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Research Article

PERFORMANCE OF VARIOUS BOTANICAL EXTRACTS AGAINST *DIAPHORINA CITRI* (HEMIPTERA: LIVIIDAE) INFECTING LEMON UNDER FIELD CONDITIONS

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Diaphorina citri is one of the major pests of citrus worldwide, primarily because it vectors the bacterium Candidatus Liberibacter asiaticus, the causal agent of huanglongbing. Due to the lack of scientific studies in Sindh addressing its management, the efficacy of various extracts, ethanol, methanol, and water, from ten indigenous plants was evaluated against *D. citri* in a lemon orchard. The plants tested included prickly chaff flower, thorn apple fruits, basil leaves, bitter apple fruits, Aloe vera leaves, siris leaves, karir, field dodder, milkweed leaves, and castor bean leaves. All plant extracts were applied at a rate of 4 Kg per acre. Three sprays were conducted during the study, with observations recorded at 24-h intervals over seven days to assess the effectiveness of the botanical extracts against D. citri. The ethanol and methanol extracts of basil, bitter apple, and milkweed were found to be the most effective, whereas field dodder and castor bean were the least effective. Water extracts of all plants were comparatively less effective than their corresponding ethanol and methanol extracts. Over time, the effectiveness of all extracts decreased. At the end of the week, the maximum corrected reductions in D. citri populations, calculated using Abbott's formula, were 87.72%, 86.67%, and 85.66% for ethanol basil, methanol basil, and methanol bitter apple extracts, respectively. Based on these findings, it is recommended that ethanol extracts of either basil or bitter apple be incorporated into the integrated management of D. *citri* in citrus orchards.

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INTRODUCTION

Diaphorina citri is recognized as a significant insect pest of citrus trees worldwide, serving as a vector for Huanglongbing (HLB), also known as citrus greening disease, the most devastating disease affecting citrus crops (Gaire et al., 2022; Rizvi et al., 2023). Both adults and nymphs of *D. citri* feed on the cell sap of young twigs and leaves, leading to twig shrinkage, curling of leaves and fruits, and premature fruit drop (Grafton-Cardwell et al., 2013; Ahmad et al., 2014; Tayyab et al., 2022). Moreover, HLB aggravates the damage caused by *D. citri*, manifesting in symptoms such as stunted tree growth, yellowing and mottling of leaves, and the production of deformed, undersized fruits that lack juice (Bové, 2006; Grafton-Cardwell et al., 2013; Hall et al., 2013; Dong et al., 2023). Severe infestations of *D. citri* can result in the death of entire citrus trees (Zaka et al., 2010; Rizvi et al., 2019; Dong et al., 2023). Since 2005, *D. citri* and HLB have devastated the citrus industries of major producers such as China, Brazil, and the USA (Singerman and Rogers, 2020; Rizvi et al., 2023).

To date, no cure has been identified for HLB. Consequently, managing D. citri populations remains the only viable approach to mitigate losses (Bové, 2006; Tavvab et al., 2022; Rizvi et al., 2023). Synthetic insecticides from various chemical groups are widely used globally for *D. citri* management (Tang et al., 2021; Yuan et al., 2021). However, repeated insecticide applications have resulted in significant environmental harm, adverse effects on non-target organisms (including humans), pest resurgence, and the development of insecticide resistance in D. citri populations (Rizvi et al., 2018; Tian et al., 2020; Chen et al., 2021a). Alarmingly, resistance to organophosphates, pyrethroids, neonicotinoids, carbamates, and diamide insecticides has been documented in D. citri populations worldwide, raising serious concerns (Pardo-Melgarejo et al., 2023; Rizvi et al., 2024). Therefore, there is a pressing need to develop alternative, safe, and sustainable control strategies to manage D. citri populations in citrus orchards effectively (Tayyab et al., 2022; Rizvi et al., 2023).

Botanical pesticides have long been used as potential alternatives to synthetic insecticides for managing major agricultural and household pests (Kayani et al., 2012; Mukhtar et al., 2013). This is primarily due to their multiple modes of action, biodegradability, and safety for humans, non-target animals, and the environment (Sa'adah et al., 2023). Among botanical pesticides, *Azadirachta indica* and *Nicotiana tabacum* are the most widely utilized for controlling pests in agriculture, horticulture, and households, including *D. citri* (Kamaraj et al., 2018; Azeem et al., 2021; Saeed et al., 2021; Lee et al., 2022).

In addition to these, several other plants have been evaluated for their efficacy in managing *D. citri* populations in citrus orchards, thereby reducing the severity of HLB disease. These include *Delphinium brunonianum* (Rizvi et al., 2024), *Cymbopogon nardus* (Rohim et al., 2023), *Artemisia absinthium* (Rizvi et al., 2023), Mentha longifolia, Melilotus officinalis, Nerium indicum, Datura alba, Salvia officinalis (Tayyab et al., 2022), and Ipomoea-periglandula (Chen et al., 2021b), among others (Rizvi et al., 2018; González-Castro et al., 2019; Rizvi et al., 2019; Reena et al., 2020).

Infestations of *D. citri* have been reported in citrusgrowing regions of Pakistan, with most studies focusing on its management (Naeem et al., 2016; Ahmad et al., 2023). However, no systematic research has been conducted on the damage caused by *D. citri* and its management in Sindh province, which is the main lemon-producing region in Pakistan.

The present study aims to evaluate the insecticidal potential of various indigenous plants for reducing *D. citri* populations in a lemon orchard. The findings will assist local growers in Sindh by providing environmentally friendly, cost-effective pest management solutions using locally available plants, thereby reducing dependence on synthetic insecticides and improving the quality and yield of lemons.

MATERIALS AND METHODS

Collection and preparation of plant materials

The indigenous plants evaluated against D. citri were prickly chaff flower (Achyranthes aspera Linn.), thorn apple fruits (Dhatura alba Linn., Syst.), basil leaves (Ocimum basilicum Linn.), bitter apple fruits (Citrullus colocynthis Linn., Schrad.), Aloe vera leaves (Aloe barbadensis Linn., Burm), siris leaves (Albizia lebbeck Linn., Benth.), karir (Capparis decidua Forssk.), field dodder (Cuscuta campestris Yuncker), milkweed leaves (Calotropis procera Forst.), castor bean leaves (Ricinus communis Linn.), and an untreated control. Fresh samples of all plant materials were obtained from the surrounding areas of the study site, Tandojam, Pakistan. Three types of extracts, ethanol, methanol, and water, were prepared for each plant. The protocols proposed by González-Castro et al. (2019), with slight modifications, were used for the preparation of ethanol and methanol extracts.

Two kilograms of each plant material were thoroughly washed with tap water and air-dried under shade for 48 h in the laboratory of Stored Grain Pests, Department of Entomology, Sindh Agriculture University, Tandojam. Afterward, the plant materials were cut into small pieces, ground to a 40 mm mesh size, sieved, and macerated with 2 L of ethanol and methanol in an Erlenmeyer flask to ensure that the entire plant material was completely submerged. The mixture was left for 24 h in the laboratory. Each plant sample was extracted three times. The obtained mixture of individual botanical extracts was then filtered using Whatman filter paper to obtain a fine extract, which was covered with aluminum foil. Subsequently, the fine mixture was heated in a glass beaker using an electric heater to evaporate the solvents (ethanol and methanol) and obtain semi-dried botanical extracts. These extracts were weighed using an electric balance for calibration. The obtained botanical extracts were stored in an electric refrigerator with proper labeling for future use in the study.

The preparation of water extracts followed the procedure outlined by Kunbhar et al. (2018) with slight modifications. After obtaining small pieces of each plant material, they were submerged in three liters of water in an Erlenmeyer flask and left for 24 h. The mixture was then sieved using fine mesh muslin cloth to obtain the stock solution of each botanical extract. The obtained extracts were stored in the Stored Grain Research Laboratory under a temperature regime of $25 \pm 3^{\circ}$ C, with proper labeling for future use in the study.

Calibration and application of botanical extracts

The calibration of water used for the experiment was based on 100 L per acre, with each plant material calibrated at a dose of 4 Kg per acre, measured either in milliliters or grams. The number of sprays against *D. citri* was scheduled based on its economic threshold level, estimated at 12 to 15 *D. citri* per flush per plant. Accordingly, three sprays were conducted during the study on April 5, 2023, May 4, 2023, and October 12, 2023.

Experimental design, data collection, and analysis

The experiment was arranged in a Completely Randomized Block Design. The selected orchard was divided into five blocks, with each botanical extract applied to an individual lemon tree. A single botanical extract was used per tree, resulting in each extract being applied to a total of five lemon trees.

Data on the performance of different botanical extracts were collected before the sprays, with subsequent observations recorded 24, 48, and 96 h, as well as one week after each spray. Young flushes (approximately 10-15 mm in length) with at least five young leaves were selected as sample units. For each sprayed lemon tree, five flushes were observed to count the number of adults and nymphs *in situ* using a 10× hand-held lens. These counts were expressed as the number of psyllids per flush (Liang et al., 2023).

The collected data on the performance of various botanical extracts against *D. citri* at different time intervals were analyzed using Analysis of Variance (ANOVA). The Least Significant Difference (LSD) test was employed to separate means with significant differences. Data collected before the sprays were excluded from the ANOVA analysis due to the increasing population of *D. citri* in the control treatment, ensuring more accurate results regarding the effectiveness of the botanical extracts. The analyses were performed using the STATISTIX 8.1 software.

The percentage reduction in the *D. citri* population due to the application of various botanical extracts was calculated using Abbott's formula, as described below:

Corrected (%) = $(1 - \frac{n \text{ in } T \text{ after treatment}}{(n \text{ in } Co \text{ after treatment})} \times 100$ Where n = psylla population per branch per tree; T = treatment; Co. = Control.

RESULTS

Performance of various botanical extracts in managing *D. citri* on lemon at different observation intervals after the first spray

Table 1 presents the population dynamics of *D. citri* on lemon at different observation intervals following the application of various botanical extracts. Before the spray, no significant difference (F = 0.07, P = 1.000) was observed in the population of *D. citri* across treatments, with counts ranging from 15.81 ± 0.83 psylla per flush (ethanol extract of prickly chaff flower) to 16.64 ± 0.98 psylla per flush (water extract of siris).

After the spray, significant differences in *D. citri* population reduction were observed across intervals: 24 h (F = 22.09, P < 0.001), 48 h (F = 26.27, P < 0.001), 96 h (F = 33.13, P < 0.001), and one week (F = 32.82, P < 0.001). Ethanol extracts were generally more effective than methanol or water extracts, with water extracts showing comparatively lower efficacy. The performance of all botanical extracts improved over time, peaking at 96 h before slightly declining by the end of the week.

At the end of the week, the lowest populations were recorded with ethanol $(3.50 \pm 0.33 \text{ psylla per flush})$ and methanol $(3.92 \pm 0.35 \text{ psylla per flush})$ extracts of basil, followed by methanol $(4.08 \pm 0.32 \text{ psylla per flush})$ and

ethanol (4.19 \pm 0.32 psylla per flush) extracts of bitter apple. Ethanol (4.58 \pm 0.49 psylla per flush) and methanol (4.67 \pm 0.51 psylla per flush) extracts of milkweed and thorn apple extracts (ethanol: 4.92 \pm 0.46; methanol: 5.00 \pm 0.44 psylla per flush) performed similarly to basil extracts. In contrast, field dodder extracts (water: 11.28 \pm 1.18; methanol: 10.81 \pm 1.25; ethanol: 10.72 \pm 1.24 psylla per flush) were the least effective.

At all intervals, the highest *D. citri* populations were recorded in the control, with counts increasing from 20.08 ± 1.08 psylla per flush (24 h) to 28.47 ± 2.38 psylla per flush (one week) (Table 1).

The results on the performance of various botanical extracts in reducing D. citri populations, calculated using Abbott's (1925) corrected percentage reduction formula, are presented in Figure 1. Basil, bitter apple, milkweed, and thorn apple were the most effective in reducing D. citri populations, while field dodder, castor bean, and prickly chaff flower were the least effective. Among the extraction solvents, ethanol and methanol extracts were more effective than their respective water extracts. After 24 h, the highest reduction in D. citri population was observed with the ethanol basil extract (79.67%), reaching 87.71% by the end of the week. Other botanicals with notable reductions included ethanol bitter apple (85.66%), ethanol milkweed (83.90%), ethanol thorn apple (82.73%), methanol aloe vera (79.61%), ethanol karir (76.10%), ethanol siris (73.07%), ethanol chaff flowers (71.61%), ethanol castor bean (68.49%), and ethanol field dodder (62.34%) (Figure 1).

Performance of various botanical extracts in managing *D. citri* on lemon at different observation intervals after the second spray

The results of the second spray of various botanical extracts against *D. citri* in a lemon orchard are presented in Table 2. A similar trend to the first spray was observed, with ethanol and methanol extracts of basil proving the most effective in reducing *D. citri* populations, followed by extracts of bitter apple and milkweed. In contrast, ethanol, methanol, and water extracts of field dodder and castor bean were the least effective.

A steady decline in *D. citri* populations was recorded across all treatments over the week. At one week postspray, the lowest mean populations were recorded with basil ethanol (2.78 ± 0.27 psylla per flush) and methanol $(3.31 \pm 0.29 \text{ psylla per flush})$ extracts, followed by bitter apple methanol $(3.47 \pm 0.32 \text{ psylla per flush})$ and milkweed ethanol $(3.69 \pm 0.31 \text{ psylla per flush})$ extracts. The performance of milkweed methanol $(3.78 \pm 0.30 \text{ psylla per flush})$ and bitter apple ethanol $(3.86 \pm 0.35 \text{ psylla per flush})$ extracts, along with thorn apple methanol $(4.22 \pm 0.32 \text{ psylla per flush})$ and ethanol $(4.25 \pm 0.33 \text{ psylla per flush})$ extracts, was comparable to basil extracts.

Field dodder extracts (ethanol: 10.25 ± 0.81 ; water: 9.69 ± 0.76 ; methanol: 9.50 ± 0.58 psylla per flush) were the least effective, while control plants had the highest *D. citri* populations at all intervals. Statistical analysis revealed significant differences among plant species (F = 57.34, P < 0.001) and extract types (F = 35.41, P < 0.001), with a significant interaction between plants and extracts (F = 1.81, P = 0.0205).

Following the 2nd spray, the highest reduction in *D. citri* population on lemon was observed with basil, bitter apple, milkweed, and thorn apple extracts at various intervals, while field dodder and castor bean were the least effective. The efficacy of botanical extracts increased over time, with ethanol and methanol extracts outperforming water-based extracts. One week after the 2nd spray, the maximum reduction (85.84%) was recorded with ethanol basil extract, and the minimum (47.73%) with ethanol field dodder extract. Among the other extracts, methanol bitter apple (82.31%), ethanol milkweed (81.16%), methanol thorn apple (78.48%), methanol aloe vera (75.07%), ethanol karir (67.42%), methanol siris (64.46%), ethanol prickly chaff flower (61.19%), ethanol castor bean (58.07%), and methanol field dodder (51.56%) showed varying effectiveness (Figure 2).

Performance of various botanical extracts in managing *D. citri* on lemon at different observation intervals after the third spray

The results of the third spray of various botanical extracts to control *D. citri* population in a lemon orchard are shown in Table 3, which indicates similar trends observed in the first and second sprays. Basil, bitter apple, and milkweed, particularly when extracted in ethanol, were the most effective, followed by methanol and water. A continuous decline in *D. citri* populations was noted across all treatments after the third spray, with the lowest mean population of 2.47 ± 0.18 and 3.19 ± 0.34 psylla per flush recorded in the ethanol basil and bitter apple extract treatments, respectively.

Observation interval	Plant	Extracts				
		Ethanol	Methanol	Water		
Before spray	Basil	16.17 ± 0.87 a	16.44 ± 0.92 a	16.33 ± 0.95 a		
	Bitter apple	16.28 ± 0.80 a	16.50 ± 0.87 a	16.44 ± 0.93 a		
	Milkweed	15.97 ± 0.91 a	16.47 ± 0.99 a	16.06 ± 0.84 a		
	Thorn apple	16.11 ± 0.92 a	15.97 ± 0.96 a	16.53 ± 0.94 a		
	Aloe vera	16.00 ± 0.80 a	16.22 ± 0.82 a	15.83 ± 0.63 a		
	Karir	16.14 ± 0.74 a	15.89 ± 0.79 a	16.44 ± 0.92 a		
	Siris	15.94 ± 0.78 a	16.28 ± 0.82 a	16.64 ± 0.98 a		
	Prickly chaff flower	15.81 ± 0.83 a	16.28 ± 0.94 a	16.06 ± 0.97 a		
	Castor bean	16.11 ± 0.86 a	16.53 ± 0.90 a	16.11 ± 0.91 a		
	Field dodder	16.14 ± 0.80 a	16.56 ± 0.94 a	16.00 ± 0.98 a		
	Control	15.83 ± 0.41 a				
24-h	Basil	4.08 ± 0.53 l	4.25 ± 0.53 l	6.92 ± 0.70 f-k		
	Bitter apple	4.83 ± 0.50 kl	4.72 ± 0.51 kl	7.11 ± 0.67 d-j		
	Milkweed	5.11 ± 0.56 jkl	5.22 ± 0.60 jkl	7.06 ± 0.72 e-j		
	Thorn Apple	5.36 ± 0.52 i-l	5.47 ± 0.55 i-l	7.17 ± 0.77 d-j		
	Aloe vera	6.25 ± 0.54 g-l	6.19 ± 0.55 h-l	7.22 ± 0.78 d-j		
	Karir	7.28 ± 0.66 d-j	7.50 ± 0.71 d-i	8.39 ± 0.90 b-h		
	Siris	7.94 ± 0.7 1 c-h	8.06 ± 0.75 c-h	9.78 ± 1.02 abc		
	Prickly Chaff flower	8.42 ± 0.88 b-g	8.47 ± 0.89 b-f	9.89 ± 0.97 abc		
	Castor bean	9.14 ± 0.98 a-e	9.28 ± 1.00 a-d	10.50 ± 1.02 ab		
	Field dodder	10.53 ± 1.23 abc	10.56 ± 1.27 ab	10.69 ± 1.34 a		
	Control	20.08 ± 1.08				
y48-h	Basil	3.58 ± 0.37 n	3.75 ± 0.40 n	6.61 ± 0.60 h-l		
	Bitter apple	4.33 ± 0.37 mn	4.19 ± 0.39 mn	6.92 ± 0.62 g-k		
	Milkweed	4.83 ± 0.55 lmn	4.94 ± 0.57 k-n	6.86 ± 0.59 h-k		
	Thorn Apple	5.25 ± 0.48 j-n	5.39 ± 0.48 j-n	7.06 ± 0.65 f-j		
	Aloe vera	6.00 ± 0.48 i-m	5.92 ± 0.46 i-m	7.11 ± 0.78 f-j		
	Karir	7.08 ± 0.65 f-j	7.22 ± 0.67 f-j	8.08 ± 0.74 d-h		
	Siris	7.64 ± 0.64 e-i	7.75 ± 0.66 e-i	9.58 ± 0.96 a-e		
	Prickly Chaff flower	8.17 ± 0.78 c-h	8.25 ± 0.82 b-h	9.61 ± 0.89 a-e		
	Castor bean	8.86 ± 0.88 a-g	8.94 ± 0.89 a-f	10.19 ± 1.03 ab		
	Field dodder	10.06 ± 1.11 a-d	10.11 ± 1.15 abc	10.31 ± 1.17 a		
	Control	23.42 ± 1.42				
96-h	Basil	3.22 ± 0.33 n	3.50 ± 0.33 mn	6.69 ± 0.60 f-j		
	Bitter apple	4.08 ± 0.35 k-n	3.97 ± 0.37 lmn	6.83 ± 0.59 f-j		
	Milkweed	4.69 ± 0.51 k-n	4.78 ± 0.53 k-n	6.72 ± 0.61 f-j		
	Thorn Apple	5.00 ± 0.47 j-n	5.11 ± 0.48 i-m	7.19 ± 0.68 d-h		
	Aloe vera	5.86 ± 0.47 g-k	5.75 ± 0.44 h-l	7.03 ± 0.68 d-h		
	Karir	6.83 ± 0.60 f-j	7.00 ± 0.64 e-i	8.00 ± 0.75 b-f		
	Siris	7.53 ± 0.57 c-h	7.64 ± 0.57 b-g	9.50 ± 0.92 ab		
	Prickly Chaff flower	8.06 ± 0.77 b-f	8.11 ± 0.73 b-f	9.39 ± 0.82 abc		
	Castor bean	8.83 ± 0.80 a-e	8.89 ± 0.83 a-d	10.33 ± 0.97 a		
	Field dodder	10.42 ± 1.14 a	10.50 ± 1.16 a	10.11 ± 0.90 a		
	Control	26.25 ± 2.13				
1-Week	Basil	3.50 ± 0.33 j	3.92 ± 0.35 j	6.78 ± 0.60 fgh		
	Bitter apple	4.19 ± 0.32 ij	4.08 ± 0.32 ij	7.06 ± 0.71 efg		
	Milkweed	4.58 ± 0.49 ij	4.67 ± 0.51 ij	6.67 ± 0.62 fgh		
	Thorn Apple	4.92 ± 0.46 hij	5.00 ± 0.44 hij	7.39 ± 0.67 efg		
	Aloe vera	5.94 ± 0.50 ghi	5.81 ± 0.47 ghi	7.28 ± 0.72 efg		
	Karir	6.81 ± 0.61 fgh	7.11 ± 0.61 efg	8.44 ± 0.89 c-f		
	Siris	7.67 ± 0.58 efg	7.92 ± 0.63 def	9.75 ± 0.92 abc		
	Prickly Chaff flower	8.08 ± 0.66 c-f	8.17 ± 0.68 c-f	9.69 ± 0.85 a-d		
	Castor bean	8.97 ± 0.90 b-e	9.31 ± 0.94 bcd	10.69 ± 1.06 ab		
	Field dodder	10.72 ± 1.24 ab	10.81 ± 1.25 ab	11.28 ± 1.18 a		
	Control	28 47 + 2 38				

Table 1. Effect of various botanical extracts on <i>D. citri</i> j	population und	ler field	conditions fo	llowing	the first s	pray.
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*Means with same letters are not significantly different from each other (LSD values: before spray = 2.4249, 24-h = 2.1169, 48-h = 1.9293, 96-h = 1.8055, 1-week = 1.8244, P < 0.05).

Observation interval	Plant	Extracts				
		Ethanol	Methanol	Water		
Before spray	Basil	14.75 ± 0.77 a	14.47 ± 0.79 a	14.11 ± 0.79 a		
1 0	Bitter apple	14.67 ± 0.77 a	14.78 ± 0.75 a	14.50 ± 0.62 a		
	Milkweed	14.50 ± 0.55 a	14.39 ± 0.52 a	14.78 ± 0.83 a		
	Thorn Apple	14.56 ± 0.80 a	14.11 ± 0.59 a	14.75 ± 0.95 a		
	Aloe vera	14.89 ± 0.39 a	14.64 ± 0.40 a	14.25 ± 0.88 a		
	Karir	14.36 ± 0.59 a	14.67 ± 0.57 a	14.81 ± 0.94 a		
	Siris	14.72 ± 0.68 a	14.19 ± 0.73 a	14.61 ± 0.93 a		
	Prickly Chaff flower	14.06 ± 0.71 a	14.53 ± 0.78 a	14.31 ± 0.86 a		
	Castor bean	14.89 ± 0.60 a	14.72 ± 0.61 a	14.22 ± 0.79 a		
	Field dodder	14.92 ± 0.71 a	14.78 ± 0.73 a	14.50 ± 0.96 a		
	Control	14.86 ± 0.61 a				
24-h	Basil	3.19 ± 0.42 n	$4.00 \pm 0.39 \mathrm{mn}$	6.61 ± 0.53 h-l		
	Bitter apple	4.36 ± 0.41 mn	4.14 ± 0.37 mn	6.86 ± 0.56 g-l		
	Milkweed	4.33 ± 0.45 mn	4.36 ± 0.41 mn	6.61 ± 0.54 h-l		
	Thorn Apple	5.36 ± 0.52 lm	4.81 ± 0.46 m	7.08 ± 0.68 f-i		
	Aloe vera	5.56 ± 0.36 i-m	5.44 ± 0.34 klm	7.11 ± 0.53 f-i		
	Karir	647 ± 0.42 i-l	7.03 ± 0.50 f-k	$7.92 \pm 0.69 d_{-i}$		
	Siris	7.31 ± 0.60 e-i	7.65 ± 0.60 f k	853 ± 0.67 c-f		
	Prickly Chaff flower	$8.00 \pm 0.63 d-i$	$8.11 \pm 0.58 d-h$	9.14 ± 0.73 a-d		
	Castor bean	$828 \pm 0.59 d_{-9}$	8 86 ± 0 49 b-e	9.89 ± 0.81 abc		
	Field dodder	10.47 ± 1.04 a	9.89 ± 0.69 abc	10.19 ± 0.01 ab		
	Control	$16.17 \pm 1.04a$ 16.53 + 1.23	5.07 ± 0.07 abc	10.17 ± 0.71 ab		
19 h	Basil	2.78 ± 0.24 m	2.67 ± 0.21 mp	6.14 ± 0.30 jik		
40-11	Bittor applo	2.70 ± 0.34 II 3.92 ± 0.40 mp	3.07 ± 0.31 IIII 3.91 ± 0.36 mp	6.14 ± 0.39 JK		
	Millawood	4.06 ± 0.281 mp	4.09 ± 0.30 lmn	6.22 ± 0.46 jil		
	Thorn Applo	4.60 ± 0.301 mm	4.00 ± 0.30 IIIII 4.53 ± 0.37 lm	6.69 ± 0.51 g i		
	Aloo vora	5.26 ± 0.25 ibl	5.00 ± 0.37 III	6.79 ± 0.42 f j		
	Karir	5.50 ± 0.55 JK	6.64 ± 0.49 g j	0.76 ± 0.42 1-1 7.64 ± 0.50 d b		
	Siric	7.19 ± 0.54 o j	7.22 ± 0.52 o i	9.14 ± 0.59 c f		
	Drickly Chaff flower	$7.19 \pm 0.54 e^{-1}$	$7.22 \pm 0.32 \text{ e}^{-1}$	0.14 ± 0.30 C-1		
	Castor boan	$7.44 \pm 0.39 \text{ e}^{-1}$	$7.69 \pm 0.32 \text{ u-g}$	$0.05 \pm 0.75 a$ -u		
	Field doddor	10.08 ± 0.32 def	0.50 ± 0.40 D-e	9.30 ± 0.70 abc		
	Control	$10.00 \pm 0.07 a$ 17.29 ± 1.22	9.01 ± 0.37 ab	9.89 ± 0.80 ab		
06 h	Pacil	267 ± 0.20	222 ± 0.20 no	6.00 ± 0.27 jilz		
90-11	Dasii Pittor applo	2.07 ± 0.300	3.53 ± 0.20 IIO	6.00 ± 0.37 IJK		
	Millawood	3.73 ± 0.30 mmo	3.01 ± 0.32 IIII0	5.25 ± 0.35 III		
	Thorn Apple	3.09 ± 0.33 IIII0	3.92 ± 0.33 IIII0	5.97 ± 0.45 IJK		
	Aloo vora	5.29 ± 0.22 ibl	4.93 ± 0.33 IIIII	6.50 ± 0.40 g j		
	Karir	5.20 ± 0.35 JKi 6.14 ± 0.27 hij	4.05 ± 0.35 Killi 6.44 ± 0.41 bij	$0.30 \pm 0.40 \text{ g}$		
	Siric	7.11 ± 0.57 mg	0.44 ± 0.41 mj	7.20 ± 0.51 C-H		
	Drickly Chaff flowor	7.11 ± 0.30 l-1 7.22 ± 0.49 o h	7.11 ± 0.491	8.60 ± 0.57 C-1		
	Castor boan	7.33 ± 0.49 e-fi	$7.72 \pm 0.30 \text{ u-g}$	0.07 ± 0.50 bcu		
	Field doddor	10.00 ± 0.70	$0.47 \pm 0.40 \text{ b}$	9.25 ± 0.59 abc		
	Control	$10.00 \pm 0.78 a$ 10.67 ± 1.00	$9.44 \pm 0.03 \text{ ab}$	$9.01 \pm 0.75 \text{ ab}$		
1 Woolr	Basil	10.07 ± 1.00	2.21 ± 0.20 or	$E 00 \pm 0.20$ ibl		
1-Week	DdSII Pittor applo	$2.76 \pm 0.27 \text{ p}$	$3.31 \pm 0.29 \text{ op}$	5.09 ± 0.30 JKI 6.21 ± 0.25 jilz		
	Millawood	3.00 ± 0.35 hop 2.60 ± 0.21 non	3.47 ± 0.32 0p 2.79 ± 0.20 non	0.51 ± 0.55 IJK		
	Thorn Apple	3.09 ± 0.31 hop	3.78 ± 0.30 http://www.sec.edu	6.00 ± 0.39 JKI		
		4.25 ± 0.35 IIIII0	4.22 ± 0.32 IIIII0 4.00 ± 0.20 lmm	0.04 ± 0.39 g-K		
	Aloe vera	5.47 ± 0.39 killi	4.89 ± 0.38 IIIII	$0.39 \pm 0.34 \text{ II-K}$		
	NdFIF Sinia	0.37 ± 0.41 N-K	0.50 ± 0.48 g-K	$7.44 \pm 0.51 \text{ e-1}$		
	SIFIS Decision Charles Charles	$7.33 \pm 0.58 \text{ e-l}$	0.97 ± 0.54 I-J	7.94 ± 0.58 eI		
	Prickly Unaff flower	$7.01 \pm 0.52 \text{ e-h}$	7.81 ± 0.53 erg	$8.50 \pm 0.60 \text{ D-e}$		
	Castor bean	8.22 ± 0.43 def	8.36 ± 0.39 cde	$9.33 \pm 0.53 a-a$		
	riela aodaer	$10.25 \pm 0.81a$	9.50 ± 0.58 abc	$9.69 \pm 0.76 \text{ab}$		
	Control	19.61 ± 2.12				

Table 2. Effect of various botanical ex	xtracts on <i>D. citri</i> por	oulation under field (conditions following	the second sr	oray.

*Means with same letters are not significantly different from each other (LSD values- before spray = 2.0309, 24-hours = 1.5902, 48-hours = 1.3807, 96-hours = 1.2543, 1-week = 1.2637, P < 0.05).



Figure 1. Effect of various botanical extracts on the corrected population reduction percentage of *D. citri* under field conditions following the first spray.



Figure 2. Effect of various botanical extracts on the corrected population reduction percentage of *D. citri* under field conditions following the second spray.

The mean *D. citri* populations in methanol basil (3.22 ± 0.26 psylla per flush), methanol bitter apple (3.39 ± 0.31 psylla per flush), methanol milkweed (3.83 ± 0.30 psylla per flush), and ethanol milkweed (4.06 ± 0.37 psylla per flush) were also comparable to these treatments. All three field dodder extracts (water: 9.67 ± 0.68 psylla per flush, ethanol: 9.33 ± 0.86 psylla per flush, and methanol: 9.33 ± 0.55 psylla per flush) were the least effective in reducing *D. citri* populations. The

control lemon treatment exhibited the highest *D. citri* population at various observation intervals (24, 48, 96 h, and one week post-spray) (Table 3). After one week, a highly significant difference was observed in the performance of various botanicals (F = 56.20, P < 0.001) and extracts (F = 39.35, P < 0.001) in controlling the *D. citri* population, while the interaction of extracts and plants showed no significant difference (F = 1.29, P = 0.1868).

Observation interval	Plant	Extracts				
		Ethanol	Methanol	Water		
Before spray	Basil	13.97 ± 0.89 a	14.11 ± 0.98 a	14.22 ± 0.83 a		
	Bitter apple	13.78 ± 0.85 a	13.92 ± 0.72 a	14.39 ± 0.70 a		
	Milkweed	13.56 ± 0.87 a	13.67 ± 0.73 a	13.67 ± 0.93 a		
	Thorn Apple	14.00 ± 0.89 a	14.14 ± 0.68 a	13.83 ± 0.91 a		
	Aloe vera	14.44 ± 0.97 a	13.84 ± 0.66 a	14.11 ± 0.94 a		
	Karir	14.22 ± 0.93 a	14.33 ± 0.73 a	13.97 ± 0.92 a		
	Siris	13.94 ± 0.91 a	13.78 ± 0.75 a	13.81 ± 1.03 a		
	Prickly Chaff flower	14.03 ± 1.01 a	13.89 ± 0.88 a	14.22 ± 0.87 a		
	Castor bean	14.64 ± 0.98 a	14.42 ± 0.77 a	14.00 ± 0.78 a		
	Field dodder	14.03 ± 0.90 a	14.17 ± 0.79 a	13.97 ± 1.04 a		
	Control	14.36 ± 0.96 a				
24-h	Basil	3.08 ± 0.36 n	3.78 ± 0.39 mn	6.06 ± 0.36 g-k		
	Bitter apple	3.69 ± 0.39 mn	3.89 ± 0.33 mn	6.56 ± 0.46 e-j		
	Milkweed	4.39 ± 0.45 lmn	4.56 ± 0.42 k-n	6.44 ± 0.44 e-j		
	Thorn Apple	4.97 ± 0.39 j-m	5.14 ± 0.42 j-m	7.22 ± 0.48 d-h		
	Aloe vera	5.50 ± 0.60 i-l	5.72 ± 0.34 h-l	6.94 ± 0.49 e-i		
	Karir	6.39 ± 0.63 e-j	6.92 ± 0.45 e-i	8.00 ± 0.68 b-e		
	Siris	7.00 ± 0.54 e-i	7.42 ± 0.58 c-g	8.67 ± 0.68 a-d		
	Prickly Chaff flower	7.44 ± 0.72 c-g	7.69 ± 0.49 b-f	9.11 ± 0.64 ab		
	Castor bean	8.97 ± 1.02 abc	8.94 ± 0.46 abc	9.86 ± 0.75 a		
	Field dodder	10.08 ± 1.17 a	9.83 ± 0.64 a	10.22 ± 0.84 a		
	Control	16.75 ± 1.35				
48-h	Basil	2.53 ± 0.28 o	3.22 ± 0.26 no	5.72 ± 0.34 h-k		
	Bitter apple	3.42 ± 0.35 no	3.50 ± 0.31 mno	6.22 ± 0.44 ghi		
	Milkweed	4.22 ± 0.34 lmn	4.14 ± 0.37 lmn	6.06 ± 0.40 g-j		
	Thorn Apple	4.53 ± 0.33 k-n	4.81 ± 0.33 j-m	6.81 ± 0.39 fgh		
	Aloe vera	5.28 ± 0.52 i-l	5.22 ± 0.30 i-l	6.56 ± 0.38 f-i		
	Karir	6.33 ± 0.61 f-i	6.53 ± 0.41 f-i	7.64 ± 0.53 c-f		
	Siris	6.78 ± 0.44 fgh	7.03 ± 0.38 e-h	8.22 ± 0.58 b-e		
	Prickly Chaff flower	7.14 ± 0.59 efg	7.31 ± 0.41 d-g	8.78 ± 0.70 abc		
	Castor bean	8.81 ± 0.83 abc	8.61 ± 0.39 bcd	9.56 ± 0.63 ab		
	Field dodder	9.56 ± 1.02 ab	9.47 ± 0.55 ab	10.00 ± 0.73 a		
	Control	17.78 ± 1.39				
96-h	Basil	2.53 ± 0.21 p	3.14 ± 0.20 op	5.58 ±0.30h -l		
	Bitter apple	3.14 ± 0.33 op	3.33 ± 0.30 op	5.89 ± 0.32 g-k		
	Milkweed	4.14 ± 0.32 mno	3.89 ± 0.33 no	5.94 ± 0.36 f-k		
	Thorn Apple	4.36 ± 0.30 l-o	4.72 ± 0.28 k-n	6.61 ± 0.37 e-h		
	Aloe vera	5.17 ± 0.38 i-m	5.03 ± 0.31 j-n	6.42 ± 0.33 e-i		
	Karir	6.22 ± 0.57 f-j	6.31 ± 0.36 e-i	7.50 ± 0.51 cde		
	Siris	$6.67 \pm 0.41 \text{ e-h}$	$6.89 \pm 0.43 \text{ d-g}$	8.11 ± 0.53 bcd		
	Prickly Chaff flower	7.19 ± 0.57 def	7.19 ± 0.42 def	8.61 ± 0.53 abc		
	Castor bean	8.75 ± 0.75 abc	8.47 ± 0.43 bc	$9.28 \pm 0.55 \text{ ab}$		
	Field dodder	9.14 ± 0.94 ab	9.33 ± 0.62 ab	9.81 ± 0.72 a		
	Control	18.14 ± 1.61	0.00			
1-Week	Basil	2.47±0.180	3.22±0.26no	5.72±0.33h-k		
	Bitter apple	3.19±0.34no	3.39±0.31mno	5.97±0.33g-j		
	Milkweed	4.06±0.37lmn	3.83±0.30lmn	5.89±0.34g-j		
	Thorn Apple	4.61±0.38klm	4.56±0.29klm	6.64±0.32t-i		
	Aloe vera	5.44±0.43ijk	4.78±0.38jkl	6.39±0.30t-i		
	Karır	6.39±0.59f-i	6.39±0.42t-i	7.39±0.46d-f		
	Siris	6.86±0.47e-h	6.81±0.47e-h	7.94±0.48cde		
	Prickly Chaff flower	7.36±0.56def	7.06±0.43efg	8.50±0.53a-d		
	Castor bean	8.61±0.69abc	8.33±0.39bcd	9.31±0.47ab		
	Field dodder	9.33±0.86ab	9.33±0.55ab	9.67±0.68a		
	Control	1833+166				

Table 3. Effect of various bo	otanical extracts on D.	. <i>citri</i> nonulati	on under field c	conditions followin	g the third si	nrav.
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*Means with same letters are not significantly different from each other (LSD values- before spray = 2.3992, 24-hours = 1.6015, 48-hours = 1.3728, 96-hours = 1.2741, 1-week = 1.2449, P < 0.05).

The corrected percentage reduction in *D. citri* due to the third spray of various botanical extracts is presented in Figure 3. Basil, bitter apple, milkweed, and thorn apple were the most effective botanicals, especially when extracted with ethanol or methanol, while their water extracts were the least effective. The effectiveness of all treatments increased with exposure time, peaking one week after application. At the end of the week, the highest reduction (86.52%) was observed with ethanol

basil extract, and the lowest (47.25%) with water field dodder extract. Among the other botanicals, notable reductions were recorded with bitter apple in ethanol (82.58%), milkweed in methanol (79.11%), thorn apple in methanol (75.13%), aloe vera in methanol (73.93%), karir in ethanol or methanol (65.15%), siris in methanol (62.85%), prickly chaff flower in methanol (61.49%), castor bean in methanol (54.56%), and field dodder in methanol (49.11%) (Figure 3).



Figure 3. Effect of various botanical extracts on the corrected population reduction percentage of *D. citri* under field conditions following the third spray.

DISCUSSION

The results of all three sprays conducted against *D. citri* in this study demonstrated that all botanical extracts were effective in reducing *D. citri* populations on lemon trees, though their effectiveness varied significantly. Among the plants tested, basil, bitter apple, and milkweed consistently showed the highest effectiveness across all three spray schedules, while field dodder and castor bean were the least effective. Of the solvents used for extraction, ethanol and methanol performed significantly better than water, particularly during the second spray.

The maximum corrected percentage reduction in *D. citri* populations during the three sprays was achieved with ethanol basil extract (87.72%, 85.84%, and 86.52%, respectively). This was followed by methanol bitter apple extract during the first and second sprays (85.66% and 82.31%, respectively) and ethanol extract during the third spray (82.58%), as well as milkweed

ethanol extract during the first two sprays (83.90% and 81.16%) and methanol extract during the third spray (79.11%). However, the results for these three plants with ethanol and methanol extracts were not significantly different from one another.

Several studies have investigated various botanicals and their essential oils for managing *D. citri* populations in citrus orchards, highlighting differential modes of action such as pesticidal, repellent, and antifeedant effects (Rizvi et al., 2018, 2019, 2023, 2024). Among these, Borad et al. (2001) evaluated eight botanicals against *D. citri* and citrus leaf miner, finding *A. indica* and *Ipomoea fistulosa* to be comparatively more effective, while *O. sanctum* (from the same genus as basil used in this study) was less effective in managing *D. citri* populations on kagzi lime trees. In contrast, the present study recorded a promising reduction of up to 87.72% in *D. citri* populations with the ethanol extract of basil.

Tayyab et al. (2022) evaluated 40 indigenous plants from Soon Valley, Punjab, for their insecticidal potential against D. citri and Aphis gossypii, reporting that Mentha *longifolia* was the most effective, causing 93% mortality of D. citri, followed by Melilotus officinalis (91%), Nerium indicum (89%), D. alba or thorn apple (88%), and Salvia officinalis (81%). The findings for thorn apple (88%) partially align with our study, where up to 80.95% D. citri mortality was observed after the first spray. Similarly, another study recorded 31.43% and 60.0% adult mortality of D. citri using the spray and leaf dip methods, respectively, and 85.71% nymphal mortality with the leaf dip method (Khan et al., 2013). Khan et al. (2014) also reported a significant reduction in adult and nymphal populations of *D. citri* using thorn apple, comparable to neem and synthetic insecticides.

Milkweed, evaluated in our study, also caused a significant reduction in *D. citri* population (82.12%), aligning partially with Ahmad et al. (2014), who found comparable effectiveness of milkweed (20%) extracts to synthetic insecticides. However, Ahmad et al. (2014) reported no significant effect of various concentrations of thorn apple and neem on *D. citri* populations, which also supports our findings. In our study, ethanol milkweed extracts caused up to 82.12% reduction in *D. citri* population after the first spray.

Although no significant studies specifically address the pesticidal effects of basil against *D. citri*, numerous research studies have demonstrated its pesticidal potential against various agricultural and stored grain pests and diseases (Chowdhary et al., 2018; Bincy et al., 2023). In stored grain pests, the fumigant and contact toxicity of *Ocimum basilicum* and its essential oil have been recorded against *Sitophilus oryzae*, with estragole (85.08%) identified as the major constituent and linalyl acetate (6.75%) as the minor constituent (Bincy et al., 2023). Similarly, the essential oil of *O. basilicum* and its components, estragole and linalool, have been found toxic to *S. zeamais* (Ouko et al., 2017; Moura et al., 2021), *Callosobruchus maculatus* (Kéita et al., 2001), and *Lasioderma serricorne* (Naveen et al., 2021).

Beyond stored grain pests, extracts and essential oils of *O. basilicum* have shown efficacy against agricultural crop pests such as *Spodoptera littoralis* (Benelli et al., 2019), *S. frugiperda* (Silva et al., 2017), *Acanthoscelides obtectus* (Rodríguez-González et al., 2019), and *Bemisia tabaci* (Aslan et al., 2004). Its potential extends to household pests like *Musca domestica* (Benelli et al., 2019) and

various mosquito species, including *Culex quinquefasciatus* (Benelli et al., 2019; Jabbar et al., 2022), *Anopheles stephensi* (Maurya et al., 2012), and *Aedes aegypti* (Tennyson et al., 2012; Khan et al., 2021). Moreover, it has demonstrated activity against pathogens like *Colletotrichum gloeosporioides* (Ogbebor et al., 2007) and ticks such as *Rhipicephalus appendiculatus*, *R. microplus*, and *Tetranychus urticae* (Aslan et al., 2004; Martinez-Velazquez et al., 2011).

The pesticidal activities of *O. basilicum* and other *Ocimum* species are attributed to their rich diversity of terpenoids, sesquiterpenes, and flavones (Chowdhary et al., 2018), which exhibit a wide range of effects, including cytotoxicity, insecticidal activity, insect repellency, allelopathy, antimicrobial, antioxidant, and anti-inflammatory properties (Dhifi et al., 2016). Moreover, studies suggest that *O. basilicum* is less toxic to natural enemies and may even act as an attractant, facilitating the management of noxious insect pest populations (Abreu et al., 2021).

Besides basil, ethanol and methanol extracts of bitter apple (*Citrullus colocynthis*) were also effective in reducing the population of *D. citri* on lemon trees. *C. colocynthis* is widely studied for its pesticidal properties against various pests and diseases affecting field crops, household settings, warehouses, and disease vectors (Larbi et al., 2021; Ali et al., 2022; Hassam et al., 2022; El-Halim et al., 2023). However, no systematic studies have yet evaluated its effectiveness against *D. citri*. The fruits of bitter apple are rich in oils containing linoleic acid (56%) and oleic acid (25%) (Khatri et al., 2021). Additionally, they contain diverse organic compounds, including amino acids, flavonoids, glucosides, alkaloids, alcohols, esters, fatty acids, and terpenoids, particularly cucurbitacins (Ahmed et al., 2020; Khatri et al., 2021).

Although basil, bitter apple, milkweed, and thorn apple were comparatively more effective in reducing *D. citri* populations on lemons in this study, neem extracts, neem-based pesticides, and neem oil remain the most extensively evaluated agents against *D. citri*. Numerous studies have demonstrated the ability of neem-based products to induce mortality in *D. citri* adults and nymphs, along with antifeedant and repellent effects, resulting in reduced populations and damage to citrus trees (Borad et al., 2001; Khan et al., 2013, 2014; Ahmad et al., 2014; Santos et al., 2015; Zia et al., 2021).

Other plant-based products evaluated for their pesticidal potential against *D. citri* include *Artemisia*

absinthium (Rizvi et al., 2018, 2023), guava (*Psidium guajava*) (Zaka et al., 2010; Poerwanto and Solichah, 2022), *Verbascum thapsus* (Reena et al., 2020), *D. brunonianum* (Rizvi et al., 2024), *Caulerpa sertularioides, Laurencia johnstonii*, and *Sargassum horridum* (González-Castro et al., 2019), *Sophora alopecuroides* (Rizvi et al., 2019), *Praxelis clematidea* (Wang et al., 2018), fir oil (Kuhns et al., 2016), *Piper aduncum* (Volpe et al., 2016), and essential oils from lavender, coriander, rose, thyme, tea tree, and rue, particularly 2-undecanone (Mann et al., 2012).

The type of extract, ethanol, methanol, or water, used in our study significantly influenced the efficacy of various plants in controlling *D. citri*. Previous studies have reported that solvents and extraction methods play a critical role in extracting active plant constituents, as well as their stability and solubility, which determine their insecticidal activity against target pests (Ahmed et al., 2019; Ahmad et al., 2021).

Sultana et al. (2009) demonstrated that aqueous ethanol and methanol solutions (containing 20% water) produced higher yields, phenolic contents, and antioxidant activity compared to absolute ethanol and methanol when evaluating *A. indica, Acacia nilotica, Eugenia jambolana, Terminalia arjuna, Moringa oleifera, Ficus religiosa,* and *A. barbadensis* using reflux and shaking extraction methods. Similarly, Sharma and Cannoo (2017) reported that methanol, as a solvent in Soxhlet extraction, yielded higher extraction efficiency, total phenolic content, total flavonoid content, and antioxidant potential from *Nepeta leucophylla* stems. Comparable findings were reported by Sharma and Cannoo (2016) and Iqbal et al. (2012).

Other studies have highlighted the significant impact of polar (e.g., ethyl acetate, ethanol, acetone, water) and non-polar solvents (e.g., n-hexane, petroleum ether, chloroform) on the extraction efficiency of phytochemicals for pesticidal or pharmacological applications. Combinations of polar and non-polar solvents often result in better extraction efficiency and antioxidant quality (Dirar et al., 2019; Truong et al., 2019; Nawaz et al., 2020; El Mannoubi, 2023).

Building on these findings, our study showed that ethanol and methanol extracts performed better than water extracts against *D. citri*. This enhanced efficacy may be attributed to the ability of ethanol and methanol to extract a greater number of active compounds responsible for the pesticidal activity of these plants.

CONCLUSION

All the botanical extracts tested were effective in controlling *D. citri* populations in lemon, with ethanol and methanol extracts of basil, bitter apple, and milkweed being significantly more effective than their water extracts. Therefore, it is recommended to use ethanol or methanol extracts of basil, bitter apple, or milkweed for managing *D. citri* in lemon. If ethanol or methanol extraction is not feasible, water extracts of these plants can also be used as an alternative. Moreover, further research on the chemical composition of basil, bitter apple, and milkweed is encouraged to identify the key compounds responsible for *D. citri* population reduction, which could aid in developing effective pesticide formulations.

AUTHORS' CONTRIBUTIONS

JAM conducted the experiments and collected data, IAN and MAK conceived and supervise study, ZP and FIN write the initial manuscript, JGMS and SHR help in data collection, AAG designed study, analyzed data and finalized the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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