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Residual toxicities of some plant essential oils against Cotton Aphid, *Aphis gossypii* Glover

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ABSTRACT

Insecticidal activities of four essential plant oils *Artemisia vulgaris (*Burn), *Cymbopogon flexuosus* (Steud), *Tagetes minuta* (Khakibush) and *Rosmarinus officinalis* (Linneaus) were evaluated against *Aphis gossypii* by using leaf dip and residue contact bioassay techniques respectively. *Artemisia vulgaris* oil was found to be most toxic in residue contact as compared to leaf dip bioassay. The LC_{50} of *A. vulgaris* was 0.118% and 0.179% at 24 hours after exposure (HAE) in residue contact and leaf dip bioassay methods respectively. The order of toxicity at 48 HAE was as follows *A. vulgaris* (0.104%) > *C. flexuosus* (0.169%) > *T. minuta* (0.272%) and *R. officinalis* (0.319%) in residue contact bioassay. In leaf dip bioassay, the order of toxicity was *A. vulgaris* (0.161%) > *C. flexuosus* (0.182%) > *T. minuta* (0.358%) and *R. officinalis* (0.481%). Relative toxicity of *A. vulgaris*, *C. flexuosus* and *T. minuta* was found to be 3.07, 1.97 and 1.17 respectively in residue contact method and 2.97, 2.64 and 1.34 respectively in leaf dip method. The lower values of LC_{50} of all the four essential oils was observed in residue contact method which indicates it's superiority over leaf dip bioassay.

Keywords: Essential oils, LC_{50}, LT_{50}, Bioassay, Toxicity, *Artemesia vulgaris*

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INTRODUCTION

Aphis gossypii is a widespread polyphagous insect that feeds on more than 700 host plant species (Wang *et al.,* 2007, Somar *et al.,* 2019, Mostafiz *et al.,*2019). It feeds on plant juices, block photosynthesis by secreting honey dew (Shannag *et al*., 1998), reduces plant vigour and serve as vectors for many plant viruses such as maize dwarf mosaic virus, cucumber mosaic virus, potato leaf roll virus, barley yellow dwarf virus, sugarcane mosaic virus, carrot mottle virus, lettuce necrotic yellow virus (Gray *et al.,*2002, Hogenhout *et al*., 2008). In literature there are around 26 species of aphids known to have different number of biotypes (Taggar and Arora., 2017, Basky., 2003, Goggin, *et al*., 2003, Wang et al., 2019). *Aphis gossypii* is reported to have two biotypes (Wang *et al*., 2016, Xu., 2014, Wang *et al.*, 2004). Aphids infestation significantly lowers the yield of the crops (Carletto *et al.,* 2010 Koo *et al.,* 2014) Aphids have long been controlled by use of chemical insecticides which have caused environmental pollution, increase in resistance to insecticides and different biotypes to the aphids population (Patima, 2019, Bass *et al.,* 2015, Ma *et al*., 2019, Zhang *et al*., 2020), higher pesticide residues in soil (Hua *et al.*, 2023), and harm to natural enemies of insects (Das and Rahman, 2023). Hence, it is important and necessary to prioritize alternate suitable methods of management for aphids other than pesticides. In this context various researchers have studied the insecticidal properties of essential oils from plants and reported them as potential botanical insecticides to control various insect pests (Kordali *et al.,* 2005, Tripathi *et al*., 2000, Mohan *et al*., 2011). Previously *Coriandrum sativum* oil, *Lavandula spica* oil, *Foeniculum vulgare* oil, *Origanum vulgare* oil, *Juniperus communis* oil and *Syzygium aromaticum* (Atanasova *et al.,* 2018), *Ocimum sanctum*, *Ocimum basilicum*, *Ocimum gratissimum*, *Mentha piperita*, *Mentha arvensis*, *Tagetes erecta* and *Lavandula angustifolia* (Wang *et al.,* 2024) have been examined as potential

controls for *A. gossypii*. Essential plant oils of *Artemisia vulgaris* (Burn), *Cymbopogon flexuosus* (Steud), *Tagetes minuta* (Khakibush) and *Rosmarinus officinalis* (Linn.) have not been much exploited for the management of *A. gossypii*. This study was proposed to study the effect of these medicinal plants oils on the aphid population.

MATERIALS AND METHODS

The experiment was conducted in Plant Protection Laboratory, Department of Entomology at College of Horticulture,Veer Chandra Singh Garhwali Uttarakhand University of Horticulture and Forestry (VCSG UUHF), Bharsar Pauri Garhwal. Dried plant parts were obtained from Medicinal Block, Bharsar and were extracted by hydro distillation method (Ray *et al.,* 2008) using Clevenger apparatus. The distilled oil was separated from water by a separating funnel and stored in refrigerator. A preliminary screening of essential oils was done at 1% and 2% after which the oils were tested to know their LC_{50} by leaf dip and residue contact methods. Based on preliminary screening, a final regime of concentrations were prepared for the four essential plant oils.

Leaf dip bioassay

Leaf dip method was followed according to Kodandaram and Dhingra (2007). The full grown matured plant leaves of *Hibiscus rosa- chinesis* were plucked from surrounding area of College campus and brought to the laboratory. They were dipped in *Artemesia vulgaris* 0.3%, *Cymbopogan flexuosus* 0.5%, *Tagetes minuta* 0.8% and *Rosmarinus officinalis* 1% of different concentrations for one minute. Excess liquid was shaken from the foliage. This was then allowed to dry at room temperature. Treated leaf was then transferred to clean petri dish of diameter 9 cm. Later 10 apterous neonate aphids of same size were carefully released on the treated leaf by using a soft camel hail brush of zero size. Each treatment including control was replicated thrice. For control, the Hibiscus leaves were dipped in water, dried and used. The petri dishes were kept in incubator at 20±5°C. The data on mortality was recorded after 12, 24 and 48 hours feeding.

Residue contact bioassay method

Residue contact bioassay method was followed according to Srivastava and Proksch (1993) and Vedhamathi (2004). One mL of each of the concentration of oils were coated as a thin film in the lower and upper lid of petri plates. The solvent was allowed to dry at room temperature. After evaporation of the solvent, apterous aphid, *Aphis gossypii* (n=10) were given contact exposure for 30 minutes. In the control, the aphids were exposed to water alone (Parvathi and Kesar, 1999). Three replications were maintained. Thereafter, the aphids were transferred to petri dishes (9 cm diameter) containing fresh Hibiscus leaves. The data on mortality was recorded at 12, 24 and 48 hours after exposure (HAE). Moribund aphids were counted as dead. The mortality data was corrected using Abbott's formula (Abbott, 1925).

STATISTICAL ANALYSIS

The experiment was conducted in completely randomized design (CRD) and LC values were determined using probit analysis (Finney, 1971) based online computer programme OPSTAT. Relative toxicity (RT) of oil was calculated based on LC_{50} value following (Ramangouda and Srivastava, 2009).

RESULTS

A.vulgaris was the most toxic oil at 12, 24 and 48 hours respectively, with LC_{50} values of 0.228, 0.179, 0.116%, followed by *C. flexuosus* and *T. minuta*. *R. officinalis* was found to be least toxic oil at 12, 24 and 48 hours after feeding in leaf dip bioassay. *C. flexuosus*is 2.09, 2.34, 2.64 and *T. minuta* were 1.32, 1.45 and 1.34 times more toxic than *R. officinalis* oil at 12, 24 and 48 hurs, respectively. The relative toxicity values (RT_{50}) in leaf dip bioassay indicates that *A. vulgaris* was 2.97, 3.30 and 2.97 times more toxic than *R. officinalis* at 12, 24 and 48 hours, respectively. In residue contact bioassay *A. vulgaris* oil was the most toxic at 12, 24 and 48 hurs respectively, the values of LC_{50} as 0.142, 0.118 and 0.104% at 12, 24 and 48HAE, followed by *C. flexuosus* and *T. minuta*. *R. officinalis* was found to be least toxic oil with LC_{50} 0.457 at 12 HAE, 0.380 at 24 HAE and 0.319% at 48 HAE values. The relative toxicity values

(RT_{50}) in residue contact bioassay indicated that *A. vulgaris* is 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* was 1.207, 1.26 and 1.17 times more toxic than *R. officinalis* oil at 12, 24 and 48 hours, respectively. It could be inferred that *A. vulgaris* was the most toxic oil against *A. gossypii*. The LC_{50} values of *A. vulgaris* in leaf dip bioassay varied from 0.228% at 12 hurs after exposure to

Table 1. Dosage dependent mortality response of selected essential oils against *Aphis gossypii* by leaf dip bioassay at 12, 24 and 48 hours after exposure

Medicinal plants	LC_{50} values in ppm (%)					
	Leaf Dip Bioassay			Residue Contact Bioassay		
	12 hrs	24 hrs	48 hrs	12 hrs	24 hrs	48 hrs
Artemicia vulgaris	2283.460 (0.2283)	1792.200 (0.1792)	1616.630 (0.1616)	1424.389 (0.1424)	1182.484 (0.1182)	1040.706 (0.1040)
Cymbopogan flexuosus	3235.150 (0.3235)	2524.680 (0.2524)	1822.370 (0.1822)	2358.892 (0.2358)	1786.192 (0.1786)	1692.249 (0.1692)
Tagetes minuta	5126.070 (0.5126)	4070.430 (0.4070)	3582.990 (0.3582)	3787.5 (0.3787)	3016.300 (0.3016)	2724.868 (0.2724)
Rosemarinus officinalis	6784.684 (0.6784)	5919.359 (0.5919)	4813.179 (0.4813)	4573.16 (0.4573)	3807.100 (0.3807)	3198.914 (0.3198)

Table 2. Relative toxicity vale of selected essential oils against *Aphis gossypii* by leaf dip bioassay and 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 hrs	24 hrs	48 hrs	12 hrs	24 hrs	48 hrs
Artemicia vulgaris	2.97	3.30	2.97	3.21	3.22	3.07
Cymbopogan flexuosus	2.09	2.34	2.64	1.93	2.13	1.97
Tagetes minuta	1.32	1.45	1.34	1.207	1.26	1.17
Rosemarinus officinalis	1.00	1.00	1.00	1.00	1.00	1.00

Table 3. Duration - mortality response of selected essential oils against cotton aphid*, Aphis gossypii* by leaf dip bioassay.

Plantses	**Concentrations (%)**	**LT values in hours**				**Chi-square**	**Regression equation**	**Fiducial limit at LC_{50}**	
		LT_{30}	**LT_{50}**	**LT_{75}**	**LT_{90}**			**Lower**	**Upper**
Artemicia vulgaris	0.3	0.213	3.084	95.770	2109.699	0.885	0.135x + 5.126	0.0001	20987.50
Cymbopogan flexuosus	0.3	1.174	9.645	144.778	1657.700	0.752	0.17x + 4.88	0.0100	8911.80
Tagetes minuta	0.3	4.096	69.727	2671.653	71100.220	0.998	0.125x + 4.54	0.0070	660214.90
	0.5	0.981	17.629	724.145	20517.086	0.882	0.13x + 4.786	0.0020	187056.40
Rosemarinus officinalis	0.3	12.919	186.790	5801.160	127792.790	0.885	0.14x + 4.32	0.0420	1356582.40
	0.5	7.900	124.520	4320.550	105161.800	0.885	0.13x + 4.416	0.0150	1012517.35
	0.6	0.981	17.629	724.145	20517.086	0.882	0.13x + 4.786	0.0020	187056.40

Table 4. Duration - mortality response of selected essential oils against cotton aphid*, Aphis gossypii* by eaf dip bioassay and residue contact bioassay at 12, 24 and 48 hours after exposure

Plant species	Concentrations (%)	LT values in hours				Chi-square	Regression equation	Fiducial limit at LC_{50}	
		LT_{30}	LT_{50}	LT_{75}	LT_{90}			Lower	Upper
Artemicia vulgaris	0.3	0.117	0.947	13.940	156.800	0.884	0.17x + 5.46	0.000	1842.2
Cymbopogan flexuosus	0.3	0.213	3.0840	95.770	2109.690	0.885	0.135x + 5.126	0.000	20987.5
Tagetes minuta	0.3	0.981	17.629	724.140	20517.080	0.882	0.13x + 4.786	0.002	187056.4
Rosemarinus officinalis	0.5	1.818	32.674	1342.130	38026.369	0.882	0.13x + 4.673	0.003	346690.2
	0.3	0.981	17.629	724.145	20517.080	0.882	0.13x + 4.786	0.002	187056.4

0.1792 and 0.1616% at 24 and 48 HAE, respectively, whereas in residue contact method it varied from 0.1424 to 0.1182 and 0.1040% at 12, 24 and 48 HAE, respectively. *Artimisia vulgaris* was found to be more toxic oil in residue contact as it took less time to kill 50% of insects i.e. LT_{50} 0.947 hours as compared to leaf dip (LT_{50}=3.08 hours). Studies on bioassay methods clearly indicates that the relative efficacies of oils vary considerably with respect to the method of bioassay.

DISCUSSIONS

Artemesia vulgaris oil was found to be most toxic followed by *Cymbopogan flexuosus, Tagetes minuta* and *Rosemarinus officinalis in residue contact and leaf dip bioassays.* Soliman (2005) tested *Artemisia herba-alba* and *Artemisia momosperma* against three sucking insect pests under laboratory and greenhouse conditions and found that both the oils gave a high toxicity on *A. gossypii* with LC_{50} of 0.023 and 0.085%. Ateyyat et al. (2012) also reported *Artimicia eiberi* as the most toxic oil with LC_{50} value of 6161 ppm at 24 hours after exposure against apple woolly aphid. Dhen et al. (2014) proved the toxicity of *Artimicia vulgaris* against stored pests and also checked the fumigant toxicity of *Artimicia absinthium* and found its strong fumigant toxicity with LC_{50} and LC_{90} of 18.23 µL and 41.74 µl/L air respectively against *Rhizopertha dominica* adults.

The results of our experiment are in tune with the results of Ahmed et al. (2020) who studied the insecticidal activity and biochemical composition of *Citrullus colocynthis*, *cannabis indica* and *Artimicia argyi* extracts against cabbage aphid (*Brevicoryne brassicae*) and observed *Artimicia argyi* was the most toxic oil with LC_{50} value i.e. 5.62, 4.28 and 0.22 ppm in residue contact and 38.6, 13.8 and 3.91 ppm in leaf dip bioassay at 24, 48 and 72 hours respectively. In a recent study done by Caixia Han et al (2023) *A.vulgaris* essential oil displayed potent insecticidal activity against *Aphis gossypii* adults with an LC50 value of 46.706µg/mL. Our studies also confirm the insecticidal activity of *A. vulgaris* oil against *A. gossypii.* Mei Yang et al (2024) also observed a decline in aphid population on Artemesia plants and reported zero feeding on the seventh day of inoculation of aphids on Artemesia plant. The above study supports our findings and it can be concluded that various essential oils can be utilised in pest management programmes and antifeedant, insecticidal, fungicidal activities of *Artemesia* species should be further explored.

AUTHORS CONTRIBUTIONS

Rohit Kumar conducted experiment, layout, collection of data, and data analysis. Vijay Kumar

Co-advisor, Manuscript writing, Re-writing, Priyanka Bhat design of experiment, supervisor, conduct of experiment, writing, Re-writing, Editing.

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