

Research

Efficacy of herbal extracts in the management of cactus pest, *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) in Tigray Region, Ethiopia

Sisay Weldegebriel Zeweld¹ · Kiros Meles Ayimut² · Mebrahtom Gebrelibanos Hiben³

Received: 3 November 2023 / Accepted: 8 May 2024

Published online: 13 May 2024

© The Author(s) 2024 **OPEN**

Abstract

Cactus (*Opuntia ficus-indica*) is a drought-resistant plant and the source of food and income for many farmers during the rainy season, and the only option to feed livestock during the dry seasons, especially in the southern part of the Tigray region of Ethiopia. The region is currently suffering from a damaging parasitoid of cactus called *Dactylopius opuntiae*- ficus biotype. An experimental study was conducted from August 2022 to February 2023 to investigate the efficacy of herbal extracts against cochineal insects. The experiment was conducted on the insecticidal property of methanol extracts of *Solanum linnaenum*, *Euphorbia tirucalli*, *Nerium oleander*, *Tephrosia vogelii*, *Calpurnia aurea*, *Argemone Mexicana*, *Datura stramonium* and *Ricinus communis*. Herbs were extracted, and their efficacy and LC₅₀ were determined. Phytochemical screening for secondary metabolites was performed. There was a statistically significant difference in the mean mortality of insects across the levels of the herbs ($F(8, 71) = 57.812, p = 0.0001, \eta^2 = 0.867$) and the experimental extract concentrations ($F(1, 71) = 4198.3, p = 0.0001, \eta^2 = 0.983$). The individual extracts of *S. linnaenum* and *N. oleander* caused average mortality rates of 99.3% and 97.4%, respectively, at 5% extract concentration. Tannins and saponins were the major metabolites found in the extracts. The present study demonstrated the promising insecticidal efficacy of herbal extracts against the damaging cacti pest *D. opuntiae*-ficus biotype, providing insights into sustainable pest control strategies for protecting vital cactus resources in the Tigray region.

Keywords Cactus · Cacti pest · Cochineals · Herbal extract · Mortality · Phytochemical screening

1 Introduction

The Tigray region is largely covered by cacti (*Opuntia ficus-indica*). This plant is traditionally grown in rural areas of Tigray, and the fruit, locally known by the vernacular name 'Beles,' belongs to the genus *Opuntia* in the family Cactaceae. Cactus is the primary source of food for many farmers during the rainy season, especially from June to September, just before the harvesting of staple crops (cereals). A cactus thrives in this environment and plays a special role in food security for both humans and livestock [1, 2]. The importance of cactus is increasing over time. Research findings have indicated that cactus pear has become an integral part of the culture and economy of Tigray, serving as a source of seasonal household food, income, and employment, as well as livestock feed and providing other environmental benefits. It is also a source of income for school

✉ Sisay Weldegebriel Zeweld, sisayatthis@gmail.com; Kiros Meles Ayimut, kiros62@yahoo.com; Mebrahtom Gebrelibanos Hiben, mmgshn@gmail.com | ¹Mekelle University College of Veterinary Sciences, Mekele, Ethiopia. ²Mekelle University College of Dry Land Agricultural and Horticultural Sciences, Mekele, Ethiopia. ³College of Health Sciences, Department of Pharmacognosy, Mekelle University, Mekele, Ethiopia.



children and has become an occupation for adults, especially women, due to a steady increase in the price of fresh fruits. In southern Tigray, much of these fruits are harvested from wild prickly pear cactus plantations [3, 4].

Despite the benefits to humans and livestock, the cactus plant is a suitable host for diseases and pests such as the cochineal insect. Cochineal insects, particularly *Dactylopius opuntiae*, are known as potentially damaging pests that grow and reproduce on pear cladodes [5]. The cochineal insect, often referred to as a mealybug, is a parasitoid of the cactus plant and is a sucking insect that feeds exclusively on certain cactus species [6]. There are various species of cochineal insects, each of which feeds on specific cactus species. *Dactylopius coccus*, also known as 'Red Gold,' is a valuable resource for producing carminic acid (a colorant) and is a significant livelihood in Mexico, Peru, and India [7]. However, the introduction of *D. opuntiae* to Ethiopia's Tigray region has led to significant damage to cactus plants [4, 8].

D. coccus is known for producing a dye of superior quality and is the only commercial species reared specifically for carminic acid production [9]. Cochineal insects cover themselves with a waxy coat, appearing as white cottony tufts attached to cactus pads and stems. These white sticky mounds serve as housing for cochineal bugs. Cochineal insects exist in two forms based on their survival in the environment: wild and domestic (commercial or cultivated) cochineals [10]. Wild cochineals are smaller in size compared to domestic cochineals and produce lower-quality dye. Domestic cochineals, such as *D. coccus*, are delicate, sensitive to cold winters, and vulnerable to insects or natural enemies. On the other hand, wild types like *D. opuntiae* have no identified natural enemies and are resistant to harsh environmental and climatic conditions [11].

The introduction of wild-type *D. opuntiae* from South Africa to the Tigray region in April 2003, supported by a FAO-Technical Cooperation Program (TCP) project hosted by Mekelle University, aimed to establish cochineal insect nurseries for commercial purposes. However, the uncontrolled spread of cochineal insects has become a serious issue due to the failure to harvest and utilize them effectively. Cochineal insects have spread rapidly across geographical areas through wind dispersal, flood transportation, human activity, and animal interactions, resulting in extensive infestation of cactus-covered areas [12–14].

The insect infestation causes damage to cactus cladodes, reduces cactus pear size, and threatens cactus plantations. Many people view this invasion as a future threat to cactus cultivation. The communities that rely on cactus plants are experiencing significant destruction, and the impact of the cochineal insect has become a serious concern in the region. The absence of effective mechanisms or quarantine laws to control the spread of cochineal insects to other regions and the lack of designated safe zones for cochineal production in Tigray have exacerbated the severity of the infestation. Additionally, the low market price of fresh cochineal (less than USD 1 per kilogram) offered by Foodsafe Company, combined with the difficulty of harvesting the insect from spiny cactus cladodes, has contributed to the damage of cactus plants and the resulting food and economic insecurity in the region [15, 16].

Scientific efforts have been made by the Tigray Agricultural Research Institute (TARI), Tigray Regional Agricultural Bureau, Mekelle University, and other sectors to control cochineal infestations using chemical sprays, liquid soap solutions, mechanical removal of infested cacti, and burning. However, these attempts have been largely unsuccessful despite ongoing efforts. There is a hypothesis that repeated spraying of macerated herbal materials may prevent cochineal infestations. Herbal crude extracts are complex mixtures of chemical components that exhibit synergistic effects. Most herbal extracts function as:

(i) Repellents that drive insects away from plants due to their smell or taste [17], (ii) Anti-feedants that cause insects to reduce food intake, leading to starvation [18], (iii) Oviposition deterrents that prevent insects from laying eggs [19], (iv) Inhibitors that disrupt the development of various insect stages [20–22].

Therefore, this research aimed to investigate effective herbal extracts against cochineal insects. The locally available herbs included in the investigation were *Solanum linnaeanum*, *Euphorbia tirucalli*, *Nerium oleander*, *Tephrosia vogelii*, *Calpurnia aurea*, *Argemone mexicana*, *Datura stramonium*, and *Ricinus communis* (Table 1).

2 Materials and methods

2.1 Experiment site, study subjects, and study design

The experiment was conducted within the compound of Mekelle University in the Tigray region of Ethiopia. The university's mountainous border is covered with various varieties of cactus. The experimental plants were sourced from the local environment, and 200 wild-type female cochineal insects (*Dactylopius opuntiae*) were used in the experiment.

A randomized block design was implemented from August 2022 to February 2023 to assess the efficacy of herbal extracts against cochineal insect. Each plant extract constituted a block, and nine concentrations (1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, and 5%) were considered within each block. Three replicates were conducted for each plant extract.

Table 1 Plant species used in the experiment, availability, and ecological status in the study area

Plant name	Common name	Availability and status
<i>Solanum linnaeanum</i>	Apple of sodom	Easily available; Invasive (alien)
<i>Euphorbia tirucalli</i>	Pencil tree, firestick	Easily available; Invasive (alien)
<i>Nerium oleander</i>	Oleander	Easily available; Invasive (alien)
<i>Tephrosia vogelii</i>	Fish poison bean	Native
<i>Calpurnia aurea</i>	Wild laburnum	Native
<i>Argemone mexicana</i>	Mexican poppy, prickly poppy	Easily available; Invasive (alien)
<i>Datura stramonium</i>	Jimsonweed, thornapple	Easily available; Invasive (alien)
<i>Ricinus communis</i>	Castor bean	Easily available; Invasive (alien)

Source: [8, 15]

2.2 Sampling method

Locally available plants, namely *S. linnaeanum* (fruits), *E. tirucalli* (leaf), *N. oleander* (leaf), *T. vogelii* (leaf), *C. aurea* (leaf), *A. mexicana* (leaf and stem), *D. stramonium* (seeds), and *R. communis* (leaf), were purposely selected for the study.

2.3 Study methods

2.3.1 Collection and extraction of herbal materials

The herbs were collected from the field, including leaves, fruits, seeds, and stems of the experimental plants, and transported freshly with clean and dry trays to the laboratory. The herbal materials were washed with tap water to remove dust and surface contaminants, then shade-dried and ground into a fine powder for maceration. Based on the nature and characteristics of the herbs described in the literature, ethanol (97%) and methanol (99.8%) were chosen as co-solvents, along with distilled water, for maceration.

Five hundred grams (500 g) of each experimental plant powder was macerated in flasks containing 2 L of solvent. Each plant powder was macerated with three different solvents. The flasks were agitated for 10 min, four times a day, over 24 h, and allowed to settle at room temperature. The supernatant was decanted into clean 3-L glass containers. Solvents were added to the sediment, and the process was repeated three times over 72 h to maximize extract yield.

The collected supernatant liquid was filtered through Whatman No. 1 filter paper using an electrical suction pump. The filtered liquid was then evaporated in a water bath at 42 °C for 6 h for methanol [23] and 12 h for ethanol [24] to obtain dried crude oil extracts, which were stored at 4 °C. The dried extracts of the experimental plants were collected and weighed to determine the yield of each herb using the following formula [25, 26]:

$$\text{Yield \%} = \frac{\text{Weight of crude oil in grams}}{\text{The initial weight of the herbal powder in grams}} \times 100$$

2.3.2 Establishment, inoculation, and insect count

Cochineal insect-free cactus cladodes were collected from the field and established in the laboratory in plastic containers filled with wet soil. The experiment with herbal extracts was conducted both indoors and outdoors to test different herbs at various concentrations. Engorged adult female cochineals (200 in number), along with their white waxy substance containing both adults and eggs, were collected from infested cladodes in the field. These cochineals were then placed on each of the established experimental cladodes indoors and outdoors. The inoculated experimental cladodes were left for 21 weeks (5 months) to achieve a high level of infestation similar to the natural environment. Viable cochineal insects were carefully counted by three individuals before spraying to obtain an average count as pre-treatment data (resulting in different average values for the 27 groups of cacti). Similarly, dead insects were counted and recorded after the application of the experimental spray preparations (post-treatment data), and mortality rates were calculated for each experimental extract solution.

2.3.3 Preparation, application of spray, and determination of LC₅₀ of extracts

Dried crude oil extracts and their combinations, extracted with methanol 99.5%, were prepared at different concentrations (1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, and 5%), and then tested against adult cochineals and their eggs. The preparations were applied to established experimental infested cactus cladodes indoors and outdoors. Cochineal colonies were counted before spraying and then sprayed with different extract solutions to determine the lethal concentration 50 (LC₅₀). At the end of week 21 (5 months), insects were carefully counted by three individuals before spraying to obtain pre-treatment data. Similarly, viable insects were counted after spraying (post-treatment data) at 24, 48, and 72 h following application. Mortality rates or percentage reduction (% R) were calculated to determine the efficiency of the extract using the formula provided by Carey [27].

$$\% \text{ Reduction (mortality)} = \frac{\text{Number of deaths (Pre – treatment – Post – treatment data)}}{\text{Total number of insects before spray (Pre – treatment data)}} \times 100$$

2.3.4 Comparison for efficiency

The effectiveness of herbal extracts was determined based on mortality percentages relative to their corresponding experimental concentrations. Equal amounts of each extract were combined and prepared at different experimental concentrations ranging from 1 to 5% at 0.5% intervals to assess their synergistic effects. Additionally, the LC₅₀ (median lethal concentration) was determined for each herbal extract.

2.3.5 Differentiation between dead and viable cochineal insects and eggs

- (i) Adult females: Experimentally infested cactus cladodes were sprayed with different experimental spray solutions. Subsequently, the cladodes were tightly covered with a mosquito net featuring a 0.6 mm pore diameter, preventing crawlers from entering or exiting. After 72 h, the cladodes were uncovered and carefully observed for the secretion of a white waxy substance on the black surface of the adult females, indicating their viability.
- (ii) Adult males and crawling stages: Moving adult male cochineals and crawlers were directly exposed to the spray and observed for any movement indicating recovery for up to 30 min after application.
- (iii) Eggs: A cochineal-infested cladode was sprayed with distilled water to remove the white waxy materials from the surface of adult females and crawling insects. All female insects were then removed from the cladode surface to expose the tiny gray-colored eggs. The cladodes were placed in shade to protect the eggs from direct sunlight. Subsequently, experimental sprays were applied to a cladode containing only eggs, which was covered with a mosquito net. After 90 days of the insect life cycle, the cladodes were thoroughly observed for insect development. The presence of any developmental stage (refer to Fig. 1) on the cladodes indicated that the eggs had survived the spray's effects and hatched into crawlers, which eventually grew into adults.

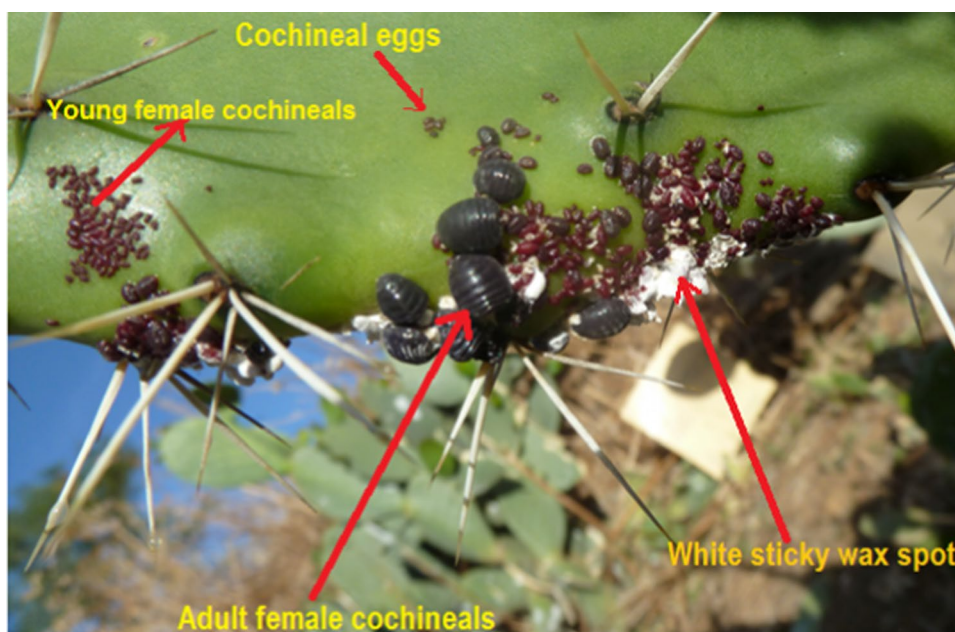
2.4 Observation for re-infestation

Two highly infested cactus cladodes were placed side by side, with one cladode sprayed using the most effective spray preparation and the other left untreated. The sprayed cladodes were observed daily for any signs of re-infestation.

2.5 Stability and persistence of extract

The stability of herbal extract products refers to their ability to maintain their physical, chemical, and insecticidal properties (efficacy) during storage and application on target plants [28, 29]. Monitoring storage period, temperature, and relative humidity is one approach used in stability testing to assess changes in chemical and physical characteristics, as well as any alteration in insecticidal effectiveness over time.

Fig. 1 A cladode infested with female cochineal and their eggs



2.5.1 Effect of storage time

The most effective sprayable extract solutions were stored at room temperature, ranging from 27 °C in April to 23 °C to 24 °C in May and June. The relative humidity (RH) during storage fluctuated between 62% (from January to June) and 75% (from July to December).

2.5.2 Physical and pH changes

The extracts were stored for a duration of 24 months, and their efficacy against cochineal insects was assessed at specific intervals: day 0, 1 month, 6 months, 1 year, and 1.5 years of storage. Short-term environmental changes resulting from opening the doors of the storage compartment were acknowledged as unavoidable. Killing percentages were determined at each storage period.

2.6 Skin patch test of the extract solution

The toxicological analysis using the Patch Test Technique (PTT) to assess the acute effects of an herbal extract solution against cochineal insects was conducted following established protocols at the Ethiopian Food, Medicine, and Health Care Administration and Control Authority (FMHACA). The test involved directly applying the final sprayable extract solution onto the skin of laboratory mice as animal models. Small patches containing the test solution were secured onto the skin of the mice using hypoallergenic adhesive tape, with careful consideration to minimize stress and discomfort to the animals. The patches remained in place for 24 h to allow for skin exposure. Following patch removal, the skin sites were closely observed for signs of acute toxicity, irritation, erythema, edema, or other adverse reactions at 24, 48, and 72 h post-exposure. This procedure was conducted by ethical guidelines and animal welfare regulations to ensure the safety and integrity of the study.

2.7 Qualitative phytochemical screening of experimental herbs

Phytochemical analysis was conducted to assess the presence of various secondary metabolites, including terpenoids, phenols, tannins, flavonoids, alkaloids, and saponins, using standard methods (Table 2).

Table 2 Preliminary qualitative phytochemical screening testing methods and observations

Phytoconstituents	Test	Observation	References
Tests for alkaloids	Mayer's test	Yellow color precipitate	Edeoga et al. [30]
Tests for flavonoids	Alkaline reagent test	Yellow color	Joshi et al. [31]
Test for saponin	Froth formation (foam) test	Layer of foam	Du et al. [32]
Test for tannins	Braymer's (ferric chloride) test	Black or blue-green precipitate	Talukdar et al. [33]
Test for phenolic compounds	Ferric chloride test	Deep blue or black color	Talukdar et al. [33]
Test for terpenoids	Salkowski test	Reddish brown color	Thite et al. [34]
Test for steroids	Salkowski test	A reddish brown ring at the junction	Yadav and Agarwala [35]

Table 3 Yield percentage of the experimental herbs macerated with different solvents

Herb	Amount of herbal powder	Amount of extract (gm) and yield % by the type of solvent used		
		Ethanol 97%	Methanol 99.8%	Distilled water
<i>S. linnaeum</i> (fruits)	500gm	64 (12.8%)	69 (13.8%)	33 (6.6%)
<i>E. tirucalli</i> (leaf)	500gm	42 (8.4%)	47 (9.4%)	29 (5.8%)
<i>N. oleander</i> (leaf)	500gm	71 (14.2%)	76 (15.2%)	35 (7.0%)
<i>T. vogelii</i> (leaf)	500gm	66 (13.2%)	74 (14.8%)	42 (8.4%)
<i>C. aurea</i> (leaf)	500gm	64 (12.8%)	69 (13.7%)	39 (7.8%)
<i>A. Mexicana</i> (leaf & stem)	500gm	48 (9.6%)	51 (10.2%)	31 (6.2%)
<i>D. stramonium</i> (seeds)	500gm	44 (8.8%)	48 (9.6%)	33 (6.6%)
<i>R. communis</i> (leaf)	500gm	54 (10.8%)	58 (11.6%)	29 (5.8%)

2.8 Data analysis

Analysis of Covariance (ANCOVA) was employed to compare the mean mortality percentages, assess the homogeneity of variance across different levels of the independent variable (experimental herbs), and determine the effect of both the main independent variable and the covariate (concentration of herbal extracts) on the dependent variable (mortality %). A significance level below 0.05 (indicating a higher *F*-value) was considered statistically significant, indicating a difference in mean mortality among the experimental herbs.

3 Results

3.1 Extraction yield of the experimental herbs

The yield percentages of the herbs were calculated for the different solvents. Among all the experimental herbal materials, relatively higher yield percentages were obtained with methanol (99.8%) maceration, while the lowest yields were obtained from distilled water maceration (Table 3).

3.2 Killing efficiency and lethal concentrations of extracts

The mean mortality percentage recorded by the admixture of extracts of *S. linnaeanum* and *N. oleander* was about 81%, whereas that of *S. linnaeanum* alone was 71.8% and *N. oleander* alone was 68.8% (Table 4). The plant *D. stramonium* caused the lowest mean mortality at 57.8% (Table 4). The actual insect mortalities of the experimental herbs at different extract concentrations are displayed in Table 5, and the LC50 of the extracts ranges between 1 and 3% (Table 5).

The test of homogeneity of variances using Levene's test showed that there was a statistically significant difference ($F(8, 72) = 4.613, p = 0.0001$) in the variances of mortality of cochineal insects across the different experimental

Table 4 Analysis of covariance (ANCOVA) results of mean mortality of herbal extracts

	N	Mean	Std. deviation	Std. error	95% confidence interval for mean		Minimum (at 1%)	Maximum (at 5%)
					Lower bound	Upper bound		
<i>S. linnaenum</i>	9	71.756	21.8010	7.2670	54.998	88.513	38.7	99.3
<i>E. tirucalli</i>	9	67.033	22.1784	7.3928	49.986	84.081	33.8	96.2
<i>N. oleander</i>	9	68.767	22.0664	7.3555	51.805	85.728	35.7	97.4
<i>T. vogelii</i>	9	60.822	21.0348	7.0116	44.653	76.991	29.2	91.0
<i>C. aurea</i>	9	62.856	21.2786	7.0929	46.499	79.212	30.6	92.8
<i>A. mexicana</i>	9	65.489	22.8117	7.6039	47.954	83.024	31.8	95.7
<i>D. stramonium</i>	9	57.844	21.0547	7.0182	41.660	74.029	27.5	88.6
<i>R. communis</i>	9	61.678	20.5617	6.8539	45.873	77.483	31.5	91.4
Admixture of Solanum & Nerium	9	80.967	16.7831	5.5944	68.066	93.867	57.2	99.8
Total	81	66.357	21.1014	2.3446	61.691	71.023	27.5	99.8
Model	Fixed effects		21.1278	2.3475	61.677	71.037		
	Random effects			2.3475 ^a	60.943 ^a	71.770 ^a		

Table 5 Herbs with their corresponding mortality (%) at different extract concentrations

Spray preparation	Cochineal mortality (%) per concentration (%) (g/100 ml of water)										
	1%	1.5%	2%	2.5%	3%	3.5%	4%	4.5%	5%	SE	SD
<i>S. linnaenum</i>	38.7	46.8	54.3*	65.7	74.2	81.8	89.4	95.6	99.3	7.3	21.8
<i>E. tirucalli</i>	33.8	41.9	50.7*	59.3	68.7	76	84.5	92.2	96.2	7.4	22.18
<i>N. oleander</i>	35.7	43	51.8*	62.1	71.4	78.5	86	93	97.4	7.35	22.07
<i>T. vogelii</i>	29.2	37.1	47	54*	61.3	68.4	74.3	85.1	91	7.01	21.03
<i>C. aurea</i>	30.6	38.4	48.3	55.4*	67.7	69.8	75.7	87	92.8	7.09	21.28
<i>A. mexicana</i>	31.8	39	48.6	57.9*	67	74.8	83.4	91.2	95.7	7.6	22.81
<i>D. stramonium</i>	27.5	34	44.2	49.3	57.8*	66	70.2	83	88.6	7.02	21.05
<i>R. communis</i>	31.5	38.4	47.7	54.6*	62	69	74.9	85.6	91.4	6.85	20.56
Admixture of Solanum and Nerium	52.7*	59.2	66.7	74.3	87.8	92	95.3	96.4	99.8	5.87	17.62

^aLethal Concentration fifty (LC50), SD standard error, SD standard deviation

concentrations of the extracts. The pair-wise comparison tests showed that there is a statistically significant difference ($p < 0.05$) in the mean mortality of insects among the different experimental concentrations of the herbal extracts except for *E. tirucalli* and *N. oleander* ($p = 0.184$), *E. tirucalli* and *A. Mexicana* ($p = 0.236$), *T. vogelii* and *C. aurea* ($p = 0.120$), *T. vogelii* and *R. communis* ($p = 0.510$), and *C. aurea* and *R. communis* ($p = 0.366$). Levene's Test of Equality of Error Variances table is not given here.

There was statistically significant difference in the mean mortality of insects across the levels of the main effect (experimental herbs) ($F(8, 71) = 57.812$, $p = 0.0001$, $\eta^2 = 0.867$) and the covariate (concentration of extracts) ($F(1, 71) = 4198.3$, $p = 0.0001$, $\eta^2 = 0.983$) with the effect sizes (Partial Eta Squared) of 86.7% and 98.3%, respectively. This tells that the different herbs and their corresponding experimental concentrations have a high effect on cochineal insect mortality (Table 6).

3.3 Physical and pH changes and effect of storage time on insect mortality

The admixture of extracts of *S. linnaenum* and *N. oleander* was kept at room temperature for 24 h, one month, 6 months, one year, and for a year and a half with mortality of 99.6%, 99.54%, 99.52%, 99.4%, and 99.21%, respectively. This showed that there was no statistically significant ($F = 2.132$, $p = 0.053$) difference in the mean mortality of insects on the cactus cladodes sprayed with the admixture. Physical and pH changes through the storage period were determined, and the initial pH values of the spray preparations fell in the acidic (pH = 4.3) and dark green, and there was no change for a year and a half period. The admixture of extracts of *Solanum* and *Nerium* plants prevented cochineal re-infestation for 93 days.

Table 6 ANCOVA tests between subjects' effects (herbs and concentration) on insect mortality

Source	Type III sum of squares	df	Mean square	F	Sig	Partial eta squared (η^2)
Corrected model	35086.922 ^a	9	3898.547	517.865	0.000	0.985
Intercept	13469.661	1	13,469.661	1789.246	0.000	0.962
Concentration	31605.210	1	31,605.210	4198.287	0.000	0.983
Herb	3481.712	8	435.214	57.812	0.000	0.867
Error	534.497	71	7.528			
Total	392282.530	81				
Corrected total	35621.419	80				

^aR Squared = 0.985 (Adjusted R Squared = 0.983)

3.4 Qualitative phytochemical screening of herbal extracts

The present study carried out on the herbal crude oil extract samples revealed the presence of active metabolites. Tannins were detected in all of the extracts and saponins were found in all of the extracts except in *E. tirucalli* (leaf) and *A. mexicana* (leaf and stem) (Table 7).

3.5 Skin patch test result

The toxicological analysis using the Patch Test Technique (PTT) assessed the acute effects of a sprayable herbal extract solution against cochineal insects. The solution was applied to laboratory mice via skin patches and monitored for 24, 48, and 72 h post-exposure. No signs of acute toxicity, irritation, or allergic reactions were observed during the observation period.

4 Discussion

The application of synthetic insecticides like organochlorine, organophosphate, and carbamates are major tools in insect pest control programs [36, 37]. Large-scale application of these chemical insecticides affects human and animal health and leaves environmental residues and these chemical insecticides contribute to biological magnification. Moreover, other non-target organisms or beneficial insects get affected and it leads to insecticide resistance [38, 39]. The biological control program is, therefore, an alternative approach and is a sustainable method of insect pest control. Plants produce numerous phytochemicals, many of which have medicinal and pesticide properties like larvicidal and repellent activities against different species of mosquitoes [40–44]. More than 1,200 plant species have been reported with potential insecticidal value [45, 46]. Secondary metabolites of the plants act as a defense mechanism against herbivores and other environmental factors. Insecticidal activities of some groups of phytochemicals such as saponins, alkaloids, tannins,

Table 7 Results of phytochemical analyses of the selected eight experimental plants

Herbal extract	Test results (+ presence, –absence)						
	Alkaloids	Flavonoids	Saponin	Phenolics	Tannins	Terpenoids	Steroids
<i>S. linnaenum</i> (fruits)	+	+	+	–	+	+	+
<i>E. tirucalli</i> (leaf)	+	+	–	–	+	–	+
<i>N. oleander</i> (leaf)	+	+	+	–	+	+	+
<i>T. vogelii</i> (leaf)	–	–	+	–	+	+	–
<i>C. aurea</i> (leaf)	+	+	+	+	+	+	–
<i>A. Mexicana</i> (leaf and stem)	+	+	–	+	+	–	–
<i>D. stramonium</i> (seeds)	+	–	+	+	+	–	+
<i>R. communis</i> (leaf)	+	–	+	+	+	–	–

+ indicates presence; –indicates absence

flavonoids, terpenoids, phenols, coumarins, quinines, and steroids have been reported previously from different plant species [39, 40, 47, 48]. Farmers may lack the capacity for methanol extraction to prepare herbal insecticides, suggesting a need for institutions like universities or agricultural bureaus to establish extraction labs for ready-made solutions. This initiative can facilitate access to herbal insecticides for local farmers, aiding in pest control efforts.

The present work investigated the effect of extracts of experimental herbs on the cochineal insect of cactus. In the investigation, the mean mortality percentage recorded by the admixture of fruit extract of *S. linnaenum* and leaf of *N. oleander* was about 81%, whereas that of *S. linnaenum* and *Nerium oleander* was 71.8% and 68.8%, respectively. Despite the difference in the killing efficacy, methods of extraction and concentration of the herbal extracts used, the present study revealed a positive effect in killing the cochineal insect and studies in the same region by Weldemariam and Welderufael [49] and Fitiwy et al. [8]) who used extracts of hydro-distillation of *E. globulus* and simple water maceration of *N. glauca*, respectively, showed similar effect in reducing the population of the insect from experimental cochineal infested cladodes.

The phytochemical screening and qualitative estimation of the plants studied showed that the leaf, seed, and fruit extracts were rich in alkaloids, flavonoids, saponins, phenolics, tannins, terpenoids, and steroids. The ethanol extracts obtained from both leaf and seed in *D. stramonium* have insecticidal, repellent, acaricidal, and oviposition deterrent properties and this is due to the phytochemical constituents of alkaloids and tannins in it [50] and this was in agreement with the current finding that the extract of *Datura* to show a killing effect on cochineal insect of cactus. Reagan et al. [44], and Warikoo and Kumar [51] reported that *A. Mexicana* extract has an effect on insects and this supports the result of the current study that the extract has a killing effect on cochineal insects. The present study shows that the *A. mexicana* plant extracts have the potential for the development of new and may be safe control products for cochineals. As naturally occurring insecticides, these plant-derived materials could be useful as an alternative to synthetic insecticides.

In the present study, leaf extract of *C. aurea* 5% caused a mean mortality of 92.8% which is similar to the findings of Hiruy and Getu [52], and Abubeker et al. [53] that the plant extract has an insecticidal effect on agricultural insect pests. Mwine and Van-Damme [54] reported that the extract and latex of *E. tirucalli* have insecticidal, nematocidal, piscicidal, and molluscicidal features and this finding is in agreement with the current study showed a killing effect on the cochineal insect of cactus. The finding of Kodjo et al. [55] is similar to the current study in that the extract of *R. communis* has a bio-insecticidal effect. Extracts from leaves and seeds of castor bean *R. communis* L. (Euphorbiaceae) have been used successfully in the management of curculionids of agricultural importance [56]. They cause death by ingestion and contact (which include clogging of the respiratory spiracles) or repel insects [57]. Ethanol extracts have been shown to have biological activity against insects [58].

The present study revealed that extract of *Tephrosia vogelii* caused high mean mortality (91%) of cochineal insects and similarly Kerebba et al. [59] and Alao and Adebayo [60] noted that it has an insecticidal effect on different agriculture pests. Moreover, WHO [61] reported that the higher mortality of agricultural insects is because of the lipophilic contents of the plant extract that is easily taken up through the respiratory spiracles. Medicinal and or toxic herbal extracts have different phytochemical constituents among which alkaloids, flavonoids, saponin, phenolics, tannins, terpenoids, and steroids have insecticidal and antimicrobial effects [62], which is in agreement with the present study findings that the phytochemical contents of the experimental herbs had an interesting killing potential on the cochineal insect pest of the cactus plant. In the present investigation, the efficacy of the extracts doesn't change with the storage periods, and this finding is similar to the study by Meena et al. [63] that shows no change in efficacy due to storage time and sunlight exposure of extracts. The present study showed three months of prevention of cochineal re-infestation of cactus plants by the herbal extracts, and a similar finding was presented by Montanucci [64] on cactus mealy bugs.

5 Conclusion and recommendations

The wild type of the cochineal insect, *D. opuntia-ficus* biotype, in the Tigray region, can be effectively controlled using environmentally friendly herbal extracts, particularly those derived from *S. linnaenum* fruit and *N. oleander* leaf, either individually or in combination with a 5% concentration. However, it is important to note that farmers may not have the capacity to undertake methanol extraction to prepare these herbal insecticides themselves. Instead, local governmental institutions such as universities or agricultural bureaus could establish extraction laboratories to provide ready-made herbal insecticides to local farmers.

Therefore, to address the issue of cactus pest management effectively, there needs to be a comprehensive approach involving education and awareness among farmers about the nature of the insect pest and the available control methods.

Local institutions should take the lead in establishing extraction facilities and distributing herbal insecticides to farmers. Additionally, farmers should be encouraged to adopt sustainable pest management practices, including creating buffer zones, manually removing infested cacti, and using organic insecticides. Collaboration between farmers, governmental institutions, and agricultural stakeholders is crucial to managing the cochineal insect pest in the region. By utilizing locally available herbal resources and establishing extraction facilities, sustainable control measures can be implemented to protect cactus plantations and ensure food security for the community.

Acknowledgements The authors are thankful to the Ethiopian Ministry of Science and Innovation for funding the project and providing full support, supervision, and encouragement during the study hosted by Mekelle University.

Author contributions Sisay Weldegebriel Zeweld (the corresponding author) collected, analyzed the data, interpreted, and wrote the manuscript. Kiros Meles Ayimut summarized the data, reviewed the manuscript, collaborated on the grammar correction, and formatted the whole text. Mebrahtom Gebrelibanos Hiben conducted the qualitative phytochemical analysis of herbal extracts and reviewed the manuscript. All authors read and approved the final manuscript.

Funding The Ethiopian Ministry of Science and Innovation funded the project.

Data availability All collected raw data are available from corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Our study sourced plants from their natural habitats, ensuring sustainable harvesting practices. No specific permissions were required, but we remain committed to ethical standards and environmental responsibility in our research.

Consent for publication All authors are aware of the submission to the journal.

Competing interests The authors declare that there is no competing interests regarding the publication of this paper.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

1. Nobel PS. Cacti: biology and uses. Berkeley: University of California Press, California, United States; 2002.
2. Makkar H. Feed demand landscape and implications of food-not-feed strategy for food security and climate change. *Animal Production and Health Division, Rome* 00153, Italy. 2018;12:1744–54.
3. Haile M, Belay T, Zimmerman H. Current and potential use of cactus in Tigray, Northern Ethiopia. *Proceedings of the IV International Congress on Cactus Pear and Cochineal, Mekelle, Ethiopia*. 2000; 581: 75–86
4. Belay T, Gebreselassie M, Abadi T. Description of cactus pear (*Opuntia ficus-indica* (L.) mill.) cultivars from Tigray, northern Ethiopia. *Mekelle, Tigray, Ethiopia, Tigray Agricultural Research Institute. Mekelle, Tigray, Ethiopia*. 2011.
5. Mazzeo G, Nucifora S, Russo A, Suma P. *Dactylopius opuntiae*, a new prickly pear cactus pest in the Mediterranean: an overview. *Entomol Exp Appl*. 2019;167:59–72.
6. Machimane Z. Evaluating different biotypes of *Dactylopius opuntiae* and *Dactylopius confusus* (cochineals) for biological control of the invasive cactus *Opuntia engelmannii* in Kenya and South Africa: Faculty of Science, University of the Witwatersrand, Johannesburg, South Africa. 2021.
7. Chávez-Moreno C, Tecante A, Casas A. The *Opuntia* (Cactaceae) and *Dactylopius* (Hemiptera: Dactylopiidae) in Mexico: a historical perspective of use, interaction and distribution. *Biodivers Conserv*. 2009;18:3337–55.
8. Fitiwy I, Gebretsadkan A, Araya A. Management of cochineal (*Dactylopius coccus* Costa) insect pest through botanical extraction in Tigray, North Ethiopia. *J Drylands*. 2016;6:499–505.
9. Nejad HE, Nejad AE. Cochineal (*Dactylopius coccus*) as one of the most important insects in industrial dyeing. *Int J Adv Biol Biomed Res*. 2013;1:1302–8.
10. Campana MG, Robles Garcia NM, Tuross N. America's red gold: multiple lineages of cultivated cochineal in Mexico. *Ecol Evol*. 2015;5:607–17.
11. Vera-Ponce León A, Dominguez-Mirazo M, Bustamante-Brito R, Higareda-Alvear V, Rosenblueth M, Martínez-Romero E. Functional genomics of a *Spiroplasma* associated with the carmine cochineals *Dactylopius coccus* and *Dactylopius opuntiae*. *BMC Genomics*. 2021;22:1–17.
12. Rodríguez LC, Méndez MA, Niemeyer HM. Direction of dispersion of cochineal (*Dactylopius coccus* Costa) within the Americas. *Antiquity*. 2001;75:73–7.
13. Portillo M, Viguera A. A review on the cochineal species in Mexico, hosts and natural enemies. *Proceedings of the V International Congress on Cactus Pear and Cochineal*. 2004; 728: 249–56.

14. Mendel Z, Protasov A, Vanegas-Rico JM, Lomeli-Flores JR, Suma P, Rodríguez-Leyva E. Classical and fortuitous biological control of the prickly pear cochineal, *Dactylopius opuntiae*, in Israel. *Biol Control*. 2020;142:104157.
15. Belay T. Carmine cochineal: fortune wasted in northern Ethiopia. *J Prof Assoc Cactus Dev*. 2015;17:61–80.
16. Nefzaoui A, Inglese P, Belay AT, 2009 Improved utilization of cactus pear for food, feed, soil and water conservation and other products in Africa. *Proceedings of the Proceedings of International Workshop, Mekelle (Ethiopia)*, 19–21 October, 224 pp, 2010.
17. Maia MF, Moore SJ. Plant-based insect repellents: a review of their efficacy, development and testing. *Malar J*. 2011;10:1–15.
18. Nnamonu LA, Onyekutu A. Green pesticides in Nigeria: an overview. *J Biol Agric Healthc*. 2015;5:48–62.
19. Kumari A, Kaushik N, 2016 Oviposition deterrents in herbivorous insects and their potential use in integrated pest management.
20. Hussain AI, 2009 Characterization and biological activities of essential oils of some species of Lamiaceae. Faisalabad: University of Agriculture.
21. Baser KHC, Buchbauer G. *Handbook of essential oils: science, technology, and applications*. Boca Raton: CRC Press; 2009.
22. Isman MB. Bridging the gap: moving botanical insecticides from the laboratory to the farm. *Ind Crops Prod*. 2017;110:10–4.
23. Lee SG, Kim B, Yang Y, et al. Berry anthocyanins suppress the expression and secretion of proinflammatory mediators in macrophages by inhibiting nuclear translocation of NF- κ B independent of NRF2-mediated mechanism. *J Nutr Biochem*. 2014;25:404–11.
24. Fang S, Li J, Xu Y, Shen C, Guo W. Evaporating potential. *Joule*. 2022;6:690–701.
25. Fabiane KC, Ferronato R, Santos ACD, Onofre SB. Physicochemical characteristics of the essential oils of *Baccharis dracunculifolia* and *Baccharis uncinella* DC (Asteraceae). *Revista Brasileira de Farmacognosia*. 2008;18:197–203.
26. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. *Int Pharm Sci*. 2011;1:98–106.
27. Carey JR. Insect biodemography. *Ann Rev Entomol*. 2001;46:79–110.
28. Pavela R. History, presence, and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects—a review. *Plant Prot Sci*. 2016;52:229–41.
29. Mohsin SMN, Raman I, Hasan ZAA, Idris Z. Influence of surfactant structure on physical stability and physicochemical properties of insecticide emulsions. *J Oil Palm Res*. 2019;31:670–80.
30. Edeoga HO, Okwu D, Mbaebie B. Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotechnol*. 2005;4:685–8.
31. Joshi N, Bhatt S, Dhyani S, Nain J. Phytochemical screening of secondary metabolites of *Argemone mexicana* Linn. flowers. *Int J Curr Pharm Res*. 2013;5:144–7.
32. Du M, Huang S, Zhang J, Wang J, Hu L, Jiang J. Toxicological test of saponins from *Sapindus mukorossi* Gaerth. *Open J For*. 2015;5:749.
33. Talukdar N, Dutta A, Chakraborty R, Das K. Screening of phytochemicals, antioxidant and inhibitory effect on alpha-amylase by ethanolic extract of *Elaeocarpus Ganitrus* (Bark). *Int J Pharm Sci Res*. 2017;8:5270–5.
34. Thite S, Chavan Y, Aparadh V, Kore B. Preliminary phytochemical screening of some medicinal plants. *Int J Pharm Chem Biol Sci*. 2013;3:87–90.
35. Yadav R, Agarwala M. Phytochemical analysis of some medicinal plants. *J Phytol*. 2011;3(12):10.
36. Madhu S, Shaikhath A, Vijayan V. Efficacy of bioactive compounds from *Curcuma aromatica* against mosquito larvae. *Acta Tropica*. 2010;113:7–11.
37. Bayen S. Occurrence, bioavailability and toxic effects of trace metals and organic contaminants in mangrove ecosystems: a review. *Environ Int*. 2012;48:84–101.
38. Russell TL, Kay BH, Skilleter GA. Environmental effects of mosquito insecticides on saltmarsh invertebrate fauna. *Aquat Biol*. 2009;6:77–90.
39. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. *Indian J Med Res*. 2012;135:581.
40. Shaalan EAS, Canyon D, Younes MWF, Abdel-Wahab H, Mansour AH. A review of botanical phytochemicals with mosquitocidal potential. *Environ Int*. 2005;31:1149–66.
41. Han Y, Li LC, Hao WB, Tang M, Wan SQ. Larvicidal activity of lansiumamide B from the seeds of *Clausena lansium* against *Aedes albopictus* (Diptera: Culicidae). *Parasitol Res*. 2013;112:511–6.
42. da Silva OS, da Silva FC, de Barros FMC, et al. Larvicidal and growth-inhibiting activities of extract and benzopyrans from *Hypericum polyanthum* (Guttiferae) against *Aedes aegypti* (Diptera: Culicidae). *Ind Crops Prod*. 2013;45:236–9.
43. Reegan AD, Gandhi MR, Paulraj MG, Ignacimuthu S. Ovicidal and oviposition deterrent activities of medicinal plant extracts against *Aedes aegypti* L. and *Culex quinquefasciatus* Say mosquitoes (Diptera: Culicidae). *Osong Public Health Res Perspect*. 2015;6:64–9.
44. Reegan AD, Gandhi MR, Sivaraman G, et al. Bioefficacy of ecobolin A and ecobolin B isolated from *Ecobolium viride* (Forsk.) Alston on dengue vector *Aedes aegypti* L. (Diptera: Culicidae). *Parasit Epidemiol Control*. 2016;1:78–84.
45. Roark R. Some promising insecticidal plants. *Econ Bot*. 1947;1:437–45.
46. Rawani A, Chowdhury N, Ghosh A, Laskar S, Chandra G. Mosquito larvicidal activity of *Solanum nigrum* berry extracts. *Indian J Med Res*. 2013;137:972.
47. Jeyachandran R, Mahesh A, Cindrella L, Sudhakar S, Pazhanichamy K. Antibacterial activity of plumbagin and root extracts of *Plumbago zeylanica* L. *Acta Biol Cracoviensia Ser Bot*. 2009;51:17–22.
48. Jagesar R, Cox M. Phytochemical screening of the chloroform and ethanol extract of stems, twigs, root and bark of *Conocarpus erectus* L. *Int J Acad Res*. 2010;2:37–45.
49. Weldemariam Y, Welderufael S. Determination of phytochemical constituents and insecticidal activity of *Eucalyptus globules* against *Cactus* pests. *Glob J Sci Res*. 2015;15:1–8.
50. Soni P, Siddiqui AA, Dwivedi J, Soni V. Pharmacological properties of *Datura stramonium* L. as a potential medicinal tree: an overview. *Asian Pac J Trop Biomed*. 2012;2:1002–8.
51. Warikoo R, Kumar S. Oviposition altering and ovicidal efficacy of root extracts of *Argemone mexicana* against dengue vector, *Aedes aegypti* (Diptera: Culicidae). *J Entomol Zool Stud*. 2014;2:11–7.
52. Hiruy B, Getu E. Evaluation of *Calpurnia aurea* leaf extracts as natural insect repellents for stored product insect pests in Ethiopia. *All Life*. 2023;16:2163303.
53. Abubeker A, Assen A, Dhuguma A. 2021 Efficacy evaluation of methanolic extracts of *Calpurnia aurea* and *Schinus molle* plants against the cattle lice *Linognathus vitulli*.

54. Mwine TJ, Van Damme P. *Euphorbia tirucalli* L. (Euphorbiaceae): the miracle tree: current status of available knowledge. *Sci Res Essays*. 2011;6:4905–14.
55. Kodjo TA, Gbénonchi M, Sadate A, et al. Bio-insecticidal effects of plant extracts and oil emulsions of *Ricinus communis* L. (Malpighiales: Euphorbiaceae) on the diamondback, *Plutella xylostella* L. (Lepidoptera: Plutellidae) under laboratory and semi-field conditions. *J Appl Biosci*. 2011;43:2899–914.
56. Tinzaara W, Tushemereirwe W, Nankinga C, Gold C, Kashaija I. The potential of using botanical insecticides for the control of the *banana weevil*, *Cosmopolites sordidus* (Coleoptera: Curculionidae). *Afr J Biotechnol*. 2006;5(20):1994.
57. Bourogâa F. Insecticidal properties of the physic nut tree *Jatropha curcas* L. (Euphorbiaceae) and potential use in pest management. Göttingen: Georg-August-Universität Göttingen; 2018.
58. Mushobozy DM, Nganilevanu G, Ruheza S, Swella GB. Plant oils as common bean (*Phaseolus vulgaris* L.) seed protectants against infestations by the Mexican bean weevil *Zabrotes subfasciatus* (Boh.). *J Plant Prot Res*. 49 (1), 2009. <https://doi.org/10.2478/v10045-009-0005-5>
59. Kerebba N, Oyedeji A, Byamukama R, Kuria S, Oyedeji O. Pesticidal activity of *Tithonia diversifolia* (Hemsl.) A. Gray and *Tephrosia vogelii* (Hook f.); phytochemical isolation and characterization: a review. *South Afr J Bot*. 2019;121:366–76.
60. Alao FO, Adebayo T. Comparative efficacy of *Tephrosia vogelii* and *Moringa oleifera* against insect pests of watermelon (*Citrullus lanatus* Thumb). *Int Lett Nat Sci*. 2015;35:71–8.
61. WHO. The WHO recommended classification of pesticides by hazard and guidelines to classification 2019. Geneva: World Health Organization; 2020.
62. Banu K, Aruna D. A novel herbal pesticide to control adult scale insects. *J Pharm Sci Innov (JPSI)*. 2013. DOI: <https://doi.org/10.7897/2277-4572.02445>.
63. Meena BR, Chittora D, Yadav J, et al. Effect of different physical factors on efficacy of *Thevetia Peruviana* leaf extract and bio-formulations. *Biochem Biophys Rep*. 2022;30:101271.
64. Montanucci RR. A safe procedure for eradicating root mealybugs from a cactus collection. *Cactus Succul J*. 2010;82:184–6.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.