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Full Length Article

Biopesticidal Effects of Essential Oils of *Pelargonium graveolens* and *Juniperus phoenicea* from Algeria

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Abstract

The consequences of the intensive use of pesticides in agriculture have caused damaging effects on human health and the natural environment. For remedy of this problem, we resorted to the use of biopesticides. The main objective of our research was to evaluate the activities of *Pelargonium graveolens* L'Hér. and *Juniperus phoenicea* Lycien essential oils on microorganisms that contaminated *Brassica napus* L. rapeseed plant harvested from the TIFC field of Khemis Miliana, Algeria. Our work consisted of isolating and identifying the microorganisms responsible for deterioration of the leaves of *B. napus*. Then, an analysis of the constituents of the essential oil, which showed *in vitro* a clear inhibitory effect on the isolated microorganisms, was carried out by GC-MS. The results showed that the microorganisms affecting rapeseed included *Alternaria alternata*, *Aspergillus niger*, *Klebsiella oxytoca*, *Klebsiella pneumoniae* and *Vibrio vulnificus*. The essential oil of *P. graveolens* gave a great inhibitory activity against these microbial strains, whose diameters of the zones of inhibition varied from 25 to 30 mm. Similarly, *J. phoenicea* essential oil caused 10 to 18 mm diameters of zones of inhibition. The IC_{50} of the two essential oils was calculated as 0.25 μg mL^{-1} and 0.23 μg mL^{-1}, respectively. Sixty-nine chemical components were identified in *P. graveolens* essential oil by GC-MS. The constituent 6-octen-1-ol, 3.7-dimethyl was predominant with a percentage of 25.50%. © 2022 Friends Science Publishers

Keywords: Antimicrobial Activity; Antioxidant Activity; Biopesticides; *Brassica napus*; Essential Oils; *Juniperus phoenicea*; TIFC of Khemis Miliana; GC-MS; *Pelargonium graveolens*

Introduction

Pesticides are indispensable in agricultural production. They have been used by farmers to control weeds and insects and their remarkable increases in agricultural products have been reported. Pesticides pertains to substances used as insecticides, fungicides, herbicides, rodenticides, molluscicides, and nematicides (Tudi *et al.* 2021). According to the FAOSTAT data (http://www.fao.org/faostat/en/ # data), during the same year, Algeria imported 17.566.404 ton of pesticides of all types (for agricultural and non-agricultural uses) the equivalent of 108,603.74*103 US $ of which only 237 ton (1.34%) were dangerous compounds while the quantity of pesticides imported for agricultural use was about 4517 ton, of which 1740 ton (39%), 323 ton (7%) and 206 ton (≈ 5%) were fungicides-bactericides, insecticides and herbicides, respectively (Bettiche *et al.* 2021).

These chemicals are considered to be the most effective means to combat pests, unfortunately they have harmful consequences. On the one hand, at the level of the environment through the accumulation of residues and soil pollution and on the other hand, the appearance and generalization of resistance mechanisms in pathogens and the ecological imbalance, due to the fact that these compounds of synthesis have a wide spectrum of action. These chemicals destroy not only harmful agents but also other populations in the ecosystem. In view of these harmful consequences, it is important to find alternative solutions which will make it possible to continue to fight against phytopathogens while reducing the use of chemicals. These may involve the rationalization of agricultural practices, the use of resistant plant varieties and / or the development of biopesticides (Fulgence *et al.* 2021). Many recent studies have shown that crude extracts of plants such as *Datura metel* (Jabeen *et al.* 2022), *Sonchus oleraceous* and *Ageratum conyzoides* (Banaras *et al.* 2020, 2021), *Cannabis sativa* (Khan and Javaid 2020), *Chenopodium murale* (Khan *et al.* 2021) and *Cassia fistula* (Akbar *et al.* 2014; Ferdosi *et al.* 2022) have the ability to control various fungal

pathogens such as *Aspergillus flavipes*, *Penicillium expansum*, *Sclerotium rolfsii* and *Macrophomina phaseolina*. There are many reports of isolation of pure compounds from plants such as lupeol acetate and coumarin for the control of fungi (Javed *et al.* 2021; Uroos *et al.* 2022) and compounds namely holadysenterine from microorganisms for the control of weeds. In addition, plant pathogens namely *Ascochyta rabiei*, *Macrophomina phaseolina* and *Sclerotium rolfsii* can effectively be controlled by soil amendment with plant materials of *D. metel* (Jabeen *et al.* 2021), *Chenopodium album* (Ali *et al.* 2020) and *Withania somnifera* (Javaid *et al.* 2020). Furthermore, biological control agents such as plant growth promoting rhizobacteria (Sharf *et al.* 2021) and various species of fungi namely *Trichoderma harzianum*, *T. viride*, *T. pseudokoningii* (Javaid *et al.* 2018; Khan and Javaid 2021; Khan *et al.* 2021), *A. versicolor* and *Penicillium italicum* (Khan and Javaid 2022a, b) have been identified for the control of soil-borne plant pathogens. The aim of the present study was to assess the antimicrobial activity of essential oils of *Pelargonium graveolens* and *Juniperus phoenicea* against various pathogens of *B. napus*.

Materials and Methods

Work place

The experiment was carried out at the level of TIFC (Experimental Station: Technical Institute of Field Crops), the laboratories of Djilali Bounaama-Khemis Miliana University. According to director Ben Taiba Bilal, the TIFC is a Public Administrative Establishment (PAE), with a scientific and technical vocation, placed under the supervision of the Ministry of Agriculture, Rural Development and Fisheries (MARDF) in 1965. This station is one of nine stations of the Technical Institute of Field Crops. It is responsible for the development of major cereals (winter and summer cereals, food legumes, fodder and industrial crops). It is located on the national road N°14 in the Wilaya of Ain Defla. Its area of action covers, in addition to the Wilaya of Ain Defla, the Wilaya of Chlef.

Brassica napus

Three untreated contaminated *B. napus* plants were harvested from the TIFC Khemis Miliana field during the month of March 2022. The samples were placed in plastic bags and transported to the university laboratory where they were stored. The three *B. napus* samples were planted in plastic pots, in order to carry out the experiment on samples of contaminated leaves.

Essential oils

In this study, two bottles of *P. graveolens* and *J. phoenicea* EO (200 mL) were provided by Dr Saifi Mounir (Djilali Bounaama-Khemis Miliana University). These rose geranium and phoenicia juniper er EOs were prepared at his Aromabiol Company located in Bordj Bou Arreridj.

Isolation and identification of microorganisms

The three contaminated rapeseed plants were named as follows: plant 1 (P1), plant 2 (P2) and plant 3 (P3). A small piece of a leaf of each contaminated plant was then cut and placed in 9 mL of sterile physiological water to prepare decimal dilutions. These mixtures are called stock solutions (SM) (SM1: tube of P1, SM2 tube of P2 and SM3 tube of P3). A series of 5 test tubes were used, each containing 9 mL of physiological water. Dilutions were made from SM1 up to 10^{-5}. The operation was repeated for SM2 and SM3. The Petri dishes containing the nutrient and Sabouraud agars were then incubated at 37°C for 24 h for the bacteria and at 25°C for 4 days for the fungi. After incubation, each colony of fungi was subcultured in the BSA medium and the bacteria in the NA medium. The purpose of this operation was to obtain pure colonies which will facilitate macroscopic and microscopic identification afterward (Mushtaq *et al.* 2022).

Physicochemical parameters of essential oils

The physical indexes (refractive index (IR), and pH) and chemical indexes (acid, saponification and ester) were studied to determine the characteristics of essential oils (Ibipiriene *et al.* 2022).

Determination of antimicrobial activity

The aromatogram is a method for evaluating whether an essential oil exhibits antifungal or antibacterial activity, *in vitro*, against a fungus or bacterium. Blotting paper discs 6 mm in diameter, previously impregnated with known quantities of essential oil with dilutions (concentrates, 1/2, 1/4, 1/8 and 1/12) prepared using DMSO (dimethyl sulfoxide) were then placed on the surface of the agar previously inoculated with a bacterial and fungal culture. After incubation, inhibition of fungal and bacterial growth was evidenced by the presence of a clear halo around the oil-impregnated disc (Bouaouina *et al.* 2022).

Antioxidant activity of essential oils

1,1-Diphenyl-2-pcrylhydrazyl (DPPH) was dissolved in absolute ethanol at a rate of 2.5 mg in 100 mL. From a stock solution of essential oil of 0.1 mg mL^{-1}, dilute solutions of different concentrations (100, 25, 50 and 75 μg mL^{-1}) were prepared by successive double dilution in ethanol. 50 μL of each extract as well as the positive control were added to 2 mL of the ethanolic solution of DPPH. Mixtures were incubated in the dark at room temperature for 30 min. The absorbances were measured at 517 nm, using a Thermo brand Genesys 10 UV-Visible type spectrophotometer. Vitamin C (0.1 mg mL^{-1}) was used as a standard.

The evaluation of antioxidant activity using the DPPH method was expressed as a percentage according to the following equation:

$$\text{Inhibition (\%)} = \frac{(\text{Abs control} - \text{Abs extrait})}{\text{Abs control}}$$

Abs control: Absorbance of the control reaction containing all the reagents except the oil (T = 0 min).

Abs extract: Absorbance of the sample containing a dose of oil tested (T = 30 min). The value of the IC_{50} inhibitory concentration represents the dose of essential oils that neutralizes 50% of DPPH radicals (Munteanu and Apetrei 2021; Sethunga *et al.* 2022).

Essential oil analysis by gas chromatography coupled with spectrometry

Gas Chromatography-Mass Spectrometry (GC-MS) is a hyphenated analytical technique that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample. GC is used to separate the volatile and thermally stable substitutes in a sample whereas GC-MS fragments the analyte to be identified on the basis of its mass (Ashish *et al.* 2014).

The chromatographic analysis of essential oil was carried out with a gas phase chromatograph type TQ 8030 coupled to a mass spectrometer. The fragmentation is carried out by electron impact at 70 eV. The operating conditions were: temperature column was from 40 to 250°C, the carrier gas was helium, the flow rate of which was fixed at 3 mL min^{-1}, injection mode was split mode and the flow control mode with pressure of 49.5 KPa. The device was connected to a computer system managing a Q3 Scan mass spectrum library to monitor the progress of the chromatographic analyses, the volume of the injected sample was 0.5 μL of pure oil. The identification of the constituents was made on the basis of the comparison of their retention indices with those of the standard compounds of the computerized database (Q3 Scan).

Results

Isolation and identification of microorganisms

We observed from Table 1 that the P2 sample contains between 60 and 113 CFU mL^{-1} compared to the other rapeseed samples. Nutrient agar is a favorable medium for the growth of bacteria that have contaminated rapeseed. There was the formation of orange, yellow and white colonies as well as fungi. On Sabouraud agar, the fungi appear black and white. We then did a second and a third subculture until we obtained pure colonies. From the contaminated rapeseed leaves, we identified cocci (B1 and B3) and a bacilli (B2) under an optical microscope. All strains examined possess the enzyme catalase. The latter decomposes the hydrogen peroxide into water and oxygen which is released in the form of gaseous bubbles. The identification of the biochemical characters of bacteria by the API 20 E Gallery allowed us to know the biochemical characteristics of the three isolated strains. The B1 and B3 strains correspond to the two species *Klebsiella oxytoca* and *K. pneumoniae*, respectively. The B2 strain is a *Vibrio vulnificus*.

Table 1: Count of colonies obtained on nutrient agar from decimal dilutions

Samples	Number of colonies (CFU mL^{-1})				
Dilutions	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Rapeseed plant 1 (P1)	105	81	76	40	22
Rapeseed plant 2 (P2)	113	100	97	46	60
Rapeseed plant 3(P3)	90	69	59	44	30

Physical indexes

The measured refractive index of *P. graveolens* and *J. phoenicea* EOs vary between 1.467 and 1.468, respectively. These indexes depend on the chemical composition which increases according to the lengths of the chains of acids, their degrees of establishment and the temperature. The value of the distilled water index is 1.33.

The IR of *P. graveolens* and *J. phoenicea* EOs is high compared to that of distilled water. The pH is used to determine the acidic, neutral or basic nature of a substance. The pH obtained indicates that our essential oils are acidic (4.5 and 5).

Chemical indexes

The acid number indicates the behavior and the quantity of free acids present in our oils. We calculate the values of the acid index and acidity of each oil, we obtain the values 5.6 and 2.8 mg g^{-1} of KOH for the essential oils of *P. graveolens* and *J. phoenicea*, respectively. *J. phoenicea* EO has a lower acid value compared to that of *P. graveolens* EO. The acidity of *P. graveolens* oil is equal to 2.8% and that of *J. phoenicea* 1.4%. The saponification index of the two oils are equal to 316.35 mg g^{-1} of KOH for *J. phoenicea* and 84.15 mg g^{-1} of KOH for *P. graveolens*.

Neutralization of the acids released by hydrolysis in a basic medium (saponification) of the esters contained in 1 g of gasoline makes it possible to calculate the ester index (EI) using the following equation:

IE = IS-IA

For *P. graveolens* IE = 78.55 mg g^{-1} of KOH.

For *J. phoenicea* IE = 313.55 mg g^{-1} of KOH.

In vitro antimicrobial activity

According to Table 2, *P. graveolens* and *J. phoenicea* EOs

Table 2: Diameters of the zones of inhibition in mm of the bacterial and fungal strains

Microbial strains	EO of *Pelargonium graveolens*					
	Inhibition zone diameters (mm)					
	HE/DMSO (SM)	1/2 dilution	1/4 dilution	1/8 dilution	1/12 dilution	control
Alternaria alternata (P1)	20	15	-	-	-	-
Alternaria alternata (P2)	-	-	-	-	-	-
Alternaria alternata (P3B)	-	-	-	-	-	-
Aspergillus niger (P3'W')	25	30	-	-	-	-
Klebsiella oxytoca (B1)	-	-	-	-	-	-
Vibrio vulnificus(B2)	15	12	14	20	-	-
Klebsiella pneumoniae spp.	10	10	10	15	15	-
	EO of *Juniperus phoenicea*					
Alternaria alternata(P1)	-	-	-	-	-	-
Alternaria alternata (P2)	-	-	-	-	-	-
Alternaria alternata(P3B)	-	-	-	-	-	-
Aspergillus niger (P3'W)	-	-	-	-	-	-
Klebsiella oxytoca (B1)	-	-	-	-	-	-
Vibrio vulnificus (B2)	15	-	10	-	-	-
Klebsiella pneumoniae sspp.	10	10	10	18	-	-

B1: Box 1; B2: Box 2; B3: Box3; P1: Plant 1; P2: Plant 2; P3' N': Plant 3 Black; P3 'B': Plant 3 White

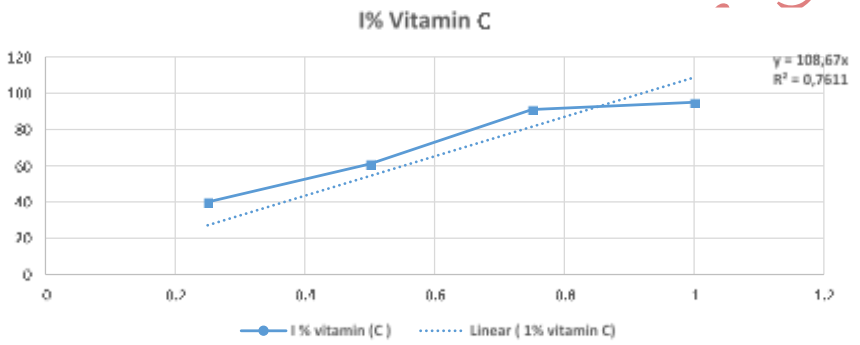

Fig. 1: Antioxidant power of ascorbic acid or Vit C.

exhibited *in vitro* the growth of certain microbial strains. If we take into consideration the diameters of inhibition, the EO of *P. graveolens* was more active on *A. niger*, *Alternaria alternata*, *V. vulnificus* and *K. pneumoniae*. However, *K. oxytoca* showed a great resistance towards the two EO. *J. phoenicea* EO has no inhibiting effect on *A. niger* and *A. alternata* fungi. On the other hand, *V. vulnificus* and *K. pneumoniae* have a sensitivity towards the EO of *J. phoenicea*.

We can say also that our *P. graveolens* EO has antifungal and antibacterial activity. This antagonistic effect results by appearance of zones of inhibition whose diameters vary between 25 and 30 mm. On the other hand, for fungi, no growth is observed with *J. phoenicea* EO. While bacteria show sensitivity to this oil. These results in zones of inhibition showed a diameter is between 10 and 18 mm.

Antioxidant activity

From the graphic representations drawn by the excel 2013, we were able to measure the IC_{50} value of the two oils and ascorbic acid (Ac asc) or vitamin C (Fig. 1, 2 and 3).

The results of the antioxidant power of the EOs tested show that the percentage of inhibition with *P. graveolens* EO is greater than 180% with a concentration of the order of 100 μg mL^{-1}. For *J. phoenicea* EO and ascorbic acid, the inhibition rate is estimated at 200 and 90%, with concentrations equal to 0.45 and 100 μg mL^{-1}, respectively.

The IC_{50} value of each sample was calculated by the following method:

For Ac asc: we use the equation of Y = 108.67X

We had IC_{50} = 108.67X

50 = 108.67X

$$X = IC50 = \frac{50}{108.67}$$

IC_{50} of ascorbic acid equals 0.46μg mL^{-1}

For EO of *P. graveolens*: Y = 193.35X

50 = 193.35X

$$X = IC50 = \frac{50}{193.35}$$

IC_{50} of *P. graveolens* oil equals 0.25 μg mL^{-1}

For EO of *J. phoenicea*: Y = 212.88X

50 = 212.88X

$$X = IC50 = \frac{50}{212.88}$$

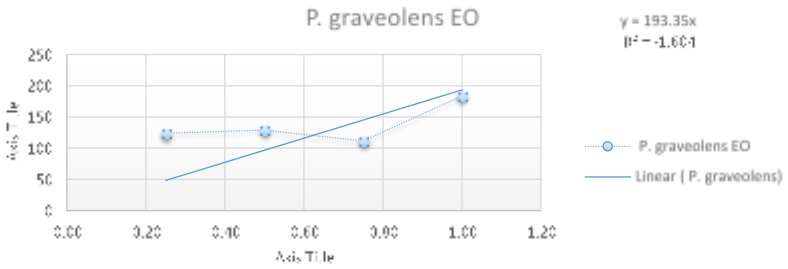

Fig. 2: Eo antioxidant power of *P. graveolens*

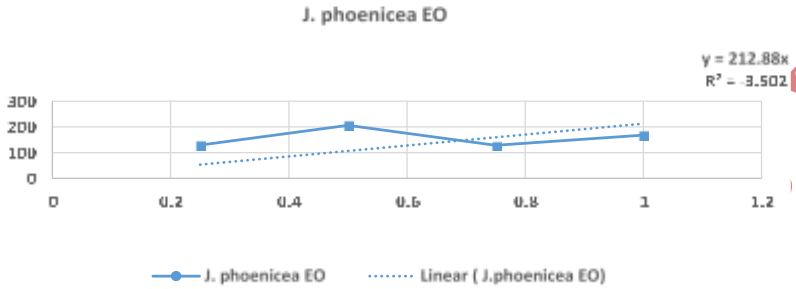

Fig. 3: Eo antioxidant power of *J. phoenicea*

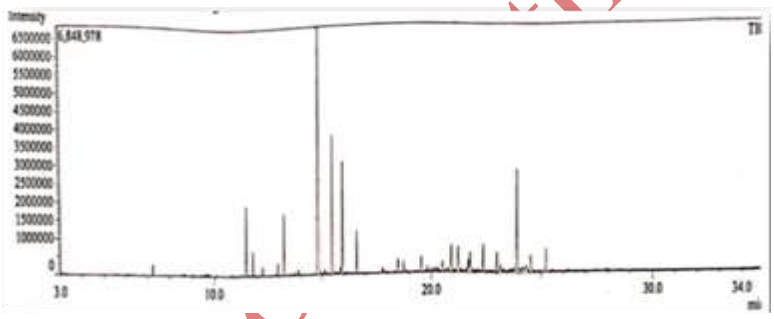

Fig. 4: Chromatographic profile of essential oil of *Pelargonium graveolens*

IC_{50} of *J. phoenicea* oil equals 0.23 μg mL^{-1}

These results show that IC_{50} of *J. phoenicea* EO is higher than that *P. graveolens* EO. The IC_{50} values of the two oils are lower than that obtained for ascorbic acid (0.46 μg mL^{-1}).

Analysis of *P. graveolens* essential oil by gas chromatography

In this part of work, we used a gas chromatography device coupled with mass spectrometry, it is used to analyze and identify chemical constituents of essential oil of *P. graveolens* which gave a clear inhibitory effect of the microorganisms responsible for deterioration of rapeseed leaves. The qualitative and quantitative analyzes of essential oil made it possible to identify and quantify 19 major chemical compounds, which were presented in Table 3 and Fig. 4. These identified compounds are listed in order of their predominance. 6-octen-1-ol, 3,7-dimethyl appears as the major constituent of EO (25.50%), followed by 2,6-octadien-1-ol3, 7-dimethyl-, (Z) - (11.78%), eudesmol (8.57%), 6-octen-1-ol, 3,7-dimethyl-, acetate (7.81%), 1,6-octadien-3-ol, 3, 7-dimethyl-(4.60%) and cyclohexanone, 5-methyl-2-(1-mrthylethyl)-, trans-(4.32%) which represent 85.65% of the total composition of our oil.

The rest of the chemical composition are minority constituents (14.35%). Chemical analysis revealed 69 chemical constituents of *P. graveolens* essential oil. Oxygenated compounds constitute an important part of the chemical composition of oil compared to hydrocarbon compounds. The chromatographic profile of absorbance as a function of time of our EO shows that it has all the constituents necessary to make it a chemotype. 6-octen-1-ol, 3,7-dimethyl-appears as the major oil constituent (25.50%).

Discussion

Brassicaceae diseases caused by *Alternaria* sp. can cause significant yield losses and are considered one of the most critical disease complexes in the world, responsible, for example, for losses of up to 47% in Indian mustard and even exceeding 70% in some species of *Brassica* (Al-Lami

Table 3: Compounds identified in essential oil of *Pelargonium graveolens* as determined by gas chromatography analysis

Sr. No	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	6-Octen-1-ol, 3,7-dimethyl-	$C_{10}H_{20}O$	156.26	14.825	25.50
2	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	$C_{10}H_{18}O$	154.24	15.471	11.78
3	Eudesmol<gamma->	C15H26O	222.37	23.907	8.57
4	6- Octen-1-ol, 3,7-dimethyl-, acetate	$C_{12}H_{22}O_2$	198.30	15.948	7.81
5	1,6-Octadien-3-ol, 3, 7-dimethyl-	$C_{10}H_{18}O$	154.24	11.490	4.60
6	Cyclohexannone, 5-methyl-2-(1-methylethyl)-, trans-	$C_{10}H_{18}O$	154.24	13.241	4.32
7	2,6- Octadien-1-ol, 3,7-dimethyl-, formate, (E)-	$C_{11}H_{18}O_2$	182.25	16.497	2.89
8	Viridiflorene	$C_{15}H_{24}$	204.35	21.219	2.79
9	1,6-Cyclidecadiene,1-methyl-5-methylene-8-(1- methylethyl)-, [s-(e, e)]	$C_{15}H_{24}$	204.35	20.914	2.22
10	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	$C_{10}H_{18}O$	154.24	22.367	2.07
11	Viridiflorol	$C_{15}H_{26}O$	222.37	24.527	1.95
12	Linalylformate	$C_{11}H_{18}O_2$	182.26	25.226	1.68
13	Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	$C_{15}H_{24}$	204.35	24.333	1.67
14	Phenylethyl tiglate 1	$C_{13}H_{16}O_2$	204.26	22.997	1.62
15	Rose oxide	$C_{10}H_{18}O$	154.25	11.800	1.54
16	Caryophyllene	$C_{15}H_{24}$	204.36	19.551	1.28
17	2,6,10- Dodecatrien-1-ol, 3, 7,11-trimethyl-	$C_{15}H_{26}O$	222.36	18.490	1.16
18	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	$C_{10}H_{18}O$	154.24	22.307	1.15
19	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methyle)	$C_{15}H_{24}$	204.35	21.601	1.05
20	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimetyl-	$C_{20}H_{32}$	272.5	7.227	0.65
21	Cyclohexene,1-methyl-4-(1-methylethenyl)-	$C_{10}H_{18}$	138.25	9.647	0.21
22	Eucalyptol	$C_{10}H_{18}O$	154.24	9.743	0.13
23	1,3,7-Octatriene, 3,7-dimethyl-, (E)-	$C_{10}H_{16}$	136.23	9.841	0.09
24	1,3,6-Octatriene, 3,7-dimethyl-, (E)-	$C_{10}H_{18}$	138.25	10.119	0.10
25	.Alpha.-methyl-.alpha.-[4-methyl -3-pentenyl]oxiranemethanol	$C_{14}H_{30}O_3Si$	274.47	10.813	0.09
26	Rose oxide	C10H18O	154.25	12.247	0.60
27	Cyclohexanone, 5-methy-2-(1-methylethyl)-, trans-	$C_{10}H_{18}O$	154.24	12.954	0.85
28	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1r-(1.alpha.,2.beta.,5.alpi)]	$C_{10}H_{20}O$	156.26	13.727	0.20
29	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-	$C_{12}H_{20}O_2$	196.29	13.903	0.39
30	2,6-Octadienal, 3,7-dimethyl-	$C_{10}H_{16}O$	152.24	15.137	0.28
31	2,6-Octadienal, 3,7-dimethyl-	$C_{10}H_{16}O$	152.24	15.849	0.42
32	2,6-Octadiene-1-ol, 3,7-dimethyl,acetate, (Z)-	$C_{12}H_{20}O_2$	196.28	16.085	0.11
33	2,6-Octadiene, 2,6-dimethyl	$C_{10}H_{18}$	138.24	17.776	0.44
34	4-Isopropyl-3,7-dimethyl-3a,3b,4,5,6,7hexahydro-1H-Cyclopenta[2,3]C	$C_{15}H_{24}$	204.35	17.843	0.17
35	Cyclobuta(1,2:3,4]dicylopentene,1,2,3,3A,3b,beta.,4,5,6,6a.beta.,6b.	$C_{10}H_{12}$	132.20	18.737	0.86
36	2-Bromopropionic acide, 2-phenylethyl ester	$C_{11}H_{14}O_2$	178.22	18.832	0.23
37	1H-cyclopenta(1,3)cyclopropa[1,2]benzene,octahydro-7-methyl-3-methylene-4-	$C_{15}H_{24}$	204.35	19.742	0.13
38	6-Octen-1-ol, 3,7-dimethyl-, propanoate	$C_{13}H_{24}O_2$	212.32	19.831	0.43
39	1H-cyclopropa[a]naphthalene,1a,2,4,5,6,7,7a,7b-octahydro-1,1,7,7a-T	$C_{15}H_{24}$	204.35	20.024	0.30
40	1,6Cyclodecadiene1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]	$C_{15}H_{24}$	204.35	20.088	0.10
41	Gurjunene<alpha->	$C_{15}H_{24}$	204.35	20.178	0.48
42	1,4,8-Cycloundecatriene, 2,6,6,9-tetramethyl-,(E,E)-	$C_{15}H_{24}$	204.35	20.317	0.37
43	Cadina-1(6),4-diene(10betah-)	$C_{15}H_{24}$	204.35	20.710	0.28
44	Azulene,1,2,3,4,5,6,7,8-octahydro-1,4dimethyl-7-(1methylethylidene)-, (1s- cI)	$C_{15}H_{24}$	204.35	20.764	0.21
45	Naphthalene,decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)	$C_{15}H_{24}$	204.35	21.045	0.20
46	Nerolidyl acetate	$C_{17}H_{28}O_2$	264.4	21.381	0.33
47	Naphtalène, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylet)	$C_{15}H_{24}$	204.35	21.601	0.26
48	Citronellyl isobutyrate	$C_{14}H_{26}O_2$	226.35	21.686	0.84
49	4-Isopropyl-1,6-dimethyl-,2,3,7,8,8a-hexahydronaphtalene	$C_{15}H_{24}$	204.35	21.837	0.25
50	Naphtalène, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	$C_{15}H_{24}$	204.35	21.981	0.15
51	5-Isopropyl-3,8-dimethyl-1,2,4,5,6,7-hexahydroazulene	$C_{33}H_{37}^{+}$	433.6	22.096	0.22
52	Cadala-1(10),3,8-triene	$C_{15}H_{22}$	202.33	22.222	0.13
53	4-(5,5-Dimethylspiro[2,5]oct-4-yl)-3-buten-2-one	$C_{14}H_{22}O$	206.32	22.495	0.10
54	3-Hexyne, 2,2,5,5-tetramethyl	C_9H_{16}	124.22	22.636	0.24
55	9-Isopropy-1-methyl-2-methylene-5-oxa-tricyclo[5.0.0 3,8]undecane	$C_{15}H_{24}O$	220.35	23.150	0.73
56	Neryl isovalerate	$C_{15}H_{26}O_2$	238.37	23.254	0.19
57	Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethyl)-,	$C_{15}H_{24}$	204.35	23.326	0.19
58	1,1,4,7-Tetramethyldecahydro-1h-cyclopropa[e]azulen-4-ol	$C_{15}H_{26}O$	222.36	23.555	0.17
59	Citronellyl valerate	$C_{15}H_{28}O_2$	240.38	23.672	0.21
60	Muurolol<alpha-,epi->	$C_{15}H_{26}O$	222.36	23.751	0.28
61	Di-epi-alpha-cedrene-(l)	$C_{15}H_{24}$	204.35	24.004	0.25
62	2-Naphtalène méthanol, 1,2,3,4,4a,5,6,7-octahydro-.alpha.,.alpha.,4a,8-tetra	$C_{15}H_{26}O$	222.36	24.079	0.21
63	2-(6,10-Dimethylspiro[4,5]dec-6-en-2-yl)-2-propanol #	$C_{15}H_{26}O$	222.3	24.174	0.43
64	(-)Globulol	$C_{15}H_{26}O$	222.37	24.680	0.10
65	Citronellyl isobutyrate	$C_{14}H_{26}O_2$	226.35	24.884	0.17
66	Butanoic acid, 3,7-dimethyl-2,6-octadienylester, (E)-	$C_{14}H_{24}O$	208.33	25.524	0.23
67	Butanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (E)-	$C_{14}H_{24}O$	208.33	26.205	0.11
68	2-Pentadecagone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268.485	27.885	0.15
69	Butanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (E)-	$C_{14}H_{24}O$	208.33	28.003	0.09
Total					100

et al. 2019). The environmental factors of the culture area of *B.napus* favored the development of this flora at the leaves. Temperature and humidity are the primary environmental factors that favor the spread of plant diseases, while mild, wet winters affect the survival of debris-borne fungi such as *Alternaria* disease (Runno-Paurson *et al*. 2021). Pathogenic fungi of *Alternaria* species produce many primary and secondary metabolites that are host-specific and non-host-specific. These toxins have various negative impacts on cell organelles including chloroplast, mitochondria, plasma membrane, nucleus, Golgi bodies, *etc*. (Meena and Samal 2019). *K. pneumoniae* and *K. oxytoca* are commonly found in carbohydrate-rich wastewater, surface water, cooling water, soil, plant products, fresh vegetables (Batt and Tortorello 2014; Rocha *et al*. 2022).

Vibrio vulnificus is a potentially deadly natural pathogen present in coastal waters. Sewage spills in coastal waters occur when infrastructure fails due to severe storms or age, and can affect bacterial populations by altering nutrient levels (Conrad and Harwood 2022). Our results are consistent with those obtained by the Algerian study of Raho *et al*. (2017) in which *P. graveolens* and *J. phoenicea* had an inhitory effect *in vitro*. In other work of Stegmayer *et al*. (2022) *P. graveolens* essential oil at a concentration of 250 ppm inhibited the growth of phytopathogenic fungi *Alternaria alternata* (100%) *in vitro*. *Juniperus* is a varied genus (about 75 species of *Juniperus* have been reported) that has been used for traditional medicinal purposes. Many species belonging to this botanical family have been used in traditional medicine in Tunisia (Boujemaa *et al*. 2022).

P. graveolens (geranium) originated from southern Africa and is widely cultivated in several countries, mainly in Russia, Egypt, Algeria, Morocco, Congo, Japan and India and some continents like Central America and Europe. Among several extracts of *P. graveolens* that may be useful as bioactive natural plant products; the essential oil has been reported to possess a wide range of biological and pharmacological properties such as antioxidant, antibacterial, antifungal, hypoglycemic, anti-inflammatory and anti-cancer properties (Al-Mijalli *et al*. 2022). These activities could be related to the presence of certain bioactive compounds including citronellol, geraniol and linalool as major compounds (Okla *et al*. 2022). *P. graveolens* is highly valued by industries for producing geranium essential oil. According to BS ISO 4371-2012, *P. graveolens* essential oil from different geographical origins should have geraniol (5–20%), citronellol (18–43%), citronellyl formate (4–12%), geranyl formate (1–8%) and linalool (2–11%) as the main components. The chemical profile of *P. graveolens* essential oil is affected from its geographical origin (Mahboubi and Valian 2019). Concerning *P. graveolens* EO at the vegetative stage, the total of the compounds identified was 92.98, divided into three classes: monoterpene hydrocarbons (20.84%), oxygenated monoterpenes (39.08%) and hydrocarbons sesquiterpenes (25.41%) (Al-Mijalli *et al*. 2022). Cebi (2021) detected a volatile compound from the essential oil of geranium (*P. graveolens*) which accounted for 99.34% of the total essential oil composition. The most abundant compounds were determined as citronellol (30.68%), geraniol (9.68%) and citronella formate (9.90%).

The mechanism action of essential oil extracts is related to changes in the permeability of the cell membrane. The fat-soluble nature of the extracts and their easy overlap with cellular structures, which have lipid constituents due to increased permeability of the cell membrane which due to electrolyte imbalance and cell lysis and then death (Kadium *et al*. 2021). Many biopesticides are based on plant extracts and secondary metabolites which, during evolution, are thought to be involved in protecting plants against biotic and abiotic stresses. Among secondary metabolites, alkaloids, phenols and terpenoids are the most common. These substances can be extracted by plant tissues by solvent or steam distillation, obtaining a complex mixture of various molecules called "essential oil". Many EOs are bioactive substances with insecticidal activity against target pest species, including toxic and repellent effects, developmental and behavioral alterations, and sterility/infertility. EOs are traditional and ancient pest control tools; several millennia ago, around 2000 BC. Medicinal plants were used in Asia, the Middle East and North Africa to control stored grain pests. The interest of using EOs as a control tool is linked to their low toxicity towards mammals, so that these substances are used as food protectants not only against insects (Palermo *et al*. 2021). In the work of Edson *et al*. (2014), in fumigation tests, the essential oil of *P. graveolens* caused 100% mortality of adults of *B. tabaci*, biotype B at concentrations from 0.5 μg L^{-1} in air. These results suggest that PG-EO and its related monoterpenes are potentially applicable to develop effective natural product-based pest-management compounds.

Conclusion

The essential oil of *P. graveolens* can be used as a biopesticide of the microorganisms responsible for the pathologies of *B. napus* plant.

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Author Contributions

GH planned the experiment and wrote first draft; KR, HR and BS performed the experiment.

Conflict of interest

No conflict

Data Availability

The data presented in this study will be available on request from the corresponding author.

Ethics Approval

Not applicable for this study.

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