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ORIGINAL RESEARCH ARTICLE

Oviposition deterrence and repellent activities of selected essential oils against *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae): laboratory and greenhouse investigations

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Abstract

Tuta absoluta, an invasive pinworm characterized by high reproductive potential and adaptation to different agroecological conditions, cause serious damage to tomato crops. Chemical control with synthetic insecticides is widely used to control this pest, although pesticides exhibit side effects on non-target organisms and negatively impact the environment, with the occurrence of resistance to some active substances in the target pest. The use of essential oils (EOs) from aromatic or officinal plants could represent an environmentally safe control method, alternative to synthetic insecticide application. In this work we investigated the effect of EOs from Spanish oregano, laurel, basil, garlic, peppermint, cypress, and eucalyptus EOs on *T. absoluta* oviposition deterrence and larval repellency. Results showed a remarkable oviposition deterrence using laurel, Spanish oregano, basil, and garlic EOs in both laboratory and greenhouse experiments. In no-choice repellence tests carried 1, 2, 6, and 22 h after treatment application, peppermint and Spanish oregano EOs showed the most lasting effects while cypress and eucalyptus EOs were not effective after 6 and 22 h, respectively. In choice tests, at either 5 or 22 h after treatment application, low preference for the test over control by larvae was observed in all treatments, except for basil EO. The most promising effects were observed in Spanish oregano and laurel treatments where no larvae were detected. Repellent effectiveness of EOs does not seem to be influenced when larvae did not get a chance to do a choice between treated and untreated leaves. This study highlights the effectiveness of EOs of aromatic or officinal plants as an alternative to synthetic insecticides for the control of *T. absoluta*.

Keywords Essential oil · Integrated pest management · Oviposition deterrence · Repellency · South American tomato pinworm

Introduction

The South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the most damaging pests of tomato (*Solanum lycopersicum* L.) crops, both in open fields and under greenhouses. This invasive pest, originating from South America, cause economic and ecological problems due to its rapid dispersion challenging pest control programs. Currently it can be found throughout Africa, Europe, Middle East and Asia (Campos et al. 2017; Biondi et al. 2018; Mansour et al. 2018; Han et al. 2019). In Italy, *T. absoluta* was reported for the first time in 2008 and rapidly spread to the tomato growing areas in all regions of the country (Speranza and Sannino 2012). In new colonization areas, the tomato pinworm has displayed great ability to adapt to different agro-ecological conditions, exhibiting high reproductive potential and significant economic damage (Desneux et al. 2010, 2011; Urbaneja et al. 2013). Under Mediterranean conditions, adults of *T. absoluta* can be detected throughout the whole year. Females generally mate only once in a day and up to six times during their lifespan. They oviposit preferentially on both sides of the leaves located in the plant apex (73%) and to a lesser extent on leaf veins and stem margin (21%), sepals (5%) or green fruits (1%) (Estay 2000). At 25–27 °C, the

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pre-oviposition period lasts for 2–3 days. A single female can lay up to 260 eggs throughout its life (Uchoa-Fernandes et al. 1995). After hatching, larvae explore the substrate for 5–10 min to about an hour, before digging a mine in the leaf parenchyma. Larvae feed and develop on leaves, stems, buds, flowers and fruits, damaging crop with yield losses that can reach 100% (Desneux et al. 2010, 2011; Biondi et al. 2018). The control of *T. absoluta* in tomato largely relies on intensive use of synthetic insecticides. However, chemical control of *T. absoluta* is very difficult, because the moth has a remarkable tendency to rapidly acquire resistance against insecticides. Furthermore, synthetic insecticides exhibit side effects on non-target organisms and negatively impact the environment (Han et al. 2018; Guedes et al. 2019). Therefore, researchers have focused on control approaches of *T. absoluta* that exhibit limited negative effects on the environment, which are based on the use of aromatic or officinal plant compounds such essential oils (EOs) (Soares et al. 2019). Indeed, plant-based bioinsecticides are cheap, biodegradable, more effective and species-specific than synthetic insecticides (Andrade-Ochoa et al. 2018). A number of different EOs shows a broad spectrum of activity against pests of many crops and stored products, exhibiting insecticidal, antifeedant, repellent, oviposition deterrent, growth regulatory, and antivector effects (Isman 2006; Said-Al Ahl et al. 2017; Bett et al. 2017; Rajkumar et al. 2019; Abdelkader et al. 2020; Chintalchere et al. 2021). In particular, the repellent and oviposition deterrence activities of EOs extracted from aromatic or officinal plants are well known. However, most of these studies were conducted on pests belonging to Coleoptera and Diptera species (Nerio et al. 2010; Mossa 2016; Marsin et al. 2020). Given the efficacy of some botanicals against *T. absoluta*, it is possible to use them in combination with soft pesticides in order to develop effective integrated pest management approaches that target this pest (Tarusikirwa et al. 2020). Currently, many studies report ovicidal, larvicidal and adulticidal activities of EOs against this pinworm (Magalhães et al. 2001; Umpiérrez et al. 2012; Alam et al. 2019; Esther et al. 2019; Lo Pinto et al. 2019, 2020; Tarusikirwa et al. 2020; Mansour and Biondi 2021); however oviposition deterrence and repellence effects are less investigated (Aynalem 2018; Marsin et al. 2020). In this perspective, the aim of the present study was to assess the effectiveness of EOs from selected plants that are understudies in terms of oviposition deterrence and repellency activity against *T. absoluta*. Specifically, the effectiveness of EOs of Spanish oregano (*Thymus capitatus* L., synonym *Thymbra capitata*, subgenus *Coridothymus capitatus* L. Reichemb. F.), laurel (*Laurus nobilis* L.), basil (*Ocimum basilicum* L.), garlic (*Allium sativum* L.), peppermint (*Mentha piperita* L.), cypress (*Cupressus sempervirens* L.), and eucalyptus (*Eucalyptus globulus* L.) was investigated. Our objective was to assess: 1) effects on the oviposition deterrence in choice tests using (i) Spanish oregano and laurel EOs in laboratory and (ii) Spanish oregano, laurel, basil, and garlic EOs in greenhouse; 2) effects on repellency against third instar larvae in laboratory, using (iii) Spanish oregano, peppermint, cypress, and eucalyptus EOs in no-choice tests and (iv) Spanish oregano, peppermint, cypress, eucalyptus, laurel, and basil EOs in choice tests.

Materials and methods

Plant essential oils

Natural EOs (100% pure industrial products obtained by steam distillation method, standardized and marketed as food supplements, ERBAMEA) of basil (leaves), Spanish oregano (flowering aerial parts), laurel (leaves), peppermint (leaves), cypress (cones), and eucalyptus (leaves), and macerate oil of garlic (bulbs), were used. Garlic macerate oil was prepared using the method described by Patil and Ravindra (2008).

To perform laboratory experiments, an emulsion of 200 µl of EOs with 2% Tween® 20 (SigmaAldrich) in distilled water (50 ml) per each treatment was used as test, and 2% Tween® 20 in distilled water (50 ml) was used as control. For the greenhouse experiment, an emulsion of 400 µl of EOs with 2% Tween® 20 (SigmaAldrich) in distilled water (100 ml) per each treatment was used as test and 2% Tween® 20 in distilled water (100 ml) was used as control. For garlic, two emulsions of 300 µl and 600 µl of EO were obtained as described above.

Tomato plants and insects

The biological material used in the bioassays was obtained from tomato seedlings kept in two greenhouses (greenhouse-1 and greenhouse-2). Tomato plants, *Solanum lycopersicum* L var. Pizzutello, were grown under greenhouse-1 (25 ± 5 °C, 50–70% relative humidity [RH], 16:08 L:D photoperiod) in plastic pots ($7 \times 7 \times 8$ cm) and were watered every 2 days. Seedlings for experiments were used when they reached about 30–40 cm in height. *Tuta absoluta* third instar larvae used in the bioassays were obtained from rearing of the insect on tomato plants under greenhouse-2 (in the aforementioned controlled climatic conditions). To obtain mated adults of *T. absoluta*, leaves with larvae were collected from plants of greenhouse-2 and placed in a plastic tray ($40 \times 30 \times 6$ cm) over a layer of sand (0.5 cm) arranged on a sheet of filter paper. Furthermore, leaves were covered with a sheet of filter paper. The tray was kept in a thermo-conditioned room in the aforementioned controlled climatic conditions. Daily, the sand was sieved and the underlying sheet of paper was observed until insect reached the pupal stage. Pupae were transferred to a Plexiglas cage (50 × 30 × 30 cm) and observed periodically until adult emergence. After mating, pairs of adults were taken from the cage to be used in tests. To obtain larvae for experiments, leaves from infested plants of greenhouse-2 were collected and brought to the laboratory. After observation under stereomicroscope, third instar larvae were carefully removed from their mines by using a fine brush and were used in the bioassays.

Experiment 1: oviposition deterrent effects in laboratory

EOs of laurel and Spanish oregano were used. Tomato leaves were taken from uninfested seedlings kept in greenhouse. In laboratory, each leaf, made up of three apical leaflets distributed along the leaf rachis, was wet with the emulsion with EOs (as test), or with water and Tween only (as control). Then leaves were singly introduced inside a glass tubes (10 × 1 cm) containing water to maintain the leaf turgor. Single pairs of compound leaves, one for the test and one for the control, were placed vertically inside Plexiglas boxes (40 × 25 × 25 cm) at a distance from each other of 30 cm. These boxes featured two side walls in tulle and a removable front window (15 × 15 cm) to facilitate transfer of leaves and insects inside the box. Subsequently, two mated pairs of *T. absoluta* were released into the box and left for 36 h. Insects were fed with a drop of honey placed centrally on the top of the box. Each treatment (laurel and Spanish oregano EOs) was replicated 40 times. Boxes were kept in a thermo-conditioned room under the aforementioned controlled climatic conditions. Observations were made after 36 h from the beginning of the experiment by counting the number of eggs laid on the leaves for each box.

Experiment 2: oviposition deterrent effects in greenhouse

EOs of Spanish oregano, basil, laurel and garlic were used, and for each treatment the following procedure was used: 40 uninfested plants were taken from greenhouse-1 and numbered from 1 to 40. At the beginning of the experiment, 20 plants were sprayed with the emulsion with EO (= test) and 20 plants with water and Tween (= control). Plants were transferred into a greenhouse-2 under a tunnel covered with tulle (200 × 150 cm and 90 cm roof height) without openings and provided of 4 removable windows (40 × 40 cm) along a side (at a distance of 40 cm from each other) to facilitate transfer of plants and insects inside the tunnel. Plants were randomly placed in 5 rows of 8 plants each, at a distance of 10 cm on the row and 20 cm between rows. Soon after, twenty mated pairs of *T. absoluta* were released into the tunnel and left for 4 days, feeding whit drops of honey placed on the top of the tunnel. After 4 days, plants were transferred to the laboratory and observed under a stereomicroscope to record the number of eggs found on leaves.

Experiment 3: repellency effects against larvae in no-choice experiments

EOs of Spanish oregano, peppermint, cypress, and eucalyptus were used. To perform experiments, uninfested compound leaves were collected from plants of the greenhouse-1 and brought to the laboratory where leaflets of similar development stage, shape and size (4 cm in length and 3 cm in width) were selected and removed from the plant. Leaflets were treated by dipping them in the solution of EOs (= test) or only in water and Tween (= control) and then left to dry for 5 min at room temperature on a non-absorbent surface. After evaporation of the access water, the leaflets were placed singly with the abaxial side into a Petri dish (5 cm diameter) containing a moist filter paper disk. For each treatment, 20 Petri dishes as tests (EOs) and 20 as controls (distilled water) were prepared. Then, one *T. absoluta* larva of the 3rd instar was put into each Petri dish. Three replicates were run for each tested oil. Petri dishes were kept in a thermo-conditioned room under the aforementioned controlled climatic conditions. To detect any larval activity on the leaf, the presence or absence of larvae producing mines were recorded after 1, 2, 6, and 22 h from the beginning of the test.

Experiment 4: repellency effects against larvae in choice experiments

EOs of laurel, Spanish oregano, peppermint, cypress, eucalyptus, and basil were tested. Treatments were prepared as described in the previous section (experiment-3). Bioassays were performed as follows: single pairs of leaflets, one for the test and one for the control, were randomly placed at a distance of 2 cm from each other in a Petri dish (9 cm diameter). For each treatment, five Petri dishes were prepared and one *T. absoluta* third instar larva was put into each Petri dish in the central area between leaflets. Five replicates were run for each tested oil under the same climatic conditions described previously. The presence or absence of larvae producing mines on the leaflets of each Petri dish was recorded after half an hour, and 5, 20, and 24 h from the beginning of the experiment.

Statistical analysis

To assess the effects of EOs on the oviposition deterrence, data were analysed using paired t-test (for dual-choice tests between test and control) in experiment-1 (laboratory). A one-way ANOVA followed by Tukey's post-hoc test ($P < 0.05$) was used for experiment-2 (greenhouse). To

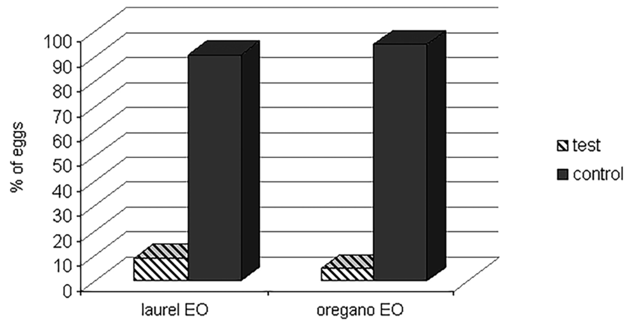

Fig. 1 Percentage of eggs laid by mated females of *T. absoluta* subjected to dual-choice test on tomato leaves treated with laurel and Spanish oregano EOs (test) or distilled water (control) after 36 h from the beginning of the experiment in laboratory

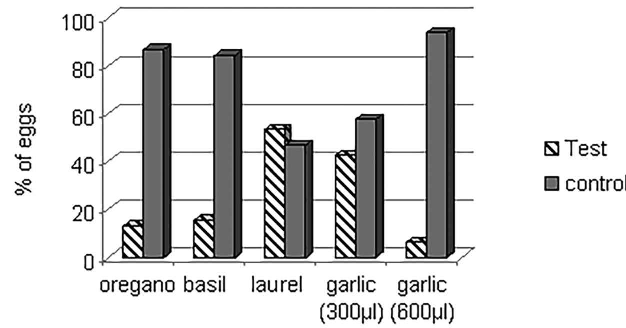

Fig. 2 Percentage of eggs laid by mated females of *T. absoluta* subjected to dual-choice test on tomato plants treated with Spanish oregano, basil, laurel, and garlic EOs (test) or distilled water (control) detected after 4 days from the beginning of each EO experiment in greenhouse

evaluate repellency effects, two-way ANOVA followed by Tukey's post-hoc test ($P<0.05$) was used for experiment-3 (no-choice test), in addition to paired t-test for experiment-4 (dual-choice test). Before analysis, a logarithmic transformation of the average values and arcsine transformation of the percentage values were carried out. Percentage repellency (PR) was calculated according to McDonald formula (McDonald et al. 1970) as follows:

$PR = [(Nc - Nt)/(Nc + Nt)]100$

Where: Nc: Number of larvae in the control.

Nt: number of larvae in the treated leaves.

Statistical analyses were performed using Statistica 7.0 for Windows Package (Stat Soft Inc. 2001).

Results

Experiment 1: oviposition deterrent effects in laboratory

The oviposition deterrent effect after 36 h from the treatment was remarkable for both Spanish oregano (4 µl/ml) and laurel (4 µl/ml) EOs (Fig. 1). The average number of eggs laid on leaves treated with Spanish oregano and laurel EOs and control is shown in Table 1. Statistical analysis (paired t-test) showed that differences between test and control were significant for both Spanish oregano (t = -7.75, df = 78, $P<0.001$) and laurel (t = -4.29, df = 78, $P<0.001$) EOs, but not significant between these two EOs (t = -0.71, df = 78, $P=0.48$).

Table 1 Number of *T. absoluta* eggs per leaf (mean ± SEM) detected in tests of choice between test (EO treatment) and control (distilled water) 36 after beginning of the experiment in laboratory

EOs	dose (µl/ml)	n	test	control
Spanish oregano	4	40	0.50 ± 0.16*	9.45 ± 1.44*
Laurel	4	40	0.75 ± 0.35*	7.68 ± 1.57*

Asterisks indicate a preference which is significantly different within a choice test

($P<0.0001$, paired t-test analysis)

Experiment 2: oviposition deterrent effects in greenhouse

The observations after 4 days from the beginning of the experiment showed oviposition deterrent effects of Spanish oregano (4 µl/ml), basil (4 µl/ml), and garlic at the dose 6 µl/ml, but not of laurel (4 µl/ml), and garlic at the dose 3 µl/ml (Fig. 2). The average number of eggs per plants for each EO is shown in Table 2. Statistical analysis (paired t-test) indicated significant differences in mean number of eggs between test and control in trials with EOs of Spanish oregano (t = -7.72, df = 38, $P<0.001$), basil (t = -4.52, df = 38, $P<0.001$), and garlic at the dose 6 µl/ml (t = -2.44, df = 38, $P=0.019$). On the contrary, non-significant differences were found in trials with EOs of laurel (t = -0.36, df = 38, $P=0.71$) and garlic at the dose 3 µl/ml (t = -1.15, df = 38, $P=0.25$). Significant differences were found among

Table 2 Number of *T. absoluta* eggs per plant (mean ± SEM) detected in tests of choice between test (EO treatment) and control (distilled water) 4 days after beginning of the experiment in greenhouse

EOs	dose (µl/ml)	n	test	control
Spanish oregano	4	20	0.65 ± 0.05**	4.30 ± 0.09**
Laurel	4	20	2.75 ± 0.17	2.40 ± 0.13
Basil	4	20	1.20 ± 0.08**	6.45 ± 0.25**
Garlic	3	20	5.40 ± 0.25	7.30 ± 0.27
	6	20	0.10 ± 0.02*	1.50 ± 0.13*

Asterisks indicate a preference which is significantly different within a choice test

(*$P<0.05$; **$P<0.0001$; paired t-test analysis)

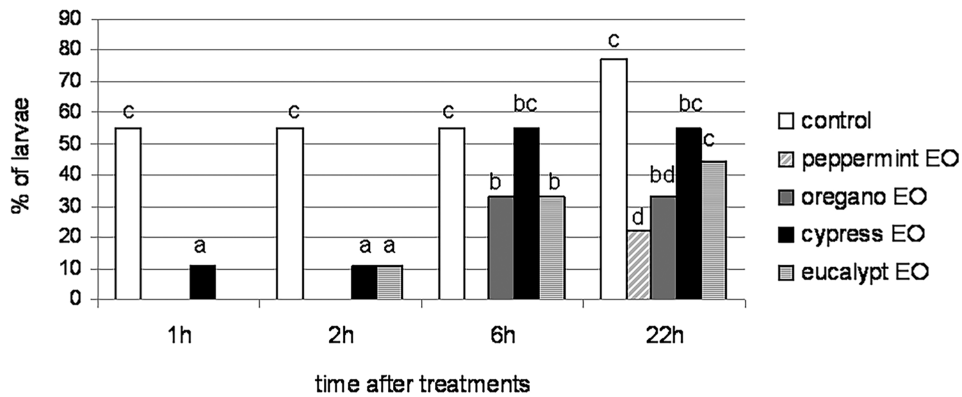

Fig. 3 Percentage of *T. absoluta* larvae on tomato leaf associated with EOs and control detected after 1, 2, 6 and 22 h from the beginning of the experiment in no-choice test (bars with the same letter are not significantly different (Tukey post hoc $P < 0.05$)

EOs (one-way ANOVA: F = 11.30, df = 4, $P < 0.001$), due to EO of garlic at the dose 3 µl/ml, that differed from the other EOs, and EO of garlic (6 µl/ml) that differed from laurel EO only (Tukey post hoc, $P = 0.005$).

Experiment 3: repellency effects against larvae in no‑choice test

The percentage of larvae detected on the leaflets treated with EOs (4 µl/ml) and control at 1, 2, 6 and 22 h after releasing the larvae are shown in Fig. 3. In the first observation, larvae were only detected on cypress EO and control, at 2 h on eucalyptus, at 6 h on Spanish oregano, and at 22 h on peppermint. In the last observation, a higher percentage of larvae was observed in the control, followed by cypress, eucalyptus, oregano, and peppermint. The percentage of repellency (PR) of EOs detected after releasing the larvae on the treated leaflets is shown in Table 3. All EOs elicited a strong repellence at 2 h from the treatment. PR of peppermint was always high, while decreased that of the other EOs, with a small increasing at 22 h. Statistical analysis (two-way ANOVA) indicated that there was a significant difference in percentage of larvae detected among EOs (F = 6.89, df = 12, $P < 0.001$). In all experiments, tests with EOs were statistically different from control, except for cypress EO at 6 and 22 h, and eucalyptus EO at 22 h. In addition, statistical differences were found at 22 h, specifically among peppermint EO and other EOs except Spanish oregano EO, and between Spanish oregano EO and eucalyptus EO. Considering time effects of each EO, significant differences were observed after 6 h for cypress and eucalyptus EOs, while no differences were detected for all EOs, except for peppermint EO, where larvae were detected on the test at 22 h (Tukey post hoc, $P = 0.005$).

Table 3 Percentage of repellency (PR) of EOs (4 µl/ml) of peppermint, Spanish oregano, cypress, and eucalyptus against third instar larvae of *T. absoluta* at 1, 2, 6 and 22 h after releasing the larvae on the treated leaflets in no-choice test

EOs	1 h	2 h	6 h	22 h
Peppermint	100	100	100	55.6
Spanish oregano	100	100	25.0	40.0
Cypress	66.7	66.7	0	16.3
Eucalyptus	100	66.7	25.0	27.3

Experiment 4: repellency effects against larvae in choice test

The response of larvae in dual-choice treatments with EOs (4 µl/ml) is shown in Fig. 4. After 5 h, all larvae in

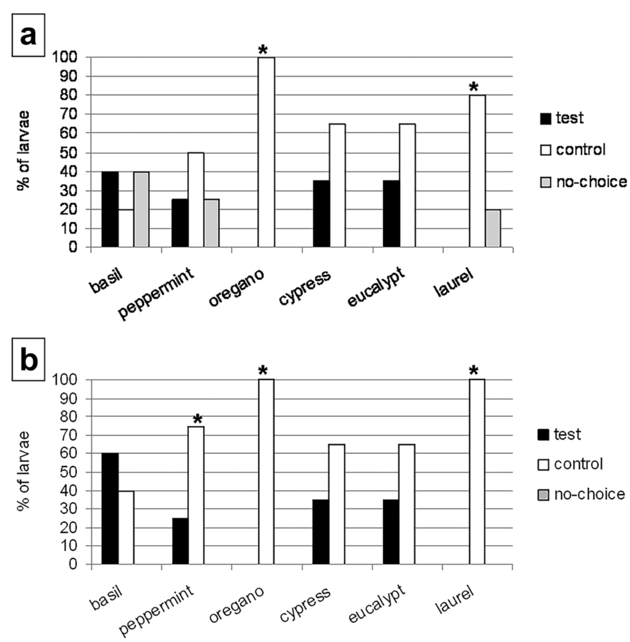

Fig. 4 Percentage of *T. absoluta* larvae on tomato leaves associated with EOs and control detected after 5 (**a**) and 20 h (**b**) from the beginning of the experiment in choice test (asterisks indicate a preference which is significantly different within a choice test, $P < 0.001$, n = 25, df = 48, paired t-test analysis)

bioassays with Spanish oregano, cypress and eucalyptus EOs, made a choice. In bioassays with basil, peppermint, and laurel EOs, larvae made a choice in 60%, 75%, and 80% of the cases respectively, while the remaining larvae exhibited a no choice. The low preference of the larvae for the test over control was observed for all EOs except basil. No larval damage was ever detected in tests with Spanish oregano and laurel EOs. After 20 h, all larvae eventually made a choice. In particular, in the treatment with basil, larvae moved towards the test in 50% of the replicates, in the treatments with peppermint and laurel EOs all larvae preferred the control condition (100%). PR of EOs detected after releasing the larvae on the treated leaflets is shown in Table 4. At 5 h from the treatment, oregano and laurel EOs showed a total repellence effect, while the others EOs showed values not exceeding 30%. PR did not change at 20 h, except for peppermint where an increase up to 50% was found. Statistical analysis (paired t-test) highlighted significant differences between test and control in Spanish oregano ($t = -4.0$, $df = 48$, $P < 0.001$) and laurel ($t = -6$, $df = 48$, $P < 0.001$) EOs at either 5 or 20 h after treatment, and in peppermint ($t = -4.52$, $df = 48$, $P < 0.001$) at 20 h. No significant differences for cypress, basil and eucalyptus EOs were found ($P < 0.05$). In addition, two-ways ANOVA indicated that there were significant differences among EOs at either 5 or 20 h after treatment ($F = 9.07$, $df = 5$, $P < 0.001$), due to peppermint EO and Spanish oregano EOs (Tukey post hoc, $P < 0.05$). No significant differences between observation were found over time ($F = 1.74$, $df = 1$, $P = 0.19$).

Discussion

Natural products of plants, especially extracts or essential oils, are traditionally used against economically important pests, and some of them have provided potential alternatives to currently used synthetic insecticides (Isman 2006). These natural products are less harmful to the environment and human health and their utilization has increased in recent years. Despite the large number of essential oils used against pest insects, only few have been tested on the tomato borer (Soares et al. 2019). Our study investigated the effectiveness in terms of oviposition deterrence and repellency of EOs of *O. basilicum* (basil), *T. capitatus*, subgenus *C. capitatus* (Spanish oregano), *L. nobilis* (laurel), *M. piperita* (peppermint), *C. sempervirens* (cypress), *E. globulus* (eucalyptus), and *A. sativum* (garlic) against *T. absoluta*. In relation to the oviposition deterrence, the control of *T. absoluta* by preventing egg deposition on plant tissues is a potentially very attractive approach in order to avoid increasing pest population on tomato fields. However, there is still little knowledge on the activity of natural products about this aspect. A literature survey revealed that some works on the oviposition deterrence of *T. absoluta* have been conducted using aqueous extracts of *Nerium oleander* L., *Olea europea* L., *Laurus nobilis* L. (Natour and Karrom 2017), EOs of *Azadirachta indica* A. Juss (Tomé et al. 2013), *Tanacetum vulgare* L., *T. parthenium* L., *Aleo vera* (L), *Melaleuca alternifolia* (Maiden and Betce) and *Juglans regia* L. (Erdogan 2019) in laboratory, EOs of *Ocimum gratissimum* L. and *O. basilicum* L. (Yarou et al. 2017) in flight tunnels, and aqueous extracts of *Allium sativum* L. and *Cymbopogon citratus* (DC.), EOs of *Eucalyptus* spp., *Ruta graveolens* L., *O. basilicum* and *Pimpinella anisum* L. (Hussein et al. 2015) in field conditions. This paper is the first record on the oviposition deterrence effects by EOs of Spanish oregano and laurel against *T. absoluta*, which highlighted a strong effectiveness of this EOs in laboratory. In greenhouse conditions, a similar deterrent activity was obtained with Spanish oregano followed by basil and garlic at dose of 6 µl/ml, but no significant effects were observed with laurel and garlic at dose of 3 µl/ml. Taken together, these results indicate that deterrence caused by garlic increased with dosage. Results about basil are in agreement with other works reporting that basil oil reduces the population of *T. absoluta* per plant up to 63% in field (Hussein et al. 2015) and caused significant ovipositional deterrent effects in a flight tunnel (Yarou et al. 2017). Moreover, our results confirm those of previous studies on other pests. For instance, basil reduced the oviposition behaviour of *Spodoptera littoralis* (Boisduval) (Abd El Aziz and El Hawary 1997), *Phthorimaea operculella* Zell. (Sharaby et al. 2009), *Callosobruchus chinensis* L. (Kiradoo and Srivastava 2010) and *Aedes aegypti* L. (Warikoo et al. 2011).

In relation to repellent effects by EOs on *T. absoluta*, some recent studies have been conducted under laboratory conditions using EOs of *O. gratissimum* and *O. kilimandscharicum* Guerke against adults (Essoung et al. 2020), and *Cinnamomum zeylanicum* Blume (Adil et al. 2015), *A. sativum*, *Syzygium aromaticum*, *Eucalyptus* spp., *M. piperita*, *Simmondsia chinensis*, *Artemisia absinthium* (Salama and Shehata 2017) and *Lantana camara* L. (Liambila et al. 2021) against larvae. In our study EOs of Spanish oregano, laurel, basil, cypress, and eucalyptus have been tested as repellents against *T. absoluta* for the first time. The results obtained provide a scientific contribution for the use of EOs to control of this pest. Peppermint, Spanish oregano and laurel EOs

Table 4 Percentage of repellency (PR) of EOs (4 µl/ml) of basil, peppermint, Spanish oregano, cypress, eucalyptus, and laurel against third instar larvae of *T. absoluta* at 5 and 20 h after releasing the larvae on the treated leaflets in choice-test

EOs	5 h	20 h
Basil	33.3	33.3
Peppermint	25.0	50.0
Spanish oregano	100	100
Cypress	30.0	30.0
Eucalyptus	30.0	30.0
Laurel	100	100

showed a strong efficacy while cypress, eucalyptus, and basil EOs were moderately repellent. In no-choice tests, repellent effects of EOs were affected by the time after treatments, showing variable responses from test larvae. In particular, the major repellency was obtained in experiments with Spanish oregano and peppermint EOs, where larvae moved on test after 6 and 22 h after treatment, respectively, and differences from the control were significant in all observations. Cypress and eucalyptus EOs were less repellent than other EOs, considering larval presence occurred on the test leaves 1 and 2 h after treatment application, respectively. The repellent activity of these two EOs decreased when time after treatment application increased, because no significant differences from the control were recorded from 6 and 22 h, respectively. The major percentage of repellency (PR) was detected with peppermint EO in all observations, pointing out its potential effectiveness in the control of *T. absoluta*. These results are in agreement with a previous study in which peppermint EO was highly repellent against first instar larvae of *T. absoluta* in laboratory (Salama and Shehata 2017). Many studies on this essential oil demonstrate deleterious effects on a wide range of insects. Indeed, peppermint EO was reported having repellent effects of ants, flies, lice, beetles and moths (Ansari et al. 2000; Hori 2003; Hussein et al. 2015; Saeidi and Mirfakhraie 2017). Our experiments on repellency under choice conditions showed variable responses from larvae in dependence on the EO tested, regardless of the time elapsed after treatments, with the exception of peppermint EO. Indeed, after 20 h, this EO caused some degree of repellency, because significant differences from the control were recorded, with PR up to 50%. The most promising effects were observed in Spanish oregano and laurel EOs treatments, where larvae were never detected in the test and differences from the control were always significant. EOs of basil, cypress and eucalyptus showed a lower efficacy in terms of larval response which never differed significantly from the control and PR levels were around 30% in both time observations. It is revealed by comparing the types of experiments (no-choice and choice test), that the repellent effectiveness of EOs does not seem to be influenced when larvae did not get a chance to make a choice between treated and untreated leaves.

The positive effects of repellency by Spanish oregano and laurel EOs against tomato pinworm observed in our work are in accordance with studies that tested these plant extracts against stored product pests (Papachristos and Stamopoulos 2002; Cosimi et al. 2009; Jemâa et al. 2011; Ncibi et al. 2019). EOs of basil, cypress and eucalyptus were weak repellent against *T. absoluta* and the lower efficacy detected in our study could due to the concentration used. Indeed, in contrast with our results, these EOs are reported as good repellents against many harmful pests such as stored product insects, moths, mosquitoes, flies and aphids (Landolt et al. 1999; Harrewijn et al. 2001; Papachristos and Stamopoulos 2002; Yang and Ma 2005; Prajapati et al. 2005; Tapondjou et al. 2005; Erler et al. 2006; Hori 2003; Mishra et al. 2012; Hasaballah et al. 2018; Madreseh-Ghahfarokhi et al. 2018; Chandel et al. 2019; Ncibi et al. 2019; Abdelkader et al. 2020). However, the low repellent activity of eucalyptus was found also against moths and mosquitoes (Landolt et al. 1999; Trongtokit et al. 2005). Overall, the results of this research indicate that EOs from plants such as Spanish oregano, basil, laurel, peppermint and garlic could be potentially implemented as biopesticides against *T. absoluta*. Further study using cypress and eucalyptus EOs should be carried out in order to evaluate their potential use against this pest.

Conclusions

EOs used in this study gave promising results against *T. absoluta*. The oviposition deterrence effect of laurel, oregano, basil and garlic EOs was remarkable both under laboratory and greenhouse conditions. Repellence tests against larvae highlighted that peppermint, Spanish oregano and laurel EOs had a strong efficacy, while cypress, eucalyptus and basil EOs displayed moderately repellent effects. To conclude, the results obtained in this work could be useful to develop pest control programs against *T. absoluta* but further studies under field conditions are needed to confirm oviposition deterrence and repellence effects.

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Declarations

Conflict of interest: The authors declare that they have no conflict of interest.

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