

Usage of agricultural DAP fertilizer and *Eucalyptus* essential oils as potential attractants against the Mediterranean fruit fly *Ceratitis capitata* (Tephritidae) [†]

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[†] Presented at the 1st International Electronic Conference on Entomology (IECE 2021), 1–15 July 2021;

Available online: <https://iece.sciforum.net/>

Abstract: This work aims to develop and evaluate an attractant based on the combination of DAP and Eucalyptus essential oils. Essential oils were isolated by steam distillation from Eucalyptus species and analyzed by GC-MS. About 12 and 22 constituents were identified with 1.8 was the major compounds in crude essential oil and formulations. The adsorption efficacy depends on the abundance of each compound in crude essential oils and the polarity of terpenic components. Results indicated the attractant potential of both essential oils (class III) and that DAP/essential oils formulations accomplished better attractant performance (class IV). Formulations were toxic against medfly adults.

Keywords: Attractant, Di-Ammonium Phosphate, Essential oil, Adsorption

Citation: Sadraoui-Ajmi, I.; Benali, N.; Soltani, A.; Chaib, S.; Limem, E.; Jallouli, S.; Boushah, E.; Fajraoui, A.; Mediouni-Ben Jemâa, J. Usage of agricultural DAP fertilizer and *Eucalyptus* essential oils as potential attractants against the Mediterranean fruit fly *Ceratitis capitata* (Tephritidae), in Proceedings of the 1st International Electronic Conference on Entomology, 1–15 July 2021, MDPI: Basel, Switzerland, doi:10.3390/IECE-10392

Published: 30 June 2021

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1. Introduction

Medfly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is among the major destructive pests of fruits worldwide, particularly in the Mediterranean regions [1,2]. In Tunisia, Medfly is a key economic pest on citrus and various crops [3]. Current control program is mainly based on applications insecticides [4]. However, their use is subject to much controversy [5]. In this respect, botanical extracts and essential oils could be suggested as alternatives [6]. Many essential oils are particularly known for their important insecticidal, pesticidal and repellent/attractant properties. [7]. Usually, the use of attractants is associated with procedure in which the pest is displaced from a protected resource (crops, fruits, area ...) and concurrently lured [8]. In this respect, it has been demonstrated that plant essential oils could act as insect attractant [9]. Indeed, various studies have been cited on attractant potential of eucalyptus essential oils against Dipdpteran pests [10,11,12]. On the other hand, Tunisia is ranked among largest phosphate rock producer in the world [13]. Fertilizers presented the major inputs in modern

agricultural production since they increased crops yields and improve soil fertility [14]. Besides, Di-Ammonium Phosphate exhibited an attractive activity towards *C. capitata* adults [15]. This paper reports the chemical composition, attractant potential and contact toxicity of two crude *Eucalyptus* essential oils (*E. cinerea* F. Muell and *E. maidenii* F. Muell) and their formulations by adsorption onto DAP fertilizers against *C. capitata*.

2. Material and methods

2.1. Insect rearing

To initiate *C. capitata* rearing, infested fruits were collected from various fields (orange, mandarin, clementin,...) of Gafsa oasis (34°23'N, 8°48'E). Rearing was conducted under the following controlled laboratory conditions: 30 ± 1 °C, 65 ± 5% RH and a continuous light [16].

2.2. Plant materials

Eucalyptus maidenii and *E. cinerea* aerial parts were collected in April 2019 from Souinet arboretum Ain Drahem, northwestern Tunisia (36°47'N 8°42'E). The harvested materials were air-dried at room temperature (20-25 °C) for one week.

2.3. Extraction, analysis and adsorption of essential oils

100 g of dry plant material was subjected to hydrodistillation for 180 min in a Clevenger-type apparatus in accordance with European Pharmacopoeia method [17]. The distilled essential oils were stored in a refrigerator at 4 °C until being analyzed and used. The analysis of the chemical composition of essential oils was realized by 6980 N Network GC system equipped with -5MS.

The adsorption percentage (%R) at an instant was calculated using the relation described by [18]:
$$\%R = \frac{C_0 - C_t}{C_0} \times 100 \quad (1)$$
 C_t: is the concentration of adsorbate at time t; C₀ is the concentration initial.

2.4. Fertilizers characterization: Ray Diffraction analysis (XRD)

The powder X-Ray Diffraction patterns of the DAP fertilizer were measured using Panalytical (X'pertPro) diffractometer equipped with a copper anticathode ($\lambda = 1.54178 \text{ \AA}$)

2.5. Repellency bioassay (Filter Paper Tests)

The repellency bioassay was evaluated against *C. capitata* adults, using the protocol described by [19]. The percent repellency (PR) was calculated according to [20].

2.6. Data analysis

Statistical analysis was performed using SPSS statistical software version 20.0. Data were subjected to two-way ANOVA. All obtained values were the mean of three replications and were expressed as the mean ± standard error. Differences in values of each oil treatments were tested by ANNOVA one way followed by Duncan test. Statistical analysis was conducted to estimate repellent concentrations (RC₅₀ and RC₉₅) (Concentrations that repelled 50 and 95% of the exposed insects). Furthermore, bioassays were considered to calculate respectively lethal concentrations LC₅₀ and LC₉₅ (doses that kill 50% and 95% of exposed insects).

3. Results

3.1. Essential oil composition

The chemical composition of essential oils extracted from *Eucalyptus* species (*E. cinerea* and *E. maidenii*) was presented in Table 1.

Table 1. Chemical composition (% , $\mu\text{g}/\mu\text{L}$) of volatile compounds of *Eucalyptus cinerea* and *Eucalyptus maidenii* essential oils

N°	Compounds	RT	<i>Eucalyptus cinerea</i>		<i>Eucalyptus maidenii</i>	
			%	C_{x1} ($\mu\text{g}/\mu\text{L}$)	%	C_{x1} ($\mu\text{g}/\mu\text{L}$)
1	α -Pinene	6.43	6.436	0.268	10.344	0.432
6	1,8-Cineole	9.027	74.586	3.103	66.708	2.783
13	α -Terpineol	13.461	2.762	0.115	7.376	0.307
15	Camphene	17.789	10.864	0.452	0.223	0.009

Results showed that qualitatively, *E. cinerea* and *E. maidenii* oils were found to possess similar compositions. The major common constituents were 1,8-cineole, α pinene, α -terpineol and camphene (Table 1).

3.2. X-Ray Diffraction analysis of DAP Fertilizer

The Diffractogram X-Ray presents characteristic peaks of pure DAP (Figure 1).

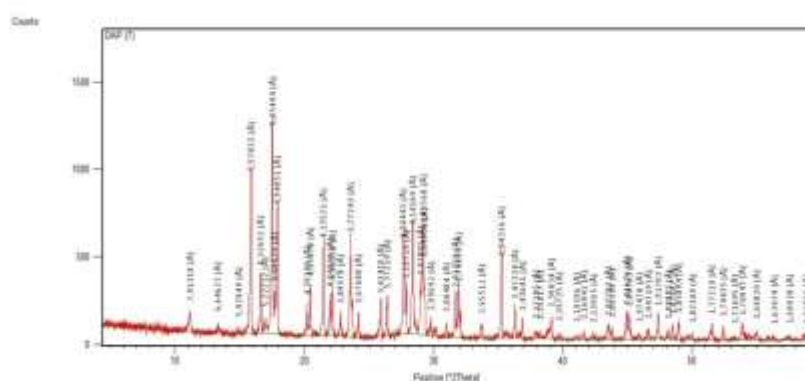


Figure 1. XRD Spectra of Diammonium Phosphate (DAP) Fertilizer .

The results revealed that in DAP sample, the main compound identified is $(\text{NH}_4)_2\text{HPO}_4$. The Diffractogram indicated three peaks at the diffraction angle $2\theta=17.424$ (d-spacing = 5.0549 Å), 16.8432 (d-spacing = 5.5783 Å) and 17.8961 (d-spacing = 4.9485 Å).

3.3. Repellent/ Attractant proprieties

The percentage repellency (PR) and indices repellency (IR) values of the crude essential oils and DAP/ essential oils based formulations against adults of *C. capitata* after 24 h exposure are given in (Table 2).

Table 2. Repellency (%) of essential oils from *Eucalyptus cinerea* and *Eucalyptus maidenii* against adults of *Ceratitis capitata*.

Formulations	Treatments	Concentration $\mu\text{l}/\text{cm}^2$	PR	Repellency class		
				IR		
Essential oils	<i>Eucalyptus cinerea</i>	0.000039	-20	I	1.20	
		0.00007	-40	II	1.40	
		0.00011	-46.66	III	1.53	
		Average repellency	-35.53	II	1.37	
	<i>Eucalyptus maidenii</i>	0.000039	-26.66	II	1.40	
		0.00007	-40	II	1.40	
		0.00011	-60	III	1.60	
		Average repellency	-42.22	III	1.46	
	DAP/ Essential oils	Crude DAP	30 mg	-20	I	1.20
		DAP/ <i>Eucalyptus cinerea</i>	110.52mg	-66.66	IV	1.66
DAP/ <i>Eucalyptus maidenii</i>		20.31mg	-73.33	IV	1.73	

It is deduced from this that the attractant effect (%) increased with the augmentation of essential oil concentration. According to [21] classification, negative Figs expressed attractancy. Consequently, both crude *E. cinerea* and *E. maidenii* oils were attractant towards

C. capitata adults. The average repellent effect of this crude oil was low attractant PR = -35.33% (class II) for *E. cinerea* and attractant effect (PR= -42.22%) class III. For both formulations, attractiveness activity belongs to class IV. Besides, for DAP, results showed that this fertilizer exhibited very low attractant effect (class I).

4. Discussion

In agreement with most cited documents, 1,8-cineole was the major compound identified in *Eucalyptus* oils [22,23,24]. Our data provides clear evidence that both essential oils were rich in compounds known to possess insecticidal activity [22-25]. Diverse reports documented that *Eucalyptus* oils exhibited high attractant and contact toxicity potential against various insects [26,27,28]. Based on this study, a synergy in the attractant efficacy of the formulations DAP/ essential oils from *E. cinerea* and *E. maidenii* was observed. The direct impact of these formulations was the improvement of DAP attractance against *C. capitata*. Indeed, crude DAP revealed very low attractant effect (class I) towards Medfly adults; while; with DAP/ essential oils formulations, attractant potential reached attractant level (class IV). The improvement of DAP/essential oil formulation attractiveness could be attributed to the insecticidal potential of essential oils previous studies pointed out the toxic effects of DAP/Eucalyptus essential oils formulations against Medfly adults.

5. Conclusion

Results from this work revealed that formulations based on the combined use of agricultural fertilizer (Di-Ammonium Phosphate) and essential oils (*Eucalyptus* oils) could be involved as a component for Medfly control strategy in oasis. This could be proposed amid current methods for an IPM approach.

Author contribution: Insaf Sadraoui Ajmi, Nejib Benali and Abir Soltani conceived, designed the experiments and wrote the manuscript with input from all authors. Emna Boushih worked for insect rearing and performed the extraction of essential oils. Selim Jallouli achieved the GC-MS analysis of the essential oils. Fajraoui Abdallah and Samira Chaib provided critical feedback on the manuscript. Essia Limem helped on fruit sampling. Jouda Mediouni Ben Jemâa devised the project, the main conceptual ideas and proof outline. All authors discussed and commented the manuscript.

Funding: "This research was funded by "MOBIDOC Promess", program of the Tunisian Ministry of Higher Education and Scientific Research, ANPR grant number 191/2019".

Acknowledgements: Authors thank Ms. Emna BOUSHIH for her technical support in the laboratory experiments.

Conflicts of Interest: "The authors declare no conflict of interest".

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