



Chemical analysis and insecticidal activity of *Cuminum cyminum* hexane extract against the cotton leaf worm *Spodoptera littoralis* (Boisd.)

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Abstract

The present work was performed to study the toxic effect of *Cuminum cyminum* hexane extract on the 2nd and 4th instar larvae of the cotton leaf worm *Spodoptera littoralis*. The extract was effective against the two instars under studying. The LC_{50} values against the 2nd and the 4th instars were 0.008 (g extract/100g diet) and 0.0134 (g extract/100g diet), respectively after 8 days post treatment. The GC-MS analysis of hexane extract of *Cuminum cyminum* seeds indicated that the extract is composed mainly of cuminaldehyde (26.61%), 3-Methylene-6-hepten-1-ol (13.83%), 7-oxabicyclo[4.1.0]heptane,1-(1,3dimethyl-1,3-butadienyl)-2,2,6-trimethyl,(E) (15.45%), Benzyl-(S)(+)-2-hydroxy-2-phenylacetate (8.71%), (1,3-Dimethyl-2-methylenecyclopentyl)-methanol (4.13%), P-cymene, (3.71%), Acoradiene (2.46%), (E)- α -Famesene (2.38%), and α -Ionone (2.22%).

Keywords: *Cuminum cyminum*- extract- *Spodoptera littoralis*- chemical constituents- GC-MS

Introduction

Currently, chemical pesticides are broadly used for pest population control. In recent decades, the application of these chemicals has caused serious problems, such as pesticides resistance, loss of pests' natural enemies and environmental pollution. In addition, they had a detrimental effect on human health due to pesticides residue in agricultural products (Othria *et al.*, 2009) [14].

Cumin (*Cuminum cyminum*) is a flowering plant in the family Apiaceae, native from the east Mediterranean to East India. Cumin seeds are oblong and yellow–grey. Cumin seeds are liberally used in several cuisines of many different food cultures since ancient times, in both whole and ground forms (Srinivasan, 2018) [19].

The objective of the present study is an investigation of toxicity of *Cuminum cyminum* hexane extract on *Spodoptera littoralis*. The chemical constituents of the extract were determined by GC-MS analysis.

Materials and Methods

Insect rearing

Spodoptera littoralis larvae used in this study were obtained from Insect Pathogen Unit-Plant Protection Research Institute-Agriculture Research Center. The culture was maintained on an artificial diet developed by Shory and Hale (1965) at 26°C, 75% RH and natural photoperiod El-Defrawi., *et al* (1964) [10] with extremely controlled condition to avert any contamination.

Plant material and extraction method

The dry seeds of *C. cyminum* were obtained from supermarket in Jeddah, Kingdom of Saudi Arabia, in September 2019. The dry seeds (250g) were grounded and then macerated in 1500ml of hexane. After leaving the solution 7 days, it was filtered through Whatman No 40 filter paper. The solvent was removed under reducing

pressure using a rotary evaporator to obtain oily dark extract (17.33g). Five concentrations 0.01, 0.03, 0.06, 0.125 and 0.25 (g extract/100g diet) were prepared to be used in the bioassay experiment to test the plant extract against *S. littoralis* (Moustafa H. Z. and Yousef H. 2020) [13].

Toxicity test for plant extract

Concentrations that have been prepared from the plant extract were tested separately against 2nd and 4th instar larvae. The diet surface treatment procedure was applied according to Addy N.D. (1969) [1], in which the larvae allowed to feed on contaminated artificial diet with plant extract concentrations for 2 days then transferred to clean cups contain untreated diet and observed daily, the diet which served to control larvae treated only with distilled water. Thirty larvae for each concentration and thirty larvae for control were triplicate. The mortality rate was recorded every 2 days till 10 days post treatment.

GC-MS analysis of hexane extract

The GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 251mm, 0.1 mm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used, Helium gas was used as the carrier gas at a constant flow rate of 1mL/min. The injector and MS transfer line temperature was set at 280 °C. Vol. oil: The oven temperature was programmed at an initial temperature 40 °C (hold 3 min) to 280 °C as a final temperature at an increasing rate of 5 °C /min (hold 5 min). The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system.

Statistical analysis

Concentrations of tested plant extract with mortality rates were computed to be analyzed and to determine the fifty percent lethal concentration (LC₅₀) by using Ldp Line software (Bakr 2000)^[3].

The differences significances were calculated by ANOVA and Duncan's multiple range tests (ANOVA of arcsine square root transformed percentages). Differences between the treatments were determined by Tukey's multiple range test ($P < 0.05$) (Snedecor and Cochran, 1989)^[18].

Results and Discussion

As shown in table (1), the GC-MS analysis of hexane extract of *Cuminum cyminum* seeds indicated that the extract is composed mainly of cuminaldehyde (26.61%), 3-Methylene-6-hepten-1-ol (13.83%), 7-oxabicyclo[4.1.0]heptane,1-(1,3dimethyl-1,3-butadienyl)-2,2,6-trimethyl,(E) (15.45%), Benzyl-(S)(+)-2-hydroxy-2-phenylacetate (8.71%), (1,3-Dimethyl-2-methylenecyclopentyl)-methanol (4.13%), P-cymene, (3.71%), Acoradiene (2.46%), (E)- α -Famesene (2.38%), and α -Ionone (2.22%).

Table 1: Determination of volatile chemicals of hexane extract of *Cuminum cyminum* seeds by GC-MS analysis.

Compound	Retention time	Area %
α -Pinene	9.59	1.08
α -Myrcene	10.12	0.24
α -Phellandrene	10.45	0.25
P-CYMENE	11.09	3.71
β -Phellandrene	11.19	0.56
3-Methylene-6-hepten-1-ol	12.18	13.83
Cis-Sabinene hydrate	12.42	0.16
Trans- Sabinene hydrate	13.34	0.91
1-Pentanol, 5-cyclopropylidene-	15.35	0.44
(-)-terpinen-4-ol	15.65	0.17
(1,3-Dimethyl-2-methylenecyclopentyl)-methanol	16.10	4.13
cuminaldehyde	17.49	26.61
p-Isopropylphenol	18.01	0.32
Safranal	18.65	0.78
Benzyl-(S)(+)-2-hydroxy-2-phenylacetate	18.85	8.71
Bicyclo(3.1.1)heptanes-2,3-diol,2,6,6-trimethyl-	19.70	0.32
p-Mentha-1,4-dien-7-ol	20.00	1.66
Calarene	21.10	1.05
Germacrene D	21.23	0.30
Trans-Caryophyllene	22.11	1.18
trans - α -Bergamotene	22.48	0.34
(E)- α -Famesene	22.99	2.38
α -Cubebene	23.37	0.91
Acoradiene	23.47	2.46
Ar-Curcumene	23.62	0.41
Δ -Chamigrene	24.05	0.28
α -Bisabolene	24.24	0.33
(-) Caryophyllene oxide	26.03	0.23
Carotol	26.34	1.30
Eudesmol	27.91	0.15
α -Phellandrene	30.50	0.18
α - Ionone	31.02	2.22
Neophytadiene	31.30	0.48
Acridine	39.36	0.45
7-Oxabicyclo[4.1.0]heptane,1-(1,3dimethyl-1,3-butadienyl)-2,2,6-trimethyl,(E)	39.89	15.45
3-Methyl-2-butenic acid, cyclobutyl ester	43.90	0.96

Wanner *et al.*, 2010^[21] reported the presence of α -Pinene, Myrcene, α -Phellandrene, *cis*-Sabinene hydrate, *trans*-Sabinene hydrate, P-Cymene, β -Phellandrene, *p*-Isopropylphenol, *p*-Mentha-1,4-dien-7-ol, Cuminaldehyde, Bergamotene, Acoradiene and Carotol in *Cuminum cyminum* L. oil. Gotmare and Tambe, 2018 showed that *Cuminum cyminum* L. oil constituents contain α -Pinene, P-Cymene, (-)-terpinen-4-ol, 3-Cyclohexene-1-carboxaldehyde, 1, 3, 4-trimethyl- and Cuminaldehyde. Rana, 2014 recorded the presence of α -Pinene, P-Cymene, Myrcene, α -Phellandrene, terpinen-4-ol and Cuminaldehyde in the *C. cyminum* oil. Boughendjioua H. 2019 found that *C. cyminum* oil constituents contain α -Pinene, Myrcene, α -

Phellandrene, *p*-cymene and Cuminaldehyde and Safranal.

The data in table (2), describe the toxic effect of *C. cyminum* against the 2nd and the 4th instar larvae of *S. littoralis*. The extract under studying showed a 96.60% mortality at concentration of 0.25 (g extract/ 100g diet) against the 2nd instar larvae, while the highest mortality against the 4th instar larvae was 86.67% at concentration of 0.25 (g extract/ 100g diet). The LC₅₀ values against the 2nd and the 4th instars were 0.008 (g extract/ 100g diet) and 0.0134 (g extract/ 100g diet), respectively after 8 days post treatment. Generally, the percentage of mortality of the 2nd and the 4th instar larvae of *S. littoralis* increased with the increasing in concentration of extract.

Table 2: Toxic effect of *Cuminum cyminum* hexane extract against the 2nd and the 4th instar larvae of *Spodoptera littoralis*.

Concentrations (%)	Mortality Percentage (%)									
	The 2 nd instar larvae					The 4 th instar larvae				
	2 days	4 days	6 days	8 days	10 days	2 days	4 days	6 days	8 days	10 days
0.250	53.33 ^a	63.33 ^a	80.00 ^a	96.6 ^a	96.6 ^a	40.00 ^a	56.67 ^a	73.33 ^a	86.67 ^a	86.67 ^a
0.125	43.33 ^b	56.67 ^b	70.00 ^b	80.00 ^b	96.6 ^b	26.67 ^b	50.00 ^b	66.67 ^b	76.67 ^b	86.67 ^b
0.060	33.33 ^c	46.67 ^c	63.33 ^c	73.33 ^c	86.67 ^c	23.33 ^c	40.00 ^c	53.33 ^c	66.67 ^c	76.67 ^c
0.030	23.33 ^d	46.67 ^d	56.67 ^d	66.67 ^d	80.00 ^d	13.33 ^d	26.67 ^d	43.33 ^d	56.67 ^d	66.67 ^d
0.010	16.67 ^e	40.00 ^e	50.00 ^e	56.67 ^e	73.33 ^e	13.33 ^e	23.33 ^e	36.67 ^e	50.00 ^e	56.67 ^e
LC ₅₀	0.2	0.05	0.01	0.008	0.0007	0.85	0.144	0.038	0.0134	0.0064

Values in the column are all significantly different from each other (ANOVA, Duncan's multiple range test, $P < 0.05$).

The results of toxicity of *C. cyminum* hexane against *S. littoralis* is in agreement with El-Sayed and Yousef, 2021^[9] who found that *C. cyminum* methylene chloride extract has highly toxic effect against the 2nd and 4th instar larvae of *S. littoralis* at all used concentrations. Al-Snafi A. E., 2018 reported that *C. cyminum* has an insecticidal activity.

Essential oil vapours distilled from cumin was recorded Fumigant activity against the eggs of two stored product insects, the confused flour beetle, *Tribolium confusum*, and the Mediterranean flour moth, *Ephestia kuehniella* (Clark, et al., 1998 & Tunc, et al., 2000)^[6, 20]. Lashgaria, et al., 2013^[12] recorded the insecticidal activity of *C. cyminum* essential oil on *Tribolium castaneum* and *Sitophilus oryzae*.

Demeter, et al., 2021 showed that *Cuminum cyminum* L. oil which contains a major components, Cuminaldehyde (28.11%), α -terpinene (20.88%), p-cymene (18.26%), β -pinene (14.18%) has a toxic effect against *S. granaries* adult. Dambolena et al, 2016 reported the toxicity of 20 natural components of essential oils to ovidal activity against *Pediculus humanus capitis* and found that hydrocarbon terpenes limonene, α -pinene, β -pinene, and α -terpinene were the most active compounds evaluated. Owolabi, et al 2020^[15], proved the insecticidal activity of *Launaea taraxacifolia* against *Sitophilus oryzae* and found that *Lactuca taraxacifolia* leaf essential oil was dominated by monoterpene hydrocarbons including limonene (48.8%), sabinene (18.8%) and (E)- β -ocimene (4.6%), along with the monoterpene aldehyde citronellal (11.0%).

Sağlam, Ö. and özder N. (2013), reported that cuminaldehyde was highly toxic to the egg stage of *Tribolium confusum* Jacquelin du Val. Barkman, B. 2012 recorded the toxic effect of cuminaldehyde, P-cymene, citral, geraniol, citronellal, citronellol, cinnamaldehyde and cinnamyl acetate against Whiteflies, *Bemisia tabaci*.

In conclusion the extract under studying is effective against the 2nd and 4th instar larvae of *S. littoralis* and could be considered as a chemical pesticide alternative. The insecticidal activity of *C. cyminum* against *S. littoralis* may be due to the major compounds identified by the GC-MS. The test of the major components against *S. littoralis* is recommended.

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