of viscosity data indicates that, new formulations are more viscous than the standard EC formulation. Viscosity of the latter is slightly affected by both storage conditions while, the new EW and ME formulations are significantly affected by the storage conditions. Particularly, viscosity of EW formulation remarkably decreased after hot storage condition only and ME after both cold and hot storage processes. Viscosity of EW formulation is 7.29, 7.18, and 4.98 cP for fresh, cold, and hot storage samples respectively while, viscosity of EW formulation reached 20.25, 14.06, and 14.49 cP for fresh, cold, and hot storage samples respectively. The difference in viscosity between standard EC and new EW and ME formulations may be due to that the former contains large quantity of organic solvent and surface active agents than the others. It may also due to the hydrolysis of polysorbate emulsifier affected by storage temperature to produce fatty acids which alter the viscosity values [53].

Flash point is an important characteristic of the commercially distributed formulations all over the world where, it is used to specify the conditions under which formulations are transported and stored as they are dangerous and can potentially cause a fire. It is a measure of the tendency of a sample formulation to form flammable mixtures with air in controlled laboratory conditions. It is also an important parameter for storage and handling consideration of flammable materials [54]. According to WHO specifications, the liquid formulations must have a flash point not less than 22.8ºC [7]. The prepared water-based formulations have higher flash point (over 70ºC) than the standard EC formulation, making them more safe to transport, handle, and storage.

Physicochemical characteristics of the spray solutions.

Data shown in Table (3) represent the physicochemical properties of eighteen spray solutions using the recommended application rate of the reference (EC) formulation. Two types of water (soft and hard) were used in dilution. Based on the type of formulation, it is obvious that the three investigated formulations have very close values for the viscosity, density and specific gravity parameters. There are non-significant differences in the values of such parameters. The viscosity ranges are 1.77-1.85, 1.66-1.82 and 1.50-1.70 cP, while the density ranges are 0.9848-0.9998, 0.9953-0.9982, and 0.9980-0.9983 g/cm^3 and specific gravity values are 1.0013-1.0185, 0.9983-1.0012, and 1.0010-1.0013 for standard EC, EW, and ME formulations respectively. Depending on the storage conditions and type of water used in dilution, the obtained data clearly show that the previous three parameters are close and non-significantly changed too. Notable change is recorded in the specific gravity of the standard EC formulation due to the storage conditions. Even though the accelerated cold and hot storage recorded the highest change in the specific gravity, but such changes did not exceed 1.7% of the original values that means non-significantly change.

High surface tension of water (about 72.0 dyne/cm) causes a hardily spreading of spray solution on foliage. On surveying the surface tension data we find that, surface tension of water reduces to half or less of its original value which increases the spreading power of spray solutions. Surface tension of spray solutions of both EC and ME formulations are close and less than that of the EW formulation before and after storage.

Accelerated storage conditions slightly increase the surface tension of the spray solutions of the standard (readymade) EC formulation. As an example, cold storage increases the surface tension of EC spray solutions from 30.583 and 28.808 dyne/cm to 31.332 and 30.206 dyne/cm for soft water and hard water respectively. An important parts of chemical stability are performances on accelerated testing and kinetic of pH profiles [55]. Data in Table (3) illustrate that, all spray solutions under investigation are weakly acidic and pH values ranged from 4.34 to 5.75. Spray solutions of both EW and ME are slightly more acidic than that of EC formulation. Accelerated storage conditions, especially the accelerated storage, slightly increases the pH of spray solutions in EW formulation. Conductivity of most spray solutions of the new formulations is slightly higher than that of the standard EC formulation. Given the values of the surface tension, pH and electrical conductivity, reducing the values of pH and surface tension with increasing the values of electrical conductivity of the new WE and EW water-based formulations may led to an enhancement in wetting, spreading and retention of spray on treated plants which improve their insecticidal activity [56,57] .

Table (3): Physicochemical properties of the studied EC, EW and ME spray solutions.

Formulation Type	Conditions	Water of dilution	Viscosity$^{(a)}$ (cP)	Density$^{(b)}$ (g/cm^3)	Specific gravity$^{(b)}$	Surface tension$^{(c)}$ (dyne/cm)	pH$^{(d)}$	Conductivity (µs)
Standard 48%EC formulation	Pre storage	S.W	1.82	0.9970	1.0060	30.583	5.34	99.9
		H.W	1.85	0.9983	1.0013	28.808	5.68	641.0
	Cold storage	S.W	1.78	0.9998	1.0032	31.332	5.38	96.7
		H.W	1.77	0.9905	1.0128	30.206	5.72	608.0
	Hot storage	S.W	1.80	0.9887	1.0145	31.395	5.35	97.6
		H.W	1.79	0.9848	1.0185	30.162	5.60	608.0
New 24% EW formulation	Pre storage	S.W	1.66	0.9980	1.0010	36.448	4.58	113.8
		H.W	1.76	0.9982	1.0012	34.520	4.60	641.0
	Cold storage	S.W	1.80	0.9953	0.9983	36.419	4.95	120.4
		H.W	1.78	0.9982	1.0012	35.958	5.75	642.0
	Hot storage	S.W	1.77	0.9979	1.0009	36.000	5.43	117.4
		H.W	1.82	0.9982	1.0012	35.771	5.61	641.0
New 24% ME formulation	Pre storage	S.W	1.64	0.9980	1.0010	30.717	4.34	119.2
		H.W	1.70	0.9983	1.0013	30.159	5.34	651.0
	Cold storage	S.W	1.69	0.9980	1.0010	30.623	4.85	113.3
		H.W	1.68	0.9982	1.0013	30.305	5.12	648.0
	Hot storage	S.W	1.50	0.9980	1.0013	30.113	4.29	116.2
		H.W	1.68	0.9983	1.0013	29.844	4.48	662.0

(**a**) = value ±0.115 (**b**)= value ±0.0009 (**c**) = value ±0.068 (**d**) = value ±0.041 These results values ± uncertainty .

Insecticidal activity of the studied formulations. The insecticidal activity of the studied chlorpyrifos formulations against Pink bollworm (*Pectinophora gossypiella*). Data presented in Table (4) and Fig. (2) emerged the insecticidal activity of the two new chloepyrifos (EW and ME) formulations against of

Pink bollworm (*P. gossypiella*) as compared with the reference EC formulation. Data revealed that, the most potent formulation against the pest is the EW formulation followed by the microemulsion ME formulation. The standard EC formulation showed the least activity. The LC_{50} values were found to be 173.77, 179.062, and 311.12 ppm for EW, ME and EC formulations respectively. Depending on toxicity index and relative potency parameters, the same result is obtained where, the activity of the tested formulations are arranged in descending order as before. The obtained values of the relative potency indicate that, the standard EC formulation is differ in result with the new ME and EW formulations.

The insecticidal activity of the studied formulations against migratory locust (*L. migratoria*).
Laboratory assessment.

Data in Table (5) and Fig. (3) represent the insecticidal potentiality of the new water-based emulsion formulations (EW and ME) and the reference Drusban H 48% EC against the 5th instar larvae of migratory locust (*L. migratoria*). The new formulations possess nearly closed activity that is lower than that of the standard insecticide. $LC_{50}$of the standard insecticide are 4.9 ppm (LC_{50}) and 0.35 hrs (LT_{50}). The LC_{50} values of the new formulations are 15.5 and 19.9 ppm for ME and EW formulations, and their average LT_{50} values are 4.71 and 7.28 hrs respectively. Slopes of the obtained Ldp lines revealed the analogous response of the treated pests to the studied formulations in the order EW > ME > EC. Consequently, standard EC formulation was found to be the most effective one and acquired 100.0 toxicity index.

Table (4): The insecticidal activity of the studied chlorpyrifos formulations against pink bollworm (*P. gossypiella*).

Concentration (ppm)	**Mortality % of** EW formulation	ME formulation	**Concentration (ppm)**	**Mortality % of** EC formulation
60	13.3	6.7	120	20.0
120	26.7	33.3	240	33.3
180	53.3	40.0	360	40.0
240	60.0	60.0	480	66.7
300	80.0	86.7	600	93.3
LC_{50} (ppm)*	173.77	179.06	LC_{50} (ppm)	311.12
Slope	2.749	3.305	Slope	2.739
Toxicity index**	100.0	97.05	Toxicity index	55.86
Relative potency	1.00	1.03	Relative potency	1.79

* Lethal Concentration (LC_{50}) is the concentration of the tested formulation that kills 50% of the treated pests.
** The toxicity of the tested formulation relative to the most efficient one that has T.I equals to 100.

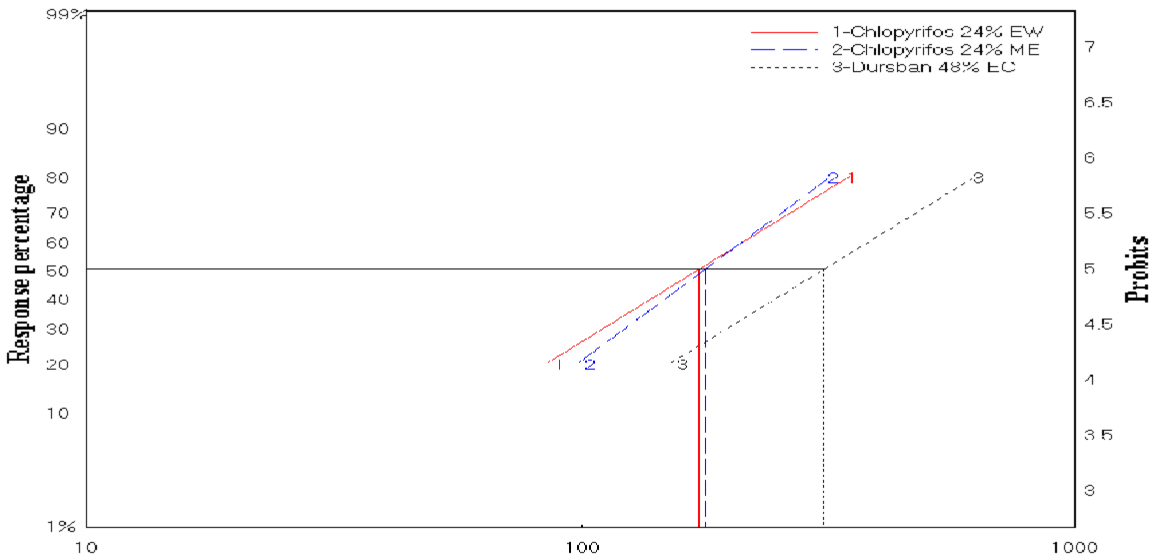


Fig. (2): Ldp lines of the tested formulations against of Pink bollworm (*P.gossypiella*).

Table (5): Insecticidal activity of the studied chlorpyrifos formulations against migratory locust (*L. migratoria*).

Concentration (ppm)	Mortality % of		Concentration (ppm)	Mortality % of the standard EC formulation
	EW formulation	ME formulation		
12	26.7	40.6	24	78.5
24	59.1	65.9	48	87.2
48	86.0	85.5	96	93.1
LC_{50} (ppm)	19.9	15.5	LC_{50} (ppm)	4.9
Slope	2.83	2.15	Slope	1.15
Toxicity index	24.8	31.7	Toxicity index	100.0
Average LT_{50}* (hrs)	4.71	7.28	Average LT_{50} (day)	0.35

*LT_{50} is the median Lethal Time (time until death) after exposure of the pest to a toxic substance or stressful conditions.

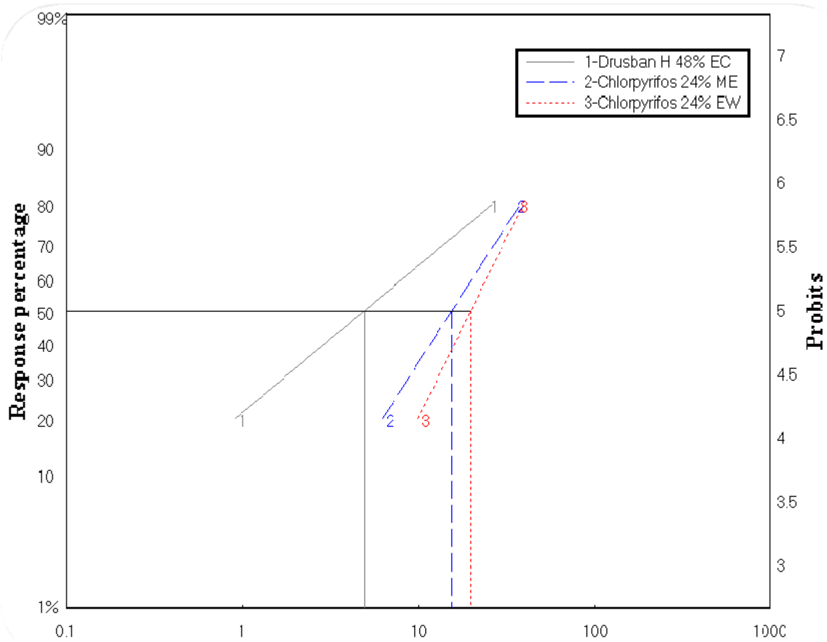


Figure (3): LDP lines of the studied formulations against the 5th instar nymphs of migratory locust (*L. migratoria*).

Field Experiment

Data presented in Table (6) and Fig. (4) showed the activity of the three studied chlorpyrifos formulations (ME, EW and EC) using two rates of application in El Baharia Oasis, Western Desert, Giza Governorate during the 2021 season. Half the recommended rate was 500 ml/fedden and the recommended rate was 1000 ml/fedden. The experiment was applied to estimate the field performance of the new EW and ME formulations as compared with that of the standard EC formulation in terms of lethal time determination against the migratory locust (*L. migratoria*) after 6, 12, 24 and 48 hrs after treatment.

Data obtained reveals a direct relationship between the mortality percentage and the time after treatment for the three tested formulations. On applying with half the recommended dosage (500 ml/feddan) of the new microemulsion (ME) that contains 24% active ingredient gave 95.9% activity of the standard EC formulation containing 48% active ingredient. Application with the recommended rate (1000 ml/feddan) showed the advantage of the new EW formulation over the new ME and the reference EC formulations. The LT_{50} values are 6.6, 7.0 and 7.6 hrs for EW, EC and ME formulations respectively.

Generally, data obtained in laboratory and field experiments showed an improvement in the insecticidal efficiency against the two pests. The improved activity of the new water-based ME and EW formulations against the two target pests, as compared with that of the reference EC one, may be interpreted in terms of the small particle size of the formulations. It may also due to the change in droplets polarity that affects the arrangement of the active ingredient and surfactants on the droplet interface which improves its absorption by the target insects [8,58–63].

Table (6): Field performance of the studied chloropyrifos formulations against the migratory locust (*L. migratoria*)

Time after treatment (hrs)	Mortality %					
	ME formulation		EW formulation		EC formulation	
	500mL/fed.	1000mL/fed	500mL/fed.	1000mL/fed	500mL/fed.	1000mL/fed
6	30.1	42.4	26.6	47.8	33.4	44.4
12	48.8	63.8	46.2	64.9	51.3	68.7
24	67.8	81.5	66.7	79.5	68.9	86.8
48	83.0	92.5	83.2	89.6	82.9	95.9
LT_{50} (hrs)	11.9	7.6	13.6	6.6	11.4	7.0
Toxicity index	95.9	85.5	84.0	100.0	100.0	93.3

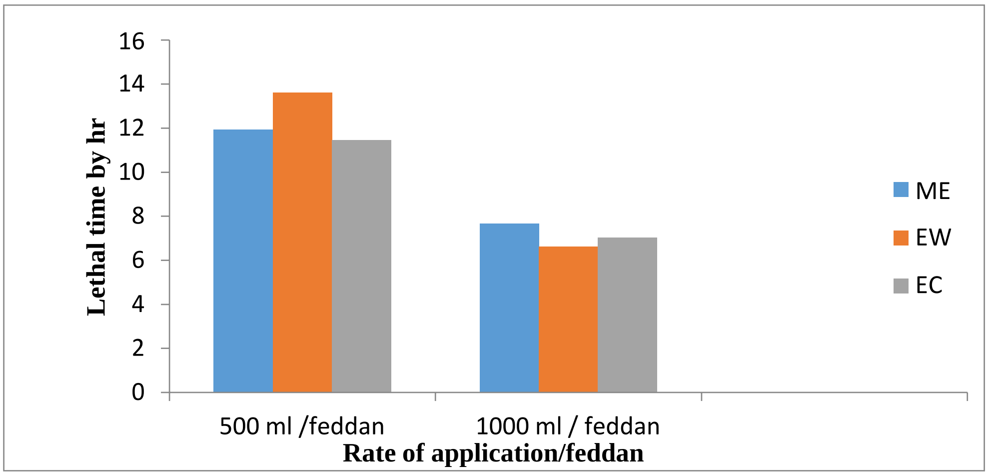


Fig. (4): The LT_{50} of the studied chlorpyrifos formulations against the migratory locust (*L. migratoria*) under the field conditions.

4. Conclusion

Pesticides in developing countries are mainly available in traditional or old technology forms. These forms have many disadvantages like dustiness, using of organic or petroleum solvents, increased dose rates and repeated applications to get the desired bio-efficacy. With growing the awareness of the dangers of such formulations on the public health and environment, they have been replaced, in advanced countries, by more safe, less toxic, high effective and eco-friendly water-based formulations. As chloropyrifos is a highly applicable and distributed insecticide, our research work aimed to prepare it in the novel and non-conventional oil/water emulsion (EW) and oil/water microemulsion (ME) loaded with chlopyrifos at a half of its common concentration (24%). The new formulations passed successfully the required tests according to the standard organizations. Insecticidal activity of the new formulations was studied against the pink bollworm (*P.gossypiella)* and the migratory locust *(L. migratoria*) in laboratory and field conditions. The activity was compared with that of the standard insecticide Dursban H 48% EC. The pink bollworm showed an advantage of the new formula 24% EW over the other formula (24% ME) and the standard formulation (48% EC). Field evaluation against the migratory locust showed relatively close values inefficacy of the new formulations and that of the standard formulation although the new ones contain the half amount of the active ingredient. To approve the registration and commercial distribution for the new formulations, toxicological studies, further field experiments on many crops and against different insects are recommended to be conducted in the near future.

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