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Original Article 1

Biochemical and toxicological effects of *Citrullus colocynthis* (L.) seed oil (extracted by three different methods) on the potato tuber moth, *Phthorimaea operculella* (Zeller)

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Background
Citrullus colocynthis (L.) is a plant of the Cucurbitaceae family, traditionally known for its medicinal properties. Recently, its seed oil has gained attention as a natural insecticidal agent.
Objective
This study aimed to compare three extraction methods: maceration, Soxhlet, and ultrasound-assisted solvent extraction for obtaining seed oil from *C. colocynthis*, and to evaluate the oils' physicochemical properties and their toxicological and biochemical effects on *Phthorimaea operculella*.
Materials and methods
Seeds were extracted using hexane via the three methods. The oils were analyzed for yield, acid value, peroxide value, iodine value, saponification value, and fatty acid composition. FTIR spectroscopy was used to identify functional groups. Toxicity (LC_{50}) was tested against *P. operculella* larvae and adults. Enzyme activities (AChE, GST, SOD) and total protein content were assessed in treated insects and compared with those of the control group.
Results and conclusion
Ultrasound-assisted extraction gave the highest oil yield. The extracted oils differed slightly in physicochemical properties. FTIR analysis showed consistent functional groups, with macerated oil displaying more absorption bands. Toxicity tests revealed that ultrasound oil had the strongest insecticidal effect. Enzyme assays showed significant inhibition in treated insects.
These findings support the potential of *C. colocynthis* seed oil as an eco-friendly insecticide.

Keywords: Bitter melon, seed oil yield, Fourier transform infrared spectroscopy, fatty acid composition, potato tuber moth, oxidative stress, enzyme activities, protein content.

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Introduction

Among global food crops, potato *(Solanum tuberosum L.)* which belongs to the Solanaceae family is considered the third most vital for human nutrition and food security [1,2]. It is cultivated in a wide variety of climates and is believed to have originated in western South America [3,4]. China and India are the leading producers worldwide, while Egypt holds the top position in Africa and ranks among the top 20 countries globally for potato production. Egypt is also the continent's largest producer and exporter of potatoes [4,5]. Nutritionally, potatoes are rich in carbohydrates and water. Although relatively low in protein, the quality of potato protein is superior to that of cereals [6]. Due to their natural absence of gluten, potatoes serve as a valuable dietary option for individuals with gluten intolerance or celiac disease [7]. They are also a rich source of nutritional elements such as amino acids, antioxidants, fibers, and a broad spectrum of essential vitamins and minerals [8]. Beyond their role as a staple food, potatoes are utilized in the production of starch, beverages, processed foods, and even in medicinal applications. Potatoes, however, are susceptible to a range of insect pests that compromise both yield and quality [9]. The potato tuber moth (*Phthorimaea operculella* (Zeller); Insecta: Lepidoptera: Gelechiidae) is one of the most damaging pests of potato and other Solanaceae plants [10]. The larvae feed on leaves and stems and tunnel into the tubers, creating galleries that not only reduce marketability but also allow for pathogen invasion. Infestations in stored tubers are particularly devastating, as rapid reproduction can compromise seed quality and lead to the spread of infestations into new fields [11].

Chemical insecticides are commonly used to manage *P. operculella* because of their quick action, effectiveness, and affordability. However, their widespread use has several serious disadvantages, including environmental pollution, health hazards to humans and animals, and the accumulation of pesticide residues on crops. The indiscriminate use of such chemicals has become a major concern [12].

Botanical insecticides offer one of the safest, most accessible, and effective tools in modern pest management. Although slower-acting, they provide a more sustainable alternative for controlling pests such as the potato tuber moth [10]. Recent research has underscored the insecticidal potential of plant extracts and essential oils due to their bioactive compounds, which can significantly influence pest populations [13]. Herbal-based products, known for their broad-spectrum insect-repelling properties, represent a promising substitute for synthetic pesticides [14]. Although research is limited, *Citrullus colocynthis* seed oil has shown potential as a novel, natural insecticidal agent [14, 15].

Citrullus colocynthis (L.), commonly known as bitter apple, is a perennial desert plant belonging to the Cucurbitaceae family [16]. It is widely distributed across arid regions of Asia, the Mediterranean Basin, North Africa, and the Near East, including historical Egyptian sites such as Neolithic Armant and Nagada [17]. The plant features trailing stems and perennial roots, with fruits containing numerous edible seeds used traditionally by desert communities. These seeds yield approximately 17–19% oil [18]; They are primarily composed of unsaturated fatty acids (80–85%) [19]. The seeds, along with other parts of the plant, are abundant in bioactive compounds such as glycosides, flavonoids, alkaloids, essential oils, and fatty acids. These components have been traditionally used in folk medicine to treat a variety of health conditions, including urinary disorders, spleen enlargement, ulcers, tumors, anemia, and joint pain [20]. Due to its broad pharmacological and nutritional profile, *C. colocynthis* is gaining recognition for its potential in medicinal, nutritional, and industrial applications [21], with increasing global interest in its benefits [22]. To enhance the yield and bioactivity of its extracts, various methods have been employed, including traditional techniques such as steam distillation, organic solvent extraction, maceration with alcohol-water mixtures [23], and enfleurage (cold or hot fat extraction) [24]. Among modern techniques, ultrasound-assisted extraction and microwave-assisted extraction have become popular due to their efficiency in isolating seed oil and other plant constituents [25,26].

This research focuses on analyzing how different extraction methods namely maceration, Soxhlet extraction, and ultrasound-assisted techniques affect the physicochemical profile of *Citrullus colocynthis* seed oil. It also assesses the impact of these oils on the biochemical and toxicological responses of the potato tuber moth, *Phthorimaea operculella.*

Materials and Methods

Collection and identification of plant material

Colocynthis fruits were collected in August 2022 from the farm of the Faculty of Pharmacy, Cairo University, Egypt. The fruits were taxonomically authenticated by the Experiment Station for Medicinal, Aromatic, and Poisonous Plants at the same faculty. After air-drying in the shade, the fruits were crushed into small pieces to separate and clean the seeds from impurities. The seeds were then ground into a fine powder using a laboratory grinder and stored in opaque, screw-capped jars until further use.

Test insect

The potato tuber moth (*Phthorimaea operculella*) was reared primarily on potato tubers. Insects were obtained from a laboratory colony maintained for several generations at the Central Agricultural Pesticides Laboratory without exposure to insecticides.

Chemicals

All experimental procedures were carried out using high-purity (analytical grade) chemicals. Hexane was selected as the solvent for extraction. Materials such as a mixture of 37 standard FAMEs, protein assay kits, and enzyme substrates were sourced from Sigma-Aldrich, USA.

Preparation of C. colocynthis seed oil using three extraction methods: Maceration extraction

Maceration is a traditional, cost-effective method widely used for extracting natural products from plant materials. It involves a solid–liquid extraction. In this process, the powdered solid material (100 gm of *C. colocynhtis* grinding seeds) was placed in a closed vessel and soaked with 1000 ml of n-hexane, it is allowed to stand for 24 hrs. with occasional shaking. The extract was filtered by Whitman No. 1 filter paper. The seed residue was re-extracted another two times by adding the same volume of Hexane. The three filtrates were combined and concentrated by Rotary evaporator (Heidolph-Germany) under vacuum at 40°C according to [27].

Continuous solvent extraction by Soxhlet apparatus

Soxhlet extraction remains the benchmark against which the performance of alternative leaching methods is evaluated. In this study, a total of 100 g of finely ground *Citrullus colocynthis* seed powder was accurately weighed and approximately divided among several cellulose thimbles for parallel Soxhlet extraction units. Each portion was extracted using *n*-hexane as a solvent, (total volume: 1000 mL, proportionally distributed). The solvent was heated to vaporization using a controlled heating mantle, condensed in the upper condenser, and dripped onto the sample to dissolve non-polar components. The solvent-oil mixture was repeatedly siphoned back into the boiling flask over 4–8 hours. After completion, the combined extracts were filtered, concentrated under reduced pressure using a rotary evaporator, and stored in amber bottles at 4°C until analysis [28].

Ultrasound-Assisted Solvent Extraction (UASE)

By generating acoustic cavitation, ultrasound-assisted extraction improves the efficiency of solvent diffusion into plant tissues and promotes the liberation of bioactive molecules by disrupting cell walls [29]. Ultrasound-assisted extraction was carried out using a water bath sonicator (Ney Ultrasonics 28 H) operating at a fixed frequency of 40 kHz and an approximate power of 200 W. A total of 100 g of finely ground *Citrullus colocynthis* seeds was weighed and divided equally into multiple extraction batches. Each portion was mixed with a measured volume of *n*-hexane, with the total volume amounting to 1000 mL, distributed across the batches to ensure a consistent solvent-to-solid ratio. The extraction flasks containing the seed-solvent mixtures were immersed in the ultrasonic water bath and subjected to sonication for 30 minutes at ambient temperature (maintained between 25 and 30°C) without the application of any external heat. Upon completion of sonication, the mixtures were filtered to separate the solid residues. The collected filtrates were pooled, and the solvent was removed under reduced pressure using a rotary evaporator until complete recovery of the crude oil. The extracted oil was then transferred into amber-colored glass bottles and stored at 4°C for further analysis [30].

Extraction yield

The total yield of extracted oil from each method was calculated using the following equation: Extraction yield (%) = m_1/m_0 x 100, where m_1 is the total mass of the extracted oil, and m_0 is the initial mass of the grinding seeds used in each extraction [31].

Physicochemical analysis of *C. colocynthis* extracted oil

Refractive index (RI) determination

The procedure was carried out according to the standard method [32].

Acid value (AV) determination

The acid value (AV) of the oil samples was determined following the standard method [33].

Iodine value (IV) determination

The iodine value (IV) of the oil was determined using the Hanus method [32], which involves the addition of iodine monobromide to quantify unsaturation in fatty acids.

Peroxide value (PV) determination

Peroxide value (PV) was determined according to a standard analytical method [32] to assess the extent of primary oxidation in the oil samples.

Saponification value (SV) determination

Saponification value (SV) was evaluated by titration to estimate the average molecular weight of fatty acids in the oil samples, following a standardized method [32].

Unsaponifiable content (%) determination

The unsaponifiable content (%) was assessed according to the standard method [34].

Fatty acid analysis of C. colocynthis oil

Fatty acid composition in oil samples underwent transmethylation — transforming the fatty acyl chains into methyl esters (FAMEs), based on a modified version of the method using GC apparatus [35].

Fourier Transform Infrared (FTIR) Spectroscopy of C. colocynthis oil

Fourier Transform Infrared (FTIR) spectroscopy was utilized to identify the functional groups present in the oil. Spectra were recorded using a Bruker VERTEX 80 spectrometer (Germany) equipped with a Platinum Diamond ATR unit, operating within 4000–400 cm^{-1} range at a resolution of 4 cm^{-1}.

Rearing of insects

Newly hatched larvae of *Phthorimaea operculella* burrow into potato tubers, creating entry holes through which they feed internally. Once fully developed, the larvae leave the tubers and pupate in the surrounding sand. Under laboratory conditions (12L:12D photoperiod, 27 ± 2°C, and 60 ± 5% relative humidity), the life cycle of the potato tuber moth (PTM) lasts approximately 21 to 25 days [36].

Preparation of Citrullus colocynthis emulsion formulation

An oil-in-water emulsion of *Citrullus colocynthis* seed oil was prepared following the method of [37], with minor modifications. The emulsion was produced using the spontaneous emulsification method, which involves the combination of an organic phase and an aqueous phase. Briefly, the formulation consisted of a mixture of *C. colocynthis* seed oil and Tween 80® (polyoxyethylene sorbitan monooleate) in a 3:1 (v/v) ratio.

Toxic effect of extracted seed oils on *P. operculella* Larvae

The contact toxicity of the seed oil extracts using filter papers impregnated with oils extracted by the three different methods, following the procedures described in [38], [39], and [40, 41]. These treated papers were used to assess the effect of each extract on first-instar larvae of *Phthorimaea operculella*.

Toxic effect of extracted seed oils on Phthorimaea operculella adult moths

The vial method described by [42] and [43], with slight modifications, was used to assess the toxicity of Citrullus colocynthis seed oil extracts obtained from three different extraction methods (applied separately) against one-day-old adult moths of Phthorimaea operculella. Mortality data were corrected using Abbott's formula [44]. The lethal concentrations (LC_{50} and LC_{90}) and slope values were determined through probit analysis using the "LdPLine®" software, following the method outlined in [45]. Additionally, the toxicity index (TI) was calculated according to [46].

Biochemical analysis

Samples preparation for biochemical analysis

Adult moths of P. operculella were exposed for 24 hours to LC_{50} concentrations of C. colocynthis seed oil extracted by three different methods: maceration (1.21%), Soxhlet extraction (3.38%), and ultrasonication (0.92%) with five replicates for each treatment. Both treated and control moths (100 mg) were homogenized in 3 mL of sodium phosphate buffer (pH 7.0) for 3 minutes using a manual Teflon homogenizer surrounded by crushed ice. The homogenates were centrifuged at 10,000 xg for 30 minutes at 4°C, and the resulting supernatants were collected into clean tubes and stored at –20°C until further biochemical analysis.

Determination of total protein content

Total protein content was measured using the Biuret method [47], employing a commercial kit supplied by DP International Laboratory. The protein concentration was calculated and expressed as micrograms of protein per milligram of insect body weight.

Determination of Glutathione S-Transferase (GST) activity

GST enzyme activity was assessed using a spectrophotometric assay at 340 nm, based on the method described in [48], which involves the formation of a GSH-CDNB conjugate. The enzymatic rate was calculated and expressed in µmol of conjugate produced per minute per mg of protein.

Determination of Acetylcholinesterase (AChE) activity

Acetylcholinesterase (AChE) activity was evaluated using a spectrophotometric method described by [49], with slight modifications from [50]. Enzymatic activity was quantified by calculating the micromoles of substrate broken down each minute per milligram of protein (µmol/min/mg protein).

Determination of superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was assessed based on the method of [51], incorporating modifications as described by [52] and [50]. The method determines superoxide dismutase (SOD) activity by assessing how effectively the enzyme inhibits the light-induced reduction of nitro blue tetrazolium (NBT) by superoxide ions. One unit of activity is defined as the enzyme amount necessary to achieve 50%

suppression of NBT reduction under standardized conditions.

Statistical analysis

The collected data were statistically analyzed using one-way analysis of variance (ANOVA) with the aid of SAS software. Differences between treatment means were assessed using the LSD test at a 5% level of significance, following the methodology reported [53].

Results and Discussions

Extraction yield

Oil extraction methods significantly influence seed oil yield, quality, and oxidative stability. Figure 1 illustrates the effect of different extraction techniques on the yield of *Citrullus colocynthis* seed oil. The ultrasound-assisted solvent extraction (Ultra) method produced the highest yield (16.13% ± 1.10), followed by the maceration method (14.00% ± 1.41). The lowest yield was obtained using continuous solvent extraction with the Soxhlet apparatus (8.70% ± 1.00). Ultrasound-assisted extraction enhances oil recovery by disrupting the cellular structure of seeds, thereby facilitating solvent penetration and release of oil. This method also requires shorter extraction time and less solvent consumption. Similar results were reported for *Caesalpinia spinosa* (tara) seed oil, where ultrasound-assisted extraction achieved a higher yield (10.53%) compared to Soxhlet extraction (9.4%) [54]. Higher ultrasonic intensity combined with elevated temperatures contributed to enhanced extraction efficiency. For *Centella asiatica*, the optimal parameters for recovering active constituents were identified as 30 minutes of ultrasound treatment, a 29:1 ratio between solvent and plant material, and a solvent purity of 90% [55]. Ultrasonic-microwave-assisted extraction is also widely recognized for efficient seed oil recovery [1]. Many researches have focused on ultrasound-assisted solvent extraction (UASE) as a sustainable, low-impact, and economically feasible alternative to conventional methods. Ultrasound waves (20 kHz to 100 MHz) penetrate the medium, causing alternating expansion and compression that induces cavitation bubble formation and collapse resulting in microturbulence, solvent agitation, and collision among particles [56]. These findings confirm the substantial impact of extraction techniques on oil quality indices. While UASE is highly efficient, maceration has also shown favorable outcomes. In some cases, maceration was reported to be the superior solvent extraction method in terms of preserving certain oil properties [57]. For reference, the yield of *C. colocynthis* seed oil extracted using ethanol-based solvent extraction was reported as 12.45% [58].

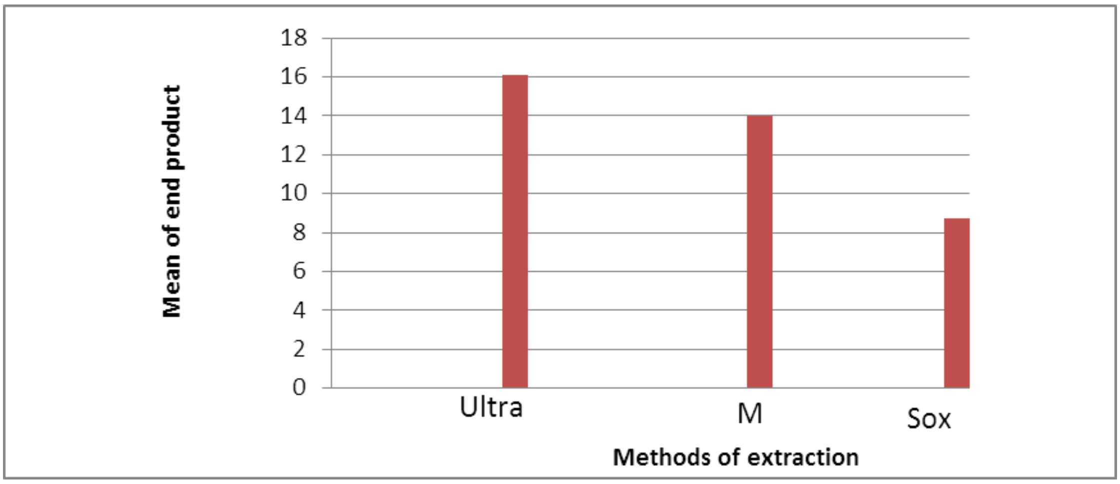

Fig. 1 Comparison of the *Citrullus colocynthis* seed oil yield produced by three extraction methods; ultrasound-assisted solvent extraction (Ultra), maceration method (M) and continuous solvent extraction by Soxhlet apparatus (Sox).

Physicochemical analysis of *Citrullus colocynthis* seed oil extracted

The extracted oils were evaluated through a range of physicochemical properties such as refractive index, acid value, peroxide value, iodine value, saponification value, and the percentage of unsaponifiable matter. Table 1 summarizes the results obtained for oils extracted via maceration (M), Soxhlet extraction (Sox), and ultrasound-assisted solvent extraction (Ultra).

The refractive index of Citrullus colocynthis seed oil showed modest variation across the various extraction techniques applied.

The acid value was highest in Ultra oil (5.43 mg KOH/g oil), followed by M oil (4.99 mg KOH/g oil), while Sox oil showed the lowest acid value

(2.79 mg KOH/g oil). A high acid value indicates the presence of free fatty acids, which can negatively impact oil quality by lowering the smoke point and oxidative stability, and by promoting rancidity and undesirable taste during storage [59].
The peroxide value, which indicates the extent of primary oxidation, was also highest in Ultra oil (23.78 meq O_2/kg oil), followed by Sox oil (21.57 meq O_2/kg oil) and M oil (20.84 meq O_2 /kg oil). The elevated peroxide value in the Ultra extract may be attributed to the frictional heat generated during ultrasound-assisted extraction, which accelerates the oxidation of lipids [60]. This value reflects the degree of oxidation of fats and fatty acids [61, 62].
The iodine value, which reflects the degree of unsaturation, was 131.11 g I_2/100 g oil for M oil, 130.99 g I_2/100 g oil for Sox oil, and 124.18 g I_2/100 g oil for Ultra oil. A high iodine value suggests a greater content of double bonds, indicating higher unsaturation levels [63]. Saponification values were 186.51, 185.44, and 182.37 mg KOH/g oil for Sox, M, and Ultra oils, respectively.
The percentage of unsaponifiable matter was 1.23% for Sox oil, 1.17% for M oil, and 1.07% for Ultra oil. The saponification value reflects the amount of alkali required to convert fats or oils into soap, it denotes the quantity of short-chain fatty acids that are more susceptible to hydrolysis and oxidation and is typically higher in palmitic (C16) and stearic (C18) acids [64,65]. These results are consistent with findings [66], who reported an unsaponifiable content of 1.07% for *Citrullus colocynthis* seed oil and 3.39% for *Coccinia grandis* seed oil.

Fatty acid composition of *Citrullus colocynthis* seed oil

Table 2 summarizes the fatty acid content of *C. colocynthis* seed oil obtained via ultrasound, Soxhlet, and maceration methods. Although the overall fatty acid composition remained consistent among the methods, linoleic acid (C18:2) dominated in all cases, reaching 64.65% in the ultrasound extract, 64.30% with Soxhlet, and 59.82% through maceration. In addition to linoleic acid, notable amounts of oleic (C18:1), palmitic (C16:0), and stearic acids (C18:0) were detected, albeit at relatively lower levels. In the maceration extract, these were found at 19.10%, 11.44%, and 9.64%, respectively. For Soxhlet extraction, the respective values were 16.88%, 10.54%, and 8.28%, while in the ultrasound-assisted extract, they were 16.83%, 10.46%, and 8.06%.
The findings are consistent with the results reported by [21], who stated that *Citrullus colocynthis* seeds contain approximately 23.16% oil, in which linoleic acid constitutes the predominant fatty acid (66.73%), followed by oleic (14.78%), palmitic (9.74%), and stearic acids (7.37%). The current findings align with earlier studies, reaffirming that *Citrullus colocynthis* seeds are abundant in essential fatty acids such as palmitic, stearic, oleic, linolenic, and linoleic acids [20].

Table 1. Physicochemical analysis of *Citrullus colocynthis* seed oil extracted by three different methods.

Physicochemical analysis	Ultra.oil Mean ± SD	M.oil Mean ± SD	Sox.oil Mean ± SD
Refractive index	$1.4705^b \pm 0.0006$	$1.4687^c \pm 0.0004$	$1.4721^a \pm 0.0003$
Acid value(mg KOH/g oil)	$5.43^a \pm 0.08$	$4.99^b \pm 0.05$	$2.79^c \pm 0.07$
Peroxide value (meq.o_2/kg oil)	$23.78^a \pm 0.12$	$20.84^c \pm 0.11$	$21.57^b \pm 0.14$
Iodine value(g I_2/100 g oil)	$124.18^c \pm 0.45$	$131.11^a \pm 0.51$	$130.96^a \pm 0.49$
Saponification value(mg KOH/g oil)	$182.37^c \pm 0.63$	$185.44^b \pm 0.58$	$186.51^a \pm 0.56$
Unsaponifiable content %	$1.07^c \pm 0.02$	$1.17^b \pm 0.01$	$1.23^a \pm 0.03$

Ultrasound-assisted solvent extraction (Ultra), maceration method (M) and continuous solvent extraction by Soxhlet apparatus (Sox), Data are shown as mean ± SD. Statistical significance at $p < 0.05$ is indicated by differing superscript letters within each row.

Table 2. The major fatty acids (%) of *Citrullus colocynthis* seed oil extracted by three different methods.

Fatty acids %	Ultra.oil Mean ± SD	M.oil Mean ± SD	Sox.oil Mean ± SD
Palmitic ($C_{16:0}$)	$10.46^b \pm 0.12$	$11.44^a \pm 0.15$	$10.54^b \pm 0.08$
Stearic ($C_{18:0}$)	$8.06^b \pm 0.10$	$9.64^a \pm 0.11$	$8.28^b \pm 0.13$
Oleic acid($C_{18:1}$)	$16.83^b \pm 0.21$	$19.10^a \pm 0.30$	$16.88^b \pm 0.19$
Linoleic acid ($C_{18:2}$)	$64.65^a \pm 0.33$	$59.82^b \pm 0.42$	$64.30^a \pm 0.28$

Ultrasound-assisted solvent extraction (Ultra), maceration method (M) and continuous solvent extraction by Soxhlet apparatus (Sox), Values are expressed as mean ± standard deviation.Different superscript letters within the same row indicate statistically significant differences ($p < 0.05$).

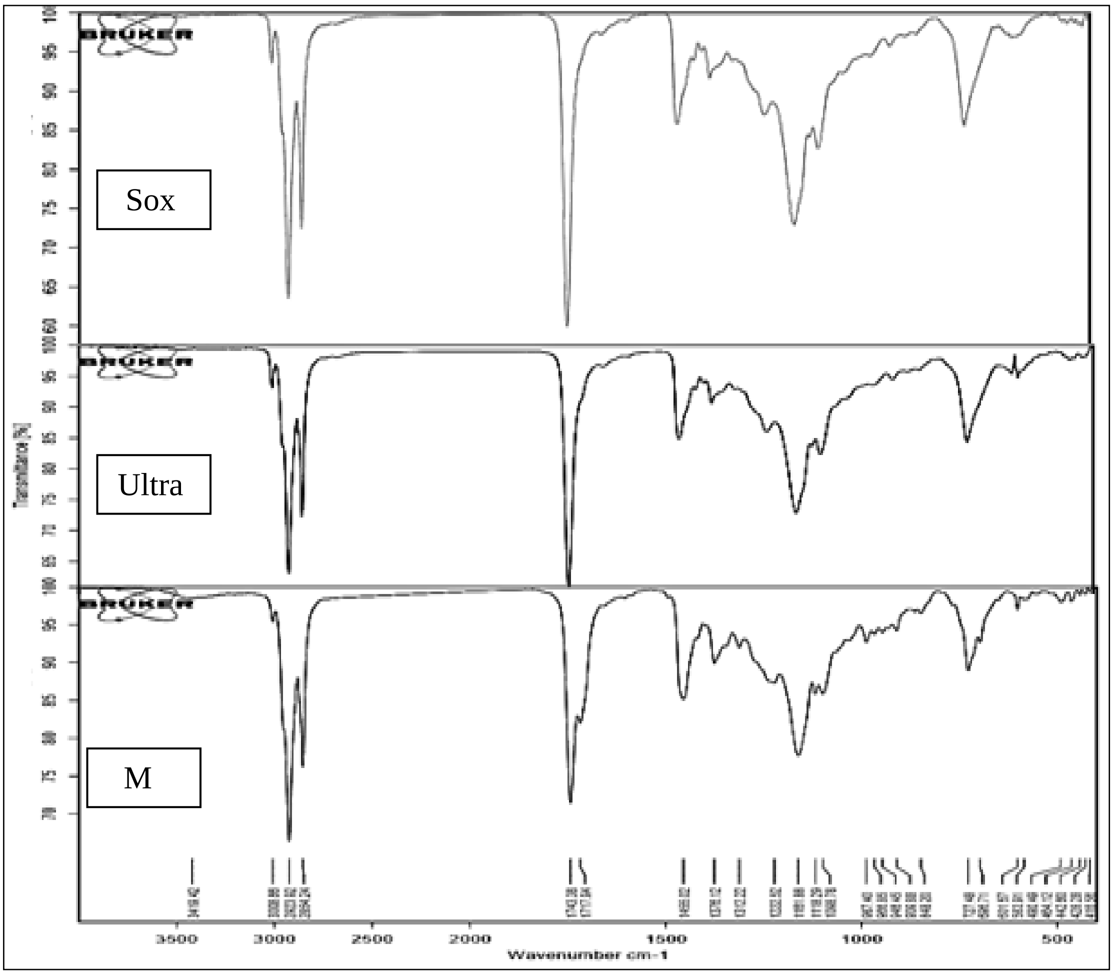

Fig. 2 FTIR of *C. colocynthis* seed oil extracted by three different methods ultrasound- assisted solvent extraction (Ultra), maceration method (M) and, continuous solvent extraction by Soxhlet apparatus (Sox).

Table 3 FTIR of *C. colocynthis* seed oil extracted by three method of extraction.

Possible function group +vibration type	**Compound class**	**Wave number range (cm^{-1})**	**Wave length detected (cm^{-1})**	**SOX**	**Ultra**	**M**
O-H stretching	Alcohol	3550-3200	3474	D	ND	ND
			3419	ND	ND	D
O-H stretching	carboxylic acid	3300-2500	3008	D	D	D
N-H stretching	Amine salt	3000-2800	2922	D	D	+1
			2853	D	D	+1
C=O stretching		1750-1735	1743	D	D	D
	Esters	1730-1715	1717	ND	ND	ND
	α,β unsaturated ester					
C-H bending	Alkane	1470-1450	1460	D	D	-5
O-H bending	carboxylic acid	1440-1395	1397	D	D	ND
O-H bending	Alcohol	1420-1330	1376	D	+1	D
O-H bending	Phenol	1390-1310	1312	ND	ND	D
C-N stretching	Amine	1250-1020	1236	D	D	-14
C-O stretching	Tertiary alcohol	1205-1124				
	Aliphatic ether	1150-1085	1160	-1	D	+1
			1118	ND	ND.	NB
			1098	D	D	D

C=C Bending	Alkene	995-985	987	ND	ND	D
		980-960	960	ND	ND	D
			940	ND		NB
			909	+4	ND +3	NB
C-CL stretching	Halo compound	850-550	848	ND D	ND	NB
C=C bending	Alkene	730-665	721	ND	+1	+6
			696		ND	NB
C-I stretching	Halo compound	600-500	600	D	+7	
			592	ND	D	+1
			583	ND	ND	ND D
O–Metal stretching	carboxylates of metallic	510- 480	490	ND	ND	D
M–S/M-O Twesting	Sulfide metal complexes	480 –460	464	ND	ND	D
M–O Rocking/ Bending	Metal oxides	470-450	456	D	+1	ND
M–S or M–O Twisting	Metal complexes	460 - 430	442	ND	ND	D
C–Cl Wagging	Aliphatic chlorides	460- 430	439	D	ND	ND
C–I or M–Cl Twisting/ Rocking	Iodoalkanes/ Metal salts	460- 420	428	ND	ND	D
C–S or M–Cl Bending/ Rocking	Thioesters/ Metal halides	460- 420	425	ND	D	ND
C–I Stretching	Alkyl iodides	500- 400	418	D	ND	ND
C–Br Bending	Organobromines	510- 410	416	ND	ND	D
C–Br Stretching	Alkyl bromides	510- 400	405	D	ND	ND

Note: NB is new band, ND is not detected and D is detected, ultrasound-assisted solvent extraction (Ultra), maceration method (M) and continuous solvent extraction by Soxhlet apparatus (Sox).

Biochemical effects of LC_{50} treatments of *C. colocynthis* seed oil

Exposure of P. operculella adult moths to LC_{50} concentrations of C. colocynthis seed oil resulted in a highly significant increase in total protein content in treated insects compared to untreated controls (Table 5). Protein content increased to 44.00 ± 6.24 µg/mg (Sox oil), 51.67 ± 7.09 µg/mg (Ultra oil), and 60.00 ± 6.00 µg/mg (M oil) versus 23.33 ± 8.39 µg/mg in untreated moths. Protein increase percentages were: M oil: +157.2%, Ultra oil: +122.5%, and Sox oil: +88.6%Similar findings were reported by [74], who observed increased protein levels in Tenebrio molitor larvae after treatment with deodar oil. However, other studies indicate that C. colocynthis oil can significantly reduce protein levels in P. operculella larvae, suggesting it may inhibit protein synthesis and impair development [75].

Effect on the activities of acetyl cholinesterase (AChE), glutathione S-transferase (GST), and superoxide dismutase (SOD)

Significant inhibition of key detoxification and nervous system enzymes acetyl cholinesterase (AChE), glutathione S-transferase (GST), and superoxide dismutase (SOD) was observed in Phthorimaea operculella moths treated with LC_{50} concentrations of Citrullus colocynthis seed oil compared with untreated insects. The activity of acetylcholinesterase (AChE) in the body tissues of Phthorimaea operculella larvae exhibited notable variations following exposure to the LC_{50} concentrations of the tested oils. In untreated larvae, AChE activity recorded a value of 0.22 ± 0.06 µmol of acetylthiocholine hydrolyzed/mg protein/min. In contrast, larvae treated with Ultra oil, Soxhlet-extracted oil, and M oil showed a marked reduction in enzyme activity, reaching 0.06 ± 0.02, 0.07 ± 0.02, and 0.16 ± 0.05 µmol/mg protein/min, respectively. The most pronounced inhibition occurred in insects treated with Ultra and Sox oils, corresponding to reductions of 72.7% and 68.2%, respectively, compared to the control. A moderate decrease in AChE activity (27.3%) was observed following M oil treatment (Table 5).

As for glutathione S-transferase (GST), the enzymatic activity in untreated larvae was 276.1 ± 99.99 µmol/µg protein/min. A substantial inhibition was evident upon treatment with Sox oil, which reduced GST activity to 52 ± 1.15 µmol/µg protein/min (an 81.2% decrease). Similarly, M oil and Ultra oil treatments resulted in significant reductions to 64.33 ± 6.89 and 87.43 ± 9.87 µmol/µg protein/min, reflecting decreases of 76.7% and 68.3%, respectively.

Superoxide dismutase (SOD) activity in the control group was measured at 1.55 ± 0.10 U/mg protein/min. A clear decline was noted after treatment with Sox and M oils, yielding activities of 0.73 ± 0.06 and 0.89 ± 0.12 U/mg protein/min, corresponding to reductions of 52.9% and 42.6%, respectively. Conversely, treatment with Ultra oil resulted in a slight elevation in SOD activity (1.59 ± 0.33 U/mg protein/min), representing an increase of 2.6% relative to the untreated group (Table 6).

When *P. operculella* insects were exposed to the extracted oil of *C. colocynthis*, the results showed a substantial suppression of AChE activity. Because AChE is essential for nerve signal transmission, inhibiting it can interfere with regular physiological processes, which may cause paralysis and even death in insects [76]. Hexane extract from the leaves of *Origanum syriacum*, *Pergularia tomentosa*, *Senna italica*, and *Otostegia fruticose* exhibits more toxic action against *Culex pipiens* larvae Treatments with LC_{50} had distinct biological effects, the overall amounts of fat, protein, and carbohydrates were reduced, the AChE activity was dramatically reduced, and the detoxification system of the tested larvae was stimulated by the increased activity of GST [77]. [78] investigated how *C. colocynthis* oil extract affected *P. operculella*'s glutathione S-transferase (GST) and superoxide dismutase (SOD) activity. In insects treated with the extract, the results showed a significant reduction in GST and SOD activity. Important enzymes for detoxification and antioxidant defense systems in insects are GST and SOD. The oil extract's inhibition of these enzymes may impair the insect's defenses against toxic substances and oxidative stress, leaving it more vulnerable to environmental stressors.

Table 4 Toxic effects of *Citrullus colocynthis* seed oil, extracted using three different methods, on adult and neonate stages of the potato tuber moth (Phthorimaea operculella).

Extracted Oil	**LC_{50} (mg/100ml)**		**Slope ± S.E**		**Toxicity Index (TI %)**	
	moths	neonate	moths	Neonate	moths	Neonate
Ultra.oil	0.9205	1.8371	2.3864 ± 0.5383	1.5729 ± 0.6508	100%	100%
M.oil	1.2121	2.7237	2.8502 ± 0.8871	1.794 ± 0.8851	76%	67.4%
Sox.oil	3.3785	10.4342	1.3311 ± 0.4440	2.1105 ± 0.5769	27.2%	17.6%

Ultrasound assisted solvent extraction (Ultra), maceration (M), and Soxhlet continuous solvent extraction (Sox).

Table 5 Effect of LC_{50} treatments of tested oil on total protein content and Acetyl cholinesterase activity in total body homogenate of *phthorimaea operculella* adult moths.

Treatments	Conc. of protein (µg protein/ mg body weight)		Activity of AChE (µm of acetyl thiocholine hydrolyzed/mg protein/min)	
	Mean ± S.D.	Difference %	Mean ± S.D.	Difference %
Ultra. oil	51.67^{b}±7.09	+121.5	0.06^{c} ±0.02	-72.7
M. oil	60.00^{a} ±6.00	+157.2	0.16^{b} ±0.05	-27.3
Sox. oil	44.00^{b} ±6.24	+88.6	0.07^{c} ±0.02	-68.2
Untreated	23.33^{d} ±8.39	0.0	0.22^{b} ±0.06	0.0

Ultrasound assisted solvent extraction (Ultra), maceration (M), and Soxhlet continuous solvent extraction (Sox). Results are reported as mean values with standard deviations. Different Superscript letters within the same row indicate significant differences at $p < 0.05$

Table 6. Effect of LC_{50} treatments of tested oil on glutathione S-transferase (GST), and superoxide dismutase (SOD) activities in total body homogenate of *Phthorimaea operculella* adult moths.

Treatments	Activity of GST (µmol/µg protein/min)		Activity of SOD (U/mg protein/min)	
	Mean ± S.D.	Difference %	Mean ± S.D.	Difference %
Ultra. oil	87.43^{c} ± 9.87	-68.3	1.59^{a} ± 0.33	+2.6
M. oil	64.33^{d} ± 6.89	-76.7	0.89^{c} ±0.12	-42.6
Sox. oil	52.00^{d} ± 1.15	-81.2	0.73^{c} ±0.06	-52.9
Untreated	276.10^{b} ±99.99	0.0	1.55^{a} ±0.10	0.0

Ultrasound assisted solvent extraction (Ultra), maceration (M), and Soxhlet continuous solvent extraction (Sox). Results are reported as mean values with standard deviations. Different Superscript letters within the same row indicate significant differences at $p < 0.05$.

Conclusion

By influencing protein synthesis, acetyl cholinesterase activity, and the activity of important detoxifying and antioxidant enzymes, *C.colocynthis* oil extract exhibits promise as a biopesticide for managing *P. operculella*. To clarify the fundamental mechanisms of action and maximize the application of the natural botanical product in pest management techniques, more investigation is necessary.

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Declaration of Competing Interest

The authors assert that they possess no identifiable competing financial interests or personal affiliations that may have seemingly influenced the work presented in this study.

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