



Plant-based insecticides and their bio-efficacy evaluation against *Myzus persicae* on capsicum Plant

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Article History	Abstract
<p>Received: 10/07/2023 Revised: 12/08/2023 Accepted: 10/09/2023</p>	<p>This study aimed to formulate plant-based insecticides to control <i>Myzus persicae</i> using essential oils extracted from four plants—<i>Artemisia vulgaris</i>, <i>Cymbopogon flexuosus</i>, <i>Tagetes minuta</i>, and <i>Rosmarinus officinalis</i>—abundantly available in the central Himalayas. The insecticidal efficacy of these essential oils was evaluated using both leaf immersion and residue contact bioassay methods. In the residue contact bioassay, <i>A. vulgaris</i> demonstrated the highest toxicity compared to the leaf dip method, with relative toxicity values of 3.07, 1.97, and 1.17 for <i>A. vulgaris</i>, <i>C. flexuosus</i>, and <i>T. minuta</i>, respectively. In the leaf immersion bioassay, the relative toxicity values were 2.97, 2.64, and 1.34 for the same plants. <i>R. officinalis</i> served as the baseline with a relative toxicity value of one in both bioassays. The order of LC₅₀ at 48 HAE (Hours After Exposure) in the residue contact bioassay was <i>A. vulgaris</i> (0.104) > <i>C. flexuosus</i> (0.166) > <i>T. minuta</i> (0.272) > <i>R. officinalis</i> (0.319%). Similarly, in the leaf immersion bioassay, the order was <i>A. vulgaris</i> (0.161) > <i>C. flexuosus</i> (0.182) > <i>T. minuta</i> (0.358) > <i>R. officinalis</i> (0.481%). While the order of LC₅₀ values remained consistent between the two bioassays, the lower LC₅₀ values in the residue contact bioassay suggest its superiority over the leaf immersion method.</p>
<p>CC License CC-BY-NC-SA 4.0</p>	<p>Keywords: essential oils, bioassay, toxicity, aphids, <i>Capsicum annum</i></p>

Introduction

Capsicum (*Capsicum annum* var. *frutescens*), also known as sweet pepper or bell pepper, stands as one of the most popular and economically rewarding vegetable crops cultivated globally. Distinguished from hot chili by its size, fruit shape, capsaicin content, and applications, Capsicum is nutritionally rich in vitamins, particularly A and C. An edible portion weighing a hundred grams provides approximately 0.99 g protein, 2.73 g dietary fiber, 133 mg vitamin C, 0.33g total fat (46.79cal), carbohydrate (10.63g), energy (195.58kj) (Durucasu et al., 2007). Facing challenges

from around 35 species of insect and mite pests, including thrips (*Scirtothrips dorsalis* Hood, *Thrips palmi* Karny), aphids (*Aphis gossypii* Glover, *Myzuspersicae* Sulzer), whitefly (*Bemisia tabaci* Gennadius), fruit borers (*Helicoverpa armigera* Hubner), and mites (*Polyphagotarsonemus latus* Banks, *Tetranychus cinnabarinus* Boisduval), along with other minor pests (Vos and Frinking, 1998; Sorensen, 2005; Berke et al., 2003). Aphids, play a significant role as major pests in capsicum (Asena 1974, Yasaraknc and Hncal 1997, and Halima and Hamouda 1994). Both adults and nymphs of *S. dorsalis* extract cell sap from leaves, resulting in leaf rolling and reduced leaf size (Sanap and Nawale 1987). In response to the current situation where farmers excessively use various pesticides to control chili pests, leading to resistance in whitefly, aphid, and mites, it is crucial to adopt a rotational approach with insecticides from different classes.

The utilization of chemical pesticides also introduces potentially toxic residues, posing risks to human and environmental health (Mondal et al., 2018) and impacting non-target organisms adversely (Antwi and Reddy, 2015). A strategic initiative is imperative to substitute synthetic pesticides with botanical alternatives, promoting sustainable agricultural development (Campos et al., 2018).

Considering environmental safety and human health as paramount concerns, the development of alternative control measures, such as biopesticides against major vegetable crop pests, becomes a necessity. Essential oils derived from plants have gained popularity due to their low-risk nature, especially among organic growers and environmentally conscious consumers. These oils, easily produced through steam distillation, contain volatile terpenes and phenolics. Major plant families for essential oil extraction include Myrtaceae, Lauraceae, Lamiaceae, and Asteraceae. Essential oils exhibit repellent, insecticidal and growth-reducing effects on various insects, effectively controlling preharvest and postharvest phytophagous insects, as well as serving as insect repellents for biting flies and home and garden insects.

The study emphasizes the significance of formulating herbal insecticides as a viable alternative to harmful synthetic chemicals, representing a substantial step forward in the development of eco-friendly technology for crop protection.

Materials & Method

The trials were executed at GBPNiHE, Kosi-Katarmal, Almora, focusing on assessing the bio-efficacy of plant essential oils against apterous aphids. The initial screening of essential oils was performed at concentrations of 1% and 2%. The experimentation comprised two distinct phases, alternating between preliminary screening and final testing, aiming to determine the lethal concentration of essential oils.

Leaf immersion bioassay method

The leaf immersion method, as per Kodandaram and Dhingra (2007), was employed. Fully matured leaves of capsicum the host plant, were harvested from the field of the RTC, GBPNiHE, Kosi-Katarmal, Almora, thoroughly washed, and immersed in the required concentration of essential oil solution (insecticides) for one minute. After removing excess liquid from the foliage, the treated leaves were left to air-dry at room temperature. Subsequently, the treated leaf was placed onto a clean petri plate, and ten apterous neonate aphids of uniform size were delicately introduced onto the treated leaf using a soft camel hairbrush (size zero). Each treatment, including the control, was replicated three times. For the control, bean leaves were dipped in water, dried, and utilized. The petri dishes were then placed in an incubator at 20±5°C, and mortality data were recorded after 12-, 24-, and 48-hours post-exposure.

Residue Contact Bioassay method

The residue contact bioassay method, as per Srivastava and Proksch (1993) and Vedhamathi (2004), was employed to assess toxicity. One milliliter of each concentration was applied as a thin film on the lower and upper lids of a petriplate (diameter: 9 cm). The solvent was allowed to dry at room temperature. After solvent evaporation, ten apterous aphids (*Myzuspersicae*) were exposed to contact for 30 minutes. In the control, aphids were exposed to water alone (Parvathi and Kesar, 1999). This process was replicated three times. Subsequently, aphids were transferred to petri dishes containing fresh capsicum leaves. Mortality data were

recorded at 12, 24, and 48 hours after exposure (HAE), with moribund aphids considered as deceased. The mortality data were corrected using Abbott's formula (Abbott, 1925).

Preliminary screening

An investigation was carried out to assess the toxicity of four essential plant oils, namely *Artemisia vulgaris* Burm., *Cymbopogon flexuosus* DC., *Tagetes minuta* Khakibush, and *Rosmarinus officinalis* L., against *Myzus persicae*, utilizing both leaf immersion and residue contact bioassay methods. Dried plant parts of the four essential oils were extracted and subjected to hydro-distillation for extraction, following the method outlined by Ray et al. (2008) using a Clevenger apparatus. The resulting distilled oil was separated from water using a separating funnel and stored in a refrigerator. Two concentrations (1.0% and 2.0%) of each oil were prepared in water and tested against neonate apterous *Myzus persicae* under controlled laboratory conditions (25±5°C and RH 75±5%).

Experiment

After conducting preliminary screening, a final set of concentrations was established for the four essential plant oils, namely T1-*Artemisia vulgaris* at 0.3% (3000 ppm), T2-*Cymbopogon flexuosus* at 0.5% (5000 ppm), T3-*Tagetes minuta* at 0.8% (8000 ppm), and T4-*Rosmarinus officinalis* at 1% (10000 ppm), for both leaf immersion and residue contact bioassay. Each treatment was replicated three times, with 10 apterous aphids per replication. Mortality observations were recorded at 12, 24, and 48 hours after exposure, considering moribund aphids as deceased. The mortality data underwent correction using Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{T - C}{100 - C} \times 100$$

Where,

T = Per cent mortality in treatment

C = Per cent mortality in control

The data so obtained was subjected to probit analysis for calculating regression equation and LC value following Finney (1971).

Relative toxicity (RT) of oil was calculated based on LC₅₀ value by using the following formula (Ramangouda and Srivastava, 2009., Basera, 2009)

$$\text{RT} = \frac{\text{LC value of least toxic oil}}{\text{LC value of candidate oil}}$$

Statistical analysis

The study followed a completely randomized design (CRD), and the determination of LC values was carried out using probit analysis (Finney, 1971) through the online computer program OPSTAT.

Results & Discussions

The concentration-dependent mortality response [LC values (%)]

The toxicity assessment of four plant essential oils, namely *A. vulgaris*, *C. flexuosus*, *T. minuta*, and *R. officinalis*, against aphids (*Myzus persicae*) was conducted using leaf immersion and residue contact bioassays. In the leaf immersion bioassay, *A. vulgaris* exhibited the highest toxicity at 12, 24, and 48 hours across all LC levels (30, 50, 75, and 90) (Table 1). The recorded values for *A. vulgaris* were maximal {LC30 (0.0998, 0.0821, 0.0778%), LC50 (0.228, 0.179, 0.116%), LC75 (0.661, 0.488, and 0.414%), and LC90 (1.723, 1.205, and 0.965%)}, surpassing *C. flexuosus* and *T. minuta* (Table 1). In contrast, *R. officinalis* displayed the least toxicity at 12, 24, and 48 hours for all four LC levels. The relative toxicity values (RT50) in the leaf immersion bioassay revealed that *A. vulgaris* is 2.97, 3.30, and 2.97 times more toxic than *R. officinalis* at 12, 24, and 48 hours, respectively. Similarly, *C. flexuosus* is 2.09, 2.34, and 2.64, and *T. minutais* 1.32, 1.45, and 1.34 times more toxic than *R. officinalis* oil at 12, 24, and 48 hours, respectively. Soliman (2005) reported a parallel outcome when assessing the impact of *A. herba-alba* (Asso) and *A. momosperma* (Delile) on three sucking insect pests in laboratory and greenhouse settings. The study revealed significant toxicity against *Aphis gossypii*, with LC50 values of 0.023 and 0.085% for the respective oils. Similarly, Ateyyat et al. (2012) also reported *A. eiberi* as the most toxic oil with LC50 value of 6161 ppm at 24 hours after exposure against Woolly apple aphid.

In residue contact bioassay *A. vulgaris* oil was observed the most toxic at 12, 24 and 48 hours at all the all four LC levels followed by *C. flexuosus* and *T. minuta*(Table2). The LC values for *A. vulgaris* were LC30 (0.0695, 0.0613 and 0.0556%), LC50 (0.142, 0.118 and 0.104%), LC75 (0.358, 0.275 and 0.232%) and LC90 (0.822, 0.588and 0.479%). *R. officinalis* was found to be least toxic oil at 12, 24 and 48 hours at all the four LC levels {(LC30 (0.211, 0.175, 0.156%), LC50 (0.457, 0.380, 0.319%), LC75 (1.233, 1.027, 0.801%) and LC90 (3.014, 2.509, 1.833)}. The relative toxicity values (RT50) in Residue Contact Bioassay indicated that *A. vulgaris* 3.21, 3.22 and 3.07 times more toxic than *R. officinalis* at 12, 24 and 48 hours, respectively. *C. flexuosus* was found to be 1.93, 2.13 and 1.97 and *T. minuta* i.e. 1.207, 1.26 and 1.17 times more toxic than *R. officinalis* oil at 12, 24 and 48 hours, respectively. Similarly, Dhen et al. (2014) demonstrated the toxicity of *A. vulgaris* against stored pests, aligning with our findings. Their investigation involved assessing the fumigant toxicity of *A. absinthium*, revealing robust fumigant toxicity with LC50 and LC90 values of 18.23 μ l and 41.74 μ l/l air, respectively, against *R. dominica* adults, a pest associated with stored products.

The experiment's results also align with Ahmed et al.'s findings (2020), where they explored the insecticidal activity and biochemical composition of extracts from *Citrulluscolocynthis*, *Cannabis indica*, and *Artemisia argyi* against the cabbage aphid (*Brevicorynebrassicae* L.). Their observations revealed that *Artemisia argyi* stood out as the most toxic oil, with LC50 values of 5.62, 4.28, and 0.22 in residue contact bioassay and 38.6, 13.8, and 3.91 ppm in leaf dip bioassay at 24, 48, and 72 hours, respectively.

The inference can be drawn that *A. vulgaris* exhibited the highest toxicity against *Myzuspersicae*. The LC50 values of *A. vulgaris* in the Leaf Dip Bioassay ranged from 0.228% at 12 hours after exposure to 0.1792% and 0.1616% at 24 and 48 HAE, respectively. In the Residue Contact method, the LC50 values varied from 0.1424% to 0.1182% and 0.1040% at 12, 24, and 48 HAE, respectively.

Duration - mortality response (LT₅₀)

A. vulgaris was found to be more toxic oil in residue contact as it took less time to kill 50% of insect i.e. LT₅₀ 0.947 hours as compared to Leaf immersion (LT₅₀=3.08 hours). Similarly, Ahmed et al. (2020) investigated the insecticidal properties and biochemical composition of extracts from *Citrulluscolocynthis*, *cannabis indica*, and *Artimiciaargyi* against cabbage aphid (*Brevicorynebrassicae*L.). They observed that *Artimiciaargyi* displayed the highest toxicity in the Residue Contact Bioassay, with LC50 values of 5.62, 4.28, and 0.22, in contrast to the Leaf Dip Bioassay where LC50 values were 38.6, 13.8, and 3.91 at 24, 48, and 72 hours, respectively. These results also supported by Ahmed et al2021 where in a study Black pepper and tea tree essential oils demonstrated high effectiveness with 80% mortality through contact application, while combinations like black pepper + tea tree and rosemary + tea tree reached 98.33% mortality.

Studies on Bioassay methods clearly indicated that the relative efficacies of oils vary considerably with respect to the method of Bioassay and primarily depends on the property and uptake of oils by the organism.

Table 1: Mortality response of essential oils against *Myzuspersicae* by leaf immersion bioassay at 12, 24 and 48 hours after exposure

Plant Species	LC ₅₀ values in ppm (%)					
	Leaf Dip Bioassay			Residue Contact Bioassay		
	12 hr	24 hours	48 hrs	12 hours	24 hours	48 hours
<i>T1-Artemisia vulgaris</i>	2283.46 (0.23)	1792.20 (0.18)	1616.63 (0.17)	1424.39 (0.14)	1182.48 (0.12)	1040.71 (0.11)
<i>T2-Cymbopogonflexuosus</i>	3235.15 (0.33)	2524.68 (0.25)	1822.37 (0.19)	2358.89 (0.24)	1786.19 (0.17)	1692.25 (0.17)
<i>T3-Tagetes minuta</i>	5126.07 (0.52)	4070.43 (0.41)	3582.99 (0.36)	3787.50 (0.38)	3016.30 (0.31)	2724.87 (0.27)
<i>T4-Rosemarinus officinalis</i>	6784.68 (0.67)	5919.35 (0.59)	4813.17 (0.48)	4573.16 (0.46)	3807.10 (0.38)	3198.92 (0.32)

Table 2: Relative toxicity vale of selected essential oils against *Myzus persicae* by Leaf immersion Bioassay & Residue Contact Bioassay at 12, 24 and 48 hours after exposure

Plant Species	Relative toxicity values at LC ₅₀					
	Leaf Dip Bioassay			Residue Contact Bioassay		
	12 hours	24 hours	48 hrs	12 hours	24 hours	48 hours
T1	2.97	3.30	2.97	3.21	3.22	3.07
T2	2.09	2.34	2.64	1.93	2.13	1.97
T3	1.32	1.45	1.34	1.207	1.26	1.17
T4	1.00	1.00	1.00	1.00	1.00	1.00

Table 3: Mortality response of selected essential oils against *Myzus persicae* by Leaf immersion Bioassay in different doses and durations

Plant Species	Concentrations ppm (%)	LT values in hours				Chi-square	Regression equation	Fiducial limit at LC ₅₀	
		LT ₃₀	LT ₅₀	LT ₇₅	LT ₉₀			Lower	Upper
T1	3000 (0.3)	0.22	3.08	95.77	2109.69	0.88	0.135x + 5.13	0.0001	20987.50
T2	3000 (0.3)	1.17	9.65	144.78	1657.70	0.75	0.17x + 4.88	0.0100	8911.80
T3	3000 (0.3)	4.09	69.73	2671.66	71100.22	0.99	0.125x + 4.54	0.0070	660214.90
	5000 (0.5)	0.98	17.63	724.15	20517.08	0.88	0.13x + 4.79	0.0020	187056.40
T4	3000 (0.3)	12.92	186.79	5801.17	127792.79	0.88	0.14x + 4.33	0.0420	1356582.40
	5000 (0.5)	7.90	124.52	4320.56	105161.80	0.88	0.13x + 4.42	0.0150	1012517.35
	6000 (0.6)	0.98	17.63	724.15	20517.08	0.88	0.13x + 4.79	0.0020	187056.40

Table 4: Mortality response of selected essential oils against *Myzus persicae* by Residue Contact Bioassay in different doses and durations

Plant species	Concentrations ppm (%)	LT values in hours				Chi-square	Regression equation	Fiducial limit at LC ₅₀	
		LT ₃₀	LT ₅₀	LT ₇₅	LT ₉₀			Lower	Upper
T1	3000 (0.3)	0.12	0.95	13.94	156.80	0.884	0.17x + 5.46	0.000	1842.2
T2	3000 (0.3)	0.22	3.08	95.77	2109.69	0.885	0.135x + 5.13	0.000	20987.5
T3	3000 (0.3)	0.98	17.62	724.15	20517.08	0.882	0.13x + 4.77	0.002	187056.4
T4	3000 (0.3)	1.82	32.68	1342.14	38026.37	0.882	0.13x + 4.67	0.003	346690.2
	5000 (0.5)	0.98	17.63	724.15	20517.08	0.882	0.13x + 4.78	0.002	187056.4

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