Side effects of essential oils of *Monarda fistulosa* L. and *M. didyma* L. on the tachinid parasitoid *Exorista larvarum* (L.): a preliminary study

by Santolo Francati and Greta Gualandi

Alma Mater Studiorum Università di Bologna – Dipartimento di Scienze Agrarie (DipSA). E-mails: santolo.francati2@unibo.it, greta.gualandi@studio.unibo.it

This study was performed in the laboratory of Entomology at DipSA (University of Bologna). This was the subject of Greta's B.Sc. thesis and was performed under the supervision of Maria Luisa Dindo and the co-supervision of Santolo Francati.

Essential oils (EOs) are secondary metabolites produced by plant flowers, resin, wood, roots, fruits, seeds, and leaves. Although they are not fundamental for plant life, they may play important roles for plant survival, including defense against bacteria and fungi (Preuss *et al*. 2005, Zhilyakova *et al*. 2009). Moreover, larvicidal, repellent, ovicidal and anti-oviposition effects of EOs on different herbivore insect species have been shown (Isman 2000, Isman 2006, Masetti 2016).They also contain "essences", which confer characteristic scents to many fragrant plant species.

In recent years, following an increasing interest in sustainable pest control methods in agriculture, EOs have received growing attention as components of natural agrochemicals. This is because, besides their properties against the target microorganisms and insects, they show high volatility, low persistence and, in general, low toxicity to non-target animals (Isman 2006). Knowledge on this issue, in particular on the side effects of EOs on beneficial insects, is, however, still limited (Tillman 2008).

Monarda is a genus in the family of Lamiaceae endemic to North America. It includes annual and perennial flowering plants, some of which can grow up to 150 cm tall (Bellardi 2014). Many species are grown as ornamentals in different countries, because the flower color varies from red to pink or light purple. *Monarda* plants produce a high quantity of EOs and several species, including *Monarda fistulosa* L. (Wild bergamot, Figs. 1–2) and *M. didyma* L. (Oswego tea, Figs. 3–4), have a long history of use as medicinal plants by Native Americans. There are still few scientific studies on this topic (Zhilyakova *et al*. 2009).

Figures 1–4. 1–2. Plant and flowers of *Monarda fistulosa*. **3–4**. Plant and flower of *Monarda didyma*. (All photos by M.G. Bellardi)

Since 2012, in the framework of a project led by the Department of Agricultural Sciences (DipSA) of the University of Bologna (Italy), research has been conducted to verify the potential of the EOs of *Monarda* spp. in different fields, including plant protection from pathogens. As regards this issue, Minardi *et al*. (2016) showed that *M. didyma* and *M. fistulosa* EOs have an antimicrobial activity against *Pseudomonas syringae* pv. *actinidiae*, the causal bacterial agent of kiwifruit canker disease.

Our preliminary study was carried out as part of the above-mentioned project and was aimed at starting the assessment of the possible side effects of *M. didyma* and *M. fistulosa* EOs, in the event that they are used in agro-eco-

Figures 5–14. Rearing of *Exorista larvarum*. **5**. Cage with *E. larvarum* adults. **6**. Greta Gualandi changes the food in rearing cage. **7**. Flies feed on sugar cubes. **8**. Mating pair of *E. larvarum*. **9–11**. Oviposition sequence. **9**. Female fly locates a *Galleria mellonella* caterpillar. **10**. Fly prepares to oviposit. **11**. Fly extends ovipositor just before depositing an egg on the caterpillar. **12**. Multiple eggs on *G. mellonella* caterpillars (two eggs indicated by arrows). **13**. A mature *E. larvarum* larva has exited its host prior to pupariation. **14**. Puparia of *E. larvarum*. (Photos by G. Gualandi except for Fig. 6 by S. Francati)

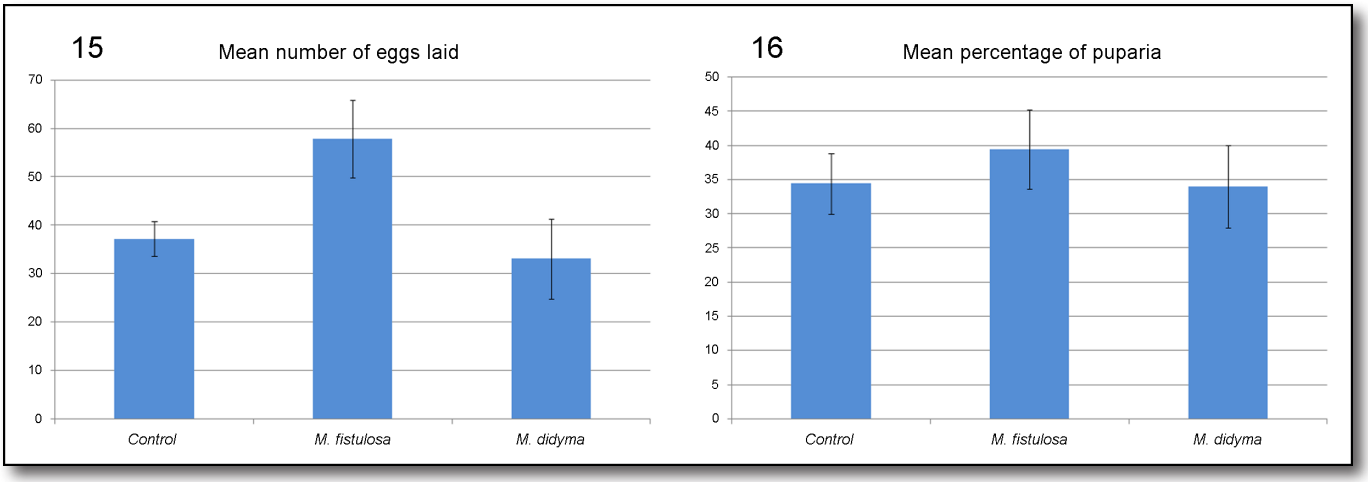
systems to control target plant pathogens or insect pests. More specifically, laboratory studies were conducted to assess the side effects on adult longevity and reproduction capacity of the tachinid *Exorista larvarum* (L.), which was selected as a model non-target species. *Exorista larvarum* is a polyphagous gregarious larval parasitoid of Lepidoptera, well distributed throughout Europe, northern Africa, and several Asian regions (Herting 1960, Cerretti & Tschorsnig 2010). In the 20th century, it was also used in inoculative releases against the gypsy moth, *Lymantria dispar* (Drury), in the northern United States and became established (Sabrosky & Reardon 1976).

A laboratory colony of *E. larvarum* was maintained in a rearing chamber (26 ± 1°C, 75 ± 5% RH, 16:8 L:D) at DipSA, using *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) as a factitious host (Figs. 5–14). The flies were fed on sugar cubes and cotton balls soaked in a honey and water solution (Dindo *et al*. 2016). *Monarda didyma* and *M. fistulosa* EOs were hydrodistilled from the aerial parts of plants grown in the "Giardino delle Erbe" (Herb Garden) located in Casola Valsenio (Emilia Romagna Region, Italy) (Contaldo *et al*. 2011) and were supplied by M.G. Bellardi of DipSA. Their composition is given in Table 1.

Compound	*M. didyma*	*M. fistulosa*
α-caryophyllene	-	0.11 ± 0.01
β-caryophyllene	-	0.16 ± 0.01
Δ 3-Carene	4.45 ± 0.09	2.67 ± 0.04
δ-Cadinene	-	0.21 ± 0.02
α-Copaene	0.11 ± 0.01	-
α-Phellandrene	0.88 ± 0.04	14.21 ± 0.09
β-phellandrene	0.15± 0.01	18.08 ± 0.09
α-Pinene	0.21 ± 0.02	1.51 ± 0.03
β-Pinene	-	2.11 ± 0.06
α-Terpineolo	-	0.25 ± 0.05
γ-Terpinolene	9.26 ± 0.19	-
α-Thujene	-	1.88 ± 0.04
α-humulene	0.11 ± 0.01	-
1-Octen-3-olo	2.01 ± 0.11	-
Camphene	3.11 ± 0.09	-
Carvacrol methyl ether	-	3.99 ± 0.04
Epi-biciclosesquifellandrene	0.12 ± 0.02	-
Germacrene D	0.35 ± 0.05	1.31 ± 0.04
Limonene	1.08 ± 0.04	-
Linalool	0.52 ± 0.03	-
myrcene	3.23 ± 0.08	8.81 ± 0.08
p-Cymene	10.57 ± 0.11	13.11 ± 0.09
Thymol	63.73 ± 0.23	31.59 ± 0.13
Thymol methyl ether	0.11 ± 0.01	-

Table 1: Chemical composition (%) of *Monarda didyma* and *M. fistulosa* essential oils (EOs). (Modified from Epifano 2014)

The experiment was conducted in 2015 and all tests were performed under the same controlled conditions described above. Newly emerged *E. larvarum* adults, obtained from the standard colony, were paired and placed inside 20x20x20 cm Plexiglas cages (1 couple per cage). For every replicate, 3 couples were tested, each corresponding to a treatment. The couples were exposed to sugar cubes respectively treated with 1 mL of either *M. didyma* (couple 1) or *M. fistulosa* (couple 2) EO solution diluted to 0.01% (Mattarelli 2014), or with distilled water (couple 3, maintained as control). Fifteen replicates were carried out, for a total of 45 couples. As the preoviposition period of *E. larvarum* lasts about three days, from the 3rd day after pairing, three *G. mellonella* mature larvae were daily exposed to each couple for 5 days, in order to verify the parasitoid female oviposition capacity (Dindo *et al*. 1999). The larvae were removed from



Figures 15–16. 15. Mean number (± SE) of eggs laid by *E. larvarum* females during the five days of observation. **16**. Percentages (mean ± SE) of *E. larvarum* puparia obtained from eggs.

cages after about 1 hr and the eggs that had been laid on their body were counted. The larvae were then placed in the rearing chamber inside 6 cm diameter plastic cups until puparium formation. The parameters considered for the result evaluation were the adult parasitoid mortality (= total number of dead adults), the mean number of eggs laid by females in the experiment period (= 5 days) as an estimate of fecundity, the percentages of puparia obtained from eggs (number of puparia/ number of eggs x 100), the percentages of adults emerged (number of adults/ number of puparia x 100) and the sex ratio (calculated as percentage of the adult females). The data for mortality were pooled for the 15 replicates and they were analyzed by 2x2 contingency tables using Yates correction for small numbers (<100). The other parameters were analyzed by One-way ANOVA. The percentages values were transformed for the analyses by the ARCSIN transformation (Mosteller & Youtz 1961). All statistical tests were done with STATISTICA 10.0 (StatSoft 2010).

The number of dead adults was the same (= 6) in the control and in the flies treated with *M. fistulosa* EO. This number was lower than that observed in the flies treated with *M. didyma* EO (= 9). We compared the data of the two EOs and the difference was not significant (χ^2 = 0.36; df = 1, P = 0.55). Moreover, no significant difference was found among the two EOs and the control for the mean number of eggs laid by females (F = 3.64; gl = 2.12; P = 0.058), although the females exposed to *M. fistulosa* EO laid more eggs (Fig. 15). Also, for the mean percentages of puparia (F = 0.29; df = 2.12; P = 0.775) (Fig. 16) and of adults (F = 0.19; df = 2.12; P = 0.83) and for sex ratio (F = 0.18; df = 2.12; P = 0.775), no significant differences were found among treatments.

In conclusion, the results of our preliminary laboratory study did not show significant side effects of *M. didyma* or *M. fistulusa* EOs on *E. larvarum*, for any of the considered parameters. Survival and reproductive capacity, however, tended to be lower for the flies supplied with *M. didyma* EO compared with those treated with *M. fistulosa* EO and control flies. This tendency may be attributed to the different chemical composition of the two EOs and, in particular, to the higher thymol content found in *M. didyma* than in *M. fistulosa* oil (Table. 1). Thymol is the most abundant individual compound in thyme oil and has been proven to have pesticidal properties (Tak *et al*. 2016) also against *Varroa destructor* (Anderson & Trueman), a parasitic mite that attacks honeybees (Leza *et al*. 2015). It cannot be excluded, therefore, that *M. didyma* EO, rich in thymol, may, in the long run, affect *E. larvarum* fitness. Further research is necessary, also at a field level.

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