The difference in the transverse tubular staining by anti-dystrophin antibodies in cardiac and skeletal muscle might be caused by a variation in antibody accessibility as a result of protein-protein interactions or a difference in dystrophin concentration. It has recently been reported that chimaeric myocytes with either cardiac or skeletal muscle calcium channel alpha-1 subunits are available [16]. It would be interesting to study the interactions of dystrophin with the two subunit proteins in order to further our understanding of the paucity of cardiac pathology in DMD.

The significance of the absent intercalated disc staining remains unclear.

To conclude, our study demonstrates the presence of dystrophin in cardiac myocyte sarcolemma and transverse tubules, but not the intercalated discs, and the resistance of this dystrophin localization to ischaemic injury.

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0014-4754/92/060614-03$1.50 + 0.20/0

Insecticidal effects of essential oils. A study of the effects of essential oils extracted from eleven Greek aromatic plants on *Drosophila auraria*

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Received 11 October 1991; accepted 23 December 1991

Abstract. Effects of the essential oils (EOs) extracted from eleven aromatic plants belonging to the Lamiaceae family (common in the Greek flora) were examined upon three different developmental stages of *Drosophila auraria*. All of the EOs examined exhibited insecticidal effects, either by preventing egg hatching, or by causing the death of larvae and adult flies. In several cases, malformation and/or prohibition of puparium formation was also observed.
Key words. Aromatic plants; essential oils; *Drosophila*; insecticides.

During the last decade, a growing body of evidence concerning the biological activity of plant-derived compounds has emerged [1, 2]. Among these compounds, essential oils (EOs) possess biological activity against prokaryotic and eukaryotic organisms (e.g., antibacterial, antifungal, insecticidal) [3, 4]. The necessity of finding safer insecticides has led to the exploitation of the mechanisms of chemical defense that plants naturally possess [5]. Recently it was shown that juvocymenes, the active ingredients of the EO of *Ocimmum basilicum*, and farnesol, a component of many EOs, exhibit insecticidal activity [5]. These compounds have a limited use on a commercial scale, but have been used as prototypes for the chemical synthesis of other, commercially available, insecticides [6, 7].

In the present study, eleven EOs, extracted from Greek aromatic plants have been tested for insecticidal activities. *Drosophila auraria* (eggs, larvae and adults) was used as an insect model system, in a controlled environment.

Materials and methods

Plant material and essential oil extraction. Using the Clevenger apparatus (constructed according to the specifications of the American Spice Trade Association) essential oils of eleven aromatic plants were extracted (table). The distillation yield (ml of EO per 100 g of dried plant material) as well as the major constituents of each EO are given in the table.

Animals. Drosophila auraria, an oriental member of the *montium* subgroup of the *melanogaster* species group [15],

List of the aromatic plants used (belonging to the Lamiaceae family), the distillation yield in essential oil (EO), and the major consituents of the EOs.

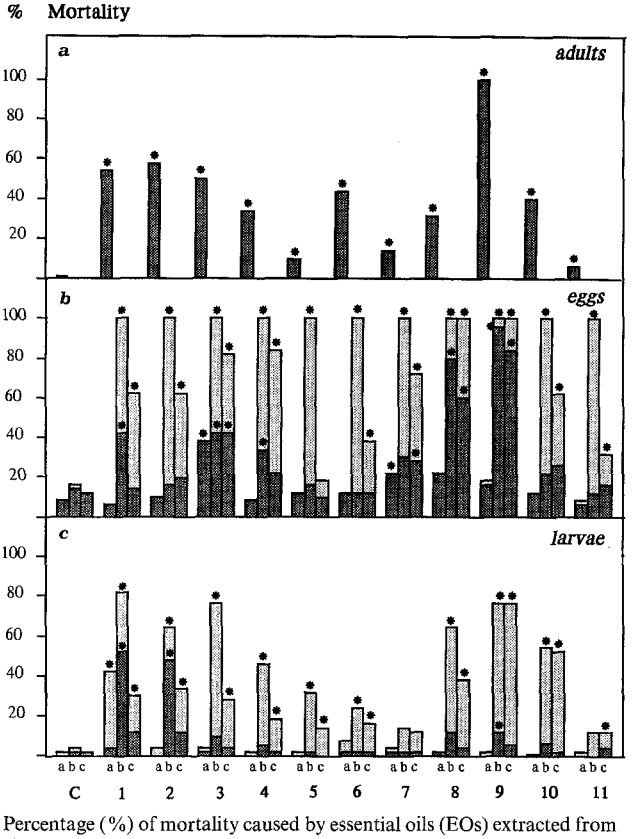
Plant species	Common name	Distillation yield in EO (ml/100 g d.wt)	Major constituents of the EO
1. *Satureja thymbra* L.	Spanish origanum	3.952	Carvacrol, thymol, γ-terpinene, π-cymene[8]
2. *Coridothymus capitatus* (L.) Reichenb. fil.	Thyme	3.891	Carvacrol, thymol, γ-terpinene, π-cymene[8]
3. *Origanum vulgare* ssp. *hirtum* (Link) leetswart	Greek origanum	2.180	Carvacrol, thymol, γ-terpinene, π-cymene[8]
4. *Origanum dictamnus* L.	Dittany of Crete	2.130	Carvacrol, linalool, γ-terpinene[9]
5. *Origanum majorana* L.	Marjoram	1.018	Carvacrol, linalool[10]
6. *Lavandula stoechas* L.	Lavender	1.695	Fenchone, camphor, pinocarvyl acetate, eucalyptol[11]
7. *Lavandula angustifolia* (Miller)	Lavender	2.579	Linalyl acetate, linalool[12]
8. *Mentha spicata* L. – carvone type	Mint	0.711	Carvone, di-hydro-carvone[8]
9. *Mentha pulegium* L.	Pennyroyal mint	2.196	Pulegone[13]
10. *Salvia fruticosa* (Miller)	Sage	3.322	Cineol, thujone, camphor, α-pinene, β-pinene[11]
11. *Rosmarinus officinalis* L.	Rosemary	0.377	Limonene, cineol, borneol, terpineol[14]

was used in this study. *D. auraria* was selected as an experimental insect model because it is well known to us[16–18]: it has a short life cycle, it can be easily cultured, and genetic, biochemical and physiological parameters are well established.

Experimental procedures. The experiments were carried out on three developmental stages: (1) freshly laid eggs (1–2 h), (2) late third instar larvae and (3) 2–4-day-old adult flies. Eggs and larvae were exposed to each individual EO in parafilm-sealed petri dishes (diam. = 9 cm) with three different substrates: (1) food: a yeast-glucose medium, (2) paper: Whatman 3 mm paper moistened with Ringer solution, and (3) paper-yeast: the same as (2), but supplemented with 3 drops of diluted baker's yeast (1 : 1 in tap water) added at the center of the dish. Adult flies were kept in *Drosophila* cultivation bottles (150 ml) with yeast-glucose medium (food). In all cases, different amounts of EO (from 1 µl to 20 µl) were applied on a small filter paper disc (diam. 4 mm) placed at the center of the petri dish or of the cultivation bottle. After that, dishes and bottles were kept at 21 ± 1 °C for the desired time. Each experiment was repeated three times, and 30 animals were screened each time.

Results and discussion

The insecticidal action of all the EOs tested was confirmed after a series of preliminary experiments with varying amounts of EOs. These experiments showed that in amounts of 5 µl or more, all eleven EOs caused severe lethal effects (mortality rates up to 100%), using the conditions described above. When 2.5 µl per dish or bottle was used, the effect on mortality appeared to be more EO-specific (fig.). Using this amount determination of the most effective EOs, as well as screening of the surviving animals for other effects, was possible.



Percentage (%) of mortality caused by essential oils (EOs) extracted from eleven Greek aromatic plants (see table) on three discrete developmental stages of *Drosophila*: adult flies, eggs and late third instar larvae. **a** ■ Mortality of adult flies after 24 h of exposure to the EOs only in the FOOD substrate. **b** Inhibition of egg hatching (■) and deaths of newly emerged larvae (▒) after 48 h of exposure of eggs to the EOs. **c** Mortality of larvae after the first 24 (■) and the second 24 h (▒) of exposure of larvae to the EOs. At the bottom: a, b and c, the three different substrates used; food, paper and paper-yeast, respectively; C, control; numbers from 1 to 11, indicate the EOs extracted from the aromatic plants listed in the table (for further explanations see text). Asterisks indicate the statistically significant results after comparing each EO-treated population with the respective control population (χ^2 test of homogeneity).

The figure (a, b and c) shows the percentage of mortality after exposure to 2.5 µl of the EOs of adult flies, eggs and larvae, respectively. Lethality on eggs is measured in two ways, as: (1) inhibition of hatching, and (2) death of newly emerged larvae (the term mortality defines the sum of 1 and 2). As can be seen in the figure (b), the percentage of mortality differs between the three different environments used; embryos are mostly affected in the paper dish, while the effect is much lower in the food dish. Newly emerged larvae did not survive in the paper dishes with any of the tested EOs, while in the food dishes, once the eggs were hatched almost all larvae survived.

There are at least two reasons for the stress being more severe in the paper dishes: first, newly-emerged larvae are deprived of food during a crucial developmental stage, and second, in the food dishes larvae have the advantage of being able to hide in the feeding medium, and so their exposure to the toxic environment created by the volatile oil may be limited. Thus, the results obtained from the paper-yeast dishes may be more representative of the effectiveness of each of the EOs, since the paper-yeast dishes can be considered as an intermediate situation. Among the EOs tested *Mentha pulegium* and *Mentha spicata* were the most effective in preventing egg hatching (fig., see also table).

Lethal effects on late third instar larvae are shown in the figure (c). Mortality was measured at 24 and at 48 h. The comments made for eggs concerning the difference in mortality between the three environments used also apply here, although third instar larvae seem to survive better after the 48-h exposure than newly-emerged larvae (fig. b, c). The results obtained show that mortality did not increase significantly after a 24-h exposure to most of the EOs (with the exception of *Satureja thymbra* and *Coridothymus capitatus*, but only in the paper dishes). After 48 h exposure, mortality in the paper and, in some cases, in the paper-yeast dishes increased dramatically, but larvae still survived in the food dishes (with the only exception of *Satureja thymbra*). The most effective EOs at this developmental stage appear to be *Satureja thymbra, Coridothymus capitatus* and *Origanum vulgare* ssp. *hirtum*; all three EOs share the same major constituents (table). Also very effective were *Mentha pulegium*, *Mentha spicata* and *Salvia fruticosa*. After 48 h exposure, almost all the surviving larvae became pupae.

When the surviving and dead larvae and pupae were examined, several kinds of malformations were observed; these included (a) blackened long narrow larvae, (b) black spots in the larval body and the puparium, (c) blackened posterior and/or anterior ends, (d) pupae with posterior ends of larval morphology, and (e) blackened salivary glands and/or gut. All the above indicate strong toxic effects of the EOs and probably some interference of the EOs with the developmental processes (e.g. pupae with posterior ends showing larval morphology) and are compatible with previous observations[19,20]. However, one should not forget that the EOs are a mixture of different compounds, and therefore more than one mechanism of action may exist. Some of the recently proposed hypotheses concerning mechanisms of action of EOs include denaturation/degradation of proteins, inhibition of enzyme action, and interference with the electron flow in the respiratory chain, or with ADP phosphorylation[21–23]. None of these hypotheses can yet provide a complete explanation for the action of the EOs.

The figure (a) presents the results obtained after a 24-h exposure of adult flies to the EOs. Equal numbers of flies of both sexes were treated. No differences in mortality between male and female flies were observed. The most striking result in this series of experiments is the great difference between mortality caused by the EO of *Mentha pulegium* and that caused by all other EOs. This difference is made even more impressive by the fact that mortality reached 100% within 30 min of exposure to *Mentha pulegium*, whereas flies exposed to the other EOs began to die only after several hours of exposure. The group of the three EOs with the same major constituents, *Satureja thymbra, Coridothymus capitatus* and *Origanum vulgare*, ssp. *hirtum* also appear very effective in treating adult flies.

The data presented in this report strongly indicate that eleven essential oils isolated from Greek aromatic plants belonging to the Lamiaceae family exhibit insecticidal activity. Of these EOs, *Mentha pulegium* and *Mentha spicata* proved to be most effective on eggs, *Satureja thymbra, Coridothymus capitatus* and *Origanum vulgare*, ssp. *hirtum* on larvae, and *Mentha pulegium* on adults. Whether the major constituents of these oils (see table) or their synergistic effects are the causal insecticidal factors is not yet clear. Work is in progress in our laboratory to screen more essential oils for incecticidal effects, to study the differential action of the EOs on different economically important insects, and to investigate the mechanism of action of the EOs on insect development.

Acknowledgments. We would like to thank Dr S. Kokkini for her help during the initial phase of this work and Drs M. Arsenakis and J. Karakoussis for their fruitful discussions.

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