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RESEARCH ARTICLE

In silico and *in vitro* study of insecticidal effect of oil extracted from hemp seeds against *Tribolium castaneum* (Herbst)

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Abstract

The protection of stored products in Morocco still relies heavily on synthetic insecticides, posing risks to human health and the environment and contributing to the emergence of resistance to these chemicals. This study explored the insecticidal potential of vegetable oils extracted from the seeds of three *Cannabis sativa* L. varieties (Beldia (Bld), Kherdala (Krd) and Critical (Crt) against *Tribolium castaneum* (Herbst), a major pest of stored grains. The chemical composition of the oils was characterised using FTIR and GC-MS, revealing a predominance of fatty acids, particularly linoleic acid (38.97-50.11 %) and oleic acid (10.82–27.26 %). Biological tests showed significant insecticidal activity by contact, with cumulative mortality dependent on dose and exposure time. The Krd variety exhibited the highest toxicity (LD_{50} = 3.75 mL kg^{-1}), followed by Bld (5.61 mL kg^{-1}) and Crt (7.39 mL kg^{-1}). Furthermore, oils from the Bld and Crt varieties demonstrated strong repellent properties, reaching 91.67 % and 68 %, respectively, at 0.63 µL/cm^2, while Krd showed weak repellency. Molecular docking analyses suggest that certain major fatty acids, particularly linoleic and oleic acids, interact strongly with acetylcholinesterase, a classic target enzyme for insecticides, reinforcing the hypothesis of their active role in the observed insecticidal effects. These results highlight the potential of *C. sativa* seed oils as effective and safe bioinsecticides, offering a sustainable alternative to chemical insecticides for the post-harvest protection of cereals in Morocco.

Keywords: bioinsecticide; *Cannabis sativa*; molecular docking; Morocco; post-harvest protection; *Tribolium castaneum*

Introduction

In Morocco, cereal cultivation occupies 62 % of the agricultural land (1) and is therefore the most important crop, making the country one of the world's leading wheat consumers (2). The average annual consumption of cereals rose from 138 kg per person in the 1960s to 255 kg per person in 2016, representing a significant increase (1). One of the main constraints to the efficient use and high production of cereal crops in Africa is the loss caused by insect pests (3).

The most dangerous insects are still beetles, which can cause up to 57 % of grain losses in African countries (4). One of the most dangerous and prevalent pests in grocery shops, flour mills and warehouses is *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (5, 6). The control of this insect is generally achieved using plant protection products, either by spraying and/or fumigation, which causes serious problems for humans and the environment and, above all, leads to the development of insect strains that are resistant to insecticides (6, 7).

Given these problems, much research has focused on alternatives to chemical control and the development of integrated pest management approaches (6). In this regard, plant extracts (essential oils, methanolic and ethanolic extracts, vegetable oil etc.) have been tested for their insecticidal potential, either by inhalation or direct contact (6, 8). Among the possible options, the use of vegetable oil for grain protection has been explored by many researchers (9). Peanut, sunflower and rapeseed vegetable oils were evaluated for the control of *Sitophilus granarius* L., resulting in mortality rates of 60-80 % after 14 days, with no significant differences among treatments (10). Vegetable oils derived from coconut, sesame, sunflower and mustard, alone and in combination with camphor, 1, 8-cineole and eugenol, were applied against *S. zeamais* (L.), *S. granarius* (Mots.), *Prostephanus truncatus* (Hom) and *T. castaneum* in wheat and maize. Compared to the controls, all vegetable oil treatments, either alone or in combination, resulted in a considerable mortality rate (11).

Commonly known as 'El kif', *Cannabis sativa* L. is a herbaceous plant classified within the family Cannabaceae (12, 13). Throughout history, cannabis has been used for religious and medicinal purposes (14). Cannabis-based preparations are used to treat certain diseases in humans and animals (treatment of respiratory conditions, wound healing, abortion, anti-emetics, etc.) (15). In recent years, *C. sativa* . has been suggested as a promising candidate for use as a pesticide (16, 17). The leaves of this species mainly contain ketones, ester compounds and terpenes, which impart a distinctive odour. This may partly explain its use as an insect repellent (18). Numerous pinenes and limonene are among the volatile compounds that are abundant in this plant and have been shown to repel insects (19).

To our knowledge, research on the insecticidal activity of *C. sativa* vegetable oil on stored-product pests remains unexplored in Morocco. The present study aimed to characterize the chemical composition of seed oils obtained from 3 varieties of *C. sativa* grown in Morocco and evaluate their insecticidal efficacy against *T. castaneum*, a major pest of stored commodities in the country. Vegetable oils are largely composed of fatty acids and therefore have a less pronounced aromatic profile than essential oils; they contain volatile organic compounds (20). These oils are recognized as safe for food contact, with the US Food and Drug Administration (FDA) classifying them as 'Generally Recognised As Safe' (GRAS). In the field of food protection, vegetable oils are commonly used to preserve cereals (21) and fruits (22). Therefore, this study aims to contribute to the identification of alternative solutions for protecting food stocks in Morocco.

Materials and Methods

Plant material

Cannabis sativa plants were collected from the Tafrant region, located in the province of Taounate, Morocco (34°39'28.4"North, 5°05'58.9" West) in September 2021 (Fig. 1). After harvesting, the plant materials were subjected to shade-drying at ambient temperature under natural aeration. Subsequently, the seeds were separated and stored for later use at room temperature (between 24 °C and 27 °C) in airtight plastic bags. After drying the seeds for 24 hr at 100 ± 2 °C, the moisture content of the seeds was 5.21 ± 0.67 %.

Plant identification

The botanical identification of the plant was confirmed by a specialist at the Scientific Institute of Rabat, Morocco. Specimens were archived in the herbarium of the institute with the following identifiers: Beldia = RAB 112735, Kherdala = RAB 112220 and Critical = RAB 113319.

Insect rearing

The strain of *T. castaneum* utilised for the purpose of biological testing was obtained from the Meknes wholesale market, after identification at the phytosanitary laboratory of the National Office for Food Safety (ONSSA) in Meknes. The insects were reared in the laboratory and were maintained without exposure to insecticides. The breeding mixture comprised a precise ratio of flour, wheat germ and yeast extract (13:6:1 by weight/weight/weight), meticulously prepared in glass jars. The ambient temperature was maintained with precision, ranging from 26 ± 1 °C, while the relative humidity was meticulously controlled

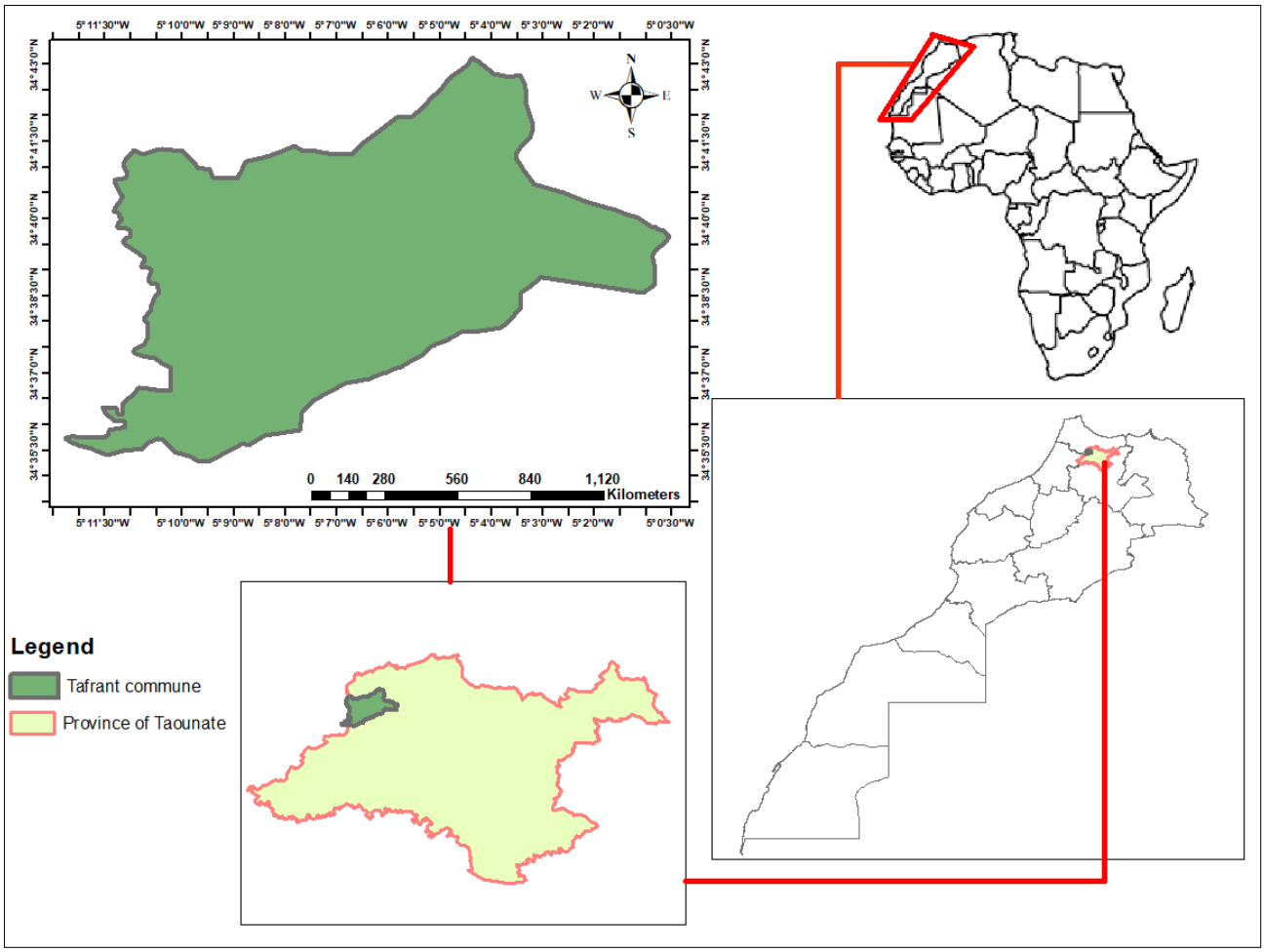


Fig. 1. Map of the geographical location of the *Cannabis sativa* collection site in Tafrant, Morocco.

to ensure it remained within the optimal range of 70 to 85 %. The photoperiod was meticulously regulated, providing a balanced cycle of 16 hr of light and 8 hr of darkness, ensuring the optimal conditions for multiple successive generations of the breeding process.

Extraction of vegetable oils

The extraction of oil from the seeds of *C. sativa* (CSO) was conducted through a mechanical cold pressing process, utilising a Cold Oil Pressing Machine (SMIR-MU-V1-70).

FTIR analysis

For the Fourier-transform infrared spectroscopy (FTIR) analysis, 20 µL of the samples were dispensed using a micropipette. The FTIR spectroscopy of CSO is obtained between the 400–4000 cm^{-1} spectral range. The samples were put directly onto the surface of a diamond ATR crystal. Measurements were taken on three separate occasions for each specimen. After that, Spectrum Quant software and OriginPro were used to conduct baseline correction and smoothing (8).

Fatty acid composition analysis of *Cannabis sativa* oil (CSO)

The official EU standard procedures outlined in Annexes II and IX of European Community Regulation EEC/2568/91 was followed in determining the fatty acid composition. In conclusion, methanolic potassium hydroxide was used for cold alkaline transesterification to create methyl esters (FAME), which were then extracted using n-heptane (23).

GC-MS analysis was conducted using a Trace GC ULTRA gas chromatograph coupled to a Polaris Q mass spectrometer equipped with a VB5 apolar column (30 m × 0.25 mm, film thickness 0.25 µm). The experiment was conducted using ultra-high purity helium as the carrier gas, with a constant flow rate of 1.0 $mLmin^{-1}$. The temperature of the injector was kept at 250 °C and both the transfer line and the ion source were set to 240 °C. Electron ionization was conducted at 70 eV. A sample volume of 1 µL was injected in split mode with a split ratio of 30:1. The oven temperature was initially set at 50 °C and held for 5 min, then increased at a rate of 4 °C/min to 250 °C, where it was held for an additional 5 min (24). Compound identification was achieved through the comparison of acquired spectra with those in established mass spectral libraries (NIST 2011 v.2.3 and Wiley, 9th edition).

Contact toxicity of *Cannabis sativa* oil (CSO)

To evaluate the effectiveness of these oils when applied to wheat grains, we used quantities of vegetable oils of 2.5, 5 and 10.0 mL kg^{-1}. For the experiment, we used untreated wheat that was pure and almost free of impurities (less than 0.9 %), with no infestation (Serein variety marketed by SONACOS). Before being used for testing, the wheat was stored at a temperature of -10 °C for one week to eliminate any insects present. Each concentration was carefully pipetted into 100 g of dry grains distributed in separate glass jars, then mixed using a glass rod. Ten adults of both sexes (aged between 2 and 4 weeks) were placed in glass jars containing various treatments. Controls were represented by untreated grains in glass jars. Three replicates of each treatment were performed. Mortality was calculated after exposure for 24 hr, 48 hr, 72 hr, 96 hr, 10 days, 15 days and 20 days and the cumulative mortality percentage was corrected using Abbott's formula (25). An insect was considered dead if it did not react in a coordinated manner when touched with a fine-bristled brush after 2 min. The LD_{50}, which corresponds to the dose required to kill 50 % of the insects, was determined using a probit analysis (3, 26).

Repellency tests of *Cannabis sativa* oil (CSO)

To determine the repellent effect of vegetable oils, we used the filter paper method (27). To do this, solutions of vegetable oil from the three varieties were prepared by dissolving 5, 7, 10, 15 and 20 µL of oil in 1 mL of acetone. Repellent activity was measured using the area preference method. Whatman No. 1 filter paper (9 cm) was cut into two halves (31.80 cm^2 each). Using a micropipette, one half of the filter paper was treated uniformly, ensuring even coverage, giving concentrations of 0.16, 0.22, 0.31, 0.47 and 0.63 µL/cm^2. As a control, the remaining half of the filter paper was treated with an equal volume of acetone. The treated and untreated halves were stuck together and placed in Petri dishes. Each filter paper disc received ten adult *T. castaneum* beetles (7–14 days old, of mixed sexes) at its centre. After that, Parafilm was used to firmly seal the Petri dishes. The number of beetles on the CSO treated portion of the discs was recorded and compared with those on the untreated portion.

Three experimental repetitions were carried out. The repulsion percentage (RP) was calculated using the formula below (8):

$$PR=(No-Nt)/(No+Nt)\times100$$

where PR is the percentage of repellency (%), N_0 is the number of adults in the control area and Nt is the number of adults in the treated area.

For each vegetable oil, the mean repellency was calculated and categorised into classes ranging from 0 % to 100 %, following the approach described by McDonald et al. (28).

Molecular docking

To explore potential interactions between the fatty acids in CSO and AChE, molecular docking studies were conducted. The AChE crystal structure (PDB ID: 4EY7) was retrieved from the Protein Data Bank (https://www.rcsb.org/, accessed 25 August 2025) and processed using AutoDock MGL Tools for refinement and preparation before docking. Ligands were retrieved from PubChem, prepared with Open Babel and molecular docking was carried out using AutoDock Vina v1.1.2 (29). The ligands included palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), γ-linolenic acid (C18:3), α-linolenic acid (C18:3), arachidic acid (C20:0) and arachidonic acid (C20:1). To elucidate molecular interactions, the top-ranked docking poses of each ligand were analysed using PyMOL (Schrödinger, New York, NY, USA) and Discovery Studio Visualizer (Dassault Systèmes, San Diego, CA, USA). Two- and three-dimensional diagrams showing interactions between the ligands and the protein active site residues were produced using Discovery Studio Analyzer (8). The docking grid was positioned at (-0.875, -51.861, 2.788) and defined with dimensions of 40 × 40 × 40 Å along x, y and z.

Statistical analysis

A completely randomized design with 3 replicates was used to examine the effects of dose, time and their interactions. Repellency and mortality data were analysed using descriptive statistics and the means and standard deviations were calculated for each treatment. Data normality was assessed before two-way ANOVA and Duncan's post hoc test (8) was applied to separate the means when significant effects were observed. Statistical analyses were conducted using SPSS Statistics version 21.

Results and Discussion

Chemical composition of *Cannabis sativa* oil (CSO)

FTIR result

The findings of the FTIR analysis are displayed in Fig. 2. Based on visual inspection, the recorded spectra are almost superimposed. Table 1 shows the vibration bands and their attribution according to the literature (30-32).

The results showed the absence of a peak in the 3300–3400 cm^{-1} band, which can be interpreted as the absence of cannabinoids in the vegetable oils derived from the seeds. The additional peaks at 2923, 2854, 1744, 1654, 1459 and 1377 cm^{-1} could correspond respectively to CH_2/CH_3 bending vibrations and C=C stretching, indicative of unsaturated compounds like carotenoids. Peaks observed at 1237, 1162 and 1097 cm^{-1} reflect C-O, C-O-C and C-C-O ester vibrations, confirming glycerol-based lipid structures (mono-, di- and triglycerides) in the oil matrix. The absorption band at 723 cm^{-1} corresponds to -CH_2- rocking vibrations in long aliphatic chains, typical of fatty acids (33).

Fatty acid composition of the *Cannabis sativa* oil (CSO)

The percentage composition (percentage by weight of the oil extracted) of the fatty acids in the oil extracted from the seeds of the three cannabis varieties was determined (Table 2) and the presence of the main fatty acids was noted: linoleic acid C18:2, the highest content of which was recorded for Bld with a value of 50.11 %, Krd with 48.81 % and Crt with 38.97 %. Oleic acid C18:1 came second with a value of 17.18 % recorded for Bld, 27.26 % for Krd and 10.82 % for Crt. For the three varieties Krd, Bld and Crt, the major fatty acids were α-linolenic acid (C18:3), palmitic acid (C16:0) and stearic acid (C18:0), while minor components such as γ-linolenic acid (C18:3) and arachidic acid (C20:0) were present at low levels (<3 %). Our results corroborate research that found the same fatty acid composition but with different percentages (23, 34).

Contact toxicity

Comparing the average concentrations, it appears that the mortality rate increases with increasing dose. The Krd variety was the most

Table 1. Major FTIR peak assignments for *Cannabis sativa* oil

Wavenumber (cm^{-1})	Functional group assignment
3010	cis double-bond stretching
2923	Asymmetrical stretching vibration of C-H in CH2
2854	Symmetrical stretching vibration of C-H in CH2
1744	C=O group Stretching
1654	Cis C=C Stretching
1459	Scissoring of C-H in CH_2 and CH_3
1377	Bending of C-H on CH_2 and CH_3
1237 and 1097	C-O group stretching
1162	C-O group stretching, bending of C-H on CH2
1097	C -O group stretching
964 and 914	Bending out of the plane of trans HC=CH
723	Rocking of CH2 and out-of-plane bending of cis disubstituted olefins
581	-------

Table 2. Fatty acid composition of *Cannabis sativa* oil

N°	Fatty acid	The content of methyl esters of fatty acids (%)		
		KHERDALA	BELDIA	CRTITICAL
1	Palmitic acid (C16:0)	7.66	7.58	9.75
2	Palmitoleic acid (C16:1)	0.63	0.51	0.18
3	Stearic acid (C18:0)	6.50	8.57	3.01
4	Oleic acid (C18:1)	27.26	17.18	10.82
5	Linoleic acid (C18:2)	48.81	50.11	38.97
6	γ -Linolenic Acid (C18:3)	2.44	3.02	2.75
7	α-Linolenic Acid (C18:3)	4.00	10.00	10.82
8	Arachidic acid (C20:0)	0.50	0.82	1.93
9	Arachidonic acid (C20:1)	0.20	0.41	2.80

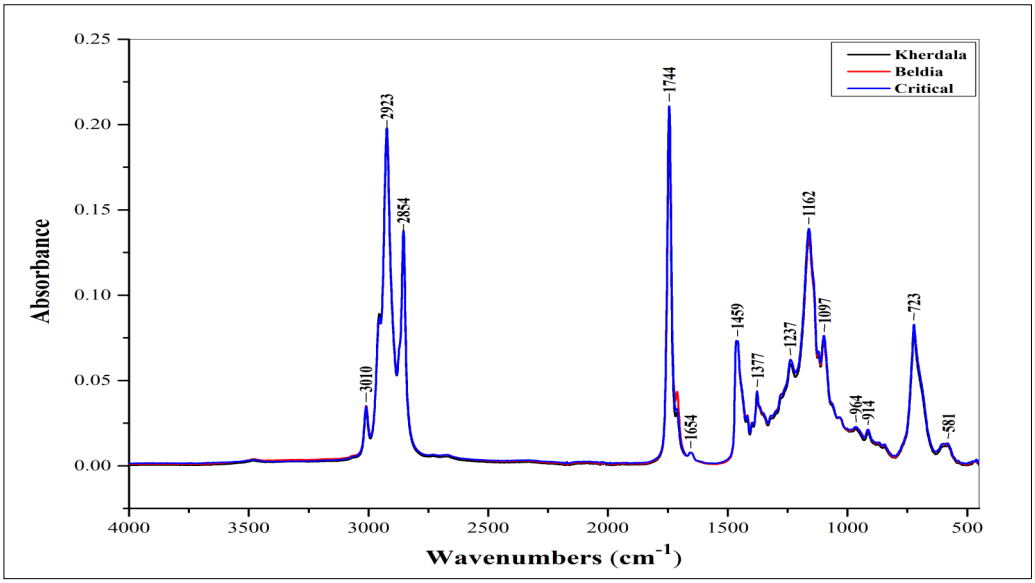


Fig. 2. Fourier transform infrared (FTIR) spectrum of *Cannabis sativa* oil showing the characteristic absorption bands.

effective, recording a mortality rate of 72 % when applying a dose of 2.5 mL kg^{-1} and almost 98 % at 10 mL kg^{-1}. The Bld and Crt varieties recorded mortality rates of 57 % and 43 % at 2.5 mL kg^{-1}, which rose to 85 % and 94 % at 10 mL kg^{-1}, respectively (Table 3).

A comparison of average times with mortality rates revealed no significant differences for the Krd variety, with mortality rates ranging from 45 % to 75 %. For the other two varieties, Bld and Crt, we recorded the highest mortality percentage at 72 hr, which was 75 % for Bld and 72 % for Crt and decreased after 72 hr.

Based on the graphs and recorded LD_{50} values, all 3 varieties demonstrated efficacy, with the Krd variety showing the highest contact toxicity (LD_{50}= 3.75 mL kg^{-1}), followed by the Bld variety (LD_{50}= 5.61 mL kg^{-1}) and the Crt variety (LD_{50} = 7.39 mL kg^{-1}) (Table 3).

This study demonstrates that the vegetable oils extracted from the Krd, Bld and Crt varieties showed significant insecticidal activity against *T. castaneum*, a major pest of wheat grains (Fig. 3-5). The effectiveness of these oils is closely linked to the dosage, duration of exposure and variety of oils evaluated. In our experiments, the mortality rate of the species evaluated was related to dosage and duration of exposure. The contact toxicity of these vegetable oils against *T. castaneum* has been demonstrated by numerous studies on other post-harvest wheat species. In this context, crude olive pomace oil, refined pomace oil and extra virgin olive oil exhibited significant contact toxicity against the stored-grain pests *S. oryzae* and *Rhyzopertha dominica* (3). A concentration of 20 mL kg^{-1} of peanut oil led to total mortality of adult *S. zeamais* after 24 hr was reported earlier (35). Vegetable oils extracted from soybean, cotton, peanut, palm and corn seeds were tested for their efficacy against *R. dominica* and *Cryptolestes pusillus* (36). Treatment of corn and sorghum seeds with a dose of 10 mL kg^{-1} of the various oils resulted in 100 % mortality within 24 hr. Similar results were obtained when sunflower, peanut and rapeseed oils were applied to wheat seeds at a concentration of 10 mL kg^{-1} against *S. granarius*, as well as with coconut, sweet almond and chamomile oils (10). A control rate exceeding 95 % was achieved after exposure of *R. dominica* to wheat treated at a dose of 10.0 mL kg^{-1} for 24 hr (21).

On other crops, a mortality rate of 99.1 % was observed after treating adult *Acanthoscelides obtectus* (Say) with a dose of castor oil (*Ricinus communis* L.) and cottonseed oil (*Gossypium hirsutum* L.) of 9 mL kg^{-1} of dry beans (LC_{50} = 2.95 mL kg^{-1} after 120 hr of exposure) (37). *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae) can be effectively controlled with a dose of 0.8 mL per 50 g of cowpea. Some researchers have demonstrated the efficacy of various treatments based on vegetable oils, including olive oil, as well as the main fatty acids present in olive oil (oleic, stearic, linoleic and palmitic) (38, 39).

In previous studies, CSOs have not been tested against pests in post-harvest stored commodities, while essential oils (40, 41), aqueous extracts (42) and cannabidiol oil have been evaluated for their insecticidal properties (43). The insecticidal activity could be attributed to the characteristics of the oils (3). It is indeed possible that the toxic effects of crude oils are due to potential synergistic activity between their components (3, 39). Another important factor that the species under study is agile, which increases contact with toxic vegetable oils (44).

About the mode of action of vegetable oils, insecticidal activity has been attributed by some authors to their fatty acid and triglyceride content (3). Oils have been reported to disrupt insect respiration or oxygen deprivation (anoxia) (45). Oils may also act as a deterrent by disrupting the feeding behaviour of insects or by altering storage conditions, making them less favourable for infestation (36, 46).

Repellent activity

Regarding the repellent activity of the CSO of the 3 varieties and comparing the different doses, we note that the Bld and Crt varieties showed high repellent power ranging from class II to IV, the Bld variety recorded the highest repellent percentage, which was around 91.67 % for the 0.63 µL/cm^2 dose, while the average repellent percentage for Krd was recorded in a range from 55 to 68 %, with most classes belonging to class IV. For the Krd variety, the average repellency ranged from a minimum of 15 % to a maximum of 40 % belonging to classes I, II and III, showing low repellency compared to other varieties (Table 4).

By comparing the fluctuation in the repellency percentage over time, we found that the repellency percentage increased when the exposure time was increased from 2 hr to 24 hr for the Bld and Crt varieties, while for Krd we initially recorded an increase from 30 % to 40 % at 2 hr and 4 hr respectively, followed by a decrease in the repellency percentage at 6 and 24 hours from 36 % to 26 %.

The graphs clearly show the evolution of the repellency percentage at different concentrations of cannabis vegetable oil for the 3 varieties, confirming that the oil from the two varieties Bld and Crt was more effective, whereas the oil from the Krd variety was classified as non-repellent to weakly repellent, according to the classification scale (28) (Fig. 6-8).

This study revealed that cannabis vegetable oil, particularly the two varieties Bld and Crt, has repellent effects on *T. castaneum*, a major cereal pest in Morocco, compared to oil extracted from the Krd variety. Nowadays, many researchers are interested in botanical products to replace chemicals that have harmful effects on human health and the environment. Much work has been done on essential

Table 3. Mortality (%) of *Tribolium castaneum* exposed to *Cannabis sativa* oil as a function of mean dose and exposure time

		BELDIA	KHERDALA	CRITICAL
Mean of concentration	Control	0.0 ± 0.0 a	0.95 ± 0.66 a	0.0 ± 0.0 a
	2.5 mL kg^{-1}	56.76 ± 7.52 b	71.90 ± 5.92 b	43.19 ± 7.00 b
	5 mL kg^{-1}	66.38 ± 6.85 b	88.10 ± 3.42 c	78.62 ± 5.21 c
	10 mL kg^{-1}	84.71 ± 4.80 c	97.62 ± 0.95 c	93.62 ± 2.70 d
Mean of time	24 h	67.00 ± 12.04 c	73.33 ± 12.81 a	63.50 ± 11.95 ab
	48 h	75.00 ± 13.06 c	75.00 ± 13.06 a	69.50 ± 12.46 b
	72 h	75.00 ± 13.06 c	76.67 ± 12.21 a	71.42 ± 12.57 b
	96 h	18.67 ± 4.95 a	45.00 ± 10.84 a	28.33 ± 9.24 a
	10 d	30.92 ± 7.30 ab	55.83 ± 10.69 a	37.25 ± 11.04 ab
	15 d	43.75 ± 10.76 abc	59.17 ± 11.45 a	50.00 ± 12.00 ab
	20 d	53.42 ± 5.8 bc	67.50 ± 12.07 a	57.00 ± 11.88 ab
p-value dose		≤ 0.0001	≤ 0.0001	≤ 0.0001
p-value Time		0.001	NS	NS
LD_{50} (mL kg^{-1})		5.61	3.75	7.39

Table 4. Repellency (%) of *Tribolium castaneum* exposed to *Cannabis sativa* oil as a function of mean dose and exposure time

	BELDIA		KHERDALA		CRITICAL	
Concentration (µL/cm²)	**Mean Repellency (%)**	**Class**	**Mean Repellency (%)**	**Class**	**Mean Repellency (%)**	**Class**
0.16	25.00 ± 7.02 a	Class II	15.00 ± 12.58 a	Class I	65.00 ± 07.83 a	Class IV
0.22	80.00 ± 4.26 b	Class IV	50.00 ± 7.18 b	Class III	63.33 ± 5.95 a	Class IV
0.31	90.00 ± 3.02 b	Class V	40.00 ± 8.52 ab	Class II	55.00 ± 6.09 a	Class III
0.47	90.00 ± 3.02 b	Class V	26.67 ± 5.69 ab	Class II	68.33 ± 6.72 a	Class IV
0.63	91.67 ± 2.97 b	Class V	35.00 ± 8.57 ab	Class II	65.00 ± 7.83 a	Class IV
Mean of Time (hr)	**BELDIA**		**KHERDALA**		**CRITICAL**	
2	69.33 ± 8.69 a		30.67 ± 9.54 a		52.00 ± 5.45 a	
4	69.33 ± 8.25 a		40.00 ± 8.28 a		64.00 ± 5.24 ab	
6	77.33 ± 7.27 a		36.00 ± 7.35 a		64.00 ± 5.24 ab	
24	85.33 ± 3.81 a		26.66 ± 7.97 a		73.33 ± 3.05 b	
p-value dose	≤ 0.0001		0.073		NS	
p-value time	NS		NS		NS	

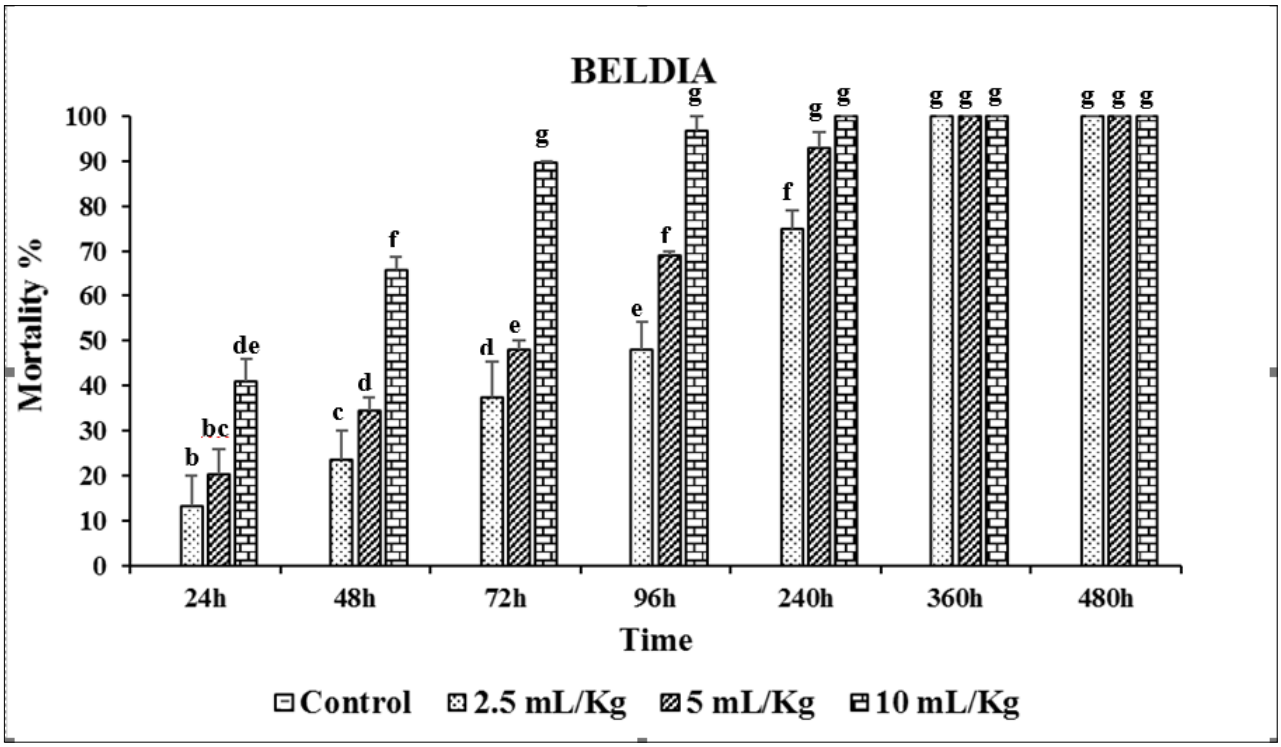


Fig. 3. Contact toxicity of different concentrations of *Cannabis sativa* vegetable oil variety BELDIA against *Tribolium castaneum* beetles.

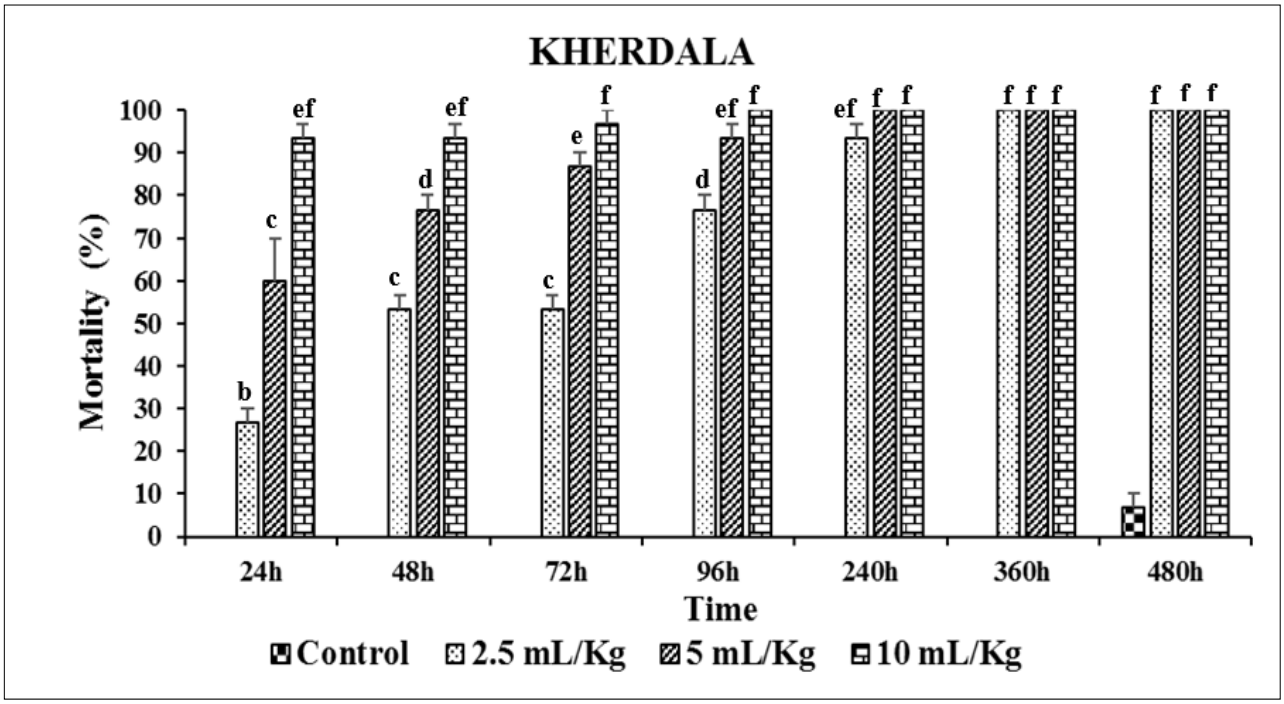


Fig. 4. Contact toxicity of different concentrations of *Cannabis sativa* vegetable oil variety KHERDALA against *Tribolium castaneum* beetles.

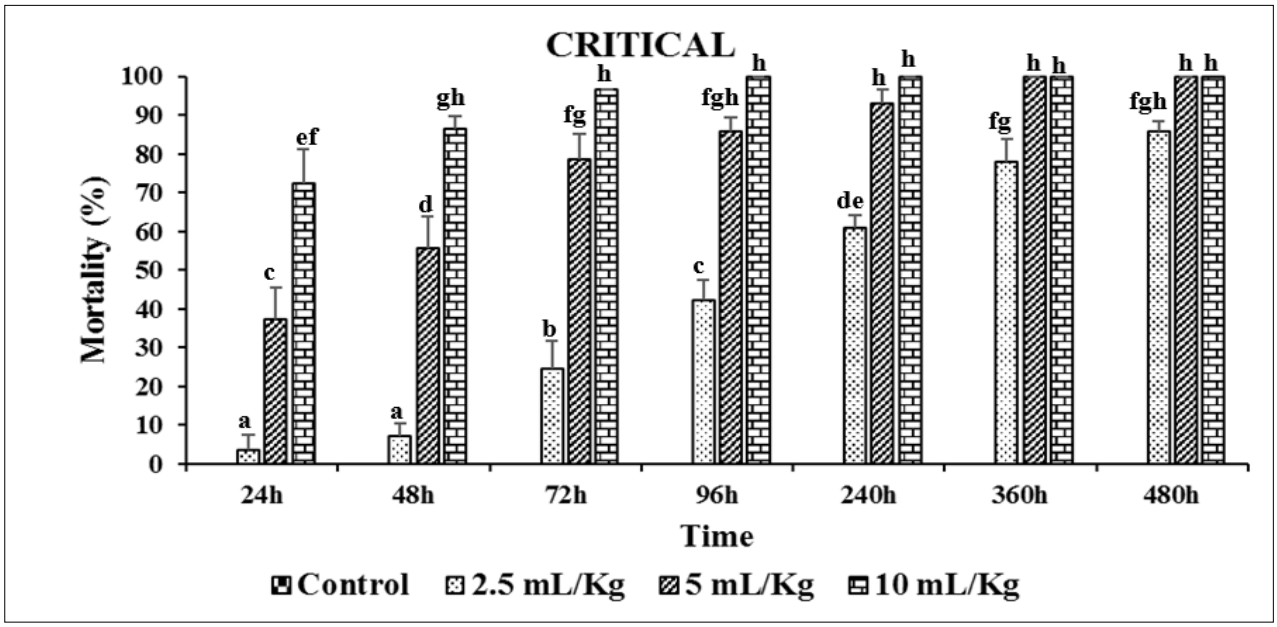


Fig. 5. Contact toxicity of different concentrations of *Cannabis sativa* vegetable oil variety CRITICAL against *Tribolium castaneum* beetles.

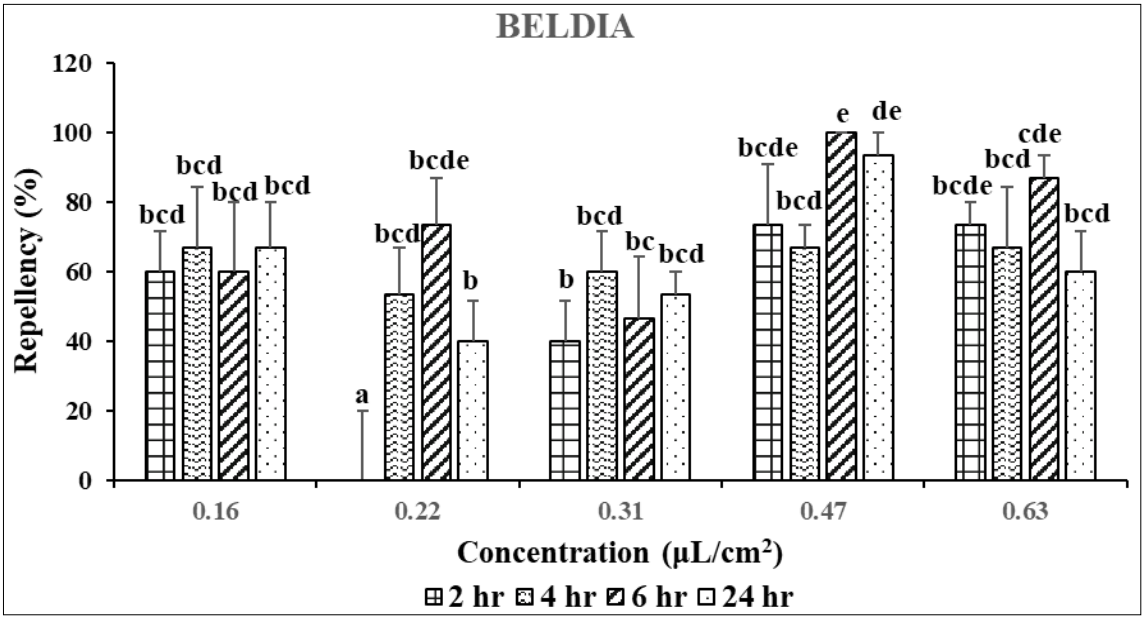


Fig. 6. Percentage repellency (PR) of the vegetable oil extracted from *Cannabis sativa* variety BELDIA against *Tribolium castaneum* at 2 hr, 4 hr, 6 hr and 24 hr after exposure.

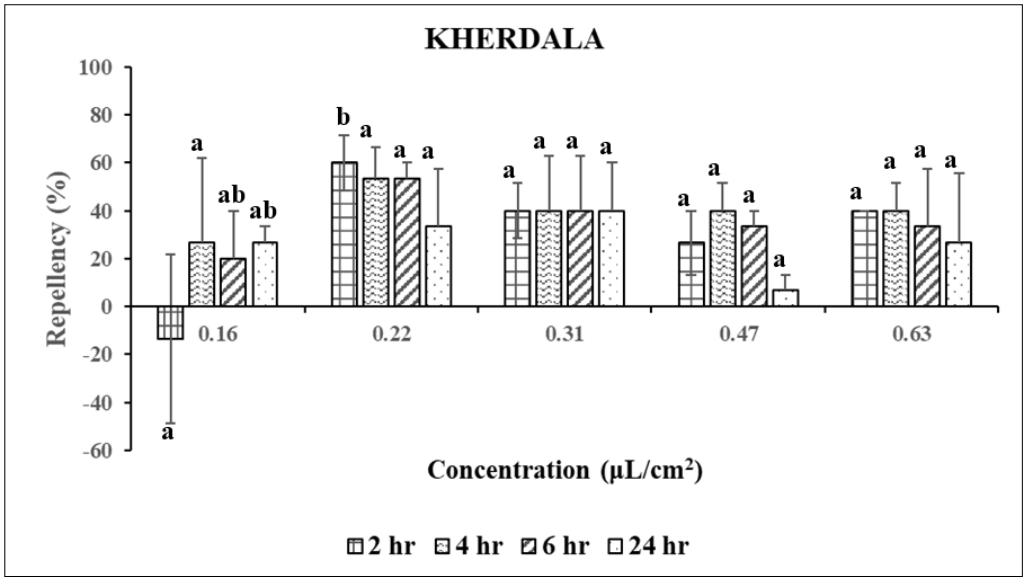


Fig. 7. Percentage repellency (PR) of the vegetable oil extracted from *Cannabis sativa* variety KHERDALA against *Tribolium castaneum* at 2 hr, 4 hr, 6 hr and 24 hr after exposure.

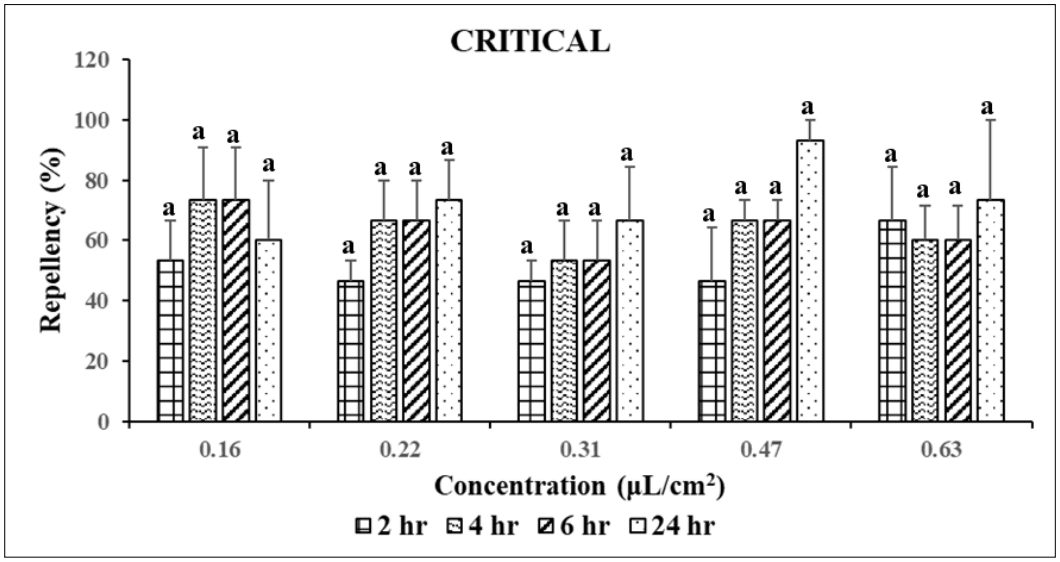


Fig. 8. Percentage repellency (PR) of the vegetable oil extracted from *Cannabis sativa* variety CRITICAL against *Tribolium castaneum* at 2 hr, 4 hr, 6 hr and 24 hr after exposure.

oils, but the problem lies in the volatility of the chemical compounds, which limits their use (6, 47). However, vegetable oils offer a better alternative given their low volatile compound content and high fatty acid content (20). Our work is oriented in this direction; our results corroborate findings on the same pest, for which soybean oil recorded a repellency rate of 40 % after exposure to a dose of 0.0019 µl/cm^2, while a dose of 0.016 µL/cm^2 resulted in a repellency rate of 80 % (27). Previous studies have shown that soybean oil exerts a repellent effect on *Neoseiulus baraki* and *Periplaneta americana* (48, 49). Similarly, a repellency rate of 90 % was achieved by applying soybean oil to the pest *S. granaries* after 48 hr of exposure.

Although the mechanism of action of plant oils remains unknown owing to the rarity of volatile compounds, the repellent effect may be mainly attributable to tactile interactions, that is, contact (27). Similarly, it has been shown that the free fatty acids present in vegetable oils act as warning signal molecules for certain insect species (50). Linoleic acid, present in relatively high concentrations in soybean and sweet almond oils, has been reported to exert harmful and repellent effects on *Stomoxys calcitrans* (biting house fly) (51). Several studies have demonstrated that vegetable oils can amplify the efficacy of essential oils and other insecticidal substances when used in combination (52).

We can also discuss the persistence of the action of vegetable oils, which, in our case, was limited to 24 hr after application. Vegetable oils can repel pests at maximum doses for up to 21 days following application, offering an advantage over essential oils, which typically exhibit only short-term repellent effects (53). This may be due to their low volatile compound content and high fatty acid content (27).

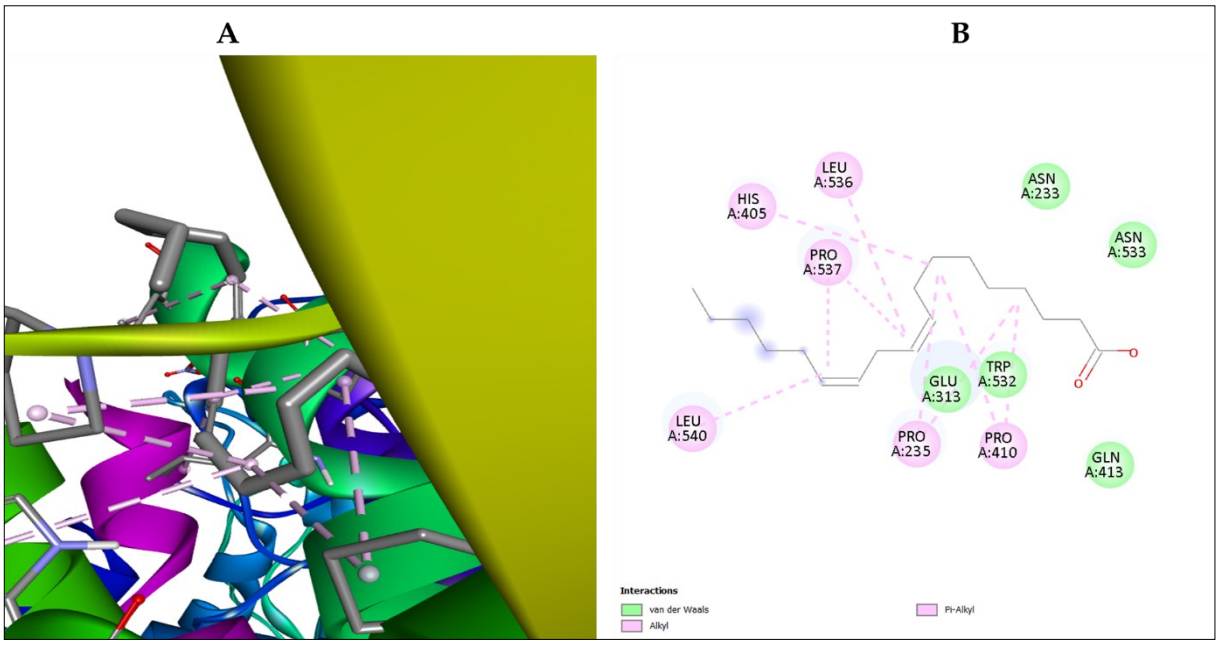
Molecular docking

The enzyme acetylcholinesterase (AChE; PDB ID: 4EY7) was selected as a virtual target to evaluate and classify the principal constituents of vegetable oils according to their binding affinities. The docking score indicates the free binding energy, with increasingly negative values denoting greater binding affinity. This bond is formed through various chemical or physical interactions between the protein and the ligand, including hydrophobic, hydrogen and electrostatic bonds. As detailed in the Table 5, molecular docking showed that γ-Linolenic Acid (C18:3) exhibited the highest activity, with a score of -6.1 kcal/mol, followed by Arachidonic acid (C20:1) (-5.7 kcal/mol),

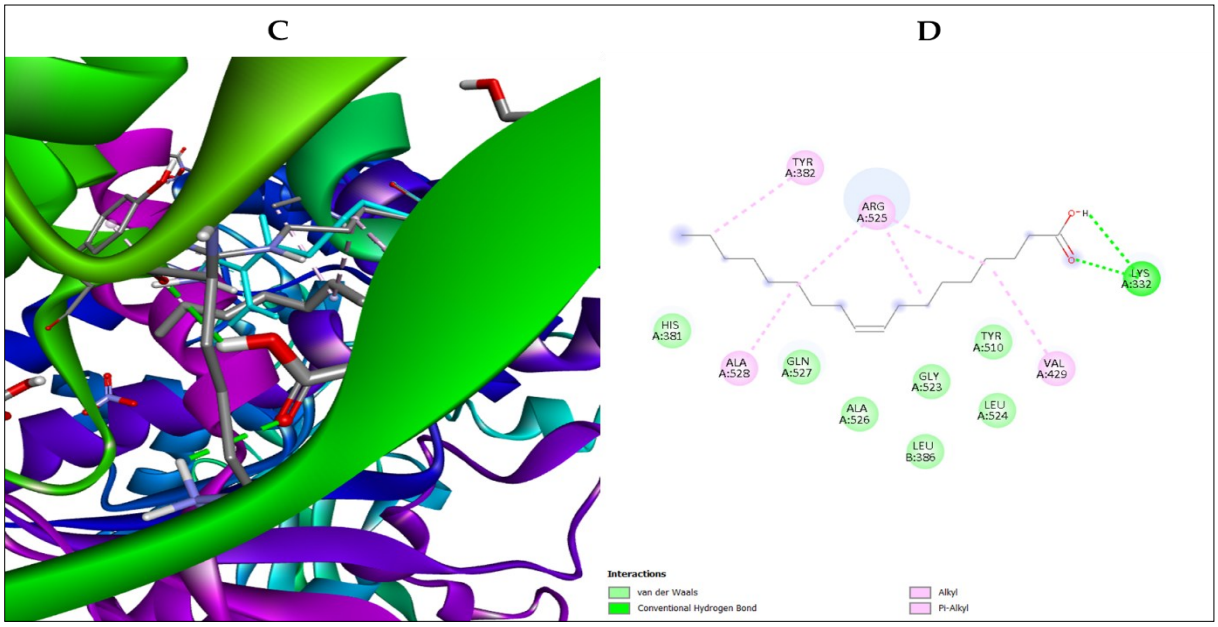
Table 5. Docking results with fatty acids identified in *Cannabis sativa* oil in the active sites of AChE (PDB: 4EY7)

Ligand	AChE binding affinity (kcal/mol)
Linoleic acid (C18:2)	-4.7 kcal/mol
Oleic acid (C18:1)	-5.3 kcal/mol
α-Linolenic Acid (C18:3)	-5.3 kcal/mol
Palmitic acid (C16:0)	-4.6 kcal/mol
Stearic acid (C18:0)	-5.2 kcal/mol
Palmitoleic acid (C16:1)	-5.2 kcal/mol
γ -Linolenic Acid (C18:3)	-6.1 kcal/mol
Arachidic acid (C20:0)	-5.6 kcal/mol
Arachidonic acid (C20:1)	-5.7 kcal/mol

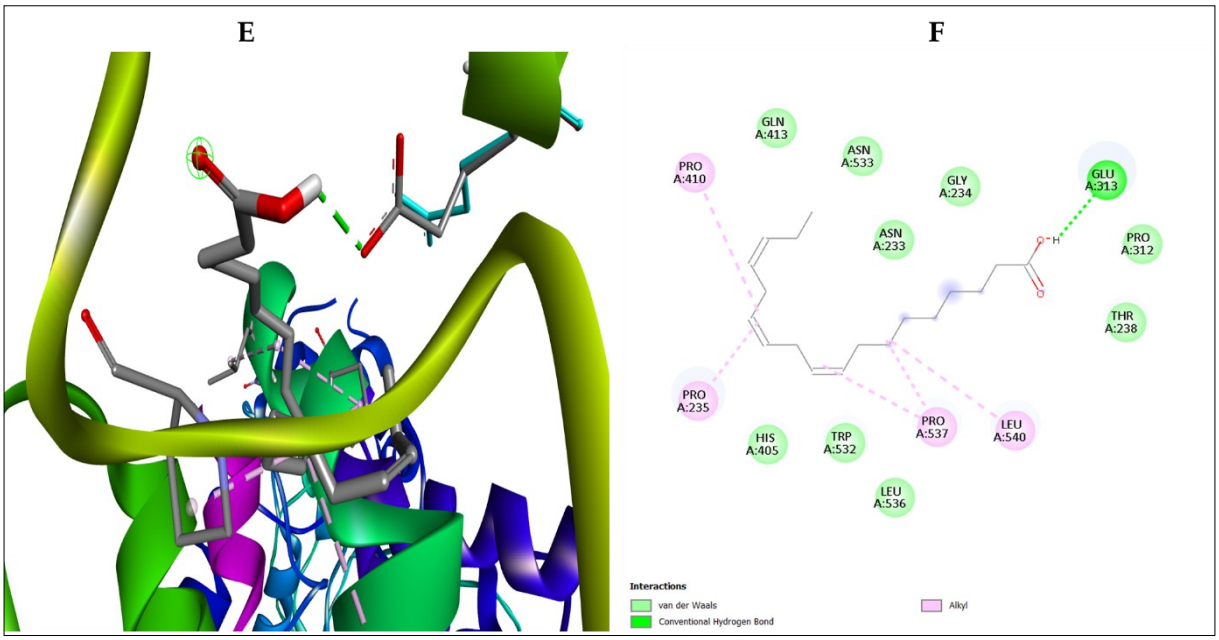
Arachidonic acid (C20:1) (-5.7 kcal/mol), Oleic acid (C18:1) and α-Linolenic Acid (C18:3) with a score of -5.3 kcal/mol, Palmitoleic acid (C16:1) and Stearic acid (C18:0) with an affinity of around -5.2 kcal/mol and finally Linoleic acid (C18:2) and Palmitic acid (C16:0) with scores of -4.7 and -4.6 kcal/mol respectively. Based on the 2D and 3D visualizations (Fig. 9) of the ligand-protein interactions, we observe that all ligands establish strong binding interactions at the protein's active site. This indicates that the compounds could serve as potential inhibitors, particularly those that are predominantly present in CSO, namely oleic acid (C18:1) and linoleic acid (C18:2). Taking the analysis further, we noted that γ-linolenic acid had the best binding score (-6.1 kcal/mol), even though it was a minor component (< 3.1 %), while linoleic acid, which is very abundant, had a relatively low interaction score (-4.7 kcal/mol). This observation highlights an apparent contradiction between the molecular docking results and the chemical composition of the oil used. However, this discrepancy is widely documented in the literature and can be explained by the fact that binding scores reflect a theoretical ligand-target affinity, independent of the actual concentration of the compound in the natural matrix. Minor constituents can exert significant biological activity when they have a favorable molecular geometry, better steric complementarity, or specific interactions (hydrogen bonds, hydrophobic interactions) with the active site of the target protein, unlike less specific major compounds (54, 55). Furthermore, the overall biological activity of an extract or vegetable oil often results from synergistic or additive effects between several compounds, which cannot be fully understood by molecular docking alone (56). Thus, our results suggest that γ-linolenic acid could contribute in a targeted manner



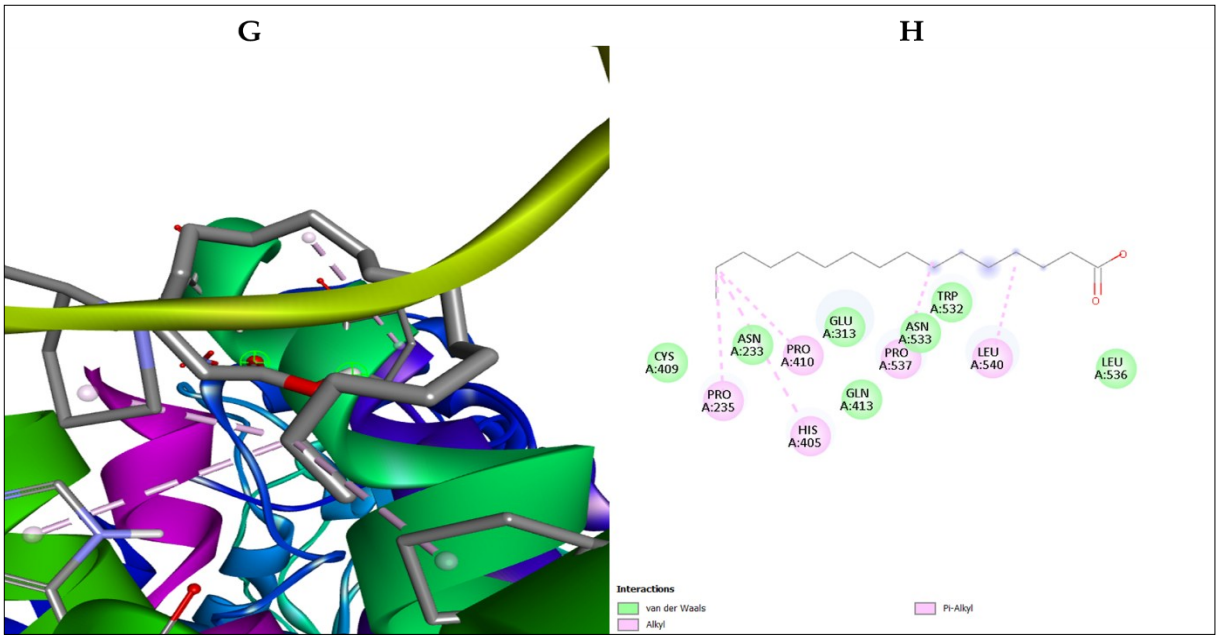
Linoleic acid (C18:2)



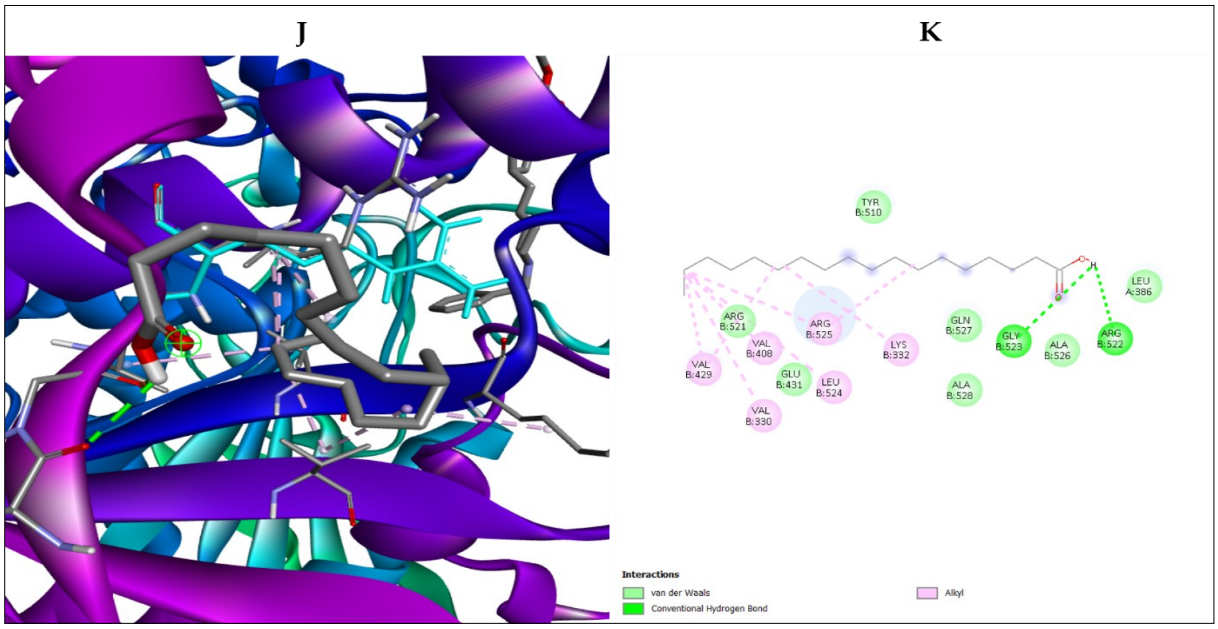
Oleic acid (C18:1)



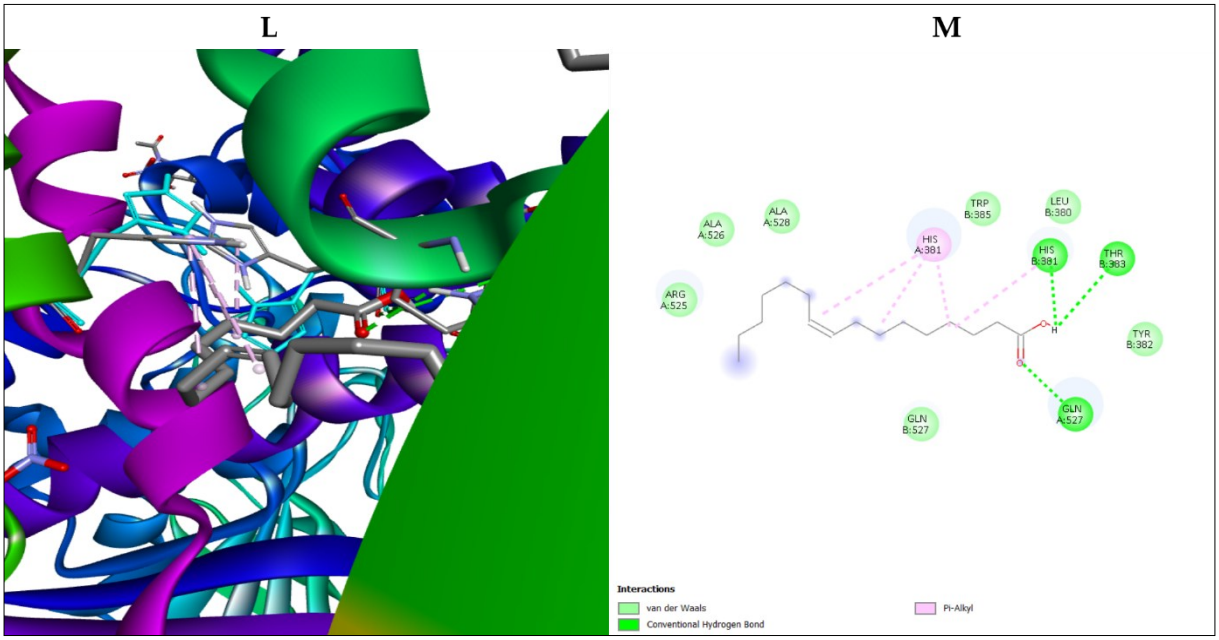
α-Linolenic Acid (C18:3)



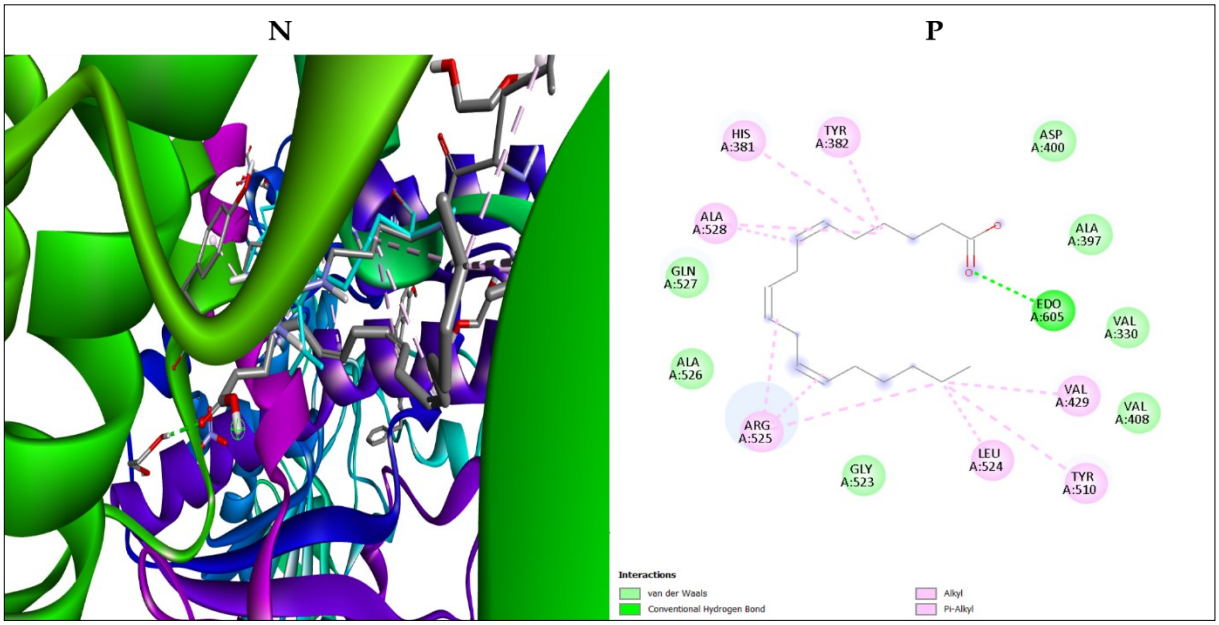
Palmitic acid (C16:0)



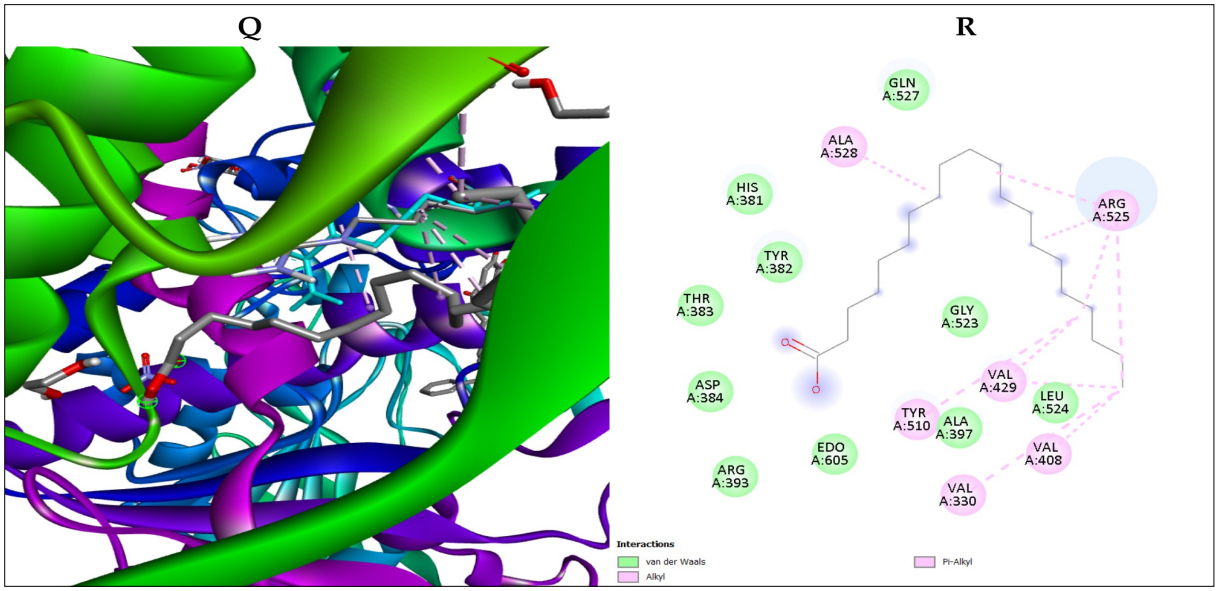
Stearic acid (C18:0)



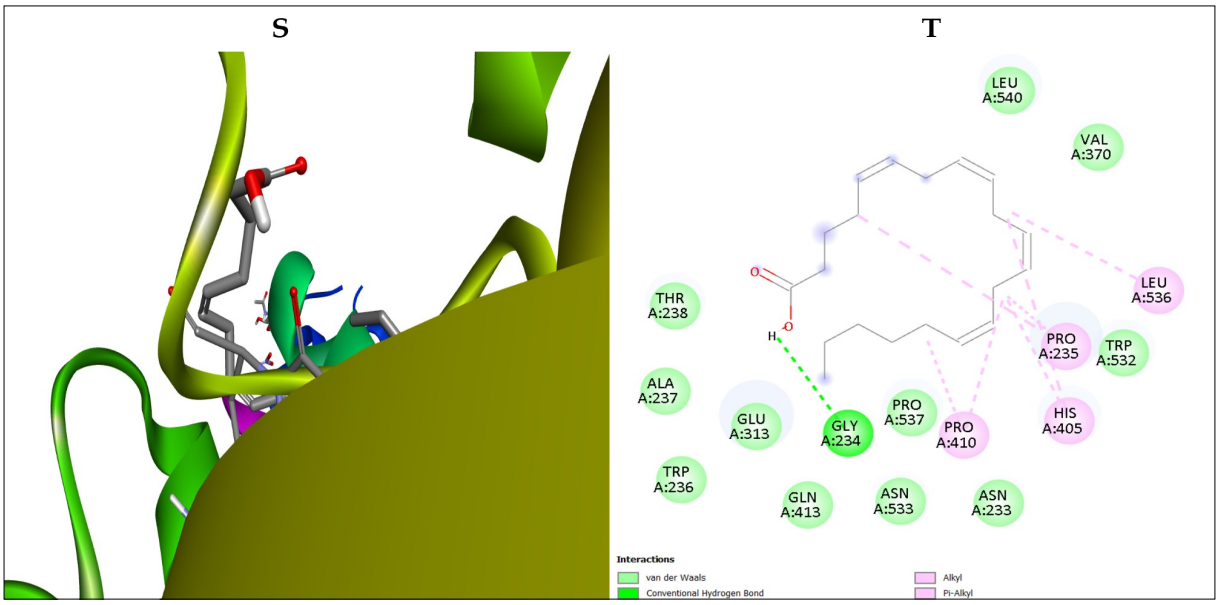
Palmitoleic acid (C16:1)



γ -Linolenic Acid (C18:3)



Arachidic acid (C20:0)



Arachidonic acid (C20:1)

Fig. 9. 3D (A-C-E-G-J-L-N-Q-S), 2D (B-D-F-H-K-M-P-R-T) diagrams from contacts of Ache active site amino acid residues with all fatty acids of CSO.

to the observed activity, while linoleic acid could contribute more to the overall effect via indirect or synergistic mechanisms, highlighting the need to combine *in silico* approaches with experimental biological studies for a more complete interpretation. Interactions such as hydrophobic contacts, hydrogen bonds and van der Waals forces are crucial for increasing ligand-binding affinity. Take oleic acid, for example, which forms hydrogen bonds with LYS 332. Van der Waals interactions are formed with the amino acid residues HIS 381, GLN 527, ALA 526, GLY 523, TYR 510, LEU 386 and LEU 524. In addition, alkyl and pi-alkyl interactions were observed with TYR 382, ARG 525, ALA 528 and VAL 429. For linoleic acid (C18:2), we observe van der Waals bonds with ASN 233, ASN 533, GLU 313, TRP 532 and GLN 413 and alkyl and pi-alkyl interactions were also formed with the amino acid residues LEU 536, HIS 405, PRO 537, LEU 540, PRO 235 and PRO 410.

Molecular docking was performed on AChE, an essential enzyme responsible for the breakdown of acetylcholine in insect synapses. Many insecticides, such as carbamates and organophosphates, inhibit this enzyme, causing acetylcholine accumulation. This accumulation leads to continuous stimulation of cholinergic receptors, causing muscle spasms, paralysis and ultimately death in insects (57). In this study, all major and minor constituents interacted with our enzyme, suggesting that the capacity of the selected vegetable oils to engage in these key molecular interactions with AChE either through individual effects or synergistic interactions between components reflects their potential inhibitory effect on the protein and, consequently, their possible insecticidal activity.

Conclusion

In conclusion, the 3 cannabis varieties (Krd, Bld and Crt) exhibited repellent activity and contact toxicity against *T. castaneum*. Fatty acids, particularly oleic and linoleic acids, are the main candidates, given their predominant presence in the three plants and their potential AChE inhibitory activity. Consequently, CSOs could be useful as an effective biopesticide for controlling adult *T. castaneum* and serve as an alternative to synthetic insecticides. However, further research is required to assess their long-term effectiveness under real storage conditions. Further studies should also explore possible synergistic interactions with other vegetables or essential oils. Another aim should also be to determine their impact on the organoleptic and nutritional quality of grains. Optimizing formulations for better stability and effectiveness is also important. Finally, consumers' safety must be verified. These prospects are instrumental in facilitating the agro-industrial development of local hemp, thereby contributing to enhanced food security and reducing the utilization of synthetic insecticides.

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Authors' contributions

REB conducted the experiment in the laboratory, collected, analysed and interpreted the data and drafted the manuscript. SB conceived of the study and participated in its design and coordination. AB performed the analysis, investigation and interpretation of the data. LN supervised all experiments and performed the statistical analysis. SEA participated in experimental analysis and contributed to the manuscript's drafting. REM performed the statistical analysis. KEO performed molecular docking and participated in writing and editing. IK performed the analysis, investigation and interpretation of the data. EHB conceptualized the study, critically revised the manuscript and supervised the research. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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