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Adulticidal activity of essential oils of *Mentha piperita* L., *Cupressus sempervirens* L., and *Eucalyptus globulus* Labill. against the tomato leafminer *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae)

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Abstract
The tomato miner *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is a Neotropical species, which cause serious damages to tomato both in open fields and under greenhouses. Chemical control using synthetic insecticides was the primary method of control of this pest, but it has showed negative implications due to high costs, and risks for human health and environment. Therefore safer alternatives are required to control of tomato leafminer as the use of natural insecticides. In this work we investigated the insecticidal activity of essential oils (EOs) of peppermint, *Mentha piperita* L., cypress, *Cupressus sempervirens* L., and eucalyptus, *Eucalyptus globulus* Labill., used for the first time against adults of *T. absoluta*. After 1 and 24 hours of exposure to EOs, the mortality rates at different concentrations were obtained. Mortality responses was variable depending on EO concentrations and time of exposure to treatments. It is remarkable that already after 1 hour of exposure all three EOs caused mortality with maximum values of 60% for peppermint (at dose of 50 µl/ml), 70% for cypress (at dose of 12 µl/ml) and 100% for eucalyptus (at dose of 7 µl/ml). At 24 hours the greatest activity, with values of 100% mortality, was detected for eucalyptus EO (at dose of 3 µl/ml) followed by cypress EO (at dose 7 µl/ml) and lastly peppermint EO (at dose 10 µl/ml). Results of the probit analysis after 24 hours revealed that adults were more sensitive to cypress EO than eucalyptus EO and peppermint EO, with corresponding LD_{50} values of 1.31 µl/ml, 1.45 µl/ml, and 3.98 µl/ml, respectively. These results suggest that *E. globulus*, *C. sempevirens* and *M. piperita* EOs have effective insecticidal activity on adults of *T. absoluta*.

Keywords: Adult toxicity, cypress, eucalyptus, inhalation effects, peppermint

1. Introduction

The use of chemicals for the control of harmful insects in agriculture has intensified over the years due to the intensify agricultural production caused by demographic expansion. The increasing of plant protection products against pests led to negative implications, such as soil and ground water contamination, toxic residues in agricultural products, risks for human health, development of resistance and resurgence in phytophagous, absence of selectivity with effects on non-target organisms [10, 13, 43, 47]. These aspects are recognized as factors that threaten the conservation of biodiversity. However, adequate agricultural management, as use of natural products of plants, can have a positive impact on the conservation of wildlife and decreasing of negative health effects, and non-target organisms and the environment [15, 30, 41]. Currently, guidelines that underlie agricultural practices are sustainability and eco-compatibility, through using natural insecticides, which have been considered eco-friendly and easily degradable products [25, 39, 40, 55]. Most of the essential oils (EOs) are known to possess ovicidal, repellent, larvicidal activities and antifeedants against various insect species [1, 2, 23, 26, 44, 49]. EOs of aromatic plants were traditionally used against economically important pests and their application on the control of phytophagous insects has a long history [30, 39].

In this work we wanted to investigate the application of essential oils of peppermint, *Mentha piperita* L., cypress *Cupressus sempervirens* L., and eucalyptus, *Eucalyptus globulus* Labill., as eco-friendly means of control against the tomato leafminer *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae). These species of plants are known to contain bioactive compounds that indicated insecticides activities [6, 7, 24]. *T. absoluta* is a species of Neotropical origin.

It is present in Africa, Europe, the Middle East and parts of Asia. In Italy it was reported for the first time in 2008 in Calabria, Campania and Sardinia [54] while in Sicily it came in 2009. In new colonization areas, the Gelechide has revealed great ability to adapt to different agro-ecological conditions, with high reproductive potential and significant economic damage. *T. absoluta* is one of the most dangerous insects for tomato both in open fields and under greenhouses. Besides the tomato, the species can also be found on other cultivated species, such as potato (*Solanum tuberosum* L.), aubergine (*S. melongena* L.), black nightshade (*S. nigrum* L.) and bittersweet nightshade (*S. dulcamara* L.) [14]. Damage is due feeding activity of the larvae on the whole aerial part of the plants (leaves, stems, fruits, buds and flowers) with losses of production up to 100% [14]. Following the moth attack, indirect damage may occur as a result of the development of pathogens (bacteria and fungi). Chemical control using synthetic insecticides was the primary method of control for *Tuta* spp. [46], but these chemicals have reduced profits due to high insecticide costs, and many negative effects (e.g. insecticide residues on tomato fruits [11], development of insecticide resistance [9, 52], etc.). Thus researchers have focused on the use of plant compounds such as essential oils to control this pest. Insecticidal activity can manifest itself in various ways as repellency or antifeedant or effects on mortality. Most of the time a plant has more than one of the insecticidal properties [8]. Many studies have dealt with use of EOs of plants for ovicidal and larvacidal activities against *T. absoluta* [4, 12, 18, 29, 37, 53] but few studies are conducted on the adult mortality. Previously we conducted a study on effects of cypress and peppermint EOs on mortality of larvae of *T. absoluta* [35]. Aim of this work was to assess insecticidal activity of these EOs and eucalyptus EO on *T. absoluta*, that are used for the first time against adults of this leafminer.

2. Materials and methods

2.1 Essential oils (EOs)

Experiments were conducted using natural EOs 100% (industrial products by steam distillation method, standardized and marketed as food supplements, ERBAMEA,) of peppermint (leaves) (*Mentha piperita* L.), cypress (cones) (*Cupressus sempervirens* L.) and eucalyptus (leaves) (*Eucalyptus globulus* Labill.). To evaluate insecticidal activity of EOs, their administration was made by inhalation against insect adults.

2.2 Insects

T. absoluta adults were obtained from a rearing in greenhouse of Department SAAF of University of Palermo (Italy). Leaves with larvae were collected from infested plants in greenhouse and transferred in laboratory where they were placed over a layer of sand (height 0.5 cm) in a plastic tray (size 40x30x6 cm) and covered with a sheet of filter paper. The tray were kept in a thermo-conditioned room (25 ± 5 °C, 50–70% RH, 16:8-h light: dark photoperiod). Daily, the sand was sieved and the underlying sheet of paper was observed until pupae differentiation. Newly formed pupae were transferred from the trays to a Plexiglas cage (size 50x30x30 cm) containing water and sucrose and observed periodically until adult emergence. Unsexed adults of 2-3 day old were taken from the cage to be used in bioassays.

2.3 Bioassays

Experiments were conducted in laboratory (25±1 ˚C, 60-70% RH, and 16:8 L/D photoperiod) using glass jars (600 ml) provided with an absorbent paper disk (Whatman No. 1) sprayed with 1 ml of solution of EO (10%) in distilled water with 2% Tween® 20 (SigmaAldrich) by means a micropipette and attached to the screw cap of glass jars before introducing to adults. Different doses of essential oil from peppermint (2, 10, 30, and 50, µl/ml), cypress (2, 5, 7, 10, and 12 µl/ml) and eucalyptus (0.3, 1, 2, 3, 5, 7, 10, and 12 µl/ml) were placed on each disk separately. Control was treated with the same quantity of distilled water and Twin alone. Glass jars were maintained at the previously mentioned laboratory conditions. 10 adults were used per each treatment. Each treatment and control was replicated two times. The number of alive and dead adults of each treatment in each jars was counted after 1 and 24 hours of exposure for peppermint and eucalyptus, also after 4 hours for cypress.

2.4 Statistical analysis. One-way analysis of variance (ANOVA) was performed on the data. A Duncan Multiple Range test was applied to detect significant differences of mortality among concentrations at the 0.05 percent level. All statistical analysis were performed using Statistica 7.0 for Windows Package [50]. Abbott's formula [3] was used to correct bioassay data for control response. LD_{50} (Lethal Dose, dose required to kill half a test sample) of each EO, at 1 and 24 hours from treatments were estimated by probit regression dose-response analysis [17].

3. Results

Bioassays on insecticidal effects on *T. absoluta* by inhalation administering of peppermint, cypress and eucalyptus EOs have highlighted their adulticidal activity. Tests with peppermint EO showed that mortality of adults were detected already after 1 hour from exposure, ranging from 3.7% at dose of 2 µl/ml to 60% at dose of 50 µl/ml; after 24 hours mortality rate increased, ranging from 16.7% at dose of 2 µl/ml to 100% at doses of 10 µl/ml, 30 µl/ml and 50 µl/ml. In control tests 10% mortality was detected in both time of observation (Fig. 1).

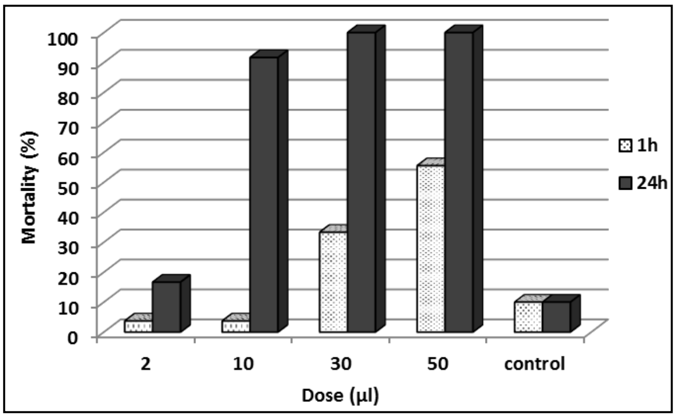

Fig 1: Mortality (%) of *T. absoluta* adults for inhalation toxicity after 1 and 24 hours of exposure to treatments with different doses (µl) of peppermint EO

Statistical analysis showed significant differences among doses after both 1 hour (F=3.5, df=4, P=0.01) and 24 hours from the treatment (F=38.6, df=4, *P*<0.001). There were also significant differences between the same doses in the different times of observation (F=19.9, df=7, *P*<0.001). Post hoc analysis highlighted that differences were imputable, after 1 hour of exposure, to control and 2 µl/ml and 10 µl/ml doses versus 30 µl/ml and 50 µl/ml doses, and, after 24 hours, to control and 2 µl/ml dose versus all doses (Table 1). Furthermore, control did not differ significantly only with doses 2 µl/ml and 10 µl/ml at 1 hour (Table 1).

Table 1: Post hoc analysis (Duncan's test) of mortality data of *T. absoluta* after 1 and 24 hours of exposure to treatments with different doses (µl) of peppermint EO

	Dose	2	10	30	50
1h	control		(**)	(*) (**)	(*) (**)
	2			(*)	(*)
	10			(*)	(*)
	30				
	50				
24h	control	(*)	(*)	(*)	(*)
	2		(*)	(*)	(*)
	10		(**)		
	30			(**)	
	50				(**)

One asterisk (*) within each rows indicates statistically significant differences (P<0.05).
Two asterisks (**) within each column indicate statistically significant differences between same dose (P<0.05).

The curve interpolating mortality percentage (expressed in probits) with doses of peppermint EO (Fig. 2) showed that the dose determining 50% mortality (LD_{50}) was at 1 hour from the treatment 43.65 µl/ml and at 24 hours 3.98 µl/ml.

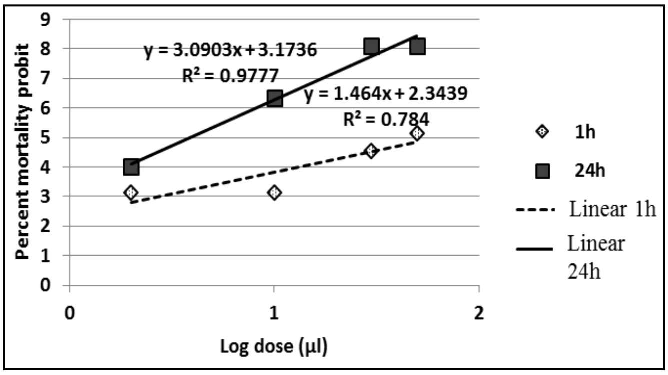

Fig 2: Dose-response analysis by probit transformation of mortality (%) of *T. absoluta* adults after 1 and 24 hours of exposure to different doses (µl) of peppermint EO

In tests with cypress EO, the highest mortality was detected after 1 hour (70%) and 4 hours (100%) from the treatment at dose 12 µl/ml, and after 24 hours (100%) starting from the dose of 7 µl/ml until 12 µl/ml. After 4 hours rate of mortality increased already at dose of 2 µl/ml achieving a high value (70%) at dose of 10 µl/ml. After 24 hours 40% mortality was detected at dose of 5 µl/ml. No mortality was caused by dose of 2 µl/ml. A low rate of mortality (10%) was detected in control after 24 hours only (Fig. 3).

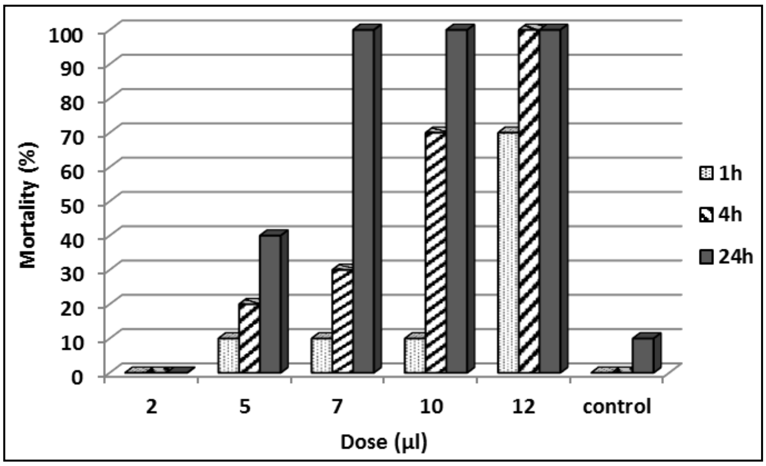

Fig 3: Mortality (%) of *T. absoluta* adults for inhalation toxicity after 1, 4, and 24 hours of exposure to treatments with different doses (µl) of cypress EO

Statistically significant differences were found among doses at 1 hour (F=7.9, df=5, P<0.001), 4 hours (F=15.1, df=5, P<0.001), and 24 hours (F=36.9, df=5, P<0.001), and between the same doses at the different times of observation (F=17.8, df=14, P<0.001). Post hoc analysis highlighted that after 1 hour differences were imputable only to dose 12 µl/ml, after 4 and 24 hours they were imputable to doses starting from 5 µl/ml. In all time of observation control did not differ significantly with 2 µl/ml dose (Table 2).

Table 2: Post hoc analysis (Duncan's test) of mortality data of *T. absoluta* after 1 and 24 hours of exposure to treatments with different doses (µl) of cypress EO

	Dose	2	5	7	10	12
1h	control				(**)	(*)
	2					(*)
	5					(*)
	7					(*)
	10					(*)
	12					
4h	control		(*)	(*)	(*)	(*)
	2		(*)	(*)	(*)	(*)
	5				(*)	(*)
	7			(**)		(*)
	10				(**)	
	12					
24h	control		(*)	(*)	(*)	(*)
	2		(*)	(*)	(*)	(*)
	5			(*)	(*)	(*)
	7			(**)	(**)	
	10					
	12					

One asterisk (*) within each rows indicates statistically significant differences ($P<0.05$). Two asterisks (**) within each column indicate statistically significant differences between same dose ($P<0.05$). The dose response analysis (Fig. 4) has showed LD_{50}= 11.48 µl/ml at 1 hour, LD_{50}= 8.91 µl/ml at 4 hours, and LD_{50}=1.31 µl/ml at 24 hours.

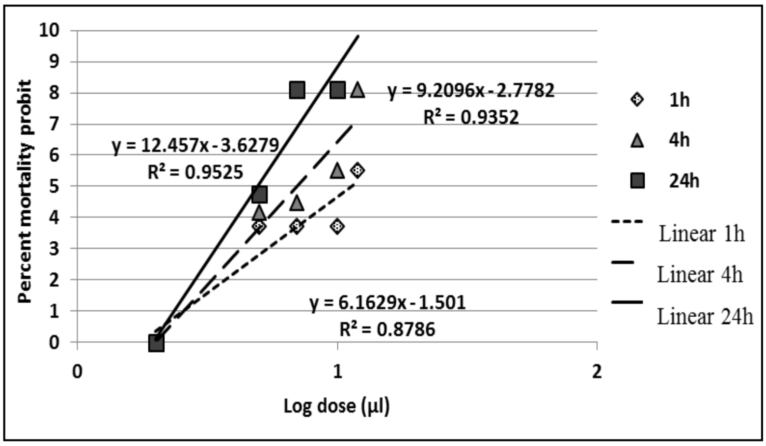

Fig 4: Dose-response analysis by probit transformation of mortality (%) of *T. absoluta* adults after 1 and 24 hours of exposure to different doses (µl) of cypress EO

Tests with EO of eucalyptus showed that after 1 hour of exposure the doses of 0.3, 1, and 2 µl/ml did not cause mortality that was detected starting from the dose of 3 µl/ml with value ranging from 33.3% to 100% at doses of 7 µl/ml, 10 µl/ml and 12 µl/ml. After 24 hours mortality was detected at all doses increasing rapidly to 90% already at dose 2 µl/ml and achieving 100% at subsequent doses. In control, after 24 hours 10% mortality was observed (Fig. 5).

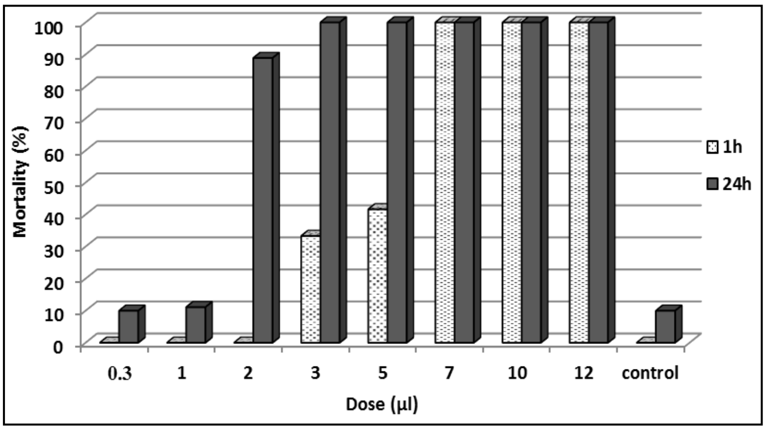

Fig 5: Mortality (%) of *T. absoluta* adults for inhalation toxicity after 1 and 24 hours of exposure to treatments with different doses (µl) of eucalyptus EO

Statistical analysis showed significant differences among doses after both 1 hour ($F=30.7$, $df=8$, $P<0.001$) and 24 hours ($F=24.8$, $df=8$, $P<0.001$) from the treatment, and between the same doses at two observation times ($F=27.3$, $df=15$, $P<0.001$). Post hoc analysis highlighted that, in both time of observation (1 and 24 hours), differences were imputable to high doses versus low doses. Control did not differ significantly with 0.3 and 1 µl/ml doses both at 1 and 24 hours, and with dose of 2 µl/ml at 1 hour (Table 3).

Table 3: Post hoc analysis (Duncan's test) of mortality data of *T. absoluta* after 1h and 24 hours of exposure to treatments with different doses (µl) of eucalyptus EO

	Dose	**0.3**	**1**	**2**	**3**	**5**	**7**	**10**	**12**
1h	Control			(**)	(*) (**)	(*) (**)	(*)	(*)	(*)
	0.3				(*)	(*)	(*)	(*)	(*)
	1				(*)	(*)	(*)	(*)	(*)
	2				(*)	(*)	(*)	(*)	(*)
	3						(*)	(*)	(*)
	5						(*)	(*)	(*)
	7								
	10								
	12								
24h	control			(*)	(*)	(*)	(*)	(*)	(*)
	0.3			(*)	(*)	(*)	(*)	(*)	(*)
	1			(*)	(*)	(*)	(*)	(*)	(*)
	2			(**)					
	3				(**)				
	5					(**)			
	7								
	10								
	12								

One asterisk (*) within each rows indicates statistically significant differences ($P<0.05$).
Two asterisks (**) within each column indicate statistically significant differences between same dose ($P<0.05$)

The dose response analysis (Fig. 6) has showed $LD_{50}=$ 5.25 µl/ml at 1 hour and $LD_{50}=1.45$ µl/ml at 24 hours from the treatment.

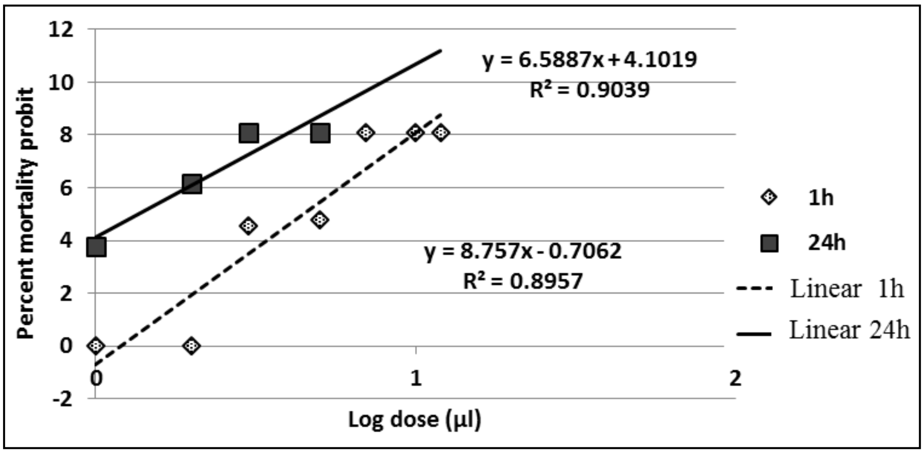

Fig 6: Dose-response analysis by probit transformation of mortality (%) of *T. absoluta* adults after 1 and 24 hours of exposure to different doses (µl) of eucalyptus EO

4. Discussion

In this study we highlighted the toxicity on *T. absoluta* by inhalation effects of peppermint, cypress and eucalyptus EOs used for the first time on adults of the tomato leafminer. *M. piperita* [7, 33, 34, 42], *C. sempervirens* [5, 19, 24, 32, 51] and *E. globulus* [6, 27, 28, 31, 36, 45, 48, 56] have previously been evaluated as bioinsecticides against some pests of agricultural importance, but they have never been used against adults of *T. absoluta*. In literature, adulticidal activity on *T. absoluta* are reported in some works on fumigation and contact effects of other plant extracts [16, 20, 21, 22, 38]. In our results, mortality responses to EOs tested was variable depending on their concentration and time of exposure to treatment. It is remarkable that already after 1 hour of exposure to treatments all three EOs caused mortality. We found that effects by inhalation of the EOs on mortality increased with increasing their doses. In agreement, similar studies to determine the fumigant toxicity of caraway (*Carum carvi* L.) and Shirazi thyme (*Zataria multiflora* Boiss.) EOs on *T. absoluta* adults reports that by increasing oil concentration, the mortality was increased [20, 21]. A work on contact toxicity of EO of bitter orange, *Citrus aurantium* L., on adults of *T. absoluta* reports that the mortality was dose dependent and increased with increasing concentrations [57]. In addition, in our results mortality increased with exposure time, from 1 to 24 hours after treatments, showing a positive correlation. This result is corroborated by other researches on *T. absoluta* adult mortality, where using EOS of cardamom (*Elettaria*

cardamomum L.) and caraway (*C. carvi*) showed strong adulticidal activity after 24 and 48 hours of exposure, respectively [20, 22]. In contrast, a work on the use of *Commiphora swynnertonii* Burtt, *Synadenium glaucescens* Pax, and *Allium sativum* L. extracts against adults of *T. absoluta* reports that each plant extract caused significant mortality but effects decreased with time after treatment [38].
Considering the efficacy on adult mortality of peppermint EO, after 1 hour of exposure we detected significant difference between control and 30 µl/ml and 50 µl/ml doses, and between these and 2 µl/ml and 10 µl/ml doses. After 24 hours, control differed significantly from all doses, as well as dose 2 µl/ml, showing that peppermint EO at low doses has showed a good effectiveness. Similar observations are reported by other authors with *C. aurantium* [57]. Peppermint use are reported against stored pests where the mortality rate of adult insects was increased with increase in EO concentration [33].
After 1 hour of exposure to cypress EO, significant differences in mortality percentage compared to control and doses were found only with dose 12 µl/ml, showing that only the highest dose was really effective. After 4 hours control significantly differed with the doses starting from 5 µl/ml and 7 µl/ml doses after 4 and 24 hours, respectively, highlighting that increasing of time of exposure increased efficacy of EO. The high doses statistically always differed from those at the lowest concentration. Similar observation was reported on *Sithophylus zeamais* (Motsch.), where using *C. sempervirens* EO, mortality increased with dose administered and time of exposure [32].
Observing effects of eucalyptus EO on adult mortality, after 1 hour we found statistical differences between control and all doses starting from dose 3 µl/ml, and between 0.3 µl/ml, 1 µl/ml and 2 µl/ml doses and the subsequent doses. Differences were also significant comparing 3 µl/ml and 5 µl/ml doses with 7 µl/ml, 10 µl/ml and 12 µl/ml doses. After 24 hours, differences were significant only comparing both control and 0.3 µl/ml and 1 µl/ml with doses starting from 2 µl/ml/ml dose. These results agree with a study, where the mortality of *Myzus persicae* (Sulzer) was increased with an increased solution concentration and exposure time of eucalyptus extract [28]. At the two exposure times, no statistical differences were found among high doses (7 µl/ml, 10 µl/ml and 12 µl/ml doses) because the highest activity, 100% mortality, was achieved at doses 3 µl/ml and 7 µl/ml, after 1 and 24 hours, respectively.
Comparing the efficacy on mortality among EOs, we highlighted that, at the two exposure times, eucalyptus EO was more effective at low doses than other two oils, and peppermint EO was always less effective than eucalyptus and cypress EOs. The greatest activity was performed by eucalyptus EO followed by cypress EO and lastly peppermint EO. Indeed, after 1 hour of exposure eucalyptus EO is the only one that has determined 100% mortality (at dose of 7µl/ml), whereas cypress and peppermint EOs caused maximum 70% (at dose of 12 µl/ml) and 50% (at dose of 50 µl/ml), respectively. Also after 24 hours of exposure to treatments, the most efficacy was showed by eucalyptus EO causing already 90% mortality at dose 2 µl/ml and 100% at dose 3 µl/ml, followed by cypress and peppermint EOs that determined 100% mortality at doses 7 µl/ml and 10 µl/ml, respectively. However, results of the probit analysis after 24 hours revealed that adults were more sensitive to cypress EO than eucalyptus EO, with corresponding LD_{50} values of 1.31 µl/ml and 1.45 µl/ml, respectively.

5. Conclusion
This study showed a good insecticidal action of EOs of peppermint, eucalypt and cypress used for the first time against adults of *T. absoluta* in laboratory confirming their usefulness for a control of the phytophagous with a low impact on the environment and human health.
The time of exposure to treatments and the increasing of concentration of EOs enhanced their effectiveness, even if at low doses EOs caused a certain rate of mortality. After 24 hours, the lowest DL_{50} was detected for cypress EO, followed by eucalyptus and peppermint EOs. On the basis of these results, EOs could constitute a good alternative to the control of the tomato leafminer, considering their insecticidal effects at low applied concentrations and short exposure times. However, this preliminary laboratory investigation should represent the basis for further study on insecticidal activity of these essential oils in the field application against tomato pests.

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