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# BIOEFFICACY AND STABLIZATION OF HYDROLYTICALLY UNSTABLE PESTICIDE IN WATER BASED MICROEMULSION AGAINST *PERIPLANETA AMERICANA*

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**Abstract:** *Chlorpyrifos microemulsion was formulated using karanja (Pongamia glabra) and jatropha (Jatropha curcas) non polar filtrate as a dispersed phase. Process of stable microemulsion development was optimized for parameters such as non-ionic surfactants concentration and chlorpyrifos concentration with high physical and chemical stability. Chlorpyrifos hydrolytical unstability was analyzed by GC-FPD Physical stability of microemulsion was optimized in terms of shelf life of the chlorpyrifos microemulsion. Particle size analysis and viscosity data also confirm the swollen miceller solubilization characteristics of the microemulsion system. The chlorpyrifos microemulsion (ME) and emulsifiable concentrate (EC) containing karanja and jatropha non-aqueous filtrate with concentration of 20-100 ppm gave 55-100 % mortality rates against Periplaneta Americana (Order: Blattodea, Family: Blattidae) within 24 hours. The $LD_{50}$ values recorded for the microemulsion and emulsifiable concentrate were 1.277 and 1.347 mgl-1 with karanja and jatropha non-polar filtrate.*

***Key Words:*** *Chlorpyrifos, Microemulsion, Emulsifiable Concentrate, Karanja & Jatropha, Micelle, Periplaneta Americana*

***Abbreviations Used:*** *Gas Chromatography (GC), Hydrophilic-lipophilic balance (HLB), Flame photometric detector (FPD), Emulsifiable concentrate (EC)*

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## INTRODUCTION

Chlorpyrifos (o, o-diethyl o-(3,5,6,-trichloro-2-pyridinyl phosphothiorate) is a moderately toxic, chlorinated organophosphate insecticide. It is used worldwide as an agricultural insecticide and belongs to the family of approximately 40 widely used organophosphate pesticides. It is used to kill a wide variety of insects including cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants, and lice by disrupting their nervous system. It is also used as a soil treatment (pre-plant and at planting), as a seed treatment and as a foliar spray, directed spray and dormant spray. The degradation pathway of chlorpyrifos in aquatic environments involves the breakdown of the thiophosphoric esters, forming 3,5,6-trichloropyridinol (TCP) and diethyl chlorpyrifos (DEC) as main metabolites (1). Importance of pesticide use in increasing agricultural production is well established, however, they can cause damage to the environment and sometimes to users. Recently the pesticide industry has made a good progress in terms of development and production of low risk environmental friendly pesticide formulations, although pesticides are still mainly available in conventional formulations such as dustable powders, wettable powders, emulsifiable concentrates, solutions, etc. Such conventional formulations could cause problems related to environmental protection, leaving residues in ecosystem, food, final products, etc. Hence, there is a growing demand for use of environmental friendly water based formulations as oil-in-water emulsions, aqueous suspension concentrates, aqueous capsule suspensions and so on instead of conventional pesticide formulations (2). These formulations are tended not only to replace toxic, non degradable ingredients from formulations, but also to increase the efficacy of products through a proper choice and a balance of all components in the formulation.

Microemulsions are defined as systems which comprise of a mixture of water, hydrocarbons and amphiphilic compounds which lead to the formation of thermodynamically stable, homogeneous (heterogeneous at molecular scale), optically isotropic solutions (3).

These are excellent vehicles for solubilization and transport of water-insoluble active compounds in food, cosmetic, and pharmaceutical applications [4,5,6]. However, most oil-based formulation concentrates will be destroyed upon dilution with the aqueous phase and will cause migration of the solubilized guest active molecule to the outer continuous phase, followed by precipitation and uncontrolled absorption (7).

Oil cakes are of two types edible and non-edible. Non edible oil cakes (NEOC) such as neem (*Azadirachta indica*), castor (*Ricinus communis*), karanja (*Pongamia pinnata*), jatropha (*Jatropha curcas*) and mahua (*Madhuca indica*) are rich source of protein, micro and macronutrients (N P K),Karinjin, Phorbol esters, saponins, tannin etc. (8,9). In these non edible oil seed cakes also contain some oil (non-edible) after mechanical extraction. In case of karanja and jatropha seed cakes the residual oil contains maximum percentage of oleic acid which is unsaturated in nature (10). Previous studies shows that the use of the edible oil seed cakes and their aqueous and methanolic extract for the termiticidal (11), insecticidal and nematicidal (12,11), larvicidal activity (13), but there is no longer studies for the shelf life of biomass and their aqueous, methanolic extract. Formulation is the best approach to convert the biomass and their aqueous /methanolic extract to suitable formulation or product to increase shelf life, cost and physico-chemical properties. Earlier the polar filtrate of deoiled cake was used as such (14) or as one of the constituents of nanoemulsion (15), but this work uses the non-polar filtrate of the cakes as dispersed phase which helps in solubilizing as well as stabilizing chlorpyrifos in water based microemulsion. The non-polar filtrate acts as a stabilizer and aids in slow release of the active ingredient, moreover it is not toxic to the insect.

Cockroaches are considered to be one of the most troublesome nuisance pests of the world. Methods of control of cockroaches include crack and crevice treatments, baseboard sprays, aerosols, foggers and baits (16). Toxic baits containing chlorpyrifos, sulfuramid, boric acid etc. have been formulated successfully in the past against Periplaneta Americana and other peridomestic cockroach species (17).

In our research work, chlorpyrifos microemulsion has been developed using a non-polar solvent, CIX and karanja and jatropha based non-polar filtrate with comparison of stability in both the cases by gas chromatography after solublization of chlorpyrifos in continuous phase. The main objective of this study was to develop environment and user friendly microemulsion of the hazardous and persistent pesticide, chlorpyrifos non polar filtrate of karanja and jatropha cakes.

## MATERIALS AND METHODS

***Chlorpyrifos and chemicals***

Chlorpyrifos (99.9% purity) and CIX were purchased from United Phosphorous India Limited, Mumbai and propylene glycol and butanol were procured from Merck, India and commercial grade Tween-80 (Polysorbate-80) was from Croda surfactants, Mumbai.

***Test Insect***

Cultures of *Periplaneta americana* were maintained at laboratory conditions, 60-70% RH, 27±1ºC and darkness i.e. 12:12 (L:D) h photocycle (16).

***Preparation of Modified Solvent and microemulsion***

Karanja and jatropha cakes (15 gm each) were taken in 70 gm of CIX. The mixture was then allowed to stand in shaker for 24 hours. at room temperature. Finally the mixture was filtered by using whatman filter paper to filter the non polar constituents present in the cake. Characterization of filtrate was done using GC-FPD (Fig.1). This non polar filtrate acts as a solvent for chlorpyrifos for microemulsion as well as for emulsifiable concentrate formulation of chlorpyrifos also.

Microemulsions were prepared using these surfactants propylene glycol and butanol from Merck, India and commercial grade Tween-80 (Polyoxyethylene 20 monooleate) and Span 80 (Sorbitan monooleate) spontaneously at 30°C. Samples were normally prepared by diluting water/surfactant mixtures with oil, or by diluting oil/surfactant mixtures with water (18).

***Droplet size measurement***

The mean droplet sizes and distribution of the microemulsions were determined by dynamic light scattering (DLS) at a scattering angle of 173 °C (Zetasizer Nano-ZS, Malvern, UK) at 25 °C, employing an argon laser (λ=633 nm).

***ATR-FTIR Analysis of Chlorpyrifos in Microemulsion Formulation***

Infrared (IR) spectra were recorded on a Bruker alpha ATR-FTIR spectrophotometer using the attenuated total reflectance (ATR) technique, and values are expressed as $\gamma_{max}$ $cm^{-1}$.

***Gas Chromatography Analysis of Chlorpyrifos in Formulation***

The samples were analyzed on GC Shimadzu make coupled with FPD detector fitted with a 30 m × 0.25 mm × 0.25 _um DB-5 MS column constituted with 95% dimethyl poly siloxanes and 5% diphenyl poly siloxanes. Carrier gas was helium with a flow rate of 1 ml/min at and column pressure of 117.2 kPa, $H_2$ and air flow were 85 ml/min and 110 ml/min respectively. Injector port and detector temperatures were maintained at 250 °C and 290 °C respectively.

The injection volume was 1.0 µl, mode of injection is splitless and column temperature program as following 150 – 200 °C at 25 °C/min held for 5 min, then rise to 230 °C at rate 4 °C/min held for 2 min, further rise up to 280 at the rate 20 °C/min and held it for 5 minute. The quantified the analyte using area normalization method assuming equal detector response.

***Viscosity***

The rheological measurements in shear flow were carried out using a rotational rheometer Physica MCR 501 produced by Anton Paar (Austria) equipped with cone-plate measuring system (60 mm, cone 1) with shear rate from 0.1 to 4000 $s^{-1}$. The measuring device was equipped with a temperature controlling unit (Peltier plate) that provided very good temperature control over an extended period of time.

*Bioefficacy of Chlorpyrifos Microemulsion against Periplaneta Americana by Contact Toxicity* ***Bioassay***

The efficacy of the microemulsions thus formulated with and without the karanja and jatropha non-polar aqueous extract was evaluated against the American Cockroach, *Periplaneta americana.* Contact toxicity bioassay was performed by applying selected doses of the microemulsions (20, 40, 60, 80, 100 ppm) in the cages with the help of a microapplicator. 10 cockroaches were released in cages containing emulsion and mortality was observed after 24 hours. Each assay for different doses of the emulsion was performed 3 times. A control set was also maintained (19).

## RESULTS

***Effect of Chlorpyrifos concentration on stability of Microemulsion***

Chlorpyrifos microemulsion was prepared by using 5-20% (Table 1) of chlorpyrifos with constant 5% (w/w) surfactant concentration. Also, these microemulsions were compared with 5% emulsifiable concentrate and degradation of emulsion at room and elevated temperature was observed. The degradation studies of chlorpyrifos were confirmed by using Gas chromatography (Fig. 2)

***Discussion***

This is understood or well known that chlorpyrifos is unstable in aqueous medium and generally breaks down in TCP (3, 5, 6-trichloro-2-pyridinol) (20). Table 3 shows the percentage degradation of chlorpyrifos with variable concentration in formulation using two different solvents i.e., CIX and biodiesel waste based non-polar filtrate or we called modified solvent in our manuscript. Table 3 clearly shows that, as the concentration of chlorpyrifos is increased in microemulsion formulation with constant surfactant concentration using CIX as a solvent, only 5% microemulsion of chlorpyrifos was possible but in other formulation having 10,15 &20 % chlorpyrifos, sedimentation occurs during storage period while in case of biodiesel waste based non-polar filtrate as a modified solvent 5, 10, 15% chlorpyrifos microemulsion were prepared and in case of 20% (F4) sedimentation occurs. Degradation analysis of microemulsion is provided in Table 3. Degradation pattern of chlorpyrifos in microemulsion formulation was directly proportional to the concentration of chlorpyrifos (Fig. 4). In the emulsifiable concentrate of chlorpyrifos, degradation occurs more in CIX.

The possibility of less degradation of chlorpyrifos in microemulsion containing biodiesel waste based non polar filtrate is because of the composition of biodiesel waste (Fig. 2). It contains some oil soluble fatty acids i.e. oleic, linoleic (21) and when chlorpyrifos was dissolved in modified solvent as compared to CIX, then these fatty acids reduce the time to make the emulsion clear and transparent as well as also help in improving the stability of chlorpyrifos in water based emulsion.

***Effect of Surfactant concentration on stability of chlorpyrifos microemulsion***

5% microemulsion of chlorpyrifos was prepared with varying surfactant concentration i.e.11, 13, 15, 17 & 20% (w/w) (Table 1) by applying low energy emulsification method at room temperature. The degradation of chlorpyrifos with different surfactant concentrations was also analyzed by gas chromatography method. Results show the degradation in CIX and modified solvent was inversely proportional to the surfactant concentration or we can say more degradation with minimum concentration of surfactants and also the solubilization of chlorpyrifos in modified solvent (Table 2).

***Discussion***

To investigate the effect of surfactant concentrations i.e. 11 to 20% on 5 % chlorpyrifos water based microemulsion formulation by incorporating propylene glycol as co-solvent with fixed inert ingredient in the system. Propylene glycol behaves as a co-solvent (Table 1) as well as capping agent in the formulation system so developed. Degradation kinetics also depends upon the surfactant concentration and this analysis was proved in our experiment by Gas chromatography and physically was proved by solubilization behavior of the non – ionic surfactant system in microemulsion formulation.

Micelles are often recognized as static structures of spherical aggregates (22) of oriented molecules. However, micelles are in dynamic equilibrium with individual surfactant molecules that are constantly being exchanged between the bulk and the micelles. These are two relaxation processes involved in micellar solutions (23). The first one is the fast relaxation process with relaxation time $\tau 1$ (generally of the order of microseconds), which is associated with the fast exchange of monomers between the micelles and the surrounding bulk phase. The second with relaxation time $\tau 2$ (usually of the order of milliseconds to minutes) is attributed to the micelle formation and dissolution process (24) Micellar relaxation kinetics show dependence upon temperature, pressure and concentration of surfactants (25). When the surfactant concentration is increased, the number of micelles increases, resulting in a decrease in intermicellar distance. Hence, the time required for a surfactant monomer to colloid with a micelle is shorter at higher surfactant concentration so the flux of surfactant monomer increases and it decreases the dynamic surface tension between the two phases (26). Due to speediness of relaxation process, the chlorpyrifos in micellar solution does not come in contact with water directly and shows less degradation in water based microemulsion formulation (Fig. 4).

***Discussion for ATR-FTIR Analysis***

Two sharp band at 2923 and 2855 $cm^{-1}$ were attributed to the asymmetric $CH_2$ and symmetric $CH_2$ stretching respectively. The intense peak at 1110 $cm^{-1}$, due to C=O stretching, which confirm the presence of oleic acid in modified solvent (Fig.5) (27). The absorption band of chlorpyrifos were located at 1459, 1022, 845 and 670 $cm^{-1}$, which are the characteristics of C=C stretching, C-O stretching, C-H bending and C-CL stretching respectively (Fig.5). In microemulsion formulation, dissolution of chlorpyrifos in modified solvent as dispersed phase was confirmed by the bands appearing at 1457, 1022, 834 and 674 $cm^{-1}$ (28). It is worth to note that the C=O (stretching band) of carboxyl group, was present at 1709 $cm^{-1}$ in modified solvent.

ATR-FTIR spectrum of oleic acid is washed from the microemulsion spectrum, because of solublization of oleic acid in tween-80 at its maximum concentration i.e. 70%. ATR-FTIR spectrum of microemulsion clearly indicate, there is no chemical interaction between disperse and continuous phase and so it can be said that it is a physical mixture of both the two phases.

***Emulsion Droplet Size and Viscosity***

All formulations were produced by using different concentrations of non-ionic surfactants and chlorpyrifos in microemulsion (Table1). The droplet size and viscosity after the preparation of microemulsion was shown in Table 3. Mean droplet size after preparation of F1 to F5 (vary with surfactant concentration) were in a range of 1 to 1.392 nm and also showed the variation when chlorpyrifos concentration was altered i.e. 10 to 15 % in the microemulsion system, mean droplet size was 2.421 & 1.555 nm (Table 3). Viscosity of the microemulsion samples increases with increase in the surfactant concentration in the microemulsion system; reducing the interfacial tension between the two phases i.e. oil and water, hence more solubilization occurs between the two phases and possibility of formation of micelles and this micellar aggregation or polydisperse system reduces the degradation of chlorpyifos in aqueous medium (29). A possible explanation of size reduction after an increased surfactant concentration may be the surfactant molecules are free to move and able to absorb the oil droplets resulting in an increase in surface to volume ratio of the particles. Moreover, surfactant molecules localized on the surface of the emulsion droplets reduce interfacial free energy and provide mechanical barrier to coalescence (30).

*Discussion for Insecticidal Bioassay*

The effect of karanja and jatropha filtrate was studied by preparing microemulsion and emulsifiable concentrate with different concentration of non-polar filtrate and determining the Lethal Dose ($LD_{50}$) of the emulsions. The emulsion were divided into four group i.e. F8 (with and without non-polar filtrate ) (Table1).

The amount of non-polar filtrate in microemulsion formulation was constant in recipe i.e. 2.5 % w/w (Table 1) but in emulsifiable concentrate it was 85% w/w. Non-polar filtrate was used to solubilize chlorpyrifos in disperse phase, it did not show any insecticidal activity alone against cockroaches. When it was used in the microemulsion (ME) as disperse phase and as a carrier solvent for EC formulation, the $LD_{50}$ values decreased i.e. 1.277 and 1.347 (Table 4) mg/l and mortality also decreased i.e. 100% for ME and 85% for EC after 24 hours. The variation of the toxicity of the ME and EC of chlorpyrifos with and without non-polar filtrate against cockroaches is shown in Figure 6. From the graph it can be seen that ME and

EC with non-polar filtrate were more toxic and effective as compared to chlorpyrifos ME and EC by using with and without non-polar filtrate as a disperse phase For ME and carrier solvent for EC (31).

## DISCUSSION

In the present work, we have developed microemulsion of chlorpyrifos with solubilization in organic solvent and biomass filtrate of the organic solvent. These microemulsions were further analyzed with gas chromatography to materialize the stability aspects of chlorpyrifos in water based formulation. Stability was also confirmed with the help of viscosity and particle size analysis.

In an attempt to utilize biomass filtrate as a disperse phase in microemulsion formulation of chlorpyrifos, this disperse phase played an important role in stabilization of hydrolytically unstable chlorpyrifos in water based microemulsion and further stability was confirmed by GC-FPD analysis and when the results were compared with emulsifiable concentrate formulation of chlorpyrifos in absence or presence of biomass filtrate in organic solvent, the stability results of chlorpyrifos were more encouraging. Biomass filtrate of organic solvent contains some fatty acids (saturated and unsaturated) and when chlorprifos is solublised in biomass filtrate, the filtrate fluxes the surfactant monomer continuously and these monomers assemble to form micelles which reduce the surface tension between the two layers and decrease the contact time with continuous phase (water) and stabilize the chlorpyrifos in water based emulsion.

The insecticidal properties of the water based microemulsions with and without karanja and jatropha non-polar filtrate make it well suited for the control of household pests i.e. cockroach in the urban areas. Based on our physicochemical characterization of the chlorpyrifos microemulsion using karanja and jatropha non-polar filtrate, this microemulsion can be used as a toxicant against *P. americana* in laboratory conditions provided it is user and environment friendly and with effective application rates.

The chlorpyrifos microemulsion thus developed can be used very conveniently in the areas having running water, where other formulations (i.e. baits, tablets) are inapplicable for the control of aquatic pests. The current work also encourages waste utilization (karanja and jatropha cakes as left over's of biodiesel process). Karanja and jatropha non-polar filtrate used in the emulsion acts as an emulsion stabilizer and slows down the degradation process.

The use of the non-polar filtrate makes t he emulsion more environment and user friendly as compared to other conventional solvents. The residue of the deoiled cake left after vaporization of the solvent can be used in other types of formulations viz. coil, tablet, granules , dust etc.

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**Table 1: Preparation of microemulsion with varying concentration of chlorpyrifos/surfactants**

| Ingredients | F 1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---|---|---|---|---|---|---|---|---|---|
| Chlorpyrifos | 5 | 10 | 15 | 20 | 5 | 5 | 5 | 5 | 5 |
| Solvent CIX/Modified Solvent | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Butanol | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| Ethylene Glycol | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 |
| Surfactants (w/w) | 15 | 15 | 15 | 15 | 11 | 13 | 15 | 17 | 20 |
| Water | Upto 100 | Upto 100 | Upto 100 | Upto 100 | Upto 100 | Upto 100 | Upto 100 | Upto 100 | Upto 100 |

* F1, F2, F3 & F4 were also prepare in the presence of CIX solvent or no extract of Karanja and Jatropha cake

**Table 2: Percentage degradation of chlorpyrifos in water based microemulsion with varying concentration of surfactants**

| S.No. | Formulation Code | Surfactants (w/w) in nano-formulation | Percent Degradation in CIX | Percent degradation in Modified Solvent | Chlorpyrifos Concentration |
|---|---|---|---|---|---|
| 1. | F1 | 15 | ----- | | 5 |
| 2. | F2 | 15 | ------ | | 10 |
| 3. | F3 | 15 | ----- | | 15 |
| 4. | F4 | 15 | ------ | | 20 |
| 1. | F5 | 11 | 17.7 | 16.4 | |
| 2. | F6 | 13 | 14.0 | 11.6 | |
| 3. | F7 | 15 | 17.59 | 9.72 | |
| 4. | F8 | 17 | 14.86 | 5.07 | |
| 5. | F9 | 20 | 11.22 | 3.64 | |

**Table 3: Mean droplet size and viscosity of the microemulsions system with varying concentration of surfactants and chlorpyrifos**

| S.No. | Formulation Code | Surfactant Concentration (W/W) | Chlorpyrifos Concentration (W/W) | Viscosity (In Cp) | Particle Size (In Nm) |
|---|---|---|---|---|---|
| 1. | F1 | 11 | 5 | 16 | 2.411 |
| 2. | F2 | 13 | 5 | 20 | 2.076 |
| 3. | F3 | 15 | 5 | 26 | 2.06 |
| 4. | F4 | 17 | 5 | 30 | 2.421 |
| 5. | F5 | 20 | 5 | 40 | 1.390 |
| 6. | F6 | 15 | 10 | 31 | 2.421 |
| 7. | F7 | 15 | 15 | 25 | 1.555 |

**Table 4: Bioefficacy of Chlorpyrifos microemulsion and estimation of $LD_{50}$ values**

| S.No. | Sample Name | Concentration | Percentage Mortality | $LD_{50}$ (mg/l) |
|---|---|---|---|---|
| 1. | ME-V | 0.02 | 80.00 | 1.441 $LD_{90}$=3.885 |
| | | 0.04 | 85.00 | |
| | | 0.06 | 90.00 | |
| | | 0.08 | 95.00 | |
| | | 0.10 | 100.00 | |
| | | Control | 0.00 | |
| 2. | ME(CPP)-CIX | 0.02 | 80.00 | 1.330 $LD_{90}$=0.0370 |
| | | 0.04 | 90.00 | |
| | | 0.06 | 90.00 | |
| | | 0.08 | 95.00 | |
| | | 0.10 | 100.00 | |
| | | Control | 0.00 | |
| 3. | ME(CPP)-MS | 0.02 | 85.00 | 1.277 $LD_{90}$=2.579 |
| | | 0.04 | 90.00 | |
| | | 0.06 | 100.00 | |
| | | 0.08 | 100.00 | |
| | | 0.10 | 100.00 | |
| | | Control | 0.00 | |
| 4. | EC(CPP)-CIX | 0.02 | 55.00 | 1.756 $LD_{90}$=0.170 |
| | | 0.04 | 65.00 | |
| | | 0.06 | 75.00 | |
| | | 0.08 | 80.00 | |
| | | 0.10 | 85.00 | |
| | | Control | 0.00 | |
| 5. | EC(CPP)-MS | 0.02 | 70.00 | 1.347 $LD_{90}$=0.130 |
| | | 0.04 | 75.00 | |
| | | 0.06 | 80.00 | |
| | | 0.08 | 85.00 | |
| | | 0.10 | 90.00 | |
| | | Control | 0.00 | |

ME V= Microemulsion V, ME(CPP)-CIX= Chlorpyrifos Microemulsion with CIX, ME(CPP)-MS= Chlorpyrifos Microemulsion with Modified Solvent (Non polar filtrate of karanja & jatropha), EC(CPP)-CIX= Emulsifiable Concentrate of Chlorpyrifos with CIX, EC(CPP)-MS= Emulsifiable Concentrate of Chlorpyrifos with Modified Solvent

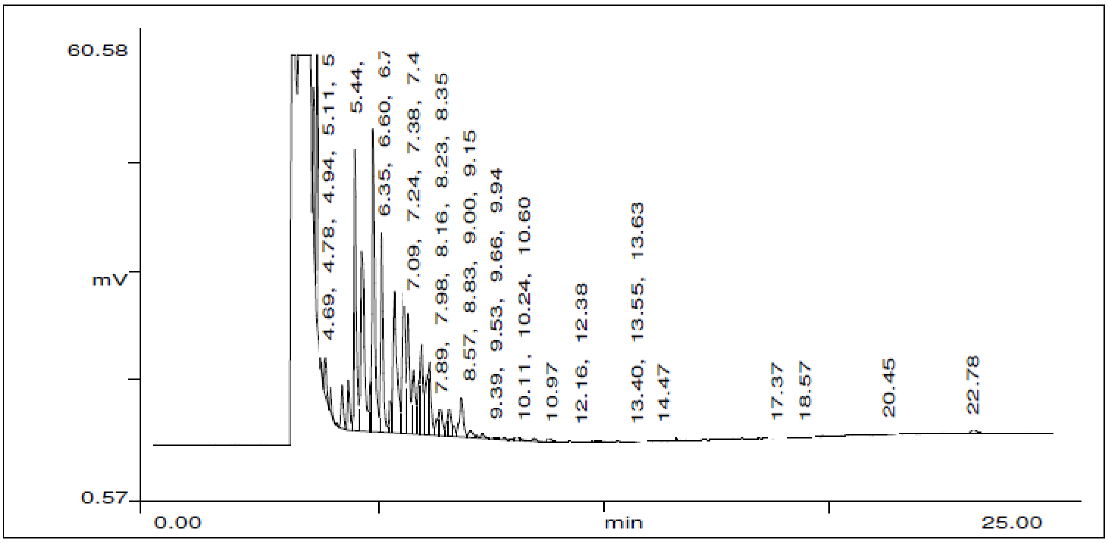


**Fig.1. GC analysis of non-polar (CIX) filtrate of karanja and jatropha cake**

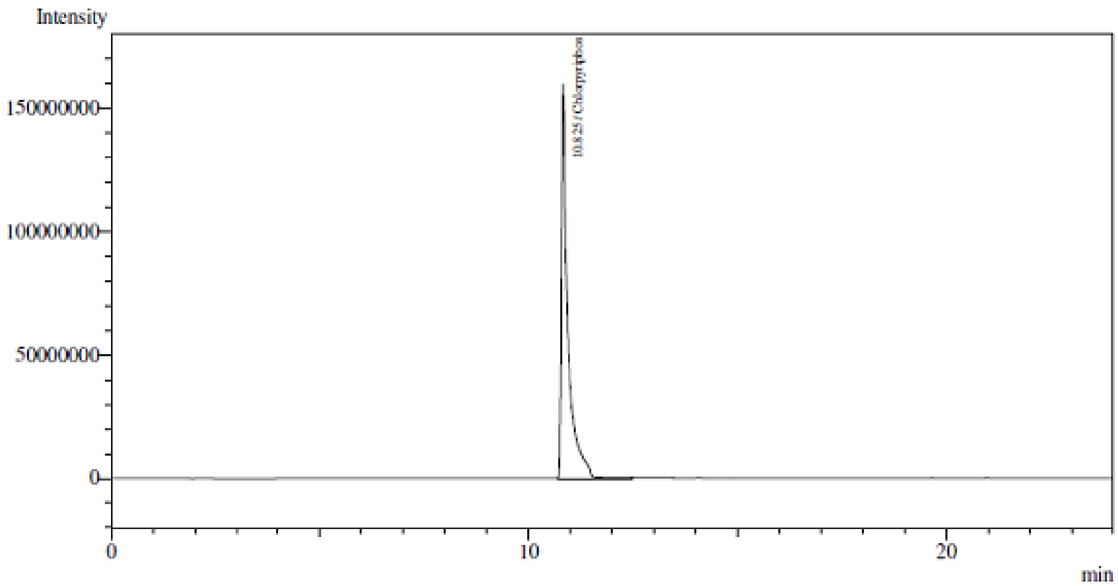


**Fig.2. GC analysis chlorpyrifos microemulsion (F7) using non-polar filtrate of karanja and jatropha cake as solvent for disperse phase**

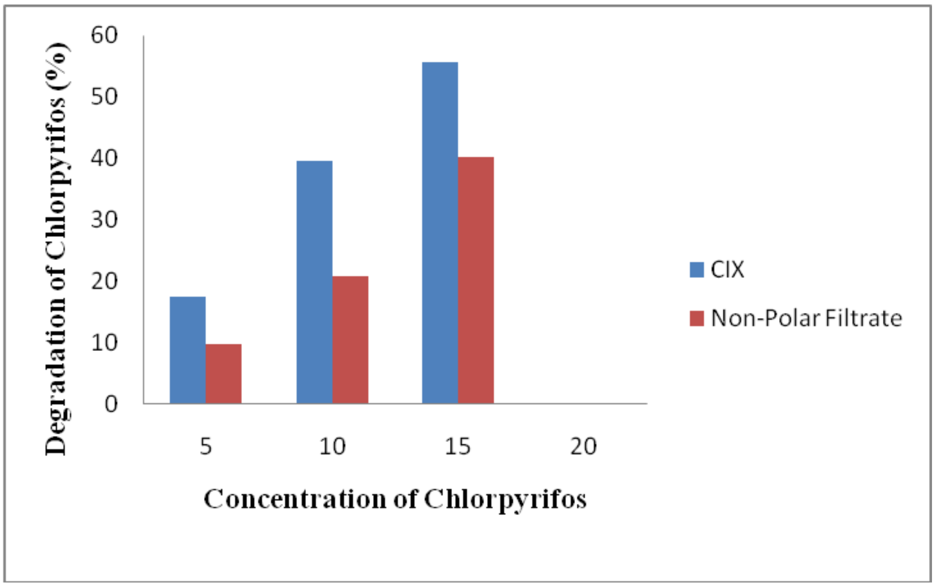


**Fig.3. Degradation of chlorpyrifos in microemulsion using CIX and non-polar filtrate of karanja and jatropha cake as a solvent for disperse phase with varying concentration of chlorpyrifos in formulation**

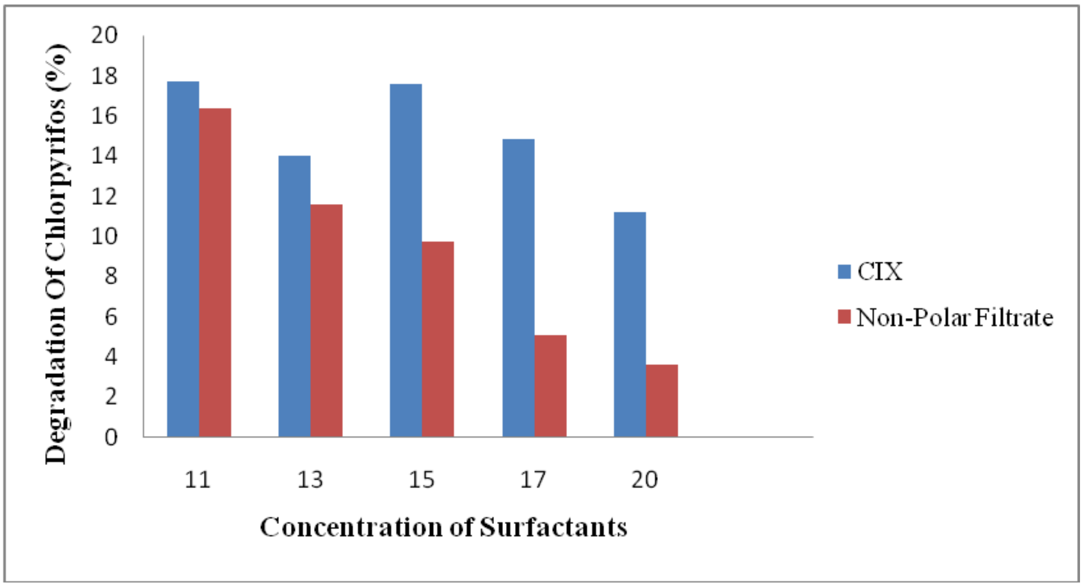


**Fig.4. Degradation of chlorpyrifos in microemulsion using CIX and non-polar filtrate of karanja and jatropha cake as a solvent for disperse phase with varying concentration of surfactants**

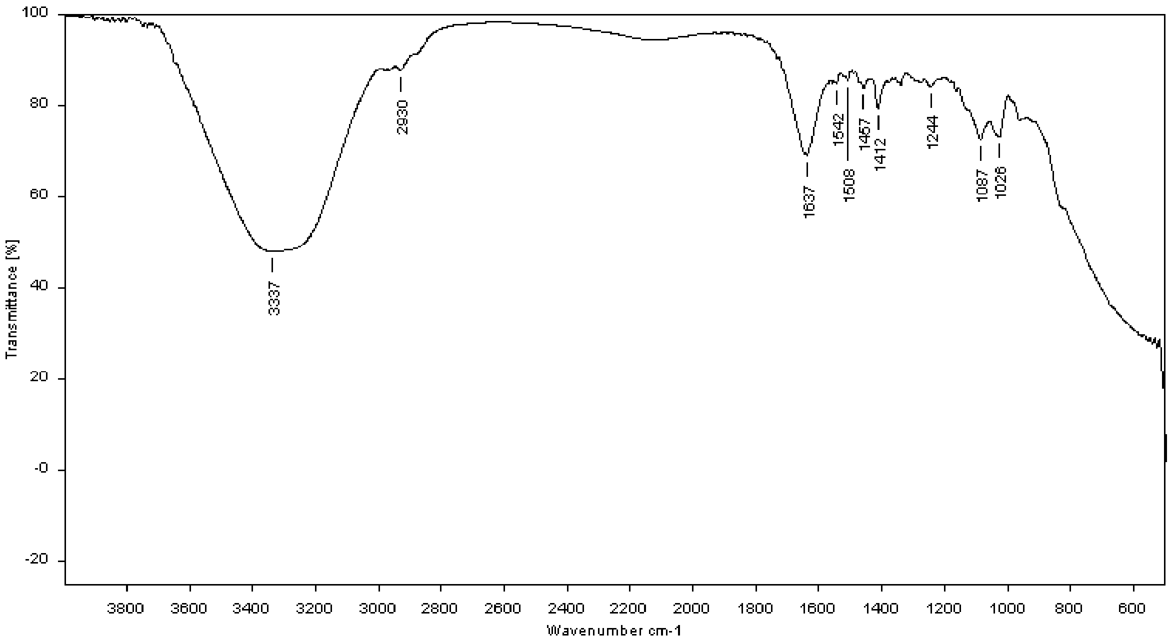


**Fig.5. ATR-FTIR analysis chlorpyrifos microemulsion using non-polar filtrate of karanja and jatropha cake as solvent for disperse phase**

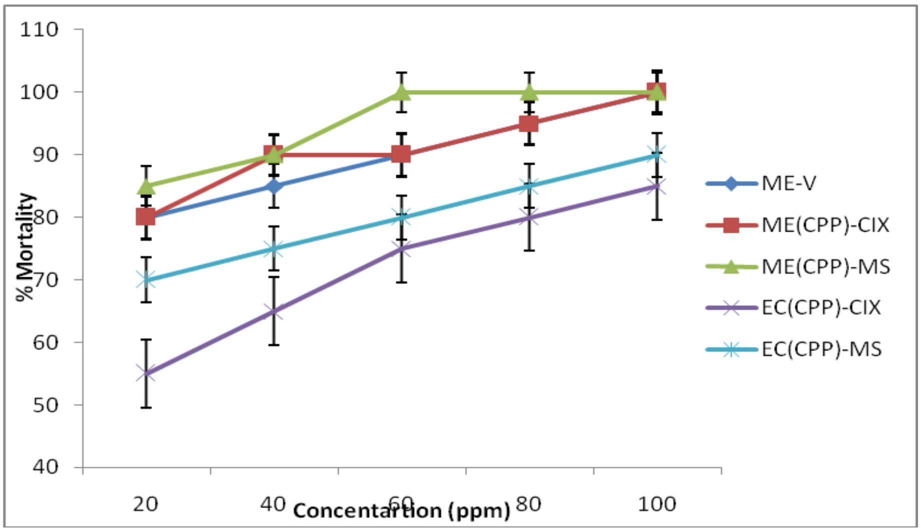


**Fig.6. Percentage mortality at different concentrations (ppm) of chlorpyrifos microemulsion & emulsifiable concentrate containing non polar filtrate of karanja and jatropha and CIX**

ME V= Microemulsion V, ME(CPP)-CIX= Chlorpyrifos Microemulsion with CIX, ME(CPP)-MS= Chlorpyrifos Microemulsion with Modified Solvent (Non polar filtrate of karanja & jatropha), EC(CPP)-CIX= Emulsifiable Concentrate of Chlorpyrifos with CIX, EC(CPP)-MS= Emulsifiable Concentrate of Chlorpyrifos with Modified Solvent