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Effect of *Myrtus communis* essential oil on the Mediterranean fruit fly mating behaviour

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ABSTRACT

The Mediterranean fruit fly *Ceratitis capitata* (Wiedmann) is a key agricultural insect pest. It has a wide range of host plants especially citrus trees. Medfly males are strongly attracted to plant semiochemicals. Exposure to such substance increased their mating success. In the current study, it was investigated whether a similar effect resulted from male exposure to the myrtle (*M. communis*) essential oil. Myrtle is a perennial shrub belonging to the Myrtaceae family and is widely distributed in the Mediterranean area. Myrtle essential oil was extracted and analysed by GC and GC-MS. The chemical composition revealed that most of the major components are monoterpenes (1,8-Cineole, alpha-pinene, Linalool, Limonene) and are similary found in citrus flavedo and orange essential oil. Laboratory trials suggest that exposure to myrtle essential oil significantly enhanced the medfly mating behaviour. It can be inferred from these tests that the myrtle essential oil can be used in insect pest management strategies such as the Sterile Insect Technique (SIT), to improve the mating competitiveness of sterile mass-reared males compared to wild males and therefore contribute to significant reduction of the medfly populations in fruit orchards.

Key words: *Myrtus communis*, monoterpenes, *Ceratitis capitata*, mating behaviour.

INTRODUCTION

Insect behaviour is tribute to a large array of environmental and ecological factors that affect in some extend their interactions with other organisms and host plants. Several research studies investigated the impact of semiochemicals, precisely, plant chemicals on the ecology and behaviour of fruit flies (Diptera:Tephritidae) (Landolt and Phylips, 1997; Katsoyannos *et al*., 1997; Papadopoulos, 2001). The Mediterranean fruit fly *Ceratitis capitata* (Wiedmann) is an agricultural key pest insect which causes serious economic losses in fruit production especially the citrus industry in the Mediterranean basin (Robinson and Hooper, 1989). Previous data has reported the effect of host plant on the mating behaviour and oviposition of this pest (Teranishi *et al*., 1987; Levinson *et al*., 1990). Several plant oils and volatiles such as citrus peel substances, ginger root oil showed significant activity in enhancing the medfly mating success. Such semiochemicals can be of a great value to the Sterile Insect Technique: an insect pest control method based on extensive large field releases of sterile males which compete with wild males. The number of offsprings decreases and therefore insect pest populations are reduced. However, the sterile males show a reduced mating performance attributed to their mass rearing process (Barry *et al*., 2001). Treating sterilized mass-reared males with specific plant essential oils would significantly enhance their mating ability and competitiveness with wild males. Similarly to citrus and ginger root oil, the effect of myrtle essential oil on medfly was investigated in laboratory essays. The aim of this work is to characterize myrtle essential oil from Tunisia and examine its effect on the mating behaviour of males of the Mediterranean fruit fly *C. capitata* (Wied.).

MATERIAL AND METHODS

Plant material

M. communis (Myrtaceae) is a perennial shrub, widely distributed in the Mediterranean area. Samples of myrtle shrubs were collected from Mateur a locality situated in the south west area of Bizerte (Northern part of Tunisia). This area belongs to the sub-humid bioclimatic stage. The myrtle leaves are dried in the laboratory and then used to extract the essential oil.

Medflies

The flies used in this study were derived from a wild colony collected from infested fruits and a laboratory colony reared from a genetic sexing strain based on a temperature sensitive lethal (*tsl*) mutation. Such strains are currently used for nearly all sterile insect technique (SIT) programs for medfly. Standard rearing procedures were employed (Tanaka *et al*., 1969), and prior to release, adults were fed a sugar/ protein (yeast hydrolysate) mixture using the ratio 3:1 per volume. Adults were separated within 24 hours of emergence, well before they attained sexual maturity (at 6-8 days of age). The flies were held in plastic cages at 25 ± 2° C and 85 % RH and a photoperiod of L12:D12 with photophase starting at 06:00.

Essential oil extraction and analysis

M. communis essential oil was extracted from dried leaves (100 g) by hydro-distillation during 2 hours followed by a liquid-liquid extraction using a diethyl ether, then dried using anhydrous sulfate sodium and stored at 20° C.The oil samples (1/100 v/v, in n pentanes) were analyzed on a Hewlett-Packard 5880A gas chromatograph equipped with a split-splitless injector, a flame-ionization detector (FID), and a 10 m × 0.53 mm i.d. HP-1 bonded-phase fused-silica capillary column. The oven temperature was maintained at 40°C for 1 min, then programmed at 10°C/min to 150°C, held at this temperature for 0.1 min, then programmed at 10°C/ min to 250°C. The carrier gas was hydrogen. Coupled GC-Mass Spectrometry (GC-MS) analysis was carried out using a capillary GC column (50 m x 0.32 mm i.d. HP-1) fitted with a cool on-column injector was directly coupled to a mass spectrometer (VG Autospec, Fisons instruments, UK). Ionization was by electron impact at 70eV, 250°C. The oven temperature was maintained at 30°C for 5 minutes, and then programmed at 5°C/minutes to 250°C. Analysis was also performed using a fused silica HP Innowax polyethylenglycol capillary column (50m x 0.2 m). The carrier gas was helium for both columns.

Mating tests

The test consists of three trials; each trial has four repetitions: two treated groups (exposed to 25µl of myrtle essential oil on cotton wick for 2 hours) and two control groups (no exposure). Each group has 100 males. One hundred females are released in every tent, between 07:30 and 08:00 am. The mating pairs were collected in vials and immediately knocked down in the freezer then scored. Individuals of both sexes were used for only one mating trial. Trial-s were usually performed under full or nearly full sunlight with air temperatures ranging between 25 and 30°C. All unmated flies were removed from the tents immediately after the test. The effect of the insect treatment with essential oil was statistically evaluated using a paired t-test ($P< 0.05$).

RESULTS AND DISCUSSION

The essential oil from Myrtle leaves was extracted and its chemical composition was determined by GC-FID and GC-MS analysis. Thirty-six components, representing approximately 74.517% of the oil, were identified. Five major components were found (considering the mean value of three analysis): 1,8-cineole (23.256%), alpha-pinene (19.449%), Linalool (14.036%), 4-methyl-3,5-heptanedione (7.217%) and limonene (3.555%). This is consistent with previous analysis of myrtle essential oil from Tunisia and other countries in the Mediterranean basin (Messaoud *et al*., 2005; Flamini *et al*., 2004).

Laboratory essays testing the effect of myrtle on the mating behaviour of medfly males show that the percentage of matings in the case of exposed laboratory-reared males reach 25.45 %; it is only 15.665 % for the control males (t-test, $P< 0.05$). Exposure of medfly sterile males to the myrtle oil conferred to them a mating advantage over untreated males from the same strain (Figure 1). However, trials using the wild medfly strain didn't show any significant improvement in the mating

frequency. It can be inferred from these experiments that the myrtle essential oil improves in some extend the mating ability of medfly laboratory mass reared males. A similar effect was previously reported involving the orange peel chemicals which enhanced the mating ability of medfly sterile males (Papadopoulos *et al.,* 2001). For instance, the chemical composition of the myrtle essential oil shows the presence in important amounts of some components found in the orange essential oil, such as monoterpenes (limonene, 1,8-cineole and linalool). It has been proven that those monoterpenes are involved in enhancing the mating competitiveness of medfly mass-reared males (Stashenko *et al*., 1996). Moreover, another major compound was found in the myrtle essential oil: the 4-methyl-3, 5-heptanedione. This compound has been reported as a major component of an

Table 1: Chemical composition of myrtle essential oil from tunisia

I [(a)]	I[(b)]	Compound	Percentage (%)
754	1044	Toluene	0.025
776	1086	hexanal	0.023
829		ethylcycohexane	0,137
900	900	Nonane	1,221
924		cumene	0,232
934	1031	alpha-pinene	19,449
946	1079	camphene	0,049
969		(+)-sabinene	0,100
984	1169	myrcene	0,040
985		Phenol	0,250
991		(R)-(-)-pantolactone	0,106
995	1280	hexyl acetate	0,199
1000	1000	Decane	0.084
1005	1888	benzyl alcohol	0,244
1011	1655	phenylacetaldehyde	0,054
1021		4-methyl-3,5-heptanedione	7,217
1022	1221	1,8-cineole	23,256
1024	1212	limonene	3.555
1029		(Z)-ocimene	0,039
1041	1262	(E)-ocimene	0,310
1052	1540	4-pentenyl isothiocyanate	0,408
1081		2-isopropyl-3-methoxypyrazine	0,367
1086	1545	linalool	14,036
1090	1293	Alpha terpinolene	0,219
1175	1796	Methyl salicylate	1,431
1278		bornyl acetate	0,503
1282		geranyl formate	0,247
1337	2181	eugenol	0,258
1347		Gamma elemene	0,729
1364		geranyl acetate	1,529
1373	1970	(Z)-jasmone	0,919
1411		cinnamyl acetate	0,067
1450	1673	(E) – beta- farnesene	0.056
1497		Ledene	0,626
1581	2000	caryophyllene oxide	0,122
1605		Ledol	0,153

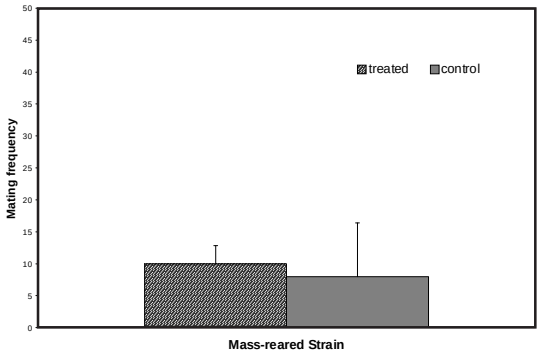

Fig. 1: Mating frequency of medfly sterile mass-reared males exposed to myrtle essential oil

aggregation pheromone for the lucerne weevil *Sitona lineatus* L. (Coleoptera: culicidae) (Nielsen and Jensen, 1992). This suggests that the 4-methyl-3, 5-heptanedione found in Myrtle essential oil may have contributed to the observed male mating behaviour enhancement. Further studies and extensive laboratory and field essays on the effect of major compounds of the myrtle essential oil are needed to confirm this evidence. The noticed effect of myrtle essential oil on the mating male behaviour can be a valuable asset to improve the efficiency of the Sterile Insect Technique as an environmentally friendly control strategy used against the Mediterranean fruit fly.

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