# Isolation and Identification of Volatile Organic Compounds from *Brassica napus* Leaves and their Insecticidal Activity against the Cotton leafworm, *Spodoptera littoralis* (Boisd.)

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**Abstract:** The volatile organic compounds (VOCs) from fresh leaves of *Brassica napus* were isolated by hydro-distillation and identified by Gas-Chromatography coupled with Mass Spectroscopy (GC-MS). The main components were terpenes (25.33%), nitrogen compounds (21.92%), esters (17.67%), aldehydes (10.54%), phenols (8.85%), alcohols (6.71%), heterocyclic compounds (4.99%), ketones (2.28%) and sulphur compounds (0.07%), where totalizing 26 compounds. Benzene propane nitrile and dill ether are the major constituents of leaves volatile oil with 21.92% and 15.51%, respectively. The isolated VOCs showed insecticidal activity against the 4th instar larvae of *Spodoptera littoralis* under laboratory conditions. The $LC_{25}$ values were 98 and 28 (mg/100 ml) after 3 and 7 days of treatment, while $LC_{50}$ values were 700 and 162 (mg/100ml) after 3 and 7 days of treatment, respectively. The $LC_{25}$ concentration of isolated VOCs caused reduction of larval and pupal weight, prolongation of larval and pupal duration, reduction of both pupation percentage with 50% and emergence percentage with 70% compared with control ones of *S. littoralis*.

**Keywords**: Volatile organic compounds (VOCs), *Brassica napus*, Gas Chromatography–Mass Spectroscopy, *Spodoptera littoralis*,

## 1.INTRODUCTION

Bio-insecticides of botanical sources as essential oils have reported as an alternative to synthetic ones in agriculture. Therefore, essential oils were studied as botanical insecticides, since its bioactive volatile organic constituents are biodegradable into non-toxic products and potentially suitable for application in integrated pest management **(Mossa, 2016)**.

Volatile organic compounds (VOCs) are organic secondary metabolites of plants with high vapor pressure at room temperature. Plants produce different VOCs including terpenes, alcohols, aldehydes, phenols, ketones, ethers, esters and carboxylic acids (**Niinemets *et al.*, 2004)**, these VOCs are emitted from leaves, flowers and fruits into the atmosphere and from roots into the soil **(Maffei, 2010**).

Volatile organic compounds play important defensive and attractive roles in the interaction between plants and herbivores, pathogens and pollinators. The VOCs were emitted as a consequence of herbivorous attack; attracting natural enemies of herbivores and helping them to find the attacked plants **(Turlings *et al.*, 1990; Agrawal *et al.*, 1999; Wallings, 2000; Mossa, 2016)**.

This study was directed for isolation and identification of the volatile organic compounds (VOCs) from the leaves of *B. napus* by using Gas Chromatography-Mass Spectroscopy technique (GC/MS) and evaluation its biological activity against the cotton leafworm, *Spodoptera littoralis* under laboratory conditions.

## 2.MATERIALS AND METHODS

### 2.1.Plant material

*Brassica napus* leaves were collected from a field of experimental farm in agricultural research institute, Ismailia Governorate.

### 2.2.Extraction of Volatile organic compounds

Fresh leaves were cut into small pieces and placed in a flask (2 liters) with 1.5 liter of water. The mixture was subjected to hydro-distillation in water for 4 h in Clevenger-type apparatus without organic solvent. The volatiles were dried over anhydrous sodium sulphate, stored in dark glass tubes and kept at 4 °C until analysis.

### 2.3.Gas Chromatography-Mass Spectroscopy

GC/MS analysis of the volatile fractions of *B. napus* leaves was carried out in Central Agric. Pesticides Laboratory, Agricultural Research Center, Dokki, Giza, Egypt. This analysis was performed on a Varian GC to Finnegan SSQ 7000 mass selective detector (SMD). The used column was cross-linked fused silica capillary column (30 m. long, 0.25 mm. internal diameter) coated with poloy dimethyl-siloxane (0.5µm. film thickness). The oven temperature was programmed isothermally from 50 °C for 3 min., then heating by 7 °C /min. to 250 °C and also for 10 min., at 250 °C. Injector temperature was 200 °C and the volume injected was 0.5µl. Transition-line and ion source temperature were 250 °C and 150 °C, respectively. The mass spectroscopy had a delay of 3 min. to avoid the solvent plead and then scanned from m/z 50 to m/z 300. Ionization energy was set at 70 eV.

### 2.4.Biological activity of isolated VOCs on *Spodoptera littoralis*

#### 2.4.1. Insect

*Spodoptera littoralis* strain used in this study is a laboratory susceptible strain reared in the plant protection research institute, Dokki, Giza, Egypt according to **EL-Defrawi *et al.*, (1964)**. The culture was maintained in laboratory conditions 25±2ºC, 75±5% R.H and (16 L: 8 D) light: dark photoperiod. It was reared on fresh castor bean leaves until the 4th instar larvae which used in this study. The newly molted 4th instar larvae were selected for this study.

**2.4.2. Insecticidal activity of isolated VOCs on *S. littoralis***

The isolated volatiles were formulated as emulsion in water containing 0.3% triton X-100. Five serial diluted concentrations were prepared and tested immediately after preparation. Leaf dipping technique was applied; castor bean leaves were dipped for 30 seconds in each concentration then left to dry. The treated leaves were offered to newly molted 4$^{th}$ instar larvae of *S. littoralis* for 48 h then replaced by untreated ones. The same procedure was followed for the control group, which consisted of water with 0.3% Triton X-100.

Mortality percentages were determined after 3 and 7 days of treatment and corrected by using **(Abott, 1925)**. The corrected mortality percentages were analyzed to estimate $LC_{25}$, $LC_{50}$ and slope values according to **Finney (1971).**

**2.4.3. Latent activity of isolated VOCs on biological parameters of *S. littoralis***

For study the residual efficacy of isolated VOCs on some biological parameters of *S. littoralis*; one hundred newly molted 4$^{th}$ instar larvae of were placed individually in glass jars and fed on castor bean leaves treated with the estimated $LC_{25}$ concentration for 48 h., then the survived larvae were transferred to fed on untreated castor leaves till pupation. Other twenty larvae were fed on castor leaves consisted of water with 0.3% Triton X-100 as for the same period as a control.

# 3.RESULTS AND DISCUSSION

Bio-insecticides of botanical sources as essential oils have reported as an alternative to synthetic ones in agriculture. Therefore, essential oils were studied as botanical insecticides, since its bioactive constituents are biodegradable into non-toxic products and potentially suitable for application in integrated pest management **(Mossa, 2016)**.

Identification of the isolated VOCs was carried out by comparing of their retention times with those of authentic compounds analyzed under the same conditions, and also with the retention indices (as Kovats indices). The comparison of MS fragmentation pattern with those of pure compounds that stored in MS databases of US National Institute of Standards and Technology (NIST) and Willey 1996 Ed. literature data (**Valette *et al.*, 2003; Adams, 2009).**

The Identification of isolated compounds was confirmed by comparison of their spectra fragmentation pattern with GC data previously published **(Davies, 1990; ESO 2000; Valette *et al.*, 2003).**

The VOCs of *B. napus* leaves, the percentage of each compound and the retention indices are showed in Table (1).

The qualitative and quantitative composition of the volatile organic components were determined in *B.napus* leaves, totalizing 26 compounds to be **eight** terpenes (25.33) %, **three** aldehydes (10.54) %, **three** Ketones (2.28) %, **one** alcohol (6.71) %, **one** phenol (8.85) %, **four** esters (17.67) %, **one** nitrogen containing compound (21.92) %, **one** sulphur containing compound (0.07) % and **four** heterocyclic compounds (4.99) % were found as shown in Table (1).

The terpenic compounds represented 25.33 % of the total content, where dill ether (3,6-epoxy-1-p-menthen) is the major compound of terpenic compounds with 15.51 % of volatile compounds. The nitrogen containing compound (Benzene propane nitrile) represented 21.92% of the total content of volatile compounds.

The $LC_{25}$, $LC_{50}$ and slope values as parameters of the insecticidal activity of isolated VOCs were obtained from probit analysis for mortality values after 3 and 7 days of treatment are shown in Table (2).

It was observed that the insecticidal activity increased with time post treatment and applied concentrations according to the descending order of $LC_{25}$, $LC_{50}$ values.

To throw a light on the mode of action of isolated VOCs of *B. napus* leaves on *S. littoralis*; the latent activity of $LC_{25}$ concentration of these VOCs on some biological parameters as; larval weight, larval duration, pupation percentages, pupal weight, pupal duration and emergence percentages of *S. littoralis* was investigated.

**3.1.. Latent activity of VOCs on larval stage of *S. littoralis***

Data in Table (3) show that after treatment of the 4$^{th}$ instar larvae of *S. littoralis* with $LC_{25}$ concentration of VOCs, the gained weights of surviving larvae were 0.26±0.02 gm which represent weight reduction of 36.58 % compared with control ones 0.41±0.04 gm. The average larval duration to reach full grown ones in treatment was 15.9±0.33 days which represent larval period prolongation by 53.92 % compared with control ones 10.33±0.33 days. It was observed that the surviving larvae from treatment resulted 50 % pupation compared with control resulted 100 % pupation.

**3.2.. Latent activity of VOCs on pupal stage of *S. littoralis***

The average weight gain of developed pupae was 0.16 ±0.01gm which represents weight reduction with 23.80 % compared with weight control pupae 0.21 ± 0.01 gm. The average pupal duration was 12.5 ± 0.6 days in treatment compared with control ones 10.5 ± 1.0 days. It means VOCs caused prolongation of pupal duration with 19.60 % compared with control pupae. The treated pupae result 30 % adult emergence; while it was 100 % for control ones, as shown in Table (3).

Reviewing the obtained results, it could be concluded that the treatment of 4$^{th}$ instar larvae of *S. littoralis* with $LC_{25}$ of isolated VOCs from *B. napus* leaves caused reduction of larval and pupal weight, prolongation of larval and pupal duration, reduction of both pupation and emergence percentages compared with control ones of *S. littoralis*.

From the previous data; the major isolated VOCs from *B. napus* leaves as terpenenic compounds especially dill ether (15.51 %), and benzene propane nitrile (21.92 %) may play important role in the insecticidal and biological activities of isolated VOCs. Our findings is in agreement with those obtained by **Gershenzon and Dudareva (2007)** who found that in lepidopteran larvae, terpenes make blocking of the stimulatory effects of glucose and inositol on chemosensory receptor cells in on the mouthparts of larvae.

Terpenes synergize the effects of other toxins by acting as solvents to facilitate their passage through larvae membrane, also they cause disturbance of the nervous system leading to paralysis and moratality of lepidopteran larvae **(Gershenzon and Dudareva, 2007)**

*Brassica* plants produce volatile organic compounds including; glucosinolates, terpenes, ketones, alcohols, aldehydes and esters. These compounds play a defense mechanism of *Brassica* plants against herbivorous attack **(Kessler and Baldwin, 2002)**.

**Table (1): Volatile Organic Constituents of *Brassica napus* leaves**

| Component Name | R.T. min. | Molecular formula | Molecular weight | Percentage % |
|---|---|---|---|---|
| **Terpenes** | | | | |
| **Dill ether** | 11.902 | $C_{10}H_{16}O$ | 152 | 15.51 |
| **β-cyclocitral** | 12.513 | $C_{10}H_{16}O$ | 152 | 0.58 |
| **Thymol** | 15.47 | $C_{10}H_{14}O$ | 150 | 0.67 |
| **Trans-Geranyl acetone** | 18.528 | $C_{13}H_{22}O$ | 194 | 0.83 |
| **β-ionone** | 19.372 | $C_{13}H_{20}O$ | 192 | 5.57 |
| **Citronella** | 19.943 | $C_{10}H_{18}O$ | 154 | 0.51 |
| **Iso-menthone** | 20.793 | $C_{10}H_{18}O$ | 154 | 0.58 |
| **phytone** | 27.203 | $C_{18}H_{36}O$ | 268 | 1.08 |
| **Total** | | | | 25.33 |
| **Aldehydes** | | | | |
| **Phenyl acetaldehyde** | 7.821 | $C_8H_8O$ | 120 | 5.68 |
| **2-phenyl-2-butenal** | 14 | $C_{10}H_{10}O$ | 146 | 4.49 |
| **3,3,4-trimethyl-1-cyclohexene-1-carboxyadehyde** | 19.174 | $C_{10}H_{16}O$ | 152 | 0.37 |
| **Total** | | | | 10.54 |
| **Ketones** | | | | |
| **6-methyl-2-heptanone** | 9.457 | $C_8H_{16}O$ | 128 | 1.54 |
| **3-ethyl-2-hydroxy-2-cyclo penten-1-one** | 18.19 | $C_7H_{10}O_2$ | 126 | 0.28 |
| **9,10-anthraquinone mono-hydrazone** | 29.159 | $C_{10}H_{10}N_2O$ | 222 | 0.46 |
| **Total** | | | | 2.28 |
| **Alcohols** | | | | |
| **Phenyl ethanol** | 9.765 | $C_8H_{10}O$ | 122 | 6.71 |
| **Total** | | | | 6.71 |
| **Phenols** | | | | |
| **4-vinyl guaiacol** | 15.163 | $C_9H_{10}O_2$ | 150 | 8.85 |
| **Total** | | | | 8.85 |
| **Esters** | | | | |
| **3-flourophenyl cyclohexane carboxylate** | 20.228 | $C_{13}H_{15}FO_2$ | 222 | 2.65 |
| **Di-butyl phthalate** | 29.613 | $C_{16}H_{22}O_4$ | 278 | 1.30 |
| **Octinoxate** | 33.345 | $C_{18}H_{26}O_3$ | 290 | 0.20 |
| **Bis-(2-ethylhexyl)phthalate** | 33.534 | $C_{24}H_{38}O_4$ | 390 | 13.52 |
| **Total** | | | | 17.67 |
| **Nitrogen compounds** | | | | |
| **Benzene propane nitrile** | 13.247 | $C_9H_9N$ | 131 | 21.92 |
| **Total** | | | | 21.92 |
| **Sulphur compounds** | | | | |
| **1,1-bis(methyl thio) ethane** | 11.151 | $C_4H_{10}S_2$ | 122 | 0.07 |
| **Total** | | | | 0.07 |
| **Heterocylic compounds** | | | | |
| **1H-Indol** | 14.831 | $C_8H_7N$ | 117 | 1.95 |
| **1-phenyl-1H-pyrazol-3-amine** | 16.979 | $C_{10}H_{11}N_3$ | 173 | 0.83 |
| **N-methyl-2-(3H)-benzothiazolethione** | 22.761 | $C_8H_7NS_2$ | 181 | 0.92 |
| **2-phenyl-3-(2-furyl) propenal** | 23.989 | $C_{13}H_{10}O_2$ | 198 | 1.29 |
| **Total** | | | | 4.99 |

Herbivorous attack caused disruption of the plant tissue; when glucosinolates come into contact with myrosinase in the presence of water during tissue cutting and chewing, the plant produce toxic degradation VOCs as nitriles and sulpher containing compounds **(Agrawal and Kurashige, 2003)**. These volatile products have important biological activities, where they protect *Brassica* plant from herbivorous attack and pathogens (**Pare and Tumlinson, 1999)**.

**Table (2): Insecticidal Activity of VOCs from *B. napus* Leaves on *S. littoralis.***

| parameter | $LC_{25}$ (mg/100 ml) (Confidence limits at 95%) | $LC_{50}$ (mg /100 ml) (Confidence limits at 95%) | Slope ± SE |
|---|---|---|---|
| **3-Days post treatment** | 98 (41 – 152) | 700 (415 – 981) | 0.889 ± 0.225 |
| **7-Days post treatment** | 28 (0.004 – 48) | 162 (48 – 218) | 0.918 ± 0.267 |

**Table (3): Latent Activity of VOCs on Biological Parameters of *S. littoralis*.**

| Biological parameters | Control | VOCS | Deviation from control (%) |
|---|---|---|---|
| **Larval stage** | | | |
| **Mean larval weight ± S.E (g)** | 0.41±0.04 | 0.26 ± 0.02 | (-) 36.58 |
| **Mean larval duration ± S.E (days)** | 10.33± 0.33 | 15.9 ± 0.33 | (+) 53.92 |
| **Pupation %** | 100 | 50 | (-) 50 |
| **Pupal stage** | | | |
| **Mean pupal weight ± S.E (g)** | 0.21 ± 0.01 | 0.16 ± 0.01 | (-) 23.80 |
| **Mean Pupal duration ± S.E (days)** | 10.5 ± 1.0 | 12.5 ± 0.6 | (+) 19.60 |
| **Emergence %** | 100 | 30 | (-) 70 |

Deviation percentage = [(Treatment – control) / control] x 100.

# REFERENCES


**Abbott, W. S. (1925`)**. A method of computing the effectiveness of an insecticide.*J. Econ. Entomol.,* 18: 265-267.

**Adams, R. P. (2009).** Identification of essential oil components by gas chromatography/mass spectroscopy. Carol Stream, IL: *Allured Publ. Corp*.

**Agrawal, A. A. and N. S. Kurashige (2003).** A role for isothiocyanatesin plant resistance against the specialist herbivorePierisrapae. *J. Chem. Ecol.,* 29: 1403–1415.

**Agrawal, A. A.; S. TUZUN and E. BENT (1999).** Induced Plant Defences Against Pathogens and Herbivores.APS Press, StPaul, MO, 403 pp.

**Davies, N. W. (1990).** Gas chromatographic retention indexes of monoterpenes and sesquiterpenes on methyl silicone andcarbowax 20M phases. *J. Chrom.,* 503: 1–24

**El-Defrawi, M. E.; A. Toppozada; N. Mansour and M. Zeid (1964).**Toxicological studies on the Egyptian cotton leafworm, *Prodenialitura* (F.) I. Susceptibility of different larval instars of *prodenia* to insecticides. *J. Econ. Entomol.,* 57: 591-593.

**Eso, (2000).** The complete database of essential oils. The Netherlands: Boelens Aroma ChemInfServ.

**Finney, D. J. (1971).** Probit Analysis. A Statisical treatment of the sigmoid response curve. 7th edition Cambridge University Press, Cambridge, England.

**Gershenzon, J. and N. Duareva (2007)**. The function of terpene natural products in the natural world.*Natural Chemical Biology* 3: 408-414.

**Kessler, A. and I. T. Baldwin (2002).** Defensive function ofherbivore-induced plant volatile emissions in nature. *Science* 291: 2141–2144.

**Maffei, M. E. (2010).** Changes in biosynthesis of aroma volatile compounds during on-tree maturation of "Pink Lady" apples.*South Afr. J. Bot*., 76: 612.

**Mossa, A. H. (2016).** Green pesticides: essential oils as biopesticides in insect-pest management. *J. Environ. Sci. Tech.,* 9(5): 354-378.

**Niinemets, U.; F. Loreto and M. Reichstein (2004).** Physiologicaland physico-chemical controls on foliar volatile organic compoundemissions. *Trends Plant Sci.,* 9: 180–186.

**Pare, P. W. and J.H. Tumlinson (1999).** Plant volatiles as a defenseagainst insect herbivores. *Plant Physiol*., 121: 325–331

**Turlings, T. C. J.; J. H. Tumlinson and W. J. Lewis (1990).**Exploitationof herbivore-induced plant odors by host-seeking parasiticwasps. *Science* 250: 1251–1253.

**Valette, L.; X. Fernandez; S. Polain; A. Loiseau; L. Lizzani-Cuvelier; R. Levieil and L. Restier (2003).** Volatile constituents from *Romanesco*-cauliflower. *Food Chem*.,.35 :80

**Walling, L. L. (2000)**. The myriad plant responses to herbivores. *J. Plant Growth Regul,* 19: 195-216.

# فصل و تعريف المركبات العضوية المتطايرة من اوراق نبات الكانولا ودراسة تأثيرها السام على دودة ورق القطن

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**الملخص العربى:**

تم فصل المركبات العضوية المتطايرة من أوراق نبات الكانولا عن طريق التقطير المائى ثم التعرف على الصيغ البنائية لهذه المركبات عن طريق كروماتوجرافيا الغاز/ طيف الكتلة. تم الفصل والتعرف على 26 مركب من التربينات بنسبة (25.33%) , المركبات النيتروجينية (21.92%), الاستيرات (17.67%), الألدهيدات (10.54%), الفينولات (8.85 %), الكحولات (6.71%), الكيتونات (2.28%) و المركبات الكبريتية (0.07%). وجد أن المركبات الاساسية هى benzene propane nitrile, dill etherيمثلان أعلى النسب وهما 21.92&15.51 % على التوالى. تم دراسة كفاءة المركبات العضوية المتطايرة ضد يرقات العمرالرابع لدودة ورق القطن معمليا. حيث أظهرت النتائج ان التركيز القاتل ل25% من الافراد المعاملة 98& 28 (مجم/100 مل) بعد مرور3 & 7 يوم من المعاملة. وأظهرت النتائج ايضا ان التركيزالقاتل ل50% من الافراد المعاملة 700 & 162 (مجم/100مل) بعد مرور 3& 7 يوم من المعاملة. أظهرت النتائج ايضا ان تغذية يرقات العمر الرابع على الاوراق المعاملة بالتركيز اللازم لقتل 25% من الافراد المعاملة من المركبات العضوية المفصولة أدى لحدوث نقص فى وزن اليرقات والعذارى, نقص فى نسبة خروج العذارى و الفراشات مقارنة بالأفراد الغير معاملة. لذلك يمكن استخدام هذه المركبات لمكافحة دودة ورق القطن.