Journal of Plant Diseases and Protection (2021) 128:1157–1166
https://doi.org/10.1007/s41348-021-00489-0

ORIGINAL ARTICLE

Biocontrol potential of essential oil from Moroccan *Ridolfia segetum* (L.) Moris

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Received: 12 March 2021 / Accepted: 7 June 2021 / Published online: 18 June 2021

Abstract

The present study characterized and evaluated the antifungal, nematicidal, acaricidal and repellent activities of *Ridolfia segetum* essential oil (EO), against the fungus *Botrytis cinerea* Pers., the nematode *Meloidogyne javanica* and the mite *Tetranychus urticae* Koch under laboratory conditions. The EO exerted inhibition of the mycelial growth of *B. cinerea* with median inhibitory concentration (IC_{50}) of 3.63 µL/mL and 0.92 µL/mL air, under contact and vapor phase conditions, respectively. EO application against *M. javanica* revealed a strong nematostatic effect on second-stage juveniles (J2) and eggs of the root knot nematode with median lethal concentration (LC_{50}) of 10.37 and 9.26 µL/mL, respectively. The oil was toxic to *T. urticae* by residual contact with median lethal dose (LD_{50}) of 6005 µL/L and exhibited a repellent effect with a repellency index (RI) of 55%. Results suggest that *R. segetum* oil has potential to be formulated in biopesticides to control the targeted pest and diseases.

Keywords *Ridolfia segetum* · Essential oil · *Botrytis cinerea* · *Meloidogyne javanica* · *Tetranychus urticae* · Biopesticide

Introduction

The Apiaceae (formerly Umbelliferae) is one of the largest families of flowering plants, with over 4000 species classified into 434 genera (Li et al. 2020). They are widely distributed, though are found predominantly in northern temperate regions and high altitudes of the tropics (Sayed-Ahmad et al. 2017). The family contains well-known plants used as foods, garnishes or spices (Knothe and Steidley 2019). Plants of the Apiaceae family also possess medicinal properties and have traditionally been used as household remedies. They are rich in phytochemicals and secondary metabolites and potential sources of flavonoids, terpenoids, triterpenoid saponins, coumarins, polyacetylenes and steroids (Sayed-Ahmad et al. 2017). Previous studies have highlighted the species of this family as natural agrochemicals, with biological activities against various plant pests and diseases (Oka et al. 2000; Tabanca et al. 2007; Ebadollahi 2013). In the family, the genus *Ridolfia* Moris is represented by one species, *Ridolfia segetum* (L.) Moris (Cabral et al. 2015).

R. segetum is an annual plant widely distributed throughout the Mediterranean basin in areas such as Morocco (Gattefossé and Igolen 1946), Tunisia (Jabrane et al. 2010), Portugal (Cabral et al. 2015), Sardinia (Marongiu et al. 2007),

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Spain and the Canaries (Palá-Paúl et al. 2002), where it grows as a weed in cereal fields (Pottier-Alapetite 1979). It is used in traditional medicine to prevent coughing, constipation, respiratory tract infections and for treatment of gastric acidity (Cabral et al. 2015). It has carminative, antispasmodic, antihaemorrhoidal and emmenagogue properties. Additionally, the dried fruits of the plants are used as a eupeptic digestive in atony and digestive difficulties (Marongiu et al. 2007).

Chemical characterization of the essential oil (EO) has been reported for the ecotypes of *R. segetum* grown in different countries and in different localities of the same country, enabling the recognition of two types of oils: those dominated by monoterpene hydrocarbons: α-phellandrene, terpinolene and p-cymene (Palá-Paúl et al. 2002, 2005; Marongiu et al. 2007; Bicchi et al. 2009; Jabrane et al. 2009; Poças et al. 2014; Cabral et al. 2015), and those which also contain phenylpropanoids (typically myristicin and dillapiol) as major compounds (Gattefossé and Igolen 1946; Gattefosse and Igolen 1951; Palá-Paúl et al. 2002, 2005; Jannet and Mighri 2007; Marongiu et al. 2007; Jabrane et al. 2010; El Karkouri et al. 2017). Biological activities of *R. segetum* EO include antioxidant (Jabrane et al. 2010; Cabral et al. 2015), antibacterial (Jannet and Mighri 2007; Jabrane et al. 2009, 2010), insecticidal (Badalamenti et al. 2021), anti-inflammatory (Cabral et al. 2015), anticancer (Poças et al. 2014) and HIV-1-inhibiting activities (Bicchi et al. 2009). The efficacy of the EO as a biopesticide against plant pathogens and pests has not been reported. Thus, the present study was designed to test the hypothesis that *R. segetum* possesses activities within the scope of crop protection against plant attacking fungi, nematodes and mites.

The gray mold agent *Botrytis cinerea*, the root knot nematode *Meloidogyne javanica* and the two-spotted spider mite *Tetranychus urticae* are the key pests that cause significant yield losses in agricultural crops, including fruits, vegetables and ornamentals (Stumpf et al. 2001; Javed et al. 2007; Angelini et al. 2016) especially in the Souss-Massa region in the south of Morocco. The aim of this study was to assess the in vitro antifungal, nematicidal, acaricidal and repellent activities of the oil against the gray mold agent *B. cinerea*, the root knot nematode *M. javanica* and the two-spotted spider mite *T. urticae*, respectively.

Materials and methods

Plant material and essential oil isolation

Aerial parts of *R. segetum* (flowers, stems, fruits) (1 kg) were collected in April, May and June of 2017, at flowering and ripening period, from the region of Souss-Massa (South-west of Morocco) coordinates (N 30° 0′ 13.6″ W 9° 38′ 19.6″). Identification of plant material was verified by one of the authors (Dr James Furze), Royal Geographical Society of London, UK. A voucher specimen (no. RS-17) was deposited in the herbarium of the Laboratory of biotechnology, National School of applied sciences, Ibn Zohr University, Agadir, Morocco.

Essential oil was obtained from dried aerial parts (250 g) by hydrodistillation using a Clevenger apparatus for 4 h until total recovery of oil. Hydrodistillation was repeated four times, and the average yield was calculated. The oil was weighed and stored in hermetically sealed dark vials at 4 °C for further analysis.

Test organisms

B. cinerea was isolated directly from infested rotten beans (*Phaseolus vulgaris*). The fungus was maintained on PDA (Biokar diagnostics) at 4 °C. Eggs of the nematode *M. javanica* were extracted from infected bean roots. Second-stage juveniles (J2) were obtained from the water suspension of infected roots containing hatched eggs and were stored at 4 °C before use. The two-spotted spider mite was obtained from a mass-rearing trial on bean plants (Microbials Production Unit, Omnium Agricole du Souss, Agadir, Morocco).

Antifungal activity

Poisoned food technique

The poisoned food technique (PF) was used according to Rhayour et al. (2003), with modifications. The EO was dispersed as an emulsion in sterile PDA using 0.1% Tween 80. The concentrations tested ranged from 0.25 to 16 µL/mL. Controls consisted of sterile PDA. The tested fungi were inoculated using a 6 mm mycelial plug from a 7-day-old culture. Three replicate plates were inoculated for each treatment, and plates were incubated for 7 days at 25 ± 2 °C. The test was conducted three times.

Volatile phase technique

Volatile activity assay (VA) was performed following Soylu et al. (2010), with modifications. Petri dishes (9 cm) were filled with 20 mL of PDA medium and seeded with a mycelial disk (6 mm diameter) cut from the periphery of a 7-day-old culture of the fungi. Petri dishes were inverted, and sterile Whatman No. 1 filter paper disks (10 mm diameter) impregnated with different volumes of EO were deposited on the inverted lids to obtain final concentrations of 0.05, 0.1, 0.2, 0.4, 0.8, 0.16 and 0.32 µL/mL air and incubated for 7 days at 25 ± 2 °C. Controls of sterilized filter paper disks

impregnated with 20 µL/disk of distilled water were used. Three replicate plates were inoculated for each treatment, and plates were incubated for 7 days at 25 ± 2 °C. The test was conducted three times.

The fungitoxicity of the EO was expressed as percent inhibition of mycelial growth (I%) and evaluated according to the formula of Pandey et al. (1982), Eq. 1.

$$I(\%) = \frac{D_c - D_t}{D_c} \times 100 \quad (1)$$

where D_c is mycelial growth diameter in control, D_t is mycelial growth diameter in treated petri plates.

Nematicidal activity

Egg hatch inhibition and nematicidal activity on second-stage juveniles (J2) of *M. javanica*

The experiment was carried out in 96-well microplates following a modified method of Sosa et al. (2012). Approximately 50 eggs or 50 freshly hatched J2s of *M. javanica* were placed in each well prior to adding the EO. The oil was diluted with water containing 0.1% Tween 20 v/v, as the surfactant to obtain the desired oil concentrations. Tested concentrations were 0.8, 8, 10, 12, 14 and 16 µL/mL. Distilled water and a 0.1% Tween 20 solution were included as controls. Microplates were maintained at 25 °C. Hatching percentage was recorded for 10 days, and the percentage of immobilized J2 was recorded for 3 days using an inverted microscope. Lack of mobility was taken as evidence of the effect of the tested solutions. Four replicates of each treatment were made, and the test was performed three times. Data of mortality and hatch inhibition (Crd) were corrected according to Abbott's formula (Abbott 1925) Eq. 2.

$$\text{Crd} = \frac{\text{Pmtr} - \text{Pmc}}{100 - \text{Pmc}} \times 100 \quad (2)$$

where Pmtr is egg hatching percentage or dead J2 percentage in treated wells, Pmc is egg hatching percentage or dead J2 percentage in the control.

Demonstration of the nematostatic effect

Second-stage juveniles of *M. javanica* (approximately 50) were exposed to the action of the EO at the highest concentration of 16 µL/mL. The suspension of the treated J2 was passed through a 50 micron sieve while being washed with distilled water to eliminate the effect of the EO. Larvae suspended in distilled water were recovered in petri plates. The vitality of immobile nematodes was evaluated using Meldola Blue staining technique as per Ogiga and Estey (1974) and Mayad et al. (2019). A few drops of Meldola blue dye were added to each plate to distinguish dead J2 from the paralyzed ones. Dead larvae were stained purple-blue, while paralyzed larvae remained uncolored. Four replicates were performed. Two controls were used: the first consisted of J2 larvae suspended in distilled water and the second were J2 treated with heat (65 °C for 24 h). After one hour, dead and paralyzed larvae were counted under an inverted microscope. The percentage of J2 resuming their mobility was determined after 24, 48 and 72 h to study the persistence of paralysis and mobility recovery of J2. The test was performed three times.

Three parameters were determined: the larval mortality rate (Mr), the paralysis rate (Pr) and the percentage of J2 which resumed their mobility (Rm). The three parameters were calculated and corrected against the control according to Abbott's formula, Eqs. 3, 4 and 5.

$$\text{Mr} = \frac{\text{number of dead J2}}{\text{total number of J2}} \times 100 \quad (3)$$

$$\text{Pr} = \frac{\text{number of paralyzed J2}}{\text{total number of J2}} \times 100 \quad (4)$$

$$\text{Rm} = \frac{\text{number of J2 having resumed mobility}}{\text{total number of J2}} \times 100 \quad (5)$$

Acaricidal activity

Toxicity effect

A modified leaf dip bioassay was used to test acaricidal activity, according to Qessaoui et al. (2020). Five concentrations including 10, 100, 1000, 5000 and 8000 µL/L of the EO were prepared with Tween-80 at 0.1% acting as an emulsifier. The bioassay was conducted in Petri dishes (9 cm diameter). Fresh bean leaves were collected from unsprayed plants growing in a greenhouse (INRA experimental farm, Agadir, Morocco). Leaves were dipped in prepared concentrations for 10 s. After drying at room temperature, each leaf was individually placed in the bottom of a Petri dish atop a 9 cm diameter disk of Whatman paper wetted with distilled water. Fifteen *T. urticae* adults were introduced into each Petri dish, put on top of the leaf and then covered. In control groups, mites were held on leaves dipped in distilled water mixed with the adjuvant (Tween-80). Four replicates for each treatment were used, and mortality rate was assessed at 1, 2, 3, 6, 7 and 8 days after treatment. The bioassay was replicated three times, and mortality rates (CrrM) were corrected using Abbott's formula (Abbott 1925), Eq. 6.

$$\mathrm{CrrM}[\%] = \frac{\mathrm{dmn} - \mathrm{dmnc}}{\mathrm{mtn} - \mathrm{dmnc}} \times 100 \quad (6)$$

where dmn is the number of dead mites in treatments, dmnc is the number of dead mites in control and mtn is the total mite number.

Repellent activity

A modified choice test was used for a repellent activity assay following Qessaoui et al. (2017). Two boxes were used. One contained the treated leaves, and the other represented the control (untreated leaves). The two boxes were connected with an 8-cm-long translucent hose (1 cm in diameter) that was pierced in the middle, to allow the introduction of *T. urticae* adults. Test solutions were prepared by diluting the EO in 0.1% Tween-80. The concentrations of 10, 100, 1000, 5000 and 8000 µL/L of the EO were tested. Fresh bean leaves were immersed for 10 s in either an EO solution or 0.1% Tween-80, air-dried for 5 min and then placed in the corresponding box. Fifteen *T. urticae* adults were transferred gently through the hole made in the center of the linked-hose, after which the hole was sealed. This structure allowed mites to move freely to both boxes (control or treated box). The repellent effect was assessed at 24, 48 and 72 h after release. Four replicates were made for each treatment, and the bioassay was replicated three times. A repellency index (RI) was determined for each treatment periodically (24, 48 and 72 h) using the formula of Qessaoui et al. (2017) (Eq. 7).

$$\mathrm{RI} = \frac{C - T}{C + T} \times 100 \quad (7)$$

where C is the number of mites in the control box and T is the number of mites in the treated box.

Statistical analysis

The data collected were subjected to one-way analysis of variance (ANOVA), followed by Newman–Keuls test. A minimum significance level of $p < 0.01$ was considered for differences between means. Data were analyzed by STATISTICA 6 software. Probit analysis was used to calculate median inhibitory concentration IC_{50}, median lethal concentration LC_{50} and median lethal dose LD_{50} values at 95% confidence limit, using the Polo-PC software (LeOra-Software 1987).

Results

Antifungal activity

The inhibitory effects of *R. segetum* EO on mycelial growth of *B. cinerea* are shown in Fig. 1 (a) and (b). Using the PF technique, concentrations equal to and above 2 µL/mL produced significant inhibition, with percent inhibition increasing with concentration. The highest inhibition (98%) was obtained when the EO was applied at 16 µL/mL. Similarly, using the VA technique, volatiles of the oil exhibited concentration-dependent inhibition of mycelial growth, with the highest inhibition of 92% achieved at 0.32 µL/mL air. IC_{50} values using PF and VA techniques were 3632.841 µL/L and 73.618 µL (0.92 µL/mL air), respectively (Table 1).

Nematicidal activity

Egg hatch inhibition and nematicidal activity against J2

Results of hatch inhibition of *M. javanica* eggs are presented in Table 2, where use of letters (a–i) denote grouped values with no significant difference using the Newman–Keuls test ($p < 0.01$). The EO strongly suppressed egg hatching

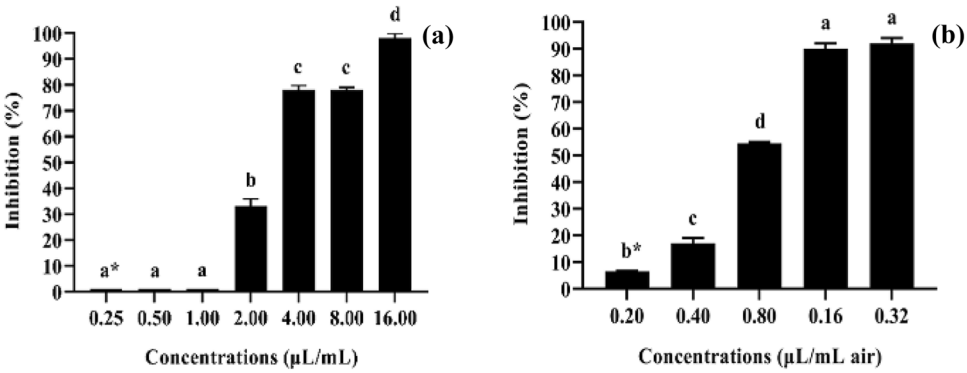

Fig. 1 Inhibition percentages of *Ridolfia segetum* essential oil on *Botrytis cinerea* at different concentrations using poisoned food (**a**) and volatile phase (**b**) methods. *Values assigned the same letter do not differ significantly, according to Newman–Keuls test ($p \leq 0.01$)

Table 1 Log-dose probit inhibition data of *Ridolfia segetum* essential oil against *Botrytis cinerea* after 7 days incubation

Method	IC_{50} (µL/L)[a]	Fiducial limits	Slope ± SE	Nat. resp	Heterogeneity	g	Log (L)	N
Poisoned food	3632.841	2890.820 to 4557.484	2.647 ± 0.331	0.000 ± 0.000	0.62	0.060	−60.11	189
Volatile phase	73.618[b]	59.425 to 91.022	3.086 ± 0.426	0.000 ± 0.000	0.40	0.073	−51.09	135

[a]IC_{50} (µL/L) the concentration at which 50% growth is inhibited

[b]IC_{50} is expressed in µL

after 10 days of incubation. The hatch inhibition rate had a linear relationship with the oil concentration, reaching up to 75.35% inhibition at 16 µL/mL concentration, with an LC_{50} value of 10,376 µL/L (Table 3). The effect of the EO on percent immobility of *M. javanica* juveniles at different concentrations and durations is summarized in Fig. 2. Low immobility of juveniles occurred after a 24 h exposure to concentrations of the oil. Juvenile immobility gradually increased with concentration and time of exposure. After 72 h, 16 µL/mL produced the highest immobility rate of 71% immobility, and an LC_{50} value of 9269 µL/L (Table 3).

Demonstration of the nematostatic effect

J2 of *M. javanica* exhibited a nematostatic response to *R. segetum* EO. Control (heat-treated J2), immobile J2 exposed to the EO at 16 µL/mL were not stained by the blue Meldola. The percentage of dead J2 was less than the paralyzed fraction, reaching 9.5% after three days of exposure. The recorded percentage of paralyzed larvae represented the majority, though showed a slight decrease over the three days of exposure. The mortality rate increased, reaching 90.5% on the third day (Fig. 3). The EO induced a direct nematostatic effect. Regarding the reversibility of J2 paralysis, juveniles subjected to treatment with the EO remained paralyzed during the three days of incubation. Dead juveniles did not recover mobility.

Acaricidal activity

Results of toxicity of the EO were presented as percentage of mortality of *T. urticae* adults at different doses and different times of exposure (Table 4). The oil exhibited contact toxicity to *T. urticae* adults. The earliest deaths occurred within 24 h after application. Response of *T. urticae* adults to treatment by the EO varied with increasing concentrations and periods after exposure. At the highest concentration (8000 ppm), the mortality rate exceeded 54% 72 h after application, and complete mortality was recorded 192 h after exposure to the oil. Probit analysis indicated an LD_{50} of 6005 µL/L (Table 5).

Repellent activity

The EO was screened for its repellent activity against *T. urticae* at doses of 10, 100, 1000, 5000 and 8000 µL/L, and the results as shown in Fig. 4. The EO exhibited moderate repellent action against *T. urticae* adults after 24, 48 and 72 h exposure. The level of repellency observed for each treatment was consistent at 24, 48 and 72 h. The EO had an attractive effect at concentrations 10, 100 and 1000 µL/L with a RI ranging from 65 to 8. After 72 h, moderate repellent action was observed on adult mites at the highest concentration tested (8000 µL/L) with a RI of 55%.

Discussion

Antifungal potency against the agent of gray mold disease has been identified in plant oils belonging to Apiaceae family members (Abo-El Seoud et al. 2005; Behdani et al. 2012; Fraternale et al. 2014). The current study is the first to investigate antifungal activity from the genus *Ridolfia*, further the oil produced different results of mycelial growth inhibition with different methods used. Greater inhibition was achieved using the volatile phase method than with the poisoned food method, in agreement with previous studies, in which the volatile fraction of *Melissa officinalis* L., *Rosmarinus officinalis* L., *Origanum syriacum* L. var. *bevanii* and *Lavandula stoechas* L. var. *stoecha* EOs showed better antifungal activity against *B. cinerea* compared to the direct contact method (Soylu et al. 2010; El Ouadi et al. 2017). Antifungal action of EO vapors depends on the presence of functional groups in the oil in a gaseous state, as well as the vapor pressure which allows them to cross through the fungal cell membranes (Belletti et al. 2007). Antifungal volatiles are penetrating and uniformly distributed. Consequently, relatively low concentrations of the oil are active at inhibiting fungal growth.

Antifungal potential of an EO depends mainly on its composition and the target fungi. The most abundant group of compounds in EOs are terpenes, which are known to be active against a wide range of fungi (Houicher et al. 2018). Further, Basaid et al. (2020a) showed that *R. segetum* EO was dominated by (z)-β-ocimene (19.7%), β-phellandrene (9.6%) and β-pinene (8.6%). In the latter study, other

Table 2 Effect of *Ridolfia segetum* essential oil on hatch inhibition of *Meloidogyne javanica* eggs

Concentration (µL/mL)	Days after incubation									
	1	2	3	4	5	6	7	8	9	10
0.8	0.00 ± 0.00 a*	0.00 ± 0.00 ab	3.85 ± 1.44 a–c	8.33 ± 3.33 a–e	5.56 ± 2.42 a–d	7.29 ± 2.12 a–e	7.41 ± 3.0 a–e	10.53 ± 2.30 a–f	12.88 ± 3.72 a–g	13.38 ± 2.70 a–h
8	7.69 ± 0.00 a–e	9.38 ± 1.97 a–e	25.00 ± 5.42 a–i	31.67 ± 8.64 a–i	40.28 ± 7.97 a–i	50.00 ± 4.53 a—i	50.93 ± 5.55 a–i	36.84 ± 7.63 a–i	39.39 ± 2.54 a–i	35.21 ± 5.71 a–i
10	7.69 ± 0.00 a–e	9.38 ± 1.97 a–e	23.08 ± 6.28 a–i	31.67 ± 3.33 a–i	43.06 ± 2.78 a–i	55.21 ± 3.99 a–i	60.19 ± 3.55 c–i	56.14 ± 3.51 a–i	58.33 ± 3.81 c–i	51.41 ± 2.70 a-i
12	57.69 ± 9.25 b–i	53.13 ± 4.37 a–i	34.62 ± 6.27 a–i	43.33 ± 2.77 a–i	50.00 ± 3.29 a–i	59.38 ± 4.44 c–i	63.89 ± 6.39 d–i	61.40 ± 5.25 c–i	65.15 ± 5.77 e–i	59.15 ± 3.06 c–i
14	76.92 ± 6.15 i	81.25 ± 7.50 i	63.46 ± 6.18 d–i	60.00 ± 13.11c–i	56.94 ± 8.86 a–i	67.71 ± 9.14 f–i	71.30 ± 5.90 g–i	64.91 ± 7.89 e–i	69.70 ± 5.45 g–i	71.83 ± 10.36 h, i
16	76.92 ± 6.65 i	81.25 ± 7.65 i	76.92 ± 9.64 i	73.33 ± 8.94 i	59.72 ± 3.13 c–i	69.79 ± 6.85 g–i	73.15 ± 8.75 i	69.30 ± 7.78 g–i	73.48 ± 6.72 i	75.35 ± 6.25 i

*Values followed by a–i are not statistically different at $p \leq 0.01$ according to the Newman–Keuls test

compounds were present in the oil at smaller percentages, such as cubenol, alloaromadendrene, γ-eudesmol, α-bisabolol and terpinen-4-ol. (Z)-β-ocimene and β-pinene have been reported to be antifungal against a wide variety of phytopathogenic fungi (Sekine et al. 2007; Saroj et al. 2015). Alternatively, Marei and Abdelgaleil (2018) reported that terpinen-4-ol had antifungal activity against *B. cinerea*, with an EC_{50} of 77 mg/L. Similarly, α-bisabolol was reported as antifungal against *B. cinerea* (Kamatou and Viljoen 2010). Terpinen-4-ol and α-bisabolol are proportionately small (2.4%) in *R. segetum* oil, but since they were reported as effective against *B. cinerea*, it is plausible that they contributed to the antifungal activity of the oil. We infer that the activity of the EO is linked to a synergism between major and minor constituents.

Essential oil constituents have different modes of action dependent on the target fungal strain (Bakkali et al. 2008). In *B. cinerea*, morphological alterations of hyphae and conidia of the fungi exposed to EOs have been reported. Treated mycelium displayed reduced hyphal diameters, shriveled hyphal aggregates and lysis of hyphal wall occurred, effecting cytoplasmic leakage (Xueuan et al. 2018). In the same context, β-pinene, a major compound of the EO was reported to destroy cellular integrity and thereby, inhibiting respiration and ion transport processes (Uribe et al. 1985), in addition to inhibiting microbial phospholipase and esterase activities (Silva et al. 2012).

Nematicidal activity of Apiaceae plant oils against *M. javanica* is limited to a few studies (Oka et al. 2000; Sousa et al. 2015; Basaid et al. 2020b). *R. segetum* oil exhibited inhibition of egg hatch and J2 survival of the root knot nematode. Lethal effects of EO include immobility of nematodes. A permanent or temporary paralysis (nematostatic effect) may also occur. Previous reports of the latter effect of plant-derived substances using the Meldola Blue staining method have centered on plant extracts (Jourand et al. 2004; Mayad et al. 2019; Seenivasan 2019). To avoid confusion, a coloring test with Meldola Blue on J2 exposed to EO treatments was performed in the current study. The EO showed a strong nematostatic effect on J2 of *M. javanica*. This study is the first to use the Meldola Blue staining method in conjunction with EOs. The nematostatic effect may be attributed to the bioactive compounds in *R. segetum*. Major compounds of the oil (z)-β-ocimene and β-pinene, have been reported to reduce hatching and J2 mobility of *Meloidogyne incognita* (Adekunle et al. 2007; Echeverrigaray et al. 2010). EO constituents may cause irreversible changes to protein structures, particularly those located on the nematode surface. EOs disrupt membrane function and cause alteration of permeability; further, they act on nematode nervous systems by inhibiting acetylcholinesterase activity (Oka 2001).

Recently, Badalamenti et al. (2021) reported toxicity of *R. segetum* EO against different pests (*Culex*

Table 3 Log-dose probit hatch inhibition and immobility data of *Ridolfia segetum* essential oil against *Meloidogyne javanica*

Test	LC_{50} (µL/L)[a]	Fiducial limits	Slope ± SE	Nat. resp	Heterogeneity	g	Log (L)	N
Hatch inhibition of eggs	10,376.240	7619.981 to 12,110.258	3.759 ± 0.913	0.667 ± 0.023	1.07	0.272	654.3	1200
Immobility of juveniles	9269.378	3108.119 to 15,542.142	0.933 ± 0.183	0.191 ± 0.031	2.51	0.416	881.0	1200

[a]LC_{50} (µL/L) the concentration at which 50% of juveniles are dead or eggs are hatched

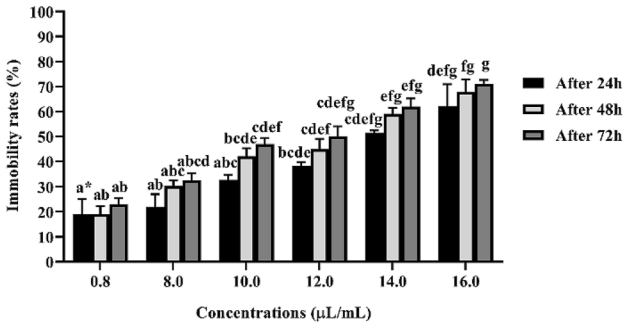

Fig. 2 In vitro effect of *Ridolfia segetum* essential oil on immobility rate of second-stage juveniles of *Meloidogyne javanica.* *Values followed by the same letter do not differ significantly according to the test of Newman–keuls ($p \leq 0.01$)

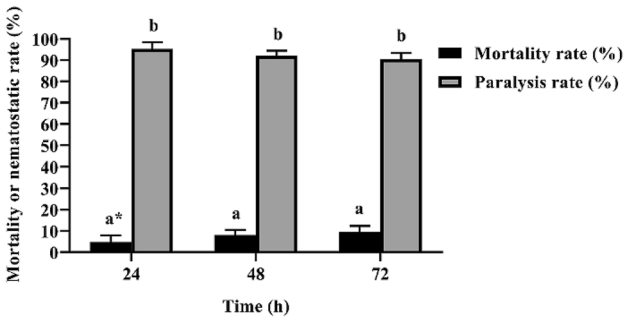

Fig. 3 In vitro mortality and nematostatic rate induced by *Ridolfia segetum* essential oil on second-stage juveniles of *Meloidogyne javanica.* *Values followed by the same letter do not differ significantly according to the test of Newman–keuls ($p \leq 0.01$)

quinquefasciatus Say, *Musca domestica* L., and *Spodoptera littoralis* (Boisduval)). Further, the EO exhibited acaricidal activity against *T. urticae*. The current study agrees with previous work, reporting the toxicity of other plant EOs from the Apiaceae family against *T. urticae* (Attia et al. 2011; Amizadeh et al. 2013; Ebadollahi et al. 2014). Although, higher concentrations and greater exposure time were required to achieve complete adult mortality. Exploring other testing methods may ascertain improved toxicity of *R. segetum* EO; Araújo et al. (2012) reported the variability of the residual effect of Piper EO through fumigation and contact against *T. urticae*. Different modes of action operate according to the method employed. In contact bioassays, toxicity may occur through the tarsi and ingestion (Araújo et al. 2012); whereas if applied as a fumigant, volatile constituents penetrate the organism via the respiratory system, resulting in enhanced efficacy (Choi et al. 2004).

The potential repellent effects of EOs against *T. urticae* have been evaluated for many plant species (Araújo et al. 2012; Motazedian et al. 2012; Reddy and Dolma 2018). In the current paper, the EO of *R. segetum* exhibited moderate repellency against adult mites at high concentrations. It is probable if adult mites detected the presence of the oil, it was not interpreted by them as a cue to avoid exploitation of the site containing the EO (de Lira et al. 2015). The low repellent effect of the EO may be linked to a relatively high volatility of the oil (Liu and Ho 1999). Effects of EOs usually dissipate relatively quickly, in contrast to when they are freshly applied, given their high volatility. This may be compensated by development of nanoformulations that

Table 4 Effects of *Ridolfia segetum* essential oil on *Tetranychus urticae* adults' mortality rates

Concentration (µL/L)	Hours after application					
	24	48	72	144	168	192
10	5.0 ± 5.2 a*	7.5 ± 5.3 ab	8.7 ± 6.2 ab	11.5 ± 6.4 a–d	10.4 ± 5.8 a–c	4.7 ± 6.1 a
100	13.3 ± 4.9 a–e	15.1 ± 5.9 a–f	16.4 ± 6.3 b–g	15.9 ± 5.5 a–g	13.4 ± 5.6 a–e	9.5 ± 4.0 a–c
1000	18.3 ± 5.7 b–g	20.2 ± 4.9 c-h	22.5 ± 7.4 d-h	26.0 ± 8.8 f–h	25.4 ± 8.1 f–h	23.8 ± 9.1 e–h
5000	21.6 ± 3.8 d–h	22.8 ± 6.0 e–h	29.5 ± 7.9 hi	37.6 ± 8.4 ij	37.3 ± 8.5 ij	39.6 ± 11.3 j
8000	26.6 ± 7.7 gh	36.3 ± 8.5 ij	54.7 ± 7.0 k	5.3 ± 4.3 l	83.6 ± 6.1 m	100 ± 0 n

*Values followed by the same letters are not statistically different at $p \leq 0.01$ according to the Newman–Keuls test

Table 5 Log-dose probit mortality data of *Ridolfia segetum* essential oil against *Tetranychus urticae*

LD_{50} (µL/L)[a]	Fiducial limits	Slope ± SE	Nat. resp	Heterogeneity	g	Log (L)	N
6005.780	5251.855 to 6617.426	7.132 ± 1.473	0.266 ± 0.025	0.42	0.164	−272.6	400

[a]LD_{50} (µL/L) the dose at which 50% of mites are dead

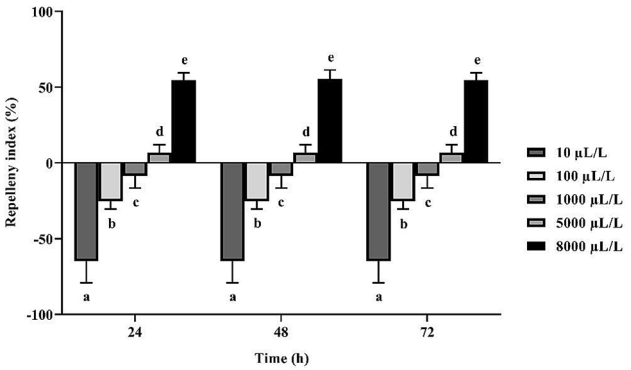

Fig. 4 Repellent effect of *Ridolfia segetum* essential oil on *Tetranychus urticae* adults. The repellency index (%) followed by the same letters do not differ at $p \leq 0.01$ according to the Newman–Keuls test

minimize loss by evaporation or degradation and establish conditions for controlled release (González et al. 2014).

Conclusion

The current investigation adds knowledge of biological activities of Moroccan *R. segetum* EO against key pest and diseases of great economic importance for many important crops. Bioassays have revealed a broad spectrum of activities of the studied EO at high concentrations. Further research is required to determine the activity of this EO against other plant pathogens and parasites, and to test the EO in combination with other biocontrol agents, keeping in view the potential synergistic effects which will allow development of sustainable tools and methods for crop protection.

Authors contributions Khadija Basaid performed the investigation and wrote the first draft; all other authors contributed equally.

Funding None.

Availability of data and materials All relevant data are within the paper.

Declarations

Conflict of interest The authors have no conflicts of interest.

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