https://doi.org/10.26651/allelo.j/2022-56-2-1399 0971-4693/94 Euro 20.00
Allelopathy Journal 56 (2): 207-220 (July, 2022) *International Allelopathy Foundation* 2022
Table: 5, Figs : 6

Insecticidal activity of oil-in-water emulsion formulations of essential oils against white fly, *Bemicia tabaci* (Gennadius) (Homoptera: Aleyrodidae)

S.M. Nebapure, D.J. Sarkar[1], S.S. Suroshe, V.S. Rana[2], N.A. Shakil[2] and S. Subramanian*
Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi-12
E. Mail: entosubra@yahoo.co.in

(Received in revised form: May 31, 2022)

ABSTRACT

Whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) is one of the global polyphagous pests in many agricultural crops. Besides, other control strategies, essential oils are being considered as one of the promising candidates for developing alternative strategies for its management. We evaluated the Oil-in-water (O/W) formulations (47.6 %) of 6-essential oils (pine oil, lemongrass oil, geranium oil, eucalyptus oil palmarosa oil and citral) against *B. tabaci* to determine their contact toxicity potential and phytotoxicity effects. The GC-MS analyses of test essential oils contained geraniol (69.8 %) in palmarosa oil, δ-3-carene (50.7 %) in pine oil, citronellal (33.4 %) in geranium oil as major constituents. The O/W emulsion formulations were physically stable at room temperature with average droplet size of 136-425 nm. Geranium oil showed high phytotoxicity at 0.125 % concentration in brinjal, *Solanum melongena* (Variety: MEBH-10) leaves, whereas, the eucalyptus and pine oils were not phytotoxic. Comparatively, palmarosa oil displayed maximum contact toxicity with LC_{50} and LC_{90} values of 0.241 and 0.658 % respectively at 24 h and 0.142 and 0.398 % at 48 h after exposure. This study revealed that O/W emulsion formulations of palmarosa and lemongrass oil can be effectively used in whitefly management.

Keywords: *Bemicia tabaci*, Brinjal, contact toxicity, essential oil, GCMS, insecticidal activity, oil-in-water formulation, phytotoxicity, white fly.

INTRODUCTION

Whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) (Fig 1) is one of the major pests in many crops in tropical and subtropical regions of the world. It causes considerable losses in cotton, brinjal, various vegetables and ornamentals (23,36). Its both nymphs and adults suck the sap from the leaves and cause yellowing and drying of plants. In addition, they also act as vector for several viral diseases. Management of whiteflies with pesticides has become difficult due to several factors: (i). Colonization habit on the undersurface of leaves which makes them less accessible to insecticide exposure, (ii). Development of resistance against many insecticides and (iii). Elimination of natural enemies due to overuse of synthetic insecticides (8,27). To overcome these problems green pesticides are one of the promising and safe alternatives for management of whitefly.

The concept of "Green Pesticides" refers to all types of nature-oriented and beneficial pest control materials that can reduce the pest population and increase food production. These pesticides are safe, eco-friendly than synthetic pesticides (19). Plant products and essential oils constitute a major chunk of green pesticides. Essential oils are considered as one of the potential candidates owing to their wide spectrum of activities [fumigants, contact toxicity, repellency, antifeedancy, growth retardation or reproductive

*Correspondence author, Correspondence author, [1]ICAR-Central Inland Fisheries Research Institute, Kolkata, West Bengal- 20, [2]Division of Agricultural chemicals, ICAR-Indian Agricultural Research Institute, New Delhi-12.

disruption properties (16,18)] with high efficacy, low mammalian toxicity and rapid degradation in the environment. Thus, the EOs are considered as alternative and effective against several insect pests (6,12,16,18). The multifaceted mode of action of EOs is due to the presence of diverse allelochemicals (19,22,40) with multiple modes of action to prevent or delay the development of resistance by pests (15). Earlier studies have shown the toxic and repellent effects of different essential oil and their constituents like Cinnamaldehyde, linalool, geraniol, citronellol use against whitefly, *B. tabaci* (7, 11,14,24,39).

Phytotoxicity is one of the limitation factors in the of essential oils as plant protection chemicals. The diverse phytochemical constituents of essential oils influences the physiological processes of plants including distortion of plant cells, inhibition of photosynthesis, suppression of mitosis leading to necrosis and plants death (38). There is limited literature on the phytotoxic effects of essential oils or the plant allelochemicals. Moreover, the threshold concentration for inducing phytotoxicity varies with the essential oil. Further, the persistence of EOs is low as some of the allelochemical compounds are volatile in nature. Essential oils are mainly composed of volatile organic compounds (VOCs), with high vapour pressure at room temperature (13). However, by adopting suitable formulation techniques, the limitations of the EOs can be overcome. Attempts have been made to develop oil-in-water formulation of certain essential oils using Amphiphilic polymer, poly [poly(oxyethylene-1000)-oxyterephthaloyl]) as a surfactant. The physico-chemical properties of these essential oil formulations were studied to understand the stability and to develop viable EOs formulation effective against *B. tabaci*. Thus safe concentrations and suitable formulations is crucial for the development of effective essential oils against insect pests. Hence, in this study we aimed to determine (i). the phytotoxicity of the chosen essential oil formulations and (ii). their biological activities against whitefly, *B. tabaci*.

MATERIAL AND METHODS

Essential oils

The EOs namely pine oil (*Pinus roxburghii* Sarg.), lemongrass (*Cymbopogon flexuosus* (Steud.) Wats), geranium oil (*Pelargonium graveolens* L. Herit), eucalyptus oil (*Eucalyptus globulus* Labill.), and palmarosa oil (*Cymbopogon martini* (Roxb.) J.F. Watson) were purchase from Fragrance and Flavour Development Centre, Kannauj, Uttar Pradesh, India and pure citral was purchased from M/s Central Drug House (P) Ltd, India.

Gas Chromatography-Mass Spectrometry Analysis

The chemo-profiling of essential oils was carried out using Focus-DSQ GC/MS (Thermo) equipped with TG-5MS capillary column (30m x 0.25mm i.d.; film thickness 0.25 µm) Chromatographic conditions were as follows: injector temperature was 250 ^{0}C, helium as carrier gas at a flow-rate of 1 ml/min and injection volume was 0.2 µl (1000 ppm in hexane),. The column temperature was held at 60 $^\circ$C and programmed at 3^{0}C/min to 250ºC and held for 5 minutes with split ratio of 1:20. The MS transfer line and source temperatures were 260º C and 230ºC, respectively. The GC column was coupled directly to single quadrupole mass spectrometer in EI mode at 70 eV with the mass range of 30-400 a.m.u at 1 scan/s. The identification of individual compound was carried out by

comparing their mass spectra with authentic samples and NIST Mass Spectral Library (Ver. 2, 2005).

Preparation of essential oil formulations

The amphiphilic polymer, Poly [poly (oxyethylene-1000)-oxyterephthaloyl] was prepared as per earlier study (33). Briefly, equimolar amounts of polyethylene glycol (avg. mol., 1000) (Sigma Aldrich, India) and dimethyl terephthalate (Sigma Aldrich, India) were taken in two-neck round-bottom flask and allowed for esterification reaction at 65°C in the presence of conc. H_2SO_4 (0.1 % of the monomer) under vacuum and constant stirring (200 rpm) for 24 h. Emulsion formulations of essential oil were prepared using spontaneous emulsification procedure (10). The essential oil and poly [poly(oxyethylene-1000)-oxyterephthaloyl] were first mixed together in equal ratio (1:1) to form a slurry (47.6 % essential oil) followed by addition of aqueous phase, while stirring (600 rpm) at room temperature to form O/W emulsion formulations.

Particle size and stability of O/W emulsion formulations

Particles size was measured using particle size analyzer (Zetatrac^{TM}) which works on the principle of Dynamic Light Scattering (DLS). The instrument detects the fluctuation of scattering intensity due to the Brownian motion of particles in suspension and determines the size. DLS measurements were performed at 25 ºC and light scattering was detected at a fixed angle. For particle size measurement 0.25 % O/W emulsion formulation was prepared by addition of deionised water into slurry formulation. Five mL of O/W emulsion was taken into a glass vial and the particle size was analyzed through Dual optical probe technology. Optical light sources were dual solid-state laser diodes in 780 nm (near-infrared) wavelength. The stability of emulsions was determined by centrifuging the emulsions at 3500 rpm for 30 min (34).

Determination of morphology of O/W emulsion formulation

The morphology of the 0.25 % O/W emulsion formulations of essential oils was studied by transmission electron microscope (TEM). A drop of O/W emulsion was placed on carbon-coated copper grids (400 mesh size) and allowed to dry in vacuum (5) and stained with a negative stain (2 % uranyl acetate). The excess water was dried gently using a filter paper and then viewed under TEM at different magnification levels and assessed the morphology of the particles of the formulations

Evaluation of Phytotoxicity

Phytotoxicity of essential oil formulations were assessed through leaf dip method under laboratory conditions (26±1°C Temperature and 65±5 % Relative Humidity). Based on preliminary studies 0.05, 0.125, 0.25, 0.5, 0.75 and 1 % concentrations were prepared by dilution of 47.6 % slurry formulations with distilled water. Freshly cut, cleaned leaves of brinjal were dipped in test concentration of O/W emulsion formulations of essential oil for 10 sec. and allowed to dry at room temperature (25±2 °C) and then the dried treated leaves were placed in plastic Petri dishes (90 mm dia). To maintain the turgidity of leaves, the leaf with petiole was inserted (Fig. 1) in the solidified 1 % agar. Each O/W formulations was replicated thrice and observed the phytotoxicity, if any, at regular interval till 48 h.

A **B**

Figure 1. Whitefly, *Bemisia tabaci* adult (**A**); Set up of Leaf dip assay (**B**)

Contact toxicity bioassay

The contact toxicity of essential oils at different concentrations (0.05 to 2 %) was assessed by leaf dip method (35). Cleaned brinjal leaves were dipped in respective concentration for 10 sec and allowed to dry on wire mesh and then transferred to Petri dishes (9 mm dia). Five replications per concentration were maintained and 30 adult whiteflies were released per replication. Whitefly mortality was recorded at 24 and 48 h after exposure. For control, leaves treated with emulsified water were used.

Statistical analysis

One-way ANOVA was used to analyze the mortality differences among all concentrations of each essential oil formulation. Before ANOVA corrected mortality (%) was calculated using Abbots correction formula (1) and values were angular transformed. Differences among the mean mortality were compared with Tukey's Honest Significant Difference (HSD) test at $\alpha = 0.05$. Further toxicity data was subjected to probit analysis using PoloPlus software (Version 2.0) (LeOra Software) to calculate lethal concentrations LC_{50} and LC_{90} (32).

RESULTS AND DISCUSSION

Oil quality analysis

The essential oils used in the study were analyzed by GC-MS and compounds were identified by comparing mass fragmentation pattern with authentic samples, kovat's index and NIST library. Twenty compounds identified from the palmarosa oil were : Geraniol (69.82 %), geranyl acetate (16.09 %) and linalool (4.39 %) as major compounds and cis-ocimene (2.82 %) and β-caryophyllene (1.49 %) as minor compounds (Table 1). Analysis of pine oil showed the presence of δ-carene (50.73 %), α-pinene (24.17 %), camphene (5.15 %), 1,8-cineole (4.07 %) and limonene (7.06 %) as major compounds, while, myrcene (1.37 %), 1,4-cineole (1.66 %) as minor compounds (Table 2). Geranium oil was complex mixture of compounds with Citronellol (31.3 %), linalool (11.9 %), geraniol

(9.74 %), isomenthone (7.85 %), 10-epi-γ-eudesmol (5.42 %), citronellyl formate (5.26 %) and menthone (3.84 %) were identified as major compounds and γ-cadinene (1.4 %), geranyl tiglate (1.14 %), geranyl formate (1.25 %), rose oxide (1.46 %), as minor constituents (Table 3). The eucalyptus oil contained 1,8-Cineole (40.23 %), α-pinene (25.85 %) and limonene (11.88 %) as major compounds (Unpublished data), while, geranial (47.3 %), neral (34.7 %) were the key constituents of lemongrass oil (25).

Table 1: Chemical composition of palmarosa (*Cymbopogon martini*) oil

Sr. No.	Compound	Kovats Index (KI)	Amount (%)
1	α-Pinene	938	0.01
2	β-Pinene	980	0.03
3	Myrcene	989	0.18
4	Limonene	1029	0.72
5	*cis*-Ocimene	1036	0.45
6	*trans*-Ocimene	1049	2.82
7	Terpinolene	1087	0.04
8	Linalool	1098	4.39
9	Menthol	1171	0.55
10	4-Terpineol	1178	0.03
11	Nerol	1230	0.18
12	Neral	1237	0.22
13	Geraniol	1252	69.82
14	Geranial	1267	0.93
15	Geranyl acetate	1381	16.09
16	β-Elemene	1390	0.05
17	β-Caryophyllene	1418	1.49
18	Humulene	1454	0.05
19	Farnesol	1698	0.25

Table 2. Chemical composition of pine (*Pinus roxburghii*) oil

Sr. No.	Compound pine oil	Kovats Index (KI)	Amount (%)
1	Tricyclene	925	0.48
2	α-Pinene	938	24.17
3	Camphene	954	5.15
4	Myrcene	989	1.37
5	α-Phellandrene	1002	0.16
6	δ-3-Carene	1011	50.73
7	Limonene	1029	7.06
8	1,8-Cineole	1031	4.07
9	γ-Terpinene	1058	0.48
10	Terpinolene	1087	1.55
11	Nonanol	1169	0.23
12	Decanal	1201	0.23

Table 3. Chemical composition of geranium (*Pelargonium graveolens*) oil

Sr. No.	Compounds	Kovats Index (KI)	Amount (%)
1	α-Pinene	938	0.87
2	β- Pinene	980	0.48
3	α-Phellandrene	1002	0.15
4	Limonene	1029	0.79
5	Cis-Ocimene	1036	0.17
6	trans-Ocimene	1049	0.33
7	Cis-Linalool oxide	1072	0.53
8	trans-Linalool oxide	1086	0.48
9	Linalool	1098	11.9
10	Rose oxide	1108	1.46
11	Menthone	1152	3.84
12	Isomenthone	1198	7.95
13	Citronellol	1224	31.3
14	Neral	1238	0.26
15	Geraniol	1252	9.74
16	Geranial	1267	0.28
17	Citronellyl formate	1273	5.26
18	Geranyl formate	1298	1.25
19	Citronellyl acetate	1352	0.2
20	α-Copaene	1376	0.36
21	β -Bourbonene	1388	0.91
22	α-Gurjunene	1409	0.42
23	Humulene	1454	0.29
24	Allo-Aromadendrene	1460	0.3
25	Geranyl propionate	1477	0.68
26	Germacrene D	1481	0.93
27	γ-Cadinene	1513	1.4
28	Geranyl butanoate	1564	0.22
29	Phenylethyltiglate	1585	1.03
30	10-epi-γ-Eudesmol	1623	5.42
31	Geranyl tiglate	1696	1.14

Physicochemical parameters of essential oil formulations

Addition of polymer in to the essential oils initially formed free flowing slurries, which after adding deionised water produced oil-in-water (O/W) emulsions. Here poly [poly(oxyethylene-1000)-oxyterephthaloyl]) having amphiphilic property behaved as non-ionic surfactant which are reported to be less affected by pH and ionic strength (30). DLS revealed that the average droplet size of essential oil emulsion formulations ranged from 136-425 nm (Table 4). The molecular structure and particle size distribution of 0.25 % O/W formulation of geraniol oil is shown in figure 2. Examination of the droplet of 0.25 % O/W emulsion formulations of EOs under Transmission Electron Microscope (TEM) revealed the presence of the spherical micelles in the emulsions (Figure 3).

Table 4. Average particle size of 0.25 % O/W emulsion formulation of essential oils

Treatments	Composition of slurry formulation (Oil: amphiphilic polymer)	Average particle size (nm)*
Lemongrass oil	1:1	156±36
Citral	1:1	369±31
Geranium oil	1:1	324±72
Palmarosa oil	1:1	136±42
Eucalyptus oil	1:1	425±68
Pine oil	1:1	253±25

* Particles size was measured using particle size analyzer (Zetatrac^{TM}); nm: Nanometer.

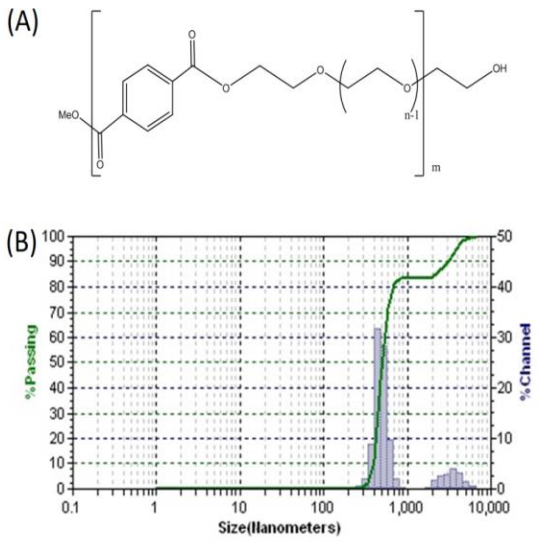

Figure 2. (A) Molecular structure of amphiphilic polymer Poly [poly (oxyethylene-1000)-oxyterephthaloyl] (B) Particle size distribution of 0.25 % O/W emulsion formulation of Geranium oil

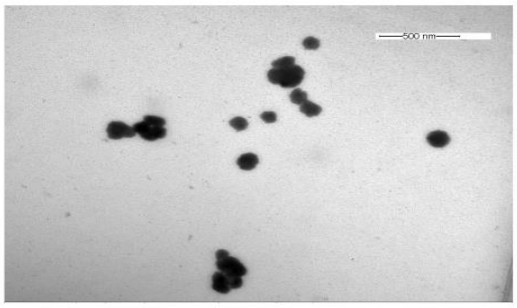

Figure 3. Morphology 0.25 % O/W emulsion formulation of Geranium oil in TEM. Spherical shapes particles of formulation is shown above.

The stability of emulsion formulations depends on the physico-chemical characteristics of its constituents including oil phase, aqueous phase and emulsifiers (21). The centrifugation is an accelerated stability test to predict the stability of the emulsion. After centrifugation at 3500 rpm for 30 min., the emulsion formulations showed sufficient resistance to the destabilization at room temperature. The stabilization of micelles in the emulsion was due to the amphiphilic polymer which behaves like surfactant reducing interfacial free energy providing mechanical barrier to coalescence (31).

Phytotoxicity

Geranium oil proved most phytotoxic, as it caused phytotoxic effects at 0.125 % concentration. Lemongrass oil was phytotoxic above 0.25 % concentration, whereas, palmarosa oil and citral were phytotoxic at concentrations above 0.5 %. Phytotoxic symptoms like necrosis, browning, drying and curling of leaf tips and margins were observed (Figs 4 and 5).

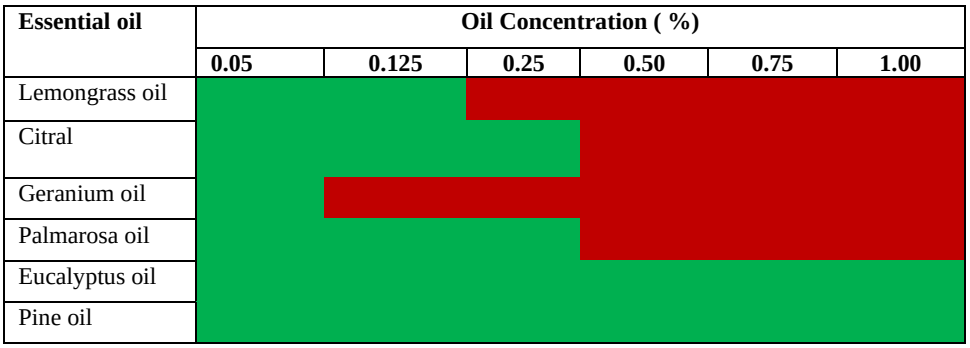

Figure 4. Phytotoxicity grading chart for essential oil formulations. Red color indicates the phytotoxic and green color indicated non-phytotoxic concentrations

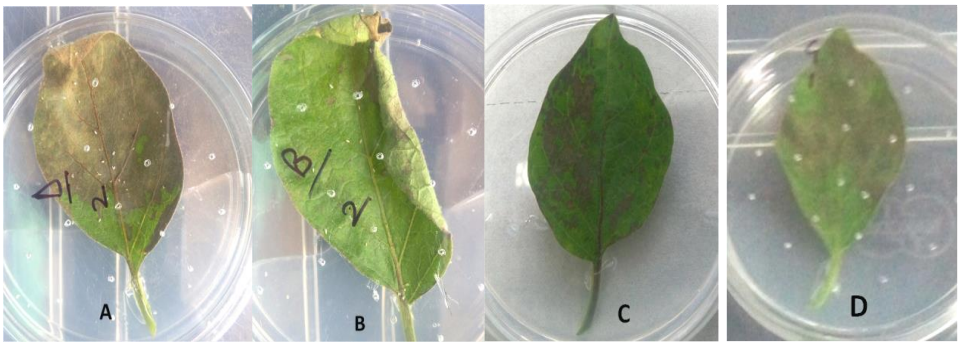

Figure 5. Brinjal leaves showing phytotoxicity in response to treatment of essential oils
A: Lemongrass oil **B:** Citral **C:** Palmarosa oil **D:** Geranium Oil

Eucalyptus and pine oils were not any phytotoxic. Very limited reports are available on the phytotoxic effects of test essential oils used in this study. The phytotoxicity of EOs, geranium oil may be attributed to the presence of the monoterpenes such as linalool and, geraniol, which is in conformity with the findings of Abd-ElGawad *et al*. (2). Phytotoxicity of lemongrass oil has been reported earlier on *Echinochloa crusgalli* (L.) (29) and Teak (*Tectona grandis* L. f.) seedlings at 1500µl/L (28). Palmarosa oil showed phytotoxic effects on seedlings of *Medicago sativa* L., *Triticum aestivum* L. and *Lactuca sativa* L. (4). Phytotoxicity observed due to geranium oil treatment above 0.05 % in the present study was similar to Yarahmadi *et al*. (41) who reported the Phytotoxic effects of geranium oil in cucumber. The threshold concentration for phytotoxicity varies with different essential oils and was influenced by the physico-chemical properties. Abd-ElGawad *et al*. (2) observed that phytotoxic activities of EOs increased with oxygenated terpenoid content). Some earlier studies showed that increasing the oxygenation of terpenoids led to an increase in phytotoxic activities (3,9,37).

Table 5. Bioassay of essential oil formulations for their contact toxicity against whitefly, *Bemicia tabaci*

Treatments	**Heterogeneity**		**b±SE**	**LC_{50} (%) (Fiducial limits (95 %)**	**LC_{90} (%) (Fiducial limits (95 %)**	**F- Value (*p*-value)**
	X^2	*Df*				
24 h Exposure period						
Lemongrass oil	1.59	5	2.12±0.39	0.433 (0.279-1.830)	1.746 (0.708-53.604)	32.86 (<0.05)
Citral	1.30	5	1.96±0.38	0.531 (0.329-2.433)	2.401 (0.90-74.086)	6.58 (<0.05)
Geranium oil	Mortality at higher concentration was <50 %, hence LC_{50}, LC_{90} could not be calculated					
Palmarosa oil	3.88	6	2.94±0.22	0.241 (0.187-0.336)	0.658 (0.440-1.446)	53.13 (<0.05)
Eucalyptus oil	0.64	5	2.82±0.34	1.374 (1.249 to 1.553)	3.914 (3.021 to 5.914)	3.58 (<0.05)
Pine oil	3.19	8	1.86±0.18	1.138 (0.891 to 1.538)	5.551 (3.238 to 18.775)	10.59 (<0.05)
48 h Exposure period						
Lemongrass oil	1.43	5	2.57±0.29	0.159 (0.131-0.206)	0.503 (0.341-1.078)	14.44 (<0.05)
Citral	1.05	5	2.07±0.27	0.219 (0.176-0.309)	0.917 (0.548-2.541)	17.77 (<0.05)
Geranium oil	1.89	4	2.40±0.35	0.159 (0.121-0.287)	0.545 (0.297-4.126)	18.08 (<0.05)
Palmarosa oil	2.71	6	2.87±0.23	0.142 (0.114-0.179)	0.398 (0.291-0.693)	41.29 (<0.05)
Eucalyptus oil	1.57	5	2.68±0.32	0.893 (0.739 to 1.037)	2.688 (1.990 to 5.068)	16.25 (<0.05)
Pine oil	7.44	8	3.51±0.33	0.631 (0.338 to 0.807)	1.461 (1.116 to 3.174)	45.40 (<0.05)

Heterogeneity (X^2): indicate goodness of fit of probit line; b: slope of regression line

Contact toxicity

The evaluation of contact toxicity of essential oils against adults of whitefly, *B. tabaci* showed dose dependent response for all tested oils. Among the EOs, the palmarosa oil showed maximum contact toxicity with LC_{50} and LC_{90} values of 0.241 and 0.658 %, respectively at 24 h after exposure time and 0.142 and 0.398 % at 48 h after exposure time. The respective LC_{50} and LC_{90} values of lemongrass oil were 0.433 and 1.746 % at 24 h and 0.159 and 0.503 % at 48 h after exposure (Table 5).

Significantly varied mortality was observed at different concentrations of palmarosa oil (F=53.13 and 41.29, $p<0.001$ at 24 and 48 h exposure time respectively), lemongrass oil (F=32.86 and 14.44, $p<0.001$ at 24 and 48 h exposure time, respectively). Although at 24 h after exposure, treatment with geranium oil resulted in non-significant mortality less than 50 % (hence LC_{50} could not be calculated), but it caused significantly higher mortality at 0.125 and 0.25 % concentration after 48 h with LC_{50} and LC_{90} values being 0.159 and 0.545 % respectively (F=18.08, $p<0.001$) (Figure 6).

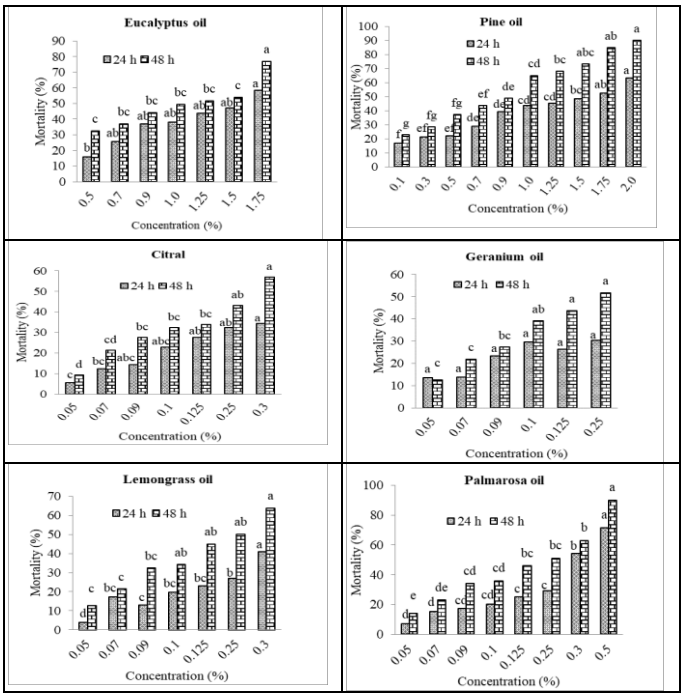

Figure 6. Contact toxicity of essential oil O/W formulations against whitefly, *Bemicia tabaci* at different concentrations after 24 h and 48 h of treatment. Bars denoted by common letter indicate non-significant difference in percent mortality (Tukey's Honest Significant Difference Test).

Management of *B. tabaci* has become difficult owing to widespread development of resistance to insecticides (9,27) leading to control failure of insecticides under field conditions. This necessitates the need for developing alternative management strategy.

Essential oils from different plant sources have been explored for their insecticidal and other properties since long, but, there is scarcity of information on stable essential oil formulations and their evaluation against whitefly, *B. tabaci*. Many earlier studies have demonstrated the contact toxicity of essential oils of Geranium, Artemisia, Thyme, Garlic, cumin, cinnamon and Lemon grass oil against whitefly, *B. tabaci* (7,11,22,41). Results of our study have shown that besides lemongrass oil, Palmarosa oil was also highly toxic against whitefly (Table 5; Figure 5). The efficacy of geraniol as contact toxicant against *B. tabaci* was demonstrated by Baldin *et al*. (6) and Devi *et al*. (12). Although the EOs are absorbed by the insects as contact poison and their volatile nature make them a fumigant toxicant. Geraniol, a key constituent of Palmarosa oils and Lemongrass oil exhibited high toxicity to *B. tabaci* in the present study. Structure toxicity relationship studies have shown that EOs bind to the GABA receptors at anesthetic site and some of the EO constituents like borneol or geraniol are structurally similar to propofol, a known ligand of anesthetic seat of GABA receptors and they potentiate the GABA induced Cl^-current (17,20). The toxicity assays of the present study were done with O/W formulations of EOs, as the stability of the EOs is greatly enhanced by appropriate formulations. Moretti *et al*. (26) demonstrated that preparation of encapsulated formulation improved the bio efficacy of certain EOs against insect pests as the formulation technique effectively entrapped the key constituents of EOS for delivery as toxicants to insects. Development of micro encapsulated formulations and nano-formulations of palmarosa oil and lemongrass oils as such or developing mixtures of key constituents of these essential oils in suitable formulations offer scope for developing novel control strategies for whitefly in future.

CONCLUSIONS

Our studies have shown promising results for contact toxicity of essential oils of palmarosa and lemongrass oils against *B. tabaci*. Oil-in-water (O/W) emulsion formulations of essential oils using poly [poly(oxyethylene-1000)-oxyterephthaloyl]) as a non-ionic surfactant was found physically stable at room temperature with the average droplet size 136 - 425 nm. A mixture of different active compounds may be used to reduce the possibility of target site resistance developed against chemical insecticides in *B. tabaci*. Development of micro encapsulated formulations and nano-formulations of these oils as such or developing mixtures of their key constituents with suitable formulations offer scope for novel control strategies against whitefly in future.

ACKNOWLEDGEMENTS

The authors acknowledge with thanks the support rendered by Division of Entomology and Division of Agricultural Chemicals, ICAR-Indian Agricultural Research Institute, New Delhi. The financial assistance for this work was partly supported by grants from ICAR-NASF and DST-SERB projects sanctioned to the corresponding author.

REFERENCES

1. Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**(2): 265-267.

2. Abd-ElGawad, A.M., El Gendy, A.E.N.G., Assaeed, A.M., Al-Rowaily, S.L., Alharthi, A.S., Mohamed, T.A., Nassar, M.I., Dewir, Y.H. and Elshamy, A.I. (2021). Phytotoxic effects of plant essential oils: A systematic review and structure-activity relationship based on chemometric analyses. *Plants* **10**(1): 36.
3. Abd-ElGawad, A.M., Elshamy, A.I., El-Nasser El Gendy, A., Al-Rowaily, S.L. and Assaeed, A.M. (2019). Preponderance of oxygenated sesquiterpenes and diterpenes in the volatile oil constituents of *Lactuca serriola* L. revealed antioxidant and allelopathic activity. *Chemistry & Biodiversity* **16**(8): e1900278.
4. Ali, I.B.E., Tajini, F., Boulila, A., Jebri, M.A., Boussaid, M., Messaoud, C. and Sebai, H. (2020). Bioactive compounds from Tunisian *Pelargonium graveolens* (LHer.) essential oils and extracts: α-amylase and acethylcholinesterase inhibitory and antioxidant, antibacterial and phytotoxic activities. *Industrial Crops and Products* **158**: 112951.
5. Baboota, S., Shakeel, F., Ahuja, A., Ali, J. and Shafiq, S. (2007). Design, development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib. *Acta pharmaceutica* **57**(3): 315-332.
6. Baldin, E.L., Aguiar, G.P., Fanela, T.L., Soares, M.C., Groppo, M. and Crotti, A.E. (2015). Bioactivity of *Pelargonium graveolens* essential oil and related monoterpenoids against sweet potato whitefly, *Bemisia tabaci* biotype B. *Journal of Pest Science* **88** (1): 191-199.
7. Barkman, B. (2013). *Repellent, Irritant and Toxic Effects of Essential Oil Constituents on Bemisia tabaci* (Gennadius). M.Sc. Thesis, Universiteit van Amsterdam.
8. Basit, M. (2019). Status of insecticide resistance in *Bemisia tabaci*: Resistance, cross-resistance, stability of resistance, genetics and fitness costs. *Phytoparasitica*, **47**(2): 207-225.
9. Bozok, F. and Ulukanli, Z. (2016). Volatiles from the aerial parts of east Mediterranean clary sage: Phytotoxic activity. *Journal of Essential Oil Bearing Plants* **19**: 1192-1198.
10. Chang, Y., McLandsborough, L. and McClements, D.J. (2013). Physicochemical properties and antimicrobial efficacy of carvacrol nanoemulsions formed by spontaneous emulsification. *Journal of Agricultural and Food Chemistry* **61**(37): 8906-8913.
11. Deletre, E., Chandre, F., Barkman, B., Menut, C. and Martin, T. (2016). Naturally occurring bioactive compounds from four repellent essential oils against *Bemisia tabaci* whiteflies. *Pest Management Science* **72(1)**: 179-189.
12. Devi, M.A., Sahoo, D., Singh, T.B. and Rajashekar, Y. (2019). Toxicity, repellency and chemical composition of essential oils from *Cymbopogon* species against red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). *Journal of Consumer Protection and Food Safety* **15(2):** 1-11.
13. Dhifi, W., Bellili, S., Jazi, S., Bahloul, N. and Mnif, W. (2016). Essential oils' chemical characterization and investigation of some biological activities: A critical review. *Medicines* **3**(4): 25.
14. Fanela, T.L.M., Baldin, E.L.L., Pannuti, L.E.R., Cruz, P.L., Crotti, A.E.M., Takeara, R. and Kato, M.J. (2016). Lethal and inhibitory activities of plant-derived essential oils against *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) biotype B in tomato. *Neotropical entomology* **45**(2): 201-210.
15. Feng R. and Isman M.B. (1995). Selection for resistance to azadirachtin in the green peach aphid, *Myzus persica*. *Experientia* **51**: 831-833.
16. Harwood, S.H., Moldenke, A.F., Berry, R.E. (1990) Toxicity of peppermint monoterpenes to the variegated cutworm (Lepidoptera: Noctuidae). *Journal of Economic Entomology* **83**:1761-1767.
17. Hossain, S.J., Aoshima, H., Koda, H. and Kiso, Y. (2004). Fragrances in oolong tea that enhance the response of GABAA receptors. *Bioscience, biotechnology, and biochemistry* **68**(9): 1842-1848.
18. Isman, M.B. (2000). Plant essential oils for pest management and disease management. *Crop Protection* **19**: 603-608.
19. Isman, M.B. and Machial, C.M. (2006). Pesticides based on plant essential oils: From traditional practice to commercialization. In: *Naturally Occurring Bioactive Compounds* (Eds. Rai M, Carpinella M.C.) pp 29-44. Elsevier, BV.
20. Jankowska, M., Rogalska, J., Wyszkowska, J. and Stankiewicz, M. (2018). Molecular targets for components of essential oils in the insect nervous system-a review. *Molecules* **23**(1): 34.
21. Karthik, P., Ezhilarasi, P.N. and Anandharamakrishnan, C. (2017). Challenges associated in stability of food grade nanoemulsions. *Critical Reviews in Food Science and Nutrition* **57**(7): 1435-1450.
22. Kim, S.I., Chae, S.H., Youn, H.S., Yeon, S.H. and Ahn, Y.J. (2011). Contact and fumigant toxicity of plant essential oils and efficacy of spray formulations containing the oils against B-and Q-biotypes of *Bemisia tabaci*. *Pest Management Science* **67**(9): 1093-1099.
23. Li, Y., Mbata, G.N., Punnuri, S., Simmons, A.M. and Shapiro-Ilan, D.I. (2021). *Bemisia tabaci* on vegetables in the Southern United States: Incidence, Impact and Management. *Insects* **12**: 198.

24. Liu, X.C., Hu, J.F., Zhou, L. and Liu, Z.L. (2014). Evaluation of fumigant toxicity of essential oils of Chinese medicinal herbs against *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). *Journal of Entomology and Zoology Studies* **2**: 164-169.
25. Mondal, P.C., Nebapure, S.M., Rajna, S., Shakil, N.A., Kaushik, P. and Rana, V.S. (2021). Chemical composition and insecticidal activity of the essential oil of *Cymbopogon flexuosus* against *Bemisia tabaci*. *Medicinal Plants-International Journal of Phytomedicines and Related Industries* **13**(1): 131-136.
26. Moretti, M.D., Sanna-Passino, G., Demontis, S. and Bazzoni, E. (2002). Essential oil formulations useful as a new tool for insect pest control. *AAPs Pharm Science Technology* **3**(2): 64-74.
27. Naveen, N.C., Chaubey, R., Kumar, D., Rebijith, K.B., Rajagopal, R., Subrahmanyam, B. and Subramanian, S. (2017). Insecticide resistance status in the whitefly, *Bemisia tabaci* genetic groups Asia-I, Asia-II-1 and Asia-II-7 on the Indian subcontinent. *Scientific reports* **7**(1): 1-15.
28. Osorio, P.R.A., Leao, E.U., Veloso, R.A., Mourao, D.D.S.C. and Santos, G.R.D. (2018). Essential oils for alternative teak rust control. *Floresta e Ambiente* **25**(2): e20160391
29. Poonpaiboonpipat, T., Pangnakorn, U., Suvunnamek, U., Teerarak, M., Charoenying, P. and Laosinwattana, C. (2013). Phytotoxic effects of essential oil from *Cymbopogon citratus* and its physiological mechanisms on barnyard grass (*Echinochloa crusgalli*). *Industrial Crops and Products* **41**:403-407.
30. Reddy, S.R. and Fogler, H.S. (1981). Emulsion stability: Determination from turbidity. *Journal of Colloid and Interface Science* **79**(1): 101-104.
31. Reiss, H. (1975). Entropy-induced dispersion of bulk liquids. *Journal of Colloids and Interface Science* **53**: 61-70.
32. Robertson, J.L., Jones, M.M., Olguin, E. and Alberts, B. (2017). *Bioassays With Arthropods*. 3rd Ed.CRC press, FL, 189pp.
33. Sarkar, D.J., Kumar, J., Shakil, N.A., Adak, T. and Watterson, A.C. (2012). Synthesis and characterization of amphiphilic PEG based aliphatic and aromatic polymers and their self-assembling behavior. *Journal of Macromolecular Science, Part A* **49**(6): 455-465.
34. Shafiq, S. and Shakeel, F. (2010). Stability and self-nanoemulsification efficiency of ramipril nanoemulsion containing labrasol and plurol oleique. *Clinical Research and Regulatory Affairs* **27**(1): 7-12.
35. Subramanian S. and Naveen N.C. (2017). Bioassay for monitoring insecticide toxicity in *Bemisia tabaci* populations. *Protocol Exchange* (2017) doi:10.1038/protex.2017.015
36. Sukhija, H.S., Butter, N.S., Jagdev, S. and Balwinder, S. (1987). Assessment of losses due to important insect pests of cotton in the Punjab. *Agricultural Science Digest, India* **7**(2): 115-118.
37. Verdeguer, M., Blazquez, M.A. and Boira, H. (2009). Phytotoxic effects of *Lantana camara*, *Eucalyptus camaldulensis* and *Eriocephalus africanus* essential oils on weeds of Mediterranean summer crops. *Biochemical Systematics and Ecology* **37**(4): 362-369.
38. Verdeguer, M., Sanchez-Moreiras, A.M. and Araniti, F. (2020). Phytotoxic effects and mechanism of action of essential oils and terpenoids. *Plants* **9**(11): 1571.
39. Wagan, T.A., Wang, W., Hua, H. and Cai, W. (2017). Chemical constituents and toxic, repellent, and oviposition-deterrent effects of ethanol-extracted *Myristica fragrans* (Myristicaceae) oil on *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Florida Entomologist* **2017:** 594-601.
40. Yang, N.W., Li, A.L., Wan, F.H., Liu, W.X. and Johnson, D. (2010). Effects of plant essential oils on immature and adult sweetpotato whitefly, *Bemisia tabaci* biotype B. *Crop protection* **29**(10): 1200-1207.
41. Yarahmadi, F., Rajabpour, A., Sohani, N.Z. and Ramezani, L. (2013). Investigating contact toxicity of Geranium and Artemisia essential oils on *Bemisia tabaci* Gen. *Avicenna journal of Phytomedicine* **3**(2): 106-111.

PUBLISHER NOTE