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Effects of essential oils of *Origanum vulgare* L. (Fam. Labiatee), *Pelargonium odoratissimum* L. (Fam. Geraniaceae) and *Syzygium aromaticum* L. (Fam. Mirtaceae) on mortality of *Aphis gossypii* Glover (Homoptera: Aphidiidae) in laboratory

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Abstract
The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphidiidae), is a cosmopolitan and highly polyphagous species causing serious economic damage to numerous plants including Cucurbitaceae. The use of synthetic insecticides represent the main method of control of this aphid, often resulting in resistance to all the major insecticide group. The use of natural products, such as essential oils (EOs), has increased in recent years because they have advantages in reduction of this resistance. Within this research area, we have implement and evaluate the applications of EOs that aim at reinforcing knowledge on the control of this aphid. We investigated the topical application of the EOs of *Origanum vulgare* L. (oregano), *Pelargonium odoratissimum* L. (cloves) and *Syzygium aromaticum* L. (geranium bourbon) by means the Potter tower on 1st instar nymphs under laboratory conditions. In our results, mortality response to EOs tested was dose-dependent and depending on the time from the treatment. It is remarkable that already after 24 hours of exposure to treatments, all three EOs caused mortality. Highest mortality was detected after 48 at doses of 1200 µl/l with values of 52%, 67% and 69% for clove, geranium and oregano, respectively. The probit analysis after 24 hours revealed that the lowest DL50 was detected for oregano (562.34 µl/l), followed by geranium (707.95 µl/l) and clove (954.99 µl/l) EOs. The results highlight a potential use of these EOs in the pest control being less toxic to the plants unlike chemical-based insecticides.

Keywords: aphid toxicity, clove, essential oils, geranium, oregano, topical effects

Introduction

The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphidiidae), is a cosmopolitan and highly polyphagous species, widely distributed in tropical, subtropical and temperate regions. The cotton aphid is present in all cotton-growing areas of the world and in temperate zones is principally a pest of vegetables and ornamentals in field and greenhouses [1]. This phytophagous insect is capable of serious economic damage due to direct feeding of the sap plant and due to the honeydew secretion and the consequent presence of sooty mold. It attacks numerous plants including Cucurbitaceae, Rutaceae and Malvaceae, and Citrus trees but it is a crucial insect pest of Cucurbitaceae plants throughout the world [2]. High populations of aphids can reduce the vigour of the plant, making it susceptible to other pests. Generally the colony invades the underside of the leaves and, on some plants (cotton, hibiscus, chrysanthemum etc.), also attacks the flowers. It causes foliar curling, vegetative slowdowns until the plants dry out [3]. The vegetation is smeared with honeydew and a black sooty mold on the substrate that reduces photosynthesis production and otherwise reduces the quality of the plant causing considerable economic injury [4]. Furthermore, the aphid is fearsome as a vector of numerous viruses such as tristeza virus, cucumber mosaic virus, celery mosaic virus, bean yellow mosaic virus, etc. [5]. The cotton aphid varies in colour and size [6] and its reproduction is mostly asexual with either alate or apterous females. In warmer environments, this aphid exhibits an anholocyclic life cycle, while in cooler areas the aphid exhibits either a heteroecious or autoecious holocyclic life cycle [7, 8].

The use of synthetic insecticides represent the main method of control of this aphid, often resulting in a number of undesirable side effects such as the development of resistant strain, environmental pollution and farmer health hazards. *A. gossypii* populations have evolved resistance to all the major insecticide groups [9]. Resistance to insecticides in field populations of *A. gossypii* has been documented world-wide including Africa, Asia, Australia, Europe and the USA [10]. In cultivated cotton systems, this aphid has emerged as primary pest because of its evolution of insecticidal resistance to novel classes of pesticides. Several mechanisms for resistance have been demonstrated in this aphid: enzymatic differences, target insensitivity, cuticular modifications, and life history modifications at the population level. The susceptibility of this aphid to insecticides is affected by plant hosts and life stage and can cause pest resurgence that results in much higher pest populations [11]. For these reasons, the use of natural products, such as essential oils (EOs), has increased in recent years. EOs are an environmentally friendly alternative to conventional insecticides showing a number of advantages that make them preferable to pesticides. Numerous studies reported the effectiveness of EOs against aphids [12, 13]). EOs of many plants, as *O. basilicum*, *Artemisia dracunculus*, *Juniperus excelsa* M. Bieb., *J. oxycedrus* L., *Foeniculum vulgare* Miller, *Pimpinella anisum*, L., *Rosmarinus officinalis* L., *Juglans regia* L., *Laurus nobilis* L. *Hyssopus officinalis* L., *Lavandula angustifolia* Miller, *Salvia officinalis* L., *Thymus vulgaris* L., *Majorana hortensis* L., *Carum carvi* L. and *Verbena officinalis* L., had interesting insecticidal activity on aphids

in laboratory studies [14, 15, 16, 17, 18]). Also, many studies reported that essential oils have good ability in management of *A. gossypii* [19].

The necessity to reduce the application of conventional insecticides due to the difficulty of managing the resistance of *A. gossypii*, leads to increase the study on EOs which have advantages in this respect. Within this research area, we have implemented and evaluated the applications of EOs that aim at reinforcing knowledge on the control of this aphid. The purpose of this work was to assess the insecticide activity of extracts obtained by three Mediterranean plants against *A. gossypii*, important pest of many cultivated plants in Sicily, to a potential control at low environmental impact. In particular, we investigated the topical application of the EOs of *Origanum vulgare* L. (oregano), *Pelargonium odoratissimum* L. (cloves) and *Syzygium aromaticum* L. (geranium bourbon) on first-instar nymphs under laboratory conditions.

Materials and methods

Essential oils (EOs)

To obtain essential oils (EOs) for experiments, leaves and inflorescences of oregano (*Origanum vulgare* L. Fam. Labiatae), buds of cloves (*Syzygium aromaticum* L. Fam. Mirtaceae), and leaves and flowers of geranium bourbon (or fragrant geranium) (*Pelargonium odoratissimum* L. Fam. Geraniaceae) were used. EOs were obtained by distillation in hexane using the Soxhlet extractor and they were used as an emulsion at concentrations of 200, 400, 600, 1000, 1200 µl/l with 2% Tween® 20 (SigmaAldrich) in distilled water (2 ml) per each treatment. Tween® 20 (2%) in distilled water (2 ml) was used as control.

Insects

The *A. gossypii* females were obtained from a rearing on young seedlings cucumber (*Cucumis sativa* L. Fam. Cucurbitaceae) in greenhouse of Department SAAF of University of Palermo (Italy). Parthenogenetic females were collected from infested plants and transferred in laboratory where they were placed singly in Petri dishes on a leaf of a cucumber plant with the underside facing upwards, over a layer of sand (height 0.5 cm). Petri dishes were kept in a thermo-conditioned room (25 ± 5 °C, 60–70% RH, 16:8-h light: dark photoperiod) and, daily, observed until deposition. Group of 5 first-instar nymphs were transferred with a fine camel hair brush to bottom side of cucumber leaf in a Petri dish to use them in bioassays.

Bioassays

Experiments to evaluate contact insecticidal effects of EOs were performed in laboratory (25 ± 5 °C, 60-70 % RH) by means the Potter tower, suitable for studying the biological effects of chemicals when applied as a direct spray on the organisms, with constant air pressure supply of 1.5 – 2 kg/cm2. This instrument reproduces the spray of an atomizer placed at a predetermined distance from the crop. Each Petri dish containing 5 nymphs was put in the tower and exposed to each treatment, using 2 ml of solution for each emulsion. For each dose, a group of 30 individuals was treated with the oil-based solution and another group with the wetting agent only (Tween® 20 and distilled water). Each test was replicated six times. After the treatment, the nymphs were transferred in new Petri dishes provided with filter paper disk (to limit the effects of any vapour released during the test) and maintained at the previously mentioned laboratory conditions. Number of live and dead nymphs after each treatment and in the control was recorded at 24 and 48 hours from the treatment. The percentage of mortality calculated at 24 and 48 hours from the treatment for each EO was assessed.

Statistical analysis

One-way analysis of variance (ANOVA) and paired *t* test were 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 [20]. Abbott's formula [21] was used to correct bioassay data for control response. LD50 (Lethal Dose, dose required to kill half a test sample) of each EO, at 24 hours from treatments were estimated by probit regression dose-response analysis [22].

Results and Discussion

In this study we highlighted the insecticidal effects on *A. gossypii* by contact of oregano, clove and geranium EOs used against 1st instar nymphs. Some EOs of different plants have previously been evaluated as bioinsecticides against this pest. Insecticidal activity of essential oils extracted from *Matricaria chamomilla* L., *Cuminum cyminum* L., *Artemisia dracunculus* L. and *Melaleuca styphelioides* Sm. were evaluated against adult stage of *A. gossypii* under laboratory conditions [23, 24, 25]. *Mentha piperita* L. and *M. pulegium* L. had toxicity on the adult females of this species [19]. An other work reported that *Coriandrum sativum* L., *Lavandula spica* L., *Foeniculum vulgare* Mill., *Juniperus communis* L., *Origanum vulgare L*. and *Syzygium aromaticum* L. showed a strong insecticidal activity against nymphs and wingless adults of cotton aphid [13]. Furthermore, high level of insecticidal activity of *Eucalyptus globules* L. [26], *Artemisia herba-alba, Laurus nobilis* L., *Eucalyptus camaldulensis* Dehn., *Azadirachta indica* Adr. Juss. [27, 28], *Thymus proximus* EOs [29], *Pistacia lentiscus* L. [30], *Thymus daenensis* Celak. [31], *Allium sativum* L. [32], *Cinnamomum camphora* L. [33], *Pimpinella anisum*, *Origanum syriacum var. bevanii*, and *Azadirachta indica* Adr. Juss. showed a strong insecticidal activity against cotton aphid [17, 34]). Lin *et al*. (2009) [35] reported that sugar apple (*Annona squamosa*) seed oil, an edible tropical fruit, was also promising in controlling *A. gossypii* on melon plant. A recent study found that an orange oil was comparable in efficacy to the conventional insecticides against this species on ornamental crops grown in glasshouse or polytunnel conditions [36].

In our results, mortality response to EOs was dose-dependent and depending on the time from the treatment. It is remarkable that already after 24 hours of exposure to treatments, all three EOs caused mortality. We found that effects by contact of the EOs on mortality increased with increasing their doses. Experiments with oregano EO showed that mortality of nymphs were detected already after 24 hour from exposure, ranging from 15.33% at dose of 200 µl/l to 40.26% at dose of 1200 µl/l; after 48 hours, mortality rate increased, ranging from 23.20% at dose of 200 µl/l to 69.05% at doses of 1200 µl/l. In control, 1.48% and 2.01% mortality was detected at 24 and 48 hours, respectively (Figure 1). Statistical analysis showed significant differences among doses after both 24 hours (F=45.47,

df=5, P=0.000) and 48 hours from the treatment (F=40.80, df=5, *P*=0.000). All treatments were statistically different from control in both times of observation. Post hoc analysis highlighted that differences were imputable, after both 24 and 48 hours of exposure, to all treatments except for 600 µl/l compared to 1000 µl/l and for 1000 µl/l compared to 1200 µl/l doses (Table 1). The curve interpolating mortality percentage (expressed in probits) with doses of oregano EO at 24h hour from the treatment (Figure 2), showed that the dose determining 50% mortality (LD50) was 562.34 µl/l. Many works reported that oregano EO has an excellent effect on the control of pentatomids [37], moths [38, 39, 40], stored product insects [41, 42], mosquitoes [43, 44]), cockroaches [45], houseflies [46, 47], and aphids [18].

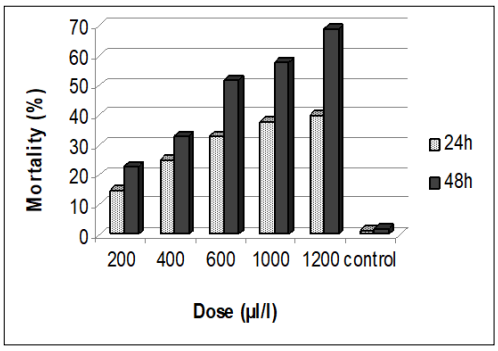

Fig 1: Mortality (%) of *A. gossypii* for contact toxicity after 24 and 48 hours of exposure to treatments with different doses of oregano EO

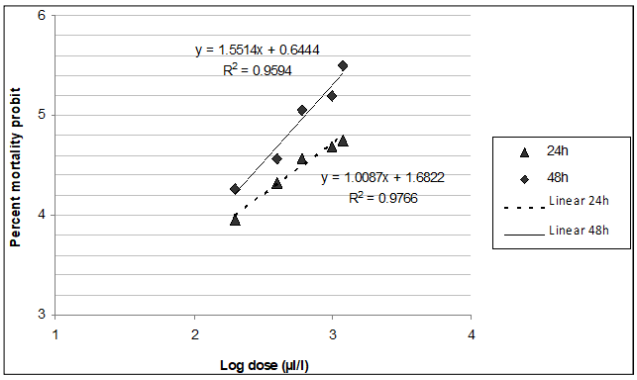

Fig 2: Dose-response analysis by probit transformation of mortality (%) of *A. gossypii* after 24h and 48 hours of exposure to different doses (µl/l) of oregano EO

Cloves EO is considered as more effective by contact against a wide range of pests [48]. Indeed, extracts of cloves showed contact activity against beetle, mosquitos and moth pests [49, 50, 51]. In our experiments with clove EO, mortality ranged from 8.5% at dose of 200 µl/l to 25% at doses of 1000 and 1200 µl/l after 24h, and from 23.9% at dose of 200 µl/l to 52% at doses of 1000 and 1200 µl/l after 48h. In control, 1.48% and 2.01% mortality was detected at 24 and 48 hours, respectively (Figure 3). Significant differences were found after both 24 hours from the treatments (F=19.27, df=5, P=0.000) between dose 200 µl/l and 400 µl/l only, whereas, after 48 hours, among 200 µl/l, 400 µl/l and 600 µl/l (F=22.07, df=5, *P*=0.000) (Table 1). All treatments were statistically different from control in both times of observations. The dose response analysis (Figure 4) has showed LD50= 954.99 µl/l at 24 hours from the treatment. These results are corroborated by an other study on the use of *S. aromaticum* and *O. vulgare* EOs against *A. gossypii* under laboratory conditions that report the LD50 is very close to the commercial synthetically insecticides on the market [13].

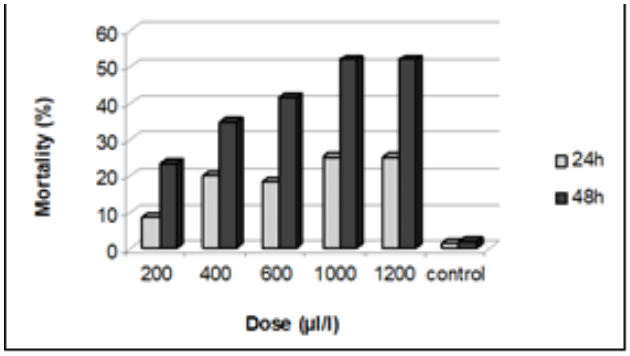

Fig 3: Mortality (%) of. *A gossypii* for contact toxicity after 24 and 48 hours of exposure to treatments with different doses of clove EO

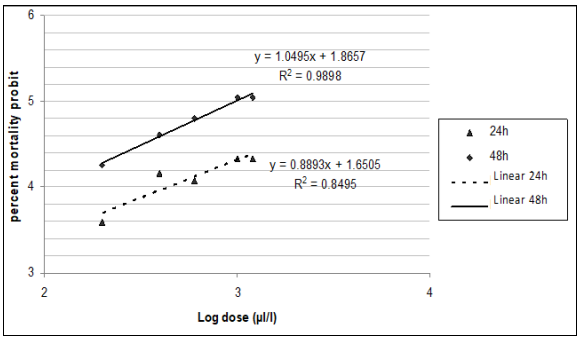

Fig 4: Dose-response analysis by probit transformation of mortality (%) of *A. gossypii* after 24h and 48 hours of exposure to different doses (µl/l) of clove EO

Tests with geranium EO showed that, after 24 hours of exposure, mortality varied from 20% at dose 200 µl/l to 35% at dose of 1200 µl/l, and after 48 h, from 29% at dose 200 µl/l to 67.5% at dose of 1200 µl/l. Mortality in the control was 1.48% and 2.01% at 24 and 48 hours, respectively (Figure 5). Statistical significant differences among doses were found after both 24 h (F=27.64, df=5, *P*=0.000) and 48 h (F=78.42, df=5, *P*=0.000) from the treatments. Post hoc analysis highlighted that no differences were found among 200 µl/l, 400 µl/l and 600 µl/l doses and between 1000 and 1200 µl/l doses (Table 1). All treatments were statistically different from control in both times of observations. The curve interpolating mortality percentage with doses at 24h hour from the treatment (Figure 6) showed that the dose determining 50% mortality (LD50) was 707.95 µl/l. Other researches showed insecticidal activity of geranium bourbon against mosquitos [52, 53].

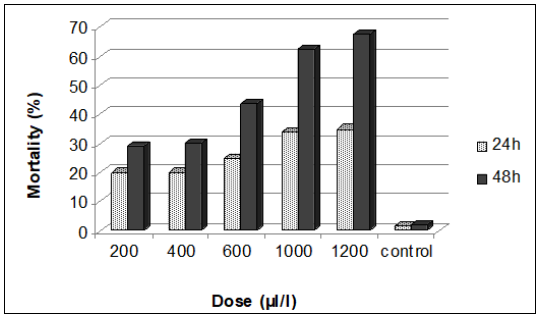

Fig 5: Mortality (%) of *A. gossypii* for contact toxicity after 24 and 48 hours of exposure to treatments with different doses of geranium EO

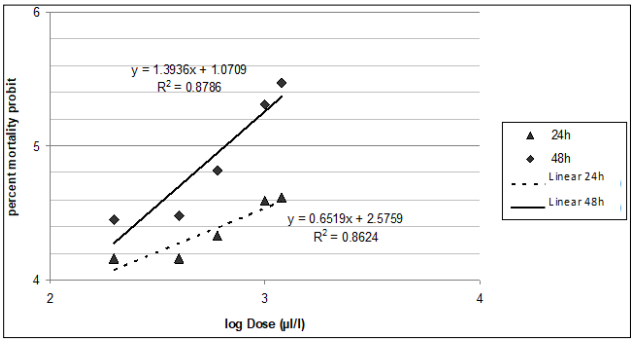

Fig 6: Dose-response analysis by probit transformation of mortality (%) of *A. gossypii* after 24h and 48 hours of exposure to different doses (µl/l) of geranium EO

Comparing mortality data detected at the same dose of each treatment after 24 h and 48 h from the start, statistical differences between two times of observation were significant for all EOs and not significant for control (Table 1). Statistical parameters from paired *t* test analysis is reported in Table 2.

Table 1: Post hoc analysis (Duncan's test) of mortality data of 1st-instar larvae of *A. gossypii* after 24 and 48 hours of exposure to treatments with different doses (µl/l) of EOs and Tween® 20 and water as control

Oil dose	Oregano		Clove		Geranium	
	24h	48h	24h	48h	24h	48h
200	(*) a	(*) a	(**) a	(**) a	(*) a	(*) a
400	(*) b	(*) b	(**) b	(**) b	(*) a	(*) ab
600	(*) c	(*) c	(**) b	(**) c	(*) b	(*) b
1000	(*) c	(*) cd	(*) b	(*) c	(**) b	(**) c
1200	(**) d	(**) d	(*) b	(*) c	(**) b	(**) c
control	(ns) e	(ns) e	(ns) c	(ns) d	(ns) c	(ns) d

Asterisks in the rows indicate statistically significant differences ((*) $P<0.05$, (**)$P<0.001$, (ns)= not significant) between 24 and 48 hours for each plant at the same dose.
Same letters within each column indicate not significant difference among treatments ($P<0.05$)

Table 2: Results of the paired *t* test analysis on mortality data of 1st-instar larvae of *A. gossypii* obtained from comparison between two times of observation of 24 and 48 hours at the same dose (µl/l) of treatment of each EO and control (Tween® 20 and water)

Doses (µl/l)	Oregano		Clove		Geranium	
200	t= -3.45	P=0.003	t= -11.38a	P=0.000	t= -3.92	P=0.0012
400	t= -2.25	P=0.002	t= -6.32	P=0.000	t= -2.96	P=0.007
600	t= -2.73	P=0.012	t= -7.86	P=0.000	t= -3.93	P=0.0017
1000	t= -3.61	P=0.002	t= -3.62	P=0.002	t= -5.97	P=0.000
1200	t= -6.38	P=0.000	t= -3.62	P=0.002	t= -6.94	P=0.000
control	t= -1.06	P=0.15	t= -1.06	P=0.15	t= -1.06	P=0.15

Conclusion

EOs used in this study gave good results in controlling *A. gossypii.* The results showed that EOs of all three plants (oregano, clove and geranium) have showed a strong effect on *A. gossypii*. Further, by increasing the concentration and duration of EO treatment, the mortality rate also increased. After 24 hours, the lowest DL50 was detected for oregano EO, followed by geranium and clove EOs. These oils can effectively control pests and be less toxic to the plants unlike chemical-based insecticides. The vantages that results by use of natural products to reduce the pest resistance of this aphids, suggest their potential employee in the pest management programmes specially under greenhouse conditions.

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