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Development of User and Environment Friendly Bio-degradable Vegetable Oil based Chlorpyrifos 20% Emulsifiable Concentrate Formulation and Evaluation it's Effectiveness against Brinjal Fruit and Shoot Borer (*Leucinodes Orbonalis* G.)

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Abstract

Emulsifiable Concentrate (EC) formulations have been criticized due to presence of large amount of solvents. In present work, user and environment friendly Chlorpyriphos 20 EC was developed using biodegradable vegetable oil to replace high-risk volatile solvents. Physico-chemical parameters of developed ECs were tested to standardize the formulations. Experimental results showed that soybean oil and combination of Nonyl Phenol Ethoxylate-13 and Calcium Alkyl Benzene Sulfonate was the best formulation. Storage stability of vegetable oil based EC formulation was higher than the organic solvent based. Developed ECs were undergone i*n-vivo* bioassay to investigate their effectiveness against brinjal fruit and shoot borer during 2013-2014 and 2014-2015. Vegetable oil based EC was found to superior over solvent based EC formulations. Vegetable oil based EC was relatively soft to prevailing natural defenders and exhibits no phytotoxicity.

Keywords: Chlorpyrifos, Economic Yield, Emulsifiable Concentrate, Flash Point, Predators

1. Introduction

Brinjal (*Solanum melongena* L.) is one of the most popular and economically important vegetables, originated from India and now grown as a vegetable throughout the tropical, subtropical and warm temperate areas of the world[1]. Shoot and fruit borer, *Leucinodes orbonalis* G, one of most the destructive pests on brinjal in Asia[2]. The shoot and fruit borer was identified as the primary limiting factor in brinjal production in South Asian region causes up to 65% yield loss[3–5]. Control measures for this pest rely mainly on the application of insecticides are presently available in Emulsifiable Concentrate (EC) formulations[6].

EC has both outstanding merits and demerits. In most conditions, the pesticide formulation produced with EC possesses a better pesticide effect than the other types

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of formulation due to its excellent biological activity. However, EC has been criticized by environmentalists over the pollution it causes due to presence of large amount of harmful benzene solvents, especially xylene, and some other harmful hydrophilic polar solvents[7]. Replacing high-risk aromatic hydrocarbon solvents with green vegetable oil may be one of the developmental directions of EC formulations due to its low cost; excellent spreading and wetting ability of leaf surface and low environmental impact.

Chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is an organophosphorous insecticide, acaricide, and nematicide used to control a broad spectrum of foliage and soil-born insect pests on a variety of food and feed crops[8]. It is ranked as one of the most extensively used insecticides (20% EC formulation) all around the world and strongly recommended for brinjal fruit and shoot borer control which is basically organic solvent based. The present investigation was, therefore, planned to develop organic solvent as well as biodegradable vegetable oil based Chlorpyriphos 20 EC.

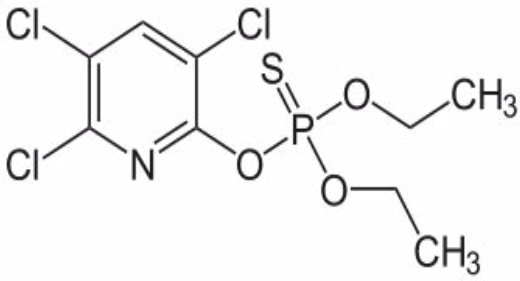


Figure 1. Structure of Chlorpyrifos (Empirical formula: $C_9H_{11}Cl_3NO_3PS$).

2. Materials and Methods

2.1 Materials

Chloropyriphos technical (96.4%-purity) was provided by Jingbo Agrochemicals Technology Co. Ltd. Vegetable oil like sunflower oil, soybean oil and sesame oil were procured from local market, Mohanpur, Nadia. Emulsifiers viz. Tween 60, Tween 80, Span 60 and Span 80 were procured from SD Fine Chem Ltd. Mumbai while CABS and NP-13 from India Glycols, Delhi. Organic solvents viz. Aromax, C-IX and Solvesso were collected from pesticide industries.

2.2 Preparation of 20EC Chloropyriphos Formulation

EC formulation of Chloropyriphos was prepared by dissolving (w/w) 20.74% Chloropyriphos in 71.26% of organic solvents or biodegradable oil and followed by mixing at 500 rpm for 5 min with 8% of suitable emulsifier or blend of emulsifiers after screening to make it clear. Prepared EC formulations were characterized and standardized by as per standard methods and results were recorded and presented in Table 1. The best solvent and oil based EC formulations were selected for bio-efficacy studies.

Table 1. Screening results of single emulsifier

Emulsifier	Blooming properties	Emulsifying properties	Cold storage stability
Tween-60	Good	Better	Separate oil
Tween-80	Good	Good	Stratification
Span-60	Poor	Good	Separate oil
Span-80	Poor	Good	Separate oil
NP-13	Better	Good	No oil separation
CABS	Better	Good	No oil separation

2.3 Physico-chemical Characteristics

Unless otherwise referred, the physico-chemical properties of the EC formulations were tested as per Collaborative International Pesticides Analytical Council (CIPAC) and Indian Standard (IS) specifications (BIS, 1997)[9]. All the experiments were done in triplicate.

2.3.1 Cold Test

50 ml of EC formulation was poured in an l00 ml transparent glass container and closed it with a cork or stopper fitted with the thermometer. Cool the formulation to 10°C by placing the container in ice cold water. When the temperature of the formulation in the container reached 10°C, a small seeding crystal of the pesticide was added with minimum opening of the stopper within the shortest possible duration of time. The formulation was stirred gently for one hour maintaining the temperature of the formulation about 10°C. At the pod of one hour, examined the formulation for any turbidity or separated solid or oily matter or both.

2.3.2 Flash Point (Abel)

Measurement of flash point of the prepared formulations was carried out by the method as described in CIPAC

MT12, (1995). The flash point was recorded as the temperature at the thermometer when a flash appeared. The flash point of the material was above 24·5°C[10].

2.3.3 Test for Emulsion Stability

75 to 80 mL of standard hard water of 342 ppm brought to a temperature of 30 ± 1°C, in the 100mL beaker. 2 mL of the developed formulation was added to the hard water by stirring with a glass rod, at about 4 rev/ sec. 100 mL volumes were made up with water with continuous stirring and immediately poured into the clean and dry cylinder. Kept at 30 ± 1°C for one hour and examined for any creaming or separation at the top or at the bottom in the cylinder if any observed[11].

2.3.4 Persistent Foam

Persistent foam is a measure the amount of foam likely to be present in a spray tank after dilution of the product with water. Specified amount of the prepared formulation is added to standard waters (95 ml) in the measuring cylinder and made up to the mark. The cylinder is stoppered and inverted 30 times. The cylinder was left to stand undisturbed for the specified time. The volume of foam was noted[12].

2.3.5 pH Measurement

The pH of the formulated samples (1% aqueous solution) was measured at 25 ±1°C using a calibrated pH meter (Systronics, model 335)[13]. The measurement was carried out in triplicates. Before the readings were observed pH meter was calibrated by using pH 4, 7.0 and 9.2 buffer solutions (Merck) respectively.

2.3.6 Test for Heat Stability

Developed EC formulation was poured into the glass bottle until it is about three-fourths full. Bottle was stoppered and sealed air-tight with sealing wax to avoid any loss of the volatile solvent. Filled bottle was placed in the thermostat maintained at 54 ± 1°C for 24 hours. Bottle was removed from the thermostat after 24 hours and cooled its contents to room temperature. The treated material was tested for compliance with the requirements of cold test, flash-point test and emulsion-stability test[14].

2.3.7 Acidity and Alkalinity Test

About 10 g of the material was accurately weighed into a dry conical flask and diluted with 100 ml of water. The content of the flask was immediately titrated with the standard sodium hydroxide solution/standard hydrochloric acid using methyl red or bromocresol purple as the indicator. Blank determination on 100 ml of water was also carried out.

2.3.8 Active Ingredient Content

Sample preparation and analysis of a.i. was carried out based on the methodology described in CIPAC Handbook 1C (1985)[15]. The content of active ingredient of chloropyriphos in the prepared formulations was determined by gas chromatography (Shimadzu 2010; Electron Capture Detector). The conditions for the analysis were: detector temperature, 300°C; injector temperature 280°C; oven temperature program starting at 150°C hold for 1 min - increased @ 7°C/min to 200°C hold for 1 min again increased @ 10°C/min to 230°C hold for 1 min again increased @ 10°C /min to 250°C, carrier gas, N_2 at 1 mL/min; injection volume 1.0 mL, in a split mode.

2.4 Bio-efficacy Test

For assessing the bio-efficacy of the developed formulations analyzed *in-vivo* condition as the method described by Pradhan *et al.,* 2013[16]. Field experiment was conducted in a progressive farmer's holdings in Ghoragacha, West Bengal during 2013–2014 and 2014–2015 with variety "*Muktakeshi*". The experiment was laid out in randomized block design (RBD) with 7 treatments including untreated control and replicated thrice. The insecticides evaluated were (i) vegetable oil based Chorpyriphos 20% EC @ 400 ml ha^{-1} (ii) 800 ml ha^{-1}; (iii) solvent based Chorpyriphos 20% EC @ 1000 ml ha^{-1} (iv) solvent based Lambda Cyhalothrin 5% EC @ 300 ml ha^{-1} along with untreated control plots. The appearance of fruit and shoot borer were keenly monitored and when the population was almost evenly distributed, the test insecticides were imposed as foliar spray (500 L ha^{-1}) by a knapsack sprayer thrice at 10 days' interval. The corrected percent reduction/ increase in field population were worked out through Henderson and Tilton equation (1995)[17]. In place of number of insects, per cent damage was substituted which was subjected to statistical analysis adopting randomized block design. The percentage values were transformed to arcsine of square root percentages and count data was converted into square root transformation. The mean values were subjected to

statistical analysis by single factor ANOVA after making necessary transformation whenever required.

3. Results

Chlorpyripos was used to develop EC formulation with the help of combination of anionic and non-ionic emulsifiers and different solvents like soyabean oil, sunflower oil, sesame oil. Among them, soybean was found compatible with technical and most of the tested emulsifiers in all aspects.

3.1 Establishment of Right Solvent System

The dissolving capacities of chorpyriphos in different solvents (Amromax, C-IX and Solvesso) and oils (sesame oil, soybean oil, and sunflower oil) were determined. The experimental effects were showed that the better compound solvents were C-IX and soybean oil. The mass ratio of TC and the compound solvent remained unchanged, stored at 0°C for 7days.

3.2 Determination of the Emulsifier

Selection of emulsifiers is the key factor for the success of EC formulation. In our EC formulation, Chlorpyiphos part (solvent/oil) was mixed with emulsifiers to reduce surface and interfacial tension. In our experiment, the emulsifiers were selected through a series of testing.

3.2.1 Selection of Single Emulsifier

Emulsifying agents such as, NP-13 Tween-60, Tween -80, Span-60, Span-80 and CABS were selected on the basis of experiments results as shown in Table 2.

As shown in Table 3, after cold storage, emulsions containing NP-13 or CABS had no oil separation, when T-60 or T-80 acted as emulsifier, the emulsion produce some layered. On contrary, when Span-60 or Span-80 used as emulsifier, separation was very high, Tween-60 had serious oil separation problems. Thus on the basis of comprehensive analysis of the results, the NP-13 and CABS were selected as the emulsifiers for further experiment.

3.2.2 Determination of Compound Emulsifiers

Results revealed that the combination of NP-13 and CABS was in accordance with the requirements after cold storage and hot storage, and was the best emulsifier combination in all combinations. Table 2 showed that the determination of stability of different combinations of NP-13 and CABS with thermal storage and cold storage. Results showed when the ratio of emulsifier NP-13: CABS was 5:5, EC has best stability.

3.2.3 LODs and LOQs

The limit of detection (LODs) and limit of quantification (LOQs) were established by constructing calibration curves for the analytes (Commission Decision, 2002). The concentration of chloropriphos was calculated (in µg/g) from the matrix spiked chromatogram which could produce the signal equal to three times of (Np-p) blank. LOQ was calculated by multiplying the LOD value by factor 3 round of to two decimal place value. LOD and LOQ values of chloropriphos in formulations are found to be 0.01 and 0.03 μgmL^{-1} respectively.

Table 2. Optimization of emulsifiers

Test sample	Proportion of emulsifier	Heat storage (54±2)°C	Cold storage (0-2)°C
A	NP-13:CABS=9:1	Oil separating,4.6%	Precipitation
B	NP-13:CABS=8:2	Oil separating,4.0%	Precipitation
C	NP-13:CABS=7:3	Oil separating,3.8%	Precipitation
D	NP-13:CABS=6:4	Oil separating,2.1%	Slight precipitation
E	NP-13:CABS=5:5	Up to standard	No precipitation
F	NP-13:CABS=4:6	Oil separating,1.7%	Slight precipitation
G	NP-13:CABS=3:7	Oil separating,2.3%	Precipitation
H	NP-13:CABS=2:8	Oil separating,3.9%	Precipitation
I	NP-13:CABS=1:9	Oil separating,4.2%	Precipitation

Table 3. Heat storage stability of Chlorpyriphos 20%EC

Sample		Before heat storage		Heat storage for 24 hrs		Decomposition (%)
		Content of Chlorpyriphos (%)	Emulsion stability	Content of Chlorpyriphos (%)	Emulsion stability	
Vegetable oil based EC	1	20.74	up to standard	20.38	up to standard	1.73
	2	20.74	up to standard	20.17	up to standard	2.65
	3	20.74	up to standard	19.89	up to standard	4.09
Organic solvent based EC	1	20.74	up to standard	19.91	up to standard	4.00
	2	20.74	up to standard	19.63	up to standard	5.35
	3	20.74	up to standard	19.37	up to standard	6.60

Table 4. Comparative Test Results of Different Physic-Chemical Parameters of Two Emulsifiable Concentrate Formulations of Chorpyrifos 20 % (FAO Specification 221/EC (October 2004*)

Parameter				Chorpyrifos 20 % EC (Biodegradable Vegetable Oil Based)	Chorpyrifos 20 % EC (Volatile Organic Solvent Based)
Description:				a mild, mercaptan-like odor of Chorpyrifos stable and homogeneous pale yellow to amber coloured liquid, free from visible suspended matter and sediment,	Strong pungent smell of Chorpyrifos and organic solvent stable and homogeneous dark yellow to amber coloured liquid, free from visible suspended matter and sediment,
Active ingredient)	Identity tests			The active ingredient complies with an identity test.	The active ingredient complies with an identity test.
	Chlorpyrifos content			20.21 Declared content in g/kg or g/l at 20 ± 2°C	19.63 Declared content in g/kg or g/l at 20 ± 2°C
Relevant impurities	Sulfotep (O,O,O',O'-tetraethyl dithiopyrophosphate,			Maximum: 3 g/kg (0.3%) of the chlorpyrifos content found under clause 2.2.	Maximum: 3 g/kg (0.3%) of the chlorpyrifos content found under clause 2.2.
Physical properties	pH range			6.4	5.8
	Emulsion and re-emulsification stability	0 h	Initial emulsification complete		Initial emulsification complete
		0.5 h	'Cream', maximum: 0ml		'Cream', maximum: 1ml
		2.0 h	'Cream', maximum: 1ml 'Oil', maximum: no oil		'Cream', maximum: 2ml ' Oil', maximum: trace
		24 h	Re-emulsification complete		Re-emulsification not complete
		24.5 h	'Cream', maximum: 1ml 'Oil', maximum: trace		'Cream', maximum: 2ml ' Oil', maximum: trace
	Persistent foam			Maximum: 1 ml after 1 minute.	Maximum: 5 ml after 1 minute.
Storage stability	Stability at 0°C			After storage at 0 ± 2°C for 7 days, the volume of solid or liquid which separates was 0 ml	After storage at 0 ± 2°C for 7 days, the volume of solid or liquid which separates was 0.3 ml
	Stability at elevated temperature			After storage at 54 ± 2°C for 14 days, the determined average active content was 98.6 %, relative to the determined average content found under before storage. The formulation continued to comply with the clauses for: pH range; Emulsion stability/ re-emulsification.	After storage at 54 ± 2°C for 14 days, the determined average active content was 95.3 %, relative to the determined average content found under before storage. The formulation continued to comply with the clauses for: pH range; Emulsion stability/re-emulsification.

4. Discussion

The formulation was consisting of technical chlorpyrifos dissolved in suitable solvents (C-IX and soybean oil) together with necessary formulants. Using vegetable oil a sable, homogeneous pale yellow to amber colored chlorpyrifos formulation was developed which was free from visible suspended matter and sediment. On the other hand, organic solvent based formulation looked like a stable and homogeneous form of dark yellow to amber coloured liquid, having strong pungent smell. Both the vegetable oil and organic solvent based Choropyriphos 20% EC were stable on accelerated temperature stability. On blooming and cold stability vegetable oil based formulations were better than organic solvent based EC. All the formulation compiled flash point test as they were above (24.5˚C).

Stability in heat was determined by decomposition rate of Chlorpriphos and emulsifying properties. A linear calibration curve was used and the calibration range was 0.01–1.0 mg kg^{-1} (Figure 2).

Under these conditions chlorpyrifos retention times were approximately 9.069 min (Figure 3). Table 3 showed that the decomposition rate of three parallel tests of EC samples (vegetable oil based) were less than 5% and the thermal stability of sample meet the requirements which was also higher than organic solvent based EC formulations. The lower amount of active ingredient in the formulations may be due to dissipation of active ingredient during storage.

The pooled data on efficacy of Chorpyriphos 20% EC along with other insecticides during 2013-2014 and 2014-2015 have been depicted in Table 5 where, the mean damage to shoot before spraying varied between 5.37 to 6.13 %.

After first spray, all the treatments gave a significant reduction of damage over control. Chorpyriphos 20% EC @ 800 ml ha^{-1}showed 7.01 % mean shoot damage which was on par with each other. Now, considering mean percent shoot damage over untreated check, it is revealed from Table 6, that Chorpyriphos 20% EC @ 800 ml ha^{-1} recorded 7.01 % reduction over control. In

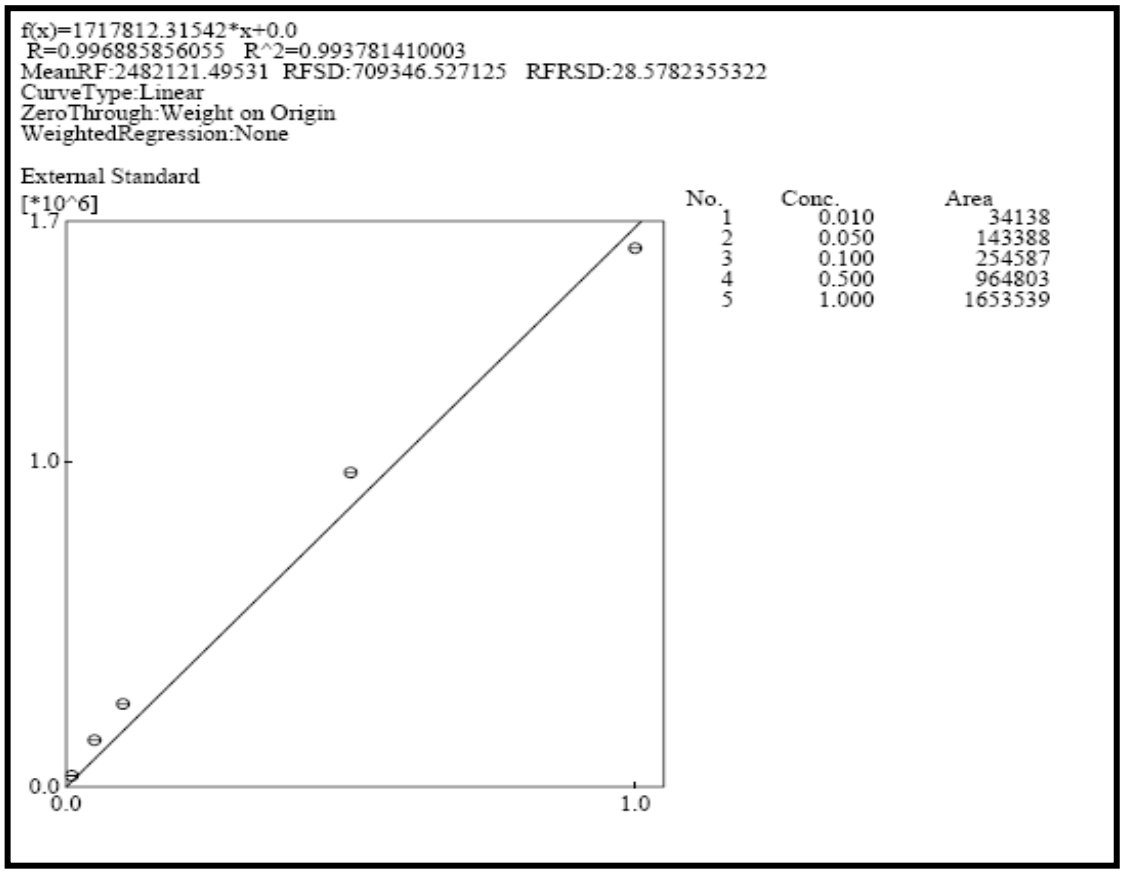


Figure 2. Detector Linearity for Chlorpyrifos with GC-ECD.

Table 5. Relative efficacy of different insecticides against shoot and fruit infestation by *Leucinodes orbonalis* G in brinjal during *kharif* season of 2014

Treatments	Dose (ml/ha)	PTD	shoot infestation at different days after spray (%)						Mean % shoot infestation	Mean % reduction over control	PTD	% fruit infestation at different days after spray						Mean % fruit infestation	Mean % reduction over control
			First Spray			Second Spray						First Spray			Second Spray				
			3 DAS	7 DAS	14 DAS	3 DAS	7 DAS	14 AS				3 DAS	7 DAS	14 DAS	3 DAS	7 DAS	14 DAS		
Chlorpyrifos 20 EC (Oil Based)	400	= 5.37	2.57 (9.23)*	1.85 (7.82)	3.90 (11.39)	2.01 (8.15)	1.77 (7.65)	2.10 (8.33)	2.37 (8.86)	77.64 (61.78)	10.48	5.32 (13.34)	3.78 (11.21)	10.25 (18.67)	4..92 (12.82)	3.10 (10.14)	8.86 (17.32)	6.04 (14.23)	80.22 (63.59)
Chlorpyrifos 20 EC (Oil Based)	800	5.82	2.02 (8.17)	0.60 (4.44)	2.89 (9.79)	1.68 (7.45)	0.42 (3.72)	1.32 (6.60)	1.49 (7.01)	85.94 (67.98)	12.75	3.85 (11.32)	2.33 (8.78)	6.10 (14.30)	2.91 (9.82)	1.25 (6.42)	4.76 (12.60)	3.53 (10.83)	88.44 (70.12)
Chlorpyrifos 20 EC (Solvent based)	1000	5.35	2.25 (8.63)	0.81 (5.16)	3.11 (10.16)	1.74 (7.58)	0.66 (4.66)	1.40 (6.80)	1.66 (7.40)	84.34 (66.69)	10.83	4.00 (11.54)	2.45 (9.01)	8.05 (16.48)	3.09 (10.12)	1.75 (7.60)	5.88 (14.03)	4.20 (11.83)	86.25 (68.23)
Lambda Cyhalothrin 5 EC	300	6.18	2.20 (8.53)	0.95 (5.59)	3.25 (10.39)	1.70 (7.49)	0.71 (4.83)	1.39 (6.77)	1.70 (7.49)	83.96 (66.39)	10.39	3.95 (11.46)	2.47 (9.04)	6.50 (14.77)	3.15 (10.22)	1.97 (8.07)	6.56 (14.84)	4.10 (11.68)	86.58 (68.51)
Untreated control	-	6.02	9.56 (18.01)	12.84 (21.00)	10.55 (18.95)	11.25 (19.60)	13.10 (21.22)	6.29 (14.53)	10.60 (19.00)	-	11.06	23.25 (28.83)	38.16 (38.15)	31.74 (34.29)	25.37 (30.24)	42.78 (40.85)	21.92 (27.92)	30.54 (33.55)	-
SEm ±		NS	0.79	1.35	0.66	1.89	2.12	0.85	0.23	NS	NS	1.23	0.45	0.96	1.57	2.18	0.74	0.52	NS
CD (0.05)		NS	2.42	3.03	3.22	4.54	5.32	3.29	1.55	NS	NS	3.78	2.25	1.60	3.64	4.89	2.55	2.46	NS
CV (%)		NS	4.10	5.28	4.69	6.40	7.18	4.55	3.29	NS	NS	5.26	4.69	3.71	5.73	6.32	4.67	4.79	NS

Table 6. Relative efficacy of different insecticides against shoot and fruit infestation by *Leucinodes orbonalis* G in brinjal during *kharif* season of 2015

Treatments	Dose (ml/ ha)	PTD	% shoot infestation at different days after spray						Mean % shoot infest ation	Mean % reduction over control	PTD	% fruit infestation at different days after spray						Mean % fruit infestation	Mean % reduction over control
			First Spray			Second Spray						First Spray			Second Spray				
			3 DAS	7 DAS	14 DAS	3 DAS	7 DAS	14 DAS				3 DAS	7 DAS	14 DAS	3 DAS	7 DAS	14 DAS		
Chlorpyrifos 20 EC (Oil Based)	400	5.82	2.35 (8.82)*	1.12 (6.08)	3.71 (11.11)	1.89 (7.90)	1.56 (7.18)	2.00 (8.13)	2.11 (8.35)	80.30 (63.65)	10.05	7.42 (15.81)	5.80 (13.94)	10.58 (18.98)	6.15 (14.36)	4.48 (12.22)	10.26 (18.68)	7.45 (15.84)	74.71 (59.80)
Chlorpyrifos 20 EC (Oil Based)	800	6.00	1.95 (8.03)	0.54 (4.21)	2.75 (9.55)	1.60 (7.27)	0.39 (3.58)	1.48 (6.99)	1.45 (6.92)	86.46 (68.41)	11.51	5.24 (13.23)	3.58 (10.91)	7.44 (15.83)	4.10 (11.68)	2.39 (8.89)	5.95 (14.12)	4.78 (12.63)	83.78 (66.25)
Chlorpyrifos 20 EC (Solvent based)	1000	6.54	2.30 (8.72)	0.69 (4.77)	2.97 (9.92)	1.64 (7.36)	0.52 (4.14)	1.50 (7.04)	1.60 (7.27)	85.06 (67.26)	9.56	5.84 (13.99)	4.00 (11.54)	8.96 (17.42)	4.17 (11.78)	3.08 (10.11)	6.92 (15.25)	5.50 (13.56)	81.33 (64.40)
Lambda Cyhalothrin 5 EC	300	6.50	2.31 (8.74)	0.89 (5.41)	3.10 (10.14)	1.68 (7.45)	0.54 (4.21)	1.49 (7.01)	1.67 (7.43)	84.41 (66.74)	11.47	5.15 (13.12)	3.73 (11.14)	7.72 (16.13)	4.25 (11.90)	3.18 (10.27)	8.05 (16.48)	5.35 (13.37)	81.84 (64.78)
Untreated control	-	7.26	10.28 (18.70)	13.48 (21.54)	9.10 (17.56)	11.75 (20.05)	12.34 (20.57)	7.29 (15.66)	10.71 (19.10)	-	11.00	20.32 (26.79)	35.84 (36.77)	29.13 (32.67)	26.75 (31.15)	41.25 (39.96)	23.48 (28.98)	29.46 (32.87)	-
SEm ±		NS	0.54	1.59	0.12	0.87	1.29	0.74	0.23	NS	NS	0.46	0.88	1.25	0.81	0.75	0.22	0.77	NS
CD (0.05)		NS	2.66	5.60	1.58	3.19	3.68	2.54	2.36	NS	NS	2.32	3.60	4.67	3.36	2.42	2.91	3.10	NS
CV (%)		NS	4.23	7.22	3.96	5.44	6.61	4.98	4.52	NS	NS	4.59	4.72	6.10	5.40	4.13	4.08	4.23	NS

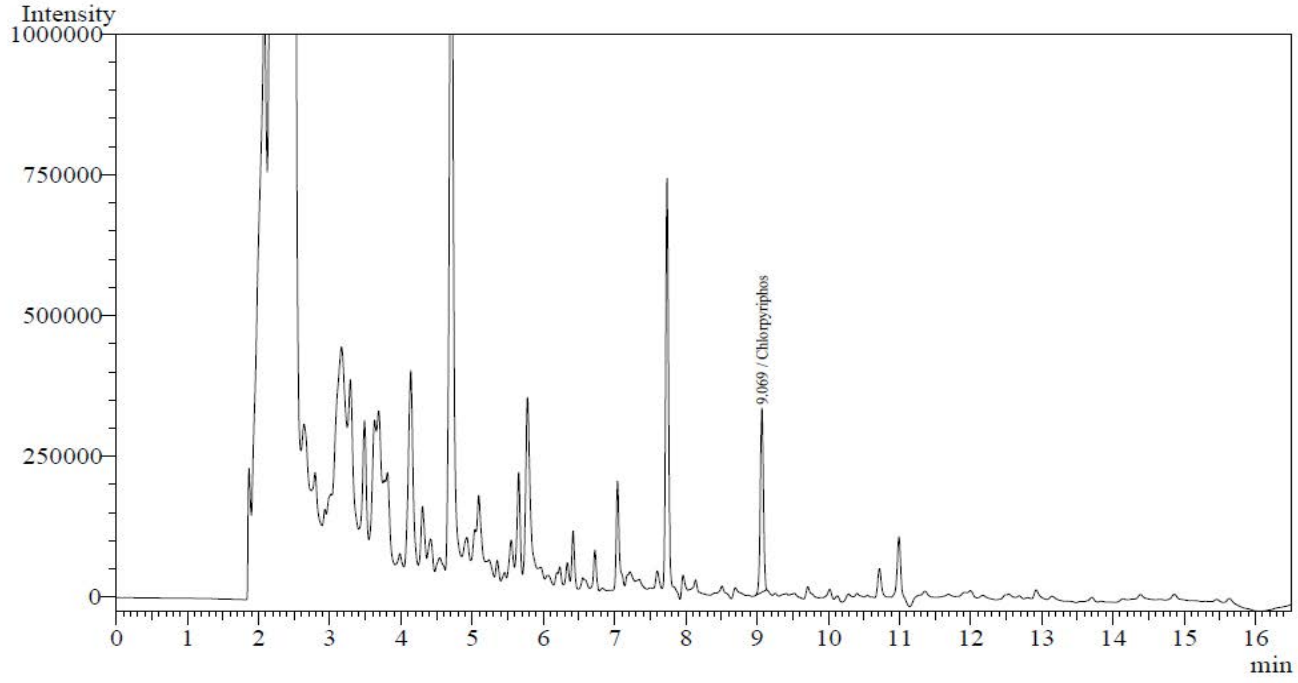


Figure 3. GC-ECD chromatogram of Chloropryphos in vegetable oil based 20% EC formulation.

Season-II, similar results were encountered. The mean percent reduction over untreated control was highest in Chorpyriphos 20% EC @ 800 ml ha^{-1} with 6.57 % reduction.

The pre-treatment population of spiders ranged from 4.75to 6.00 per 10 plants in different treatments during 2013-2014 (Table 7). Kranthi *et al.,* (2004) reported that Chorpyriphos 20% EC also acts as insecticide, selective and less toxic to beneficial insects like many predators and parasitoids[18].

Regarding coccinellid population, Table 7 reveals that in Season-I the population was highest in the untreated control plots (2.96/10 plants) followed by Chorpyriphos 20% EC 400 ml ha^{-1} (2.60/10 plants). The results are in agreement with the findings of Preetha *et al.,* (2009)[19] where chloropyriphos has declared as a harmless insecticide to coccinellid. It was also reported that low and recommended dose of chloropyriphos was harmless to *Chrysoperla carnea* and higher dose was slightly harmful[20]. This leads further support to the findings of present authors.

Table 8 reveals that three applications of Chorpyriphos 20% EC at the rate of 400, 800 ml ha^{-1} for the control of fruit and shoot borer in brinjal provided maximum biological and economic yield at the dose of 800 ml ha^{-1} (55.81 and 53.42 quintal ha^{-1}) in two seasons. Rajabaskar and Regupathy, (2013) selected Chorpyriphos 20% EC as a cost effective tool of IPM module to manage brinjal fruit and shoot borer which fully supports the ultimate results of this experiment[21].

5. Conclusion

In this study, stable oil based Chlorpyriphos 20% EC formulation was developed through vigorous optimization procedures. Moreover, the products have good cold storage, heat storage stability in accordance with EC quality standard. Vegetable oil based chlorpyrifos formulation was highly effective for the management of shoot and fruit borer of brijal under West Bengal condition with a promising cost: Benefit ratio of 1: 18.41 and was extremely soft against the prevailing natural enemies in brinjal eco-system. A detailed development study of vegetable oil based formulation was a significant step forward towards development of promising eco-friendly technology for crop protection.

Table 7. Relative effect of different insecticidal treatments on prevailing predatory fauna in brinjal agro-ecosystem during *kharif* season of 2014 and 2015

Treatments	Dose (ml/ ha)	% reduction/increase (+) of predators population during 2014								% reduction/increase (+) of predators population during 2015							
		PTC (No. per 5 plants)		15 DAFS		PTC (No. per 5 plants)		15 DASS		PTC (No. per 5 plants)		15 DAFS		PTC (No. per 5 plants)		15 DASS	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Chlorpyrifos 20 EC (Oil Based)	400	4.50 (2.24)*	6.25 (2.60)	4.62 (12.41)#	3.82 (11.27)	5.00 (2.35)	5.50 (2.45)	5.45 (13.50)	4.76 (12.60)	6.25 (2.60)	8.00 (2.92)	5.15 (13.12)	4.54 (12.30)	5.50 (2.45)	5.00 (2.35)	6.70 (15.00)	5.87 (14.02)
Chlorpyrifos 20 EC (Oil Based)	800	5.25 (2.40)	8.00 (2.92)	7.81 (16.23)	5.25 (13.25)	3.50 (2.00)	6.50 (2.65)	8.26 (16.70)	5.97 (14.14)	5.25 (2.40)	6.50 (2.65)	7.23 (15.60)	6.47 (14.74)	4.75 (2.29)	6.75 (2.69)	8.59 (17.04)	7.83 (16.25)
Chlorpyrifos 20 EC (Solvent based)	1000	4.75 (2.29)	8.25 (2.96)	13.64 (21.67)	10.96 (19.33)	4.00 (2.12)	7.25 (2.78)	15.16 (22.92)	11.31 (19.65)	6.50 (2.65)	8.25 (2.96)	10.88 (19.26)	9.25 (17.71)	5.00 (2.35)	6.25 (2.60)	12.10 (20.36)	11.56 (19.88)
Lambda Cyhalothrin 5 EC	300	5.25 (2.40)	6.00 (2.55)	11.79 (20.08)	11.15 (19.51)	4.50 (2.24)	6.50 (2.65)	12.75 (20.92)	12.12 (20.37)	5.25 (2.40)	5.00 (2.35)	9.22 (17.68)	9.21 (17.67)	6.25 (2.60)	5.00 (2.35)	11.95 (20.22)	12.13 (20.38)
Untreated control	-	6.00 (2.55)	8.25 (2.96)	+19.45 (0.00)	+23.85 (0.00)	5.75 (2.50)	6.00 (2.55)	+28.30 (0.00)	+34.29 (0.00)	4.75 (2.29)	7.75 (2.87)	+15.96 (0.00)	+20.11 (0.00)	5.25 (2.40)	6.50 (2.65)	+25.45 (0.00)	+31.71 (0.00)
SEm ±		NS	NS	0.58	0.76	NS	NS	1.24	0.78	NS	NS	0.52	0.85	NS	NS	1.12	1.09
CD (0.05)		NS	NS	3.32	3.78	NS	NS	4.75	3.69	NS	NS	3.67	3.53	NS	NS	3.33	4.45
CV (%)		NS	NS	4.96	5.07	NS	NS	5.88	6.25	NS	NS	5.11	5.56	NS	NS	5.27	6.73

A = Spider complex; B = Coccinellid predators; PTC = Pre Treatment Count; DAFS = Days After First Spray; DASS = Days After Second Spray *Data in these parentheses are √n+0.5 transformed values; #Data in these parentheses are $\sin^{-1}$ transformed values.

Table 8. Yield of brinjal and economics of different treatments against brinjal shoot and fruit borer *Leucinodes orbonalis* G. during *kharif* season of 2014 and 2015

Treatments	Dose (ml/ ha)	Marketable yield of brinjal (q/ ha)		Mean marketable yield (q/ ha)	Cost of test chemicals (Rs./ lit)	Cost of labour including spray charges (Rs./ ha)	Total cost of different treatments (Rs./ ha)	Gross return (Rs./ ha)	Net return (Rs./ ha)	Net profit over control (Rs./ ha)	B:C ratio
		2014	2015								
Chlorpyrifos 20 EC (Oil Based)	400	45.36	41.86	43.61	450.00	1750.00	1930.00	65415.00	63485.00	22500.00	11.66: 1
Chlorpyrifos 20 EC (Oil Based)	800	55.81	53.42	54.62	450.00	1750.00	2110.00	81930.00	79820.00	38835.00	18.41: 1
Chlorpyrifos 20 EC (Solvent based)	1000	53.82	50.35	52.09	400.00	1750.00	2150.00	78135.00	75985.00	35000.00	16.28: 1
Lambda Cyhalothrin 5 EC	300	53.24	50.10	51.67	550.00	1750.00	1915.00	77505.00	75590.00	34605.00	18.07: 1
Untreated control	-	27.59	29.38	28.49	0.00	1750.00	1750.00	42735.00	40985.00	-	-

Cost of fresh marketable brinjal: Rs. 15.00 per kg; Labour charges (skilled): Rs. 193.00 per day as per Govt. of West Bengal Labour Commission Circular, 2013u

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7. References

1. Maru NK, Kumar A. Comparison of bio-rational and bio-intensive IPM modules for safe pesticide residue and management of brinjal shoot and fruit borer Leucinodes orbonalis Guenee with economics. Journal of Entomology and Zoology. 2018. 6:256–65.
2. Dwivedi RK, Tripathi A, Pal RK, Singh DK. Effect and eco-friendly management of brinjal shoot and fruit borer (Leucinodes orbonalis Guenee) on brinjal. Journal of Plant Protection Research. 2014; 7:287–91. https://doi.org/10.15740/HAS/IJPP/7.2/287-291
3. Anil PC, Sharma. Bioefficacy of insecticides against Leucinodes orbonalis on brinjal. Journal of Environmental Biology. 2010; 31:399–402.
4. Mall NP, Pandey RS, Singh SV, Singh SK. Seasonal incidence of insect pests and estimation of losses caused by shoot and fruit borer on brinjal. Indian J. Entomology. 1992; 54.
5. Patil PD. Technique for mass rearing of the rearing of the brinjal shoot and fruit borer, Leucinodes orbonalis Guen. Journal of the Entomological Research Society. 1990; 14:164–72.
6. CIBRC, Registered Products [Internet]. [cited 2019 Apr]. Available from: http://ppqs.gov.in/divisions/cib-rc/registered-products.
7. Chin CP, Lan CW, Wu HS. Application of biodiesel as carrier for insecticide emulsifiable concentrate formulation. Journal of the Taiwan Institute of Chemical Engineers. 2012; 43:78–584. https://doi.org/10.1016/j.jtice.2012.02.003
8. FAO. Pesticide residues in food. Report of the joint meeting of the FAO panel of experts on pesticide residues in food and the environment and the WHO core assessment group on pesticide residues, Geneva, Switzerland; 2000. p. 45–59.
9. CIPAC MT12. Flash Point. In: Dobrat W, Martijn A, ed. CIPAC handbook F. Physico-chemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd.; 1995. p. 1.
10. CIPAC MT 18. Preparation of Standard waters A and D. In: Dobrat 255 W, Martijn A, eds. CIPAC handbook F. Physico-chemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides AnalyticalCouncil Ltd.; 1995. p. 59–62
11. CIPAC MT 36.3. Emulsion stability and re-emulsification. In: Dobrat W, Martijn A, eds. CIPAC handbook K. Physico-

chemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd.; 2003. p. 137.
12. CIPAC MT 47.2. Persistent foaming. In: Dobrat W, Martijn A, eds. CIPAC handbook F. Physico-chemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd.; 1995. p. 152–3.
13. CIPAC MT 75.3. Determination of pH. In: Dobrat W, Martijn A, eds. CIPAC handbook J. Physico-chemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd.; 2000. p. 131.
14. CIPAC MT 46.3. Accelerated storage procedure. In: Dobrat W, Martijn A, eds. CIPAC handbook J. Physico-chemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd.; 2000. p. 128.
15. CIPAC 221.b/EC/M/3. Handbook 1C, Collaborative International Pesticides Analytical Council, Harpenden, UK; 1985. p. 2028–31.
16. Pradhan S, Roy I, Lodh G, Patra P, Choudhury SR, Samanta A et al. Entomotoxicity and biosafety assessment of PEGylated acephate nanoparticles: A biologically safe alternative to neurotoxic pesticides. Journal of Environmental Science and Health. Part B. 2013; 48:559–69. https://doi.org/10.1080/03601234.2013.774891. PMid:23581688
17. Henderson CF, Tilton EW. Tests with acaricides against the brow wheat mite. Journal of Economic Entomology. 1995; 48:157–61. https://doi.org/10.1093/jee/48.2.157
18. Kranthi KR, Kranthi S, Banerjee SK, Raj S, Chaudhary A, Narula AM, et al. IRM- Revolutionising cotton pest management in India. Resistance Management Newsletter. 2004; 14:33–6.
19. Preetha G, Stanley J, Manoharan T, Chandrasekaran S, Kuttalam S. Toxicity of imidacloprid and diafenthiuron to Chrysoperla carnea Stephens Neuroptera: Chrysopidae in the laboratory conditions. Journal of Plant Protection Research. 2009; 49:3. https://doi.org/10.2478/v10045-009-0047-8
20. Nasreen A, Mustafa G, Ashfaq M. Mortality of Chrysoperla carnea Stephens Neuroptera: Chrysopidae after exposure to some insecticides; laboratory studies. South Pac. Studs. 2005; 26:1–6.
21. Rajabaskar D, Regupathy A. Neem based IPM modules for Control of Sciothrips cardamomi Ramk. and Conogethes punctiferalis Guen. in small cardamom. Asian Journal of Biological Sciences. 2013; 6:142–52. https://doi.org/10.3923/ajbs.2013.142.152