

1 **Dates Fruit Allelochemicals Compounds and their Effect on *Ectomyelois***  
2 ***ceratoniae* Zeller (Lepidoptera: Pyralidae) Oviposition Behaviour**

3  
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5 **ABSTRACT**

6 The incidence of allelochemical substances on the oviposition behaviour of *Ectomyelois*  
7 *ceratoniae* Zeller (Lepidoptera: Pyralidae), to select appropriate site for oviposition and food  
8 sources for his future offspring, on three Algerian date varieties (Ghars, Deglet-Nour, and  
9 Degla-Beidha) was investigated. The use of Headspace collection via Solid Phase Micro-  
10 Extraction followed by Gas Chromatography-Mass Spectrometry method allowed the detection  
11 of 68 compounds of which only 22 were identified in all date varieties studied. The behavioural  
12 test with wind tunnel explored the response of *E. ceratoniae* adult females to the various sources  
13 of odours, coming from different combinations of synthetic substances identified in the three  
14 date varieties. The pure substances (mono-compounds) presented the highest rates of laid eggs  
15 compared to those in the mixture. Ketones stimuli were the most attractive with about 41% of  
16 the total eggs laid rate, followed by the alcohols (15%) and terpene (7.44%). The esters and  
17 phenols showed 6.38% and 5.58% eggs laid, respectively. These results could open up other  
18 research paths to manage this pest and their impact on it host plants.

19 **KEYWORDS:** HS-SPME-GC-MS, Date palm, Deglet Nour, Degla Beidha, Ghars.

20  
21 **INTRODUCTION**

22 Plant chemistry plays a major role in plant-insect ecological interactions. The recognition of  
23 plants by herbivores is mostly rely on chemoreception and frequently depends on plant  
24 allelochemicals compounds released into air and detected by insects before landing or tasted  
25 after contact or during feeding, to locate suitable food sources, mating partners, oviposition  
26 sites and/or social interaction (Schoonhoven et al. 1998 and Bernays and Chapman, 1994).  
27 Allelochemicals are substances which transmit chemical messages between different species,

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28 known as interspecific communication (Vilela and Della Lucia 2001). They produced by  
29 individuals of one species, modify the behavior of individuals of a different species (El-Ghany,  
30 2019). They have been divided into five categories: allomones (the response is beneficial to the  
31 emitter), kairomones (the response is beneficial to the recipient), synomones (beneficial to both  
32 the releaser and receiver), antimonies (maladaptive for both the releaser and receiver), and  
33 apneumones (causing a favorable behavioral or physiological reaction to a receiving organism,  
34 but harmful to other species that may be found either in or on the non-living material) (Vilela  
35 and Della Lucia 2001). The response of insects to plant volatiles differs, they can be attracted  
36 to them (adapted herbivores), or repellent (non-adapted herbivore). The categorization of plant  
37 volatiles as attractants and repellents is not standardized due to fluctuation of insect behaviour  
38 responses to such biotic or abiotic factors (El-Ghany, 2019). For most species of insects and for  
39 moths in particular, olfactory cues provide information about biologically relevant resources  
40 such as food, mates, and oviposition sites (Mechaber et al., 2002); That is especially evident in  
41 the case of *Ectomyelois ceratoniae*, when the volatile compounds emitted by dates or carobs  
42 infested with the fungus *Phomopsis* sp., stimulate the oviposition of this species (Gothilf, 1975  
43 and Cossé et al. 1994).

44 *Ectomyelois ceratoniae* is a polyphagous on many crops in the coastal (North) and oasian  
45 (South) regions of Algeria, especially on dates, citrus, some rosaceae and ornamental plants. Its  
46 caterpillar attacks a multitude of crops and native plants in very different bio-climatic stages  
47 (Arif, 2011). The number of host plants recognized worldwide is 49 species, 32 of which exist  
48 in Algeria (Doumandji, 1981). However, according the greatest damage has been reported on  
49 date palm, *Phoenix dactylifera* (Idder et al., 2009). In Algeria, *E. ceratoniae* is the most  
50 economically damaging pest of date palm fruits, where up to 80% of the fruits are damaged by  
51 this pest (Arif et al., 2018). The behaviour exhibited by *E. Ceratoniae* towards dates'  
52 allelochemicals compounds could focus scientific research toward establishing sustainable  
53 management systems.

54 In this study, the aim was to know the oviposition behaviour of *E. ceratoniae* females, exposing  
55 them to volatile substances in the wind tunnel (under laboratory conditions).

56

## 57 MATERIALS AND METHODS

### 58 Chemical Analysis of Date Allelochemicals

59 Headspace date allelochemicals collection by solid phase micro-extraction followed by gas  
60 chromatography-mass spectrometry method (HS-SPME-GC-MS), As in El Arem et al. (2011),  
61 the date allelochemicals were sampled statically by the exposure of the SPME fibre for 50 min,

62 to the headspace above the fresh date, consisted of three varieties (Degla-Beidha, Deglet-Nour,  
63 and Ghars), collected in the palm grove of National Agricultural Research Institute station in  
64 Touggourt, Southern Algeria (33CW+M4W), during the 2018-2019 crop-year, and stored at –  
65 20 °C until analysis. A sample of five fresh dates from each cultivar was inserted into a glass  
66 vial each time. The weight of each was, 40 g for D. Nour, 35 g for Ghars and 30 g for D. Beidha.  
67 The samples were heated at 50 °C for 30 min. SPME extraction of empty vials was also  
68 performed as control for any volatile organic compound contaminants.

69 Analyses of the headspace date allelochemicals were performed using 100 µm  
70 polydimethylsiloxane (PDMS) fibre (PROCHIMA-SIGMA Tlemcen; Algeria). Analyses were  
71 carried out on a GC-MS system, SPME fibres were thermal desorbed in a gas chromatograph  
72 (GC) injection chamber, (Hp 6890, HP-5Ms capillary column (30 m × 0.25 mm × 0.25 µm);  
73 Helium at 2 mL/min; splitless injection). The oven temperature gradient was programmed at  
74 50°C for 2 min, 3°C every minute up to 240°C, isothermal for 3 minutes. The analysis took 68  
75 minutes. As for the mass spectrometer (MS), Agilent quadrupole model, functioning at a 70 eV  
76 electronic impact, 230 °C, and quadrupole mass analyzer. The data bank NIST002, and the data  
77 analysis collection program MSD ChemStation G1701DA D-02.00.275 were used to identify  
78 the allelochemicals.

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## 80 Insects

81 *E. ceratoniae* females were obtained from a laboratory rearing, started from individuals  
82 collected during the 2018/ 2019 crop-year, from infested dates in Touggourt experimentation  
83 station palm grove; then identified via the identification key developed by Dhouibi (1991) and  
84 Gilligan and Passoa (2014). They were reared at the National Institute of Agronomic Research  
85 of Algeria (INRAA), entomology laboratory station, Touggourt, Algeria. The larvae rearing  
86 conditions were described by Mediouni and Dhouibi (2007) and Arif (2011). To obtain mated  
87 females for the experiments, the rearing was carried out in an incubator set at 28 ± 1°C, 15:10  
88 (light: dark) photoperiod and 75± 5% relative humidity.

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## 90 Allelochemicals

91 The volatile compounds used in this study as stimuli were highly purified synthetic chemical  
92 compounds obtained from PROCHIMA-SIGMA Tlemcen, (Algeria). These compounds were  
93 identified in the dates studied that it was indeed a α -thujone (C<sub>10</sub>H<sub>16</sub>O); ≥ 96%, was identified  
94 in Ghars cultivar, while β-thujone (C<sub>10</sub>H<sub>16</sub>O) ; ≥ 96%, Limonene (C<sub>10</sub>H<sub>16</sub>) ; ≥ 95%, Linalool  
95 (C<sub>10</sub>H<sub>18</sub>O); ≥ 97%, Methyl N-methyl anthranilate (C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub>); ≥ 97, Phenol, 2,6-bis(1,1-

96 dimethylethyl)-4-methyl (C<sub>15</sub>H<sub>24</sub>O); ≥ 99%, 2-Undecanone (C<sub>11</sub>H<sub>22</sub>O); ≥ 98%, and α-Isomethyl  
97 ionone (C<sub>14</sub>H<sub>22</sub>O); ≥ 95%, were identified in *D. Beidha*.

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### 99 **Oviposition rate**

100 Behavioural experiments were conducted in a wind horizontal glass gallery tunnel (L x W x H:  
101 180 x 50 x 50 cm). according to Kuenen and Baker (1982); Cossé et al. (1994), and Arif (2011).  
102 An air pump ensures the air flow circulation at a constant speed (0.5 m/s). This air flow passes  
103 through a plastic pipe (Ø: 9.6 mm) to a flow meter (rotameter), equipped with an active charcoal  
104 filter to control its flow and purify it. Then, the air through the vacuum flask filled 2/3 of its  
105 volume with distilled water, for humidification. The pipe coming out of the flask is connected  
106 to a cylindrical box (ventilation box) (H x Ø : 7 x 3.7 cm,) allows the distribution of air by  
107 diffusing it into the 10 pipes (Ø: 0.37 mm) which in turn transport the air to the 10 jars  
108 containing the stimuli placed in the wind tunnel. To ensure a good circulation of the air flow,  
109 an air extractor has been placed at the other end of the wind tunnel.

110 The tests were conducted according to the methods proposed by Gothilf et al. (1975); Baker et  
111 al. (1991); Cossé et al. (1994); Mechaber et al. (2002); Dallaire (2003) and Masante-Roca et al.  
112 (2007). Tests involve exposing mated *E. ceratoniae* females to 10 stimuli at the same time in  
113 the wind tunnel. Each stimulus was in a glass jars, its composition depends on different  
114 combinations of the eight compounds mentioned above. The chemicals tested were placed in  
115 undiluted form in open capsules (H x Ø: 2 x 4 cm) coated with filter paper strips (Whatman  
116 N°1). A volume of 10 µL of each chemical is added to each capsule. Then, the capsules were  
117 placed in open glass jars (H x Ø: 8 x 7 cm), covered entirely with a piece of perforated green  
118 fabric (insect proof) to ensure the visibility of the eggs laid. Next, the 10 jars were placed on  
119 the bottom of the wind tunnel, arranged in two rows, 10 cm apart and 120 cm from the opposite  
120 side of the *E. ceratoniae* females release point. Then each jar was connected to a pipe that  
121 conducts the air flow. That finally, sweeps the surface of the filter paper, and leaves the jar  
122 opening, crossing the perforated fabric that covers it, to finally disseminate inside the wind  
123 tunnel. In each test, nine jars containing compounds (8 jars that contain a different compounds  
124 with different combinations) + 1 jar mixture of all compounds) and 1 jar was empty as control.  
125 The tests were carried out according to 130 possible combinations, arranged into 8 groups in  
126 each test (3 repetitions were performed) according to the following arrangement (Table 1).

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132**Table 1.** Composition of *E. ceratoniae* oviposition stimulus tested in each group according to substances combinations.

Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
Each volatile compound was tested independently (single compound)	Each stimulus was composed of 2 volatile compounds	Each stimulus was composed of 3 volatile compounds	Each stimulus was composed of 4 volatile compounds	Each stimulus was composed of 5 volatile compounds	Each stimulus was composed of 6 volatile compounds	Each stimulus was composed of 7 volatile compounds	Each stimulus was composed of 8 volatile compounds
10 combinations	39 combination	30 combinations	22 combinations	15 combinations	9 combinations	3 combinations	2 combinations
Total	130 combinations						
Stimulus	Substances combinations	Stimulus	Substances combinations	Stimulus	Substances combinations	Stimulus	Substances combinations
Sb1	2-Undecanone;	Sb32	Sb5+Sb7	Sb63	Sb2+Sb3 +Sb4+Sb5	Sb2	Limonene;
Sb2		Sb33	Sb5+Sb8	Sb64	Sb2+Sb3 +Sb4+Sb6	Sb3	Butylated Hydroxytoluene;
Sb3	Butylated Hydroxytoluene;	Sb34	Sb6+Sb7	Sb65	Sb2+Sb3 +Sb4+Sb7	Sb4	Linalool;
Sb4	Linalool;	Sb35	Sb6+Sb8	Sb66	Sb2+Sb3 +Sb4+Sb8	Sb5	Methyl N-methyl anthranilate;
Sb5	Methyl N-methyl anthranilate;	Sb36	Sb7+Sb8	Sb67	Sb3+Sb4 +Sb5+Sb6	Sb6	$\beta$ -thujone;
Sb6	$\beta$ -thujone;	Sb37	Sb1+Sb2 +Sb3	Sb68	Sb3+Sb4 +Sb5+Sb7	Sb7	$\alpha$ -Isomethyl ionone;
Sb7	$\alpha$ -Isomethyl ionone;	Sb38	Sb1+Sb2 +Sb4	Sb69	Sb3+Sb4 +Sb5+Sb8	Sb8	$\alpha$ -thujone
Sb8	$\alpha$ -thujone	Sb39	Sb1+Sb2 +Sb5	Sb70	Sb4+Sb5 +Sb6+Sb7	Sb9	Sb1+Sb2
Sb9	Sb1+Sb2	Sb40	Sb1+Sb2+Sb6	Sb71	Sb4+Sb5 +Sb6+Sb8	Sb10	Sb1+Sb3
Sb10	Sb1+Sb3	Sb41	Sb1+Sb2 +Sb7	Sb72	Sb5 +Sb6+ Sb7+Sb8	Sb11	Sb1+Sb4
Sb11	Sb1+Sb4	Sb42	Sb1+Sb2 +Sb8	Sb73	Sb1+Sb2 +Sb3+Sb4+Sb5	Sb12	Sb1+Sb5
Sb12	Sb1+Sb5	Sb43	Sb2+Sb3 +Sb4	Sb74	Sb1+Sb2 +Sb3+Sb4+Sb6	Sb13	Sb1+Sb6
Sb13	Sb1+Sb6	Sb44	Sb2+Sb3 +Sb5	Sb75	Sb1+Sb2 +Sb3+Sb4+Sb7	Sb14	Sb1+Sb7
Sb14	Sb1+Sb7	Sb45	Sb2+Sb3+Sb6	Sb76	Sb1+Sb2 +Sb3+Sb4+Sb8	Sb15	Sb1+Sb8
Sb15	Sb1+Sb8	Sb46	Sb2 +Sb3+Sb7	Sb77	Sb2+Sb3 +Sb4+Sb5+Sb6	Sb16	Sb2+Sb3
Sb16	Sb2+Sb3	Sb47	Sb2 +Sb3+Sb8	Sb78	Sb2+Sb3 +Sb4+Sb5+Sb7	Sb17	Sb2+Sb4
Sb17	Sb2+Sb4	Sb48	Sb3 +Sb4+Sb5	Sb79	Sb2+Sb3 +Sb4+Sb5+Sb8	Sb18	Sb2+Sb5
Sb18	Sb2+Sb5	Sb49	Sb3 + Sb4+Sb6	Sb80	Sb3+Sb4 +Sb5+Sb6+Sb7	Sb19	Sb2+Sb6
Sb19	Sb2+Sb6	Sb50	Sb3 +Sb4+Sb7	Sb81	Sb3+Sb4 +Sb5+Sb6+Sb8	Sb20	Sb2+Sb7
Sb20	Sb2+Sb7	Sb51	Sb3 +Sb4+Sb8	Sb82	Sb4 +Sb5+Sb6+Sb7+Sb8	Sb21	Sb2+Sb8
Sb21	Sb2+Sb8	Sb52	Sb4 +Sb5+Sb6	Sb83	Sb1+Sb2 +Sb3+Sb4+Sb5+Sb6	Sb22	Sb3+Sb4
Sb22	Sb3+Sb4	Sb53	Sb4 +Sb5+Sb7	Sb84	Sb1+Sb2 +Sb3+Sb4+Sb5+Sb7	Sb23	Sb3+Sb5
Sb23	Sb3+Sb5	Sb54	Sb4 +Sb5+Sb8	Sb85	Sb1+Sb2 +Sb3+Sb4+Sb5+Sb8	Sb24	Sb3+Sb6
Sb24	Sb3+Sb6	Sb55	Sb5 +Sb6+Sb7	Sb86	Sb2 +Sb3+Sb4+Sb5+Sb6+Sb7	Sb25	Sb3+Sb7
Sb25	Sb3+Sb7	Sb56	Sb5 +Sb6+Sb8	Sb87	Sb2 +Sb3+Sb4+Sb5+Sb6+Sb8	Sb26	Sb3+Sb8
Sb26	Sb3+Sb8	Sb57	Sb6+Sb7+Sb8	Sb88	Sb3+Sb4 +Sb5+Sb6+Sb7+Sb8	Sb27	Sb4+Sb5
Sb27	Sb4+Sb5	Sb58	Sb1+Sb2 +Sb3+Sb4	Sb89	Sb1+Sb2 +Sb3+Sb4+Sb5+Sb6+Sb7	Sb28	Sb4+Sb6
Sb28	Sb4+Sb6	Sb59	Sb1+Sb2 +Sb3+Sb5	Mix	Sb1+Sb2 +Sb3+Sb4+Sb5+Sb6+Sb7		
Sb29	Sb4+Sb7	Sb60	Sb1+Sb2 +Sb3+Sb6	Ctrl	+Sb8		
Sb30	Sb4+Sb8	Sb61	Sb1+Sb2 +Sb3+Sb7		Control		
Sb31	Sb5+Sb6	Sb62	Sb1+Sb2 +Sb3+Sb8				

133

134 In addition, as proposed by Cossé et al.(1994), a group of 10 females aged 4 – 6 days was  
 135 transferred from the rearing incubator in a cylindrical box (H x Ø: 7 x 5 cm) to the wind tunnel,  
 136 2 hours before starting the experiment; so that, the females could acclimatize to the wind tunnel  
 137 conditions (24-26°C, 30 Lux, 60-70% relative humidity and 0.5 m/s air speed).The moths were  
 138 placed on a high metal platform 15 cm above the bottom of the wind tunnel and 120 cm from  
 139 the nearest stimulus. The bio-tests were conducted during scotophase; optimal oviposition  
 140 period for *E. ceratoniae*(Cossé et al. 1994).After the acclimatization period, 10 females were  
 141 released at the same time into the wind tunnel. The duration of each experiment was one night.

142 The next morning of each test, the jars were removed to count the number of eggs laid on the  
143 perforated tissue covering the jar. In order, the wind tunnel must be cleaned with 70% ethanol  
144 before each test to avoid any kind of pollution by undesirable compounds.

#### 145 146 **Statistics**

147 The non-parametric Kruskal-Wallis H test was used to determine significance among the *E.*  
148 *ceratoniae* choices, the impact of number and nature of the volatile compounds on its oviposition  
149 rate responses. The statistical analysis was performed using IBM SPSS Statistics, version: 20,  
150 software, completed by pairwise post-hoc comparisons.

#### 151 152 **RESULTS**

153 The date samples presented 68 compounds, with 22 volatile compounds identified (Table 2).  
154 D. Beidha was the cultivar with the highest number of identified compounds (20), whereas D.  
155 Nour and Ghars cultivars only one compound each was identified, against 16 and 14  
156 unidentified, respectively. Furthermore, the identified compounds were classified into 9  
157 chemical classes, including amine, aromatic hydrocarbons, ester, ketones, phenols, saturated  
158 aliphatic hydrocarbons, terpenic alcohols, terpenic hydrocarbons, and unsaturated cyclic  
159 hydrocarbons.

160 **Table 2.** Date allelochemicals identified in three Algerian varieties (Degla Beidha, Degla Nour and Ghars) via  
161 Headspace SPME-GC-MS method.

Chemical class	Compounds	Degla-Beidha	Deglet-Nour	Ghars
Amine	"Benzene ethanamine, 3-benzyloxy-2-fluoro-.beta.-hydroxy-N-methyl-"		+	
Aromatic hydrocarbons	"Furan, tetrahydro-"	+		
	"Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-"	+		
Ester	"1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate"	+		
	"methyl N-methylanthranilate"	+		
Ketones	"Thujone"	+		
	"2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-"	+		
	"2-Undecanone"	+		
	"alpha.-iso-methyl ionone"	+		
	".BETA.-THUJONE"	+		
	".alpha.-Thujone "			+
Phénols	"Phenol, 2,6-bis (1,1-dimethylethyl)-4-methyl-"	+		
Saturated aliphatic Hydrocarbons	"Undecane"	+		
	"Dodecane"	+		
	"Tridecane"	+		
	"Hexatriacontane"	+		
	"Heneicosane"	+		
	"Tetradecane"	+		
Terpenic alcohols	"Linalool L"	+		
Terpenic hydrocarbons	"dl-Limonene"	+		

	"Nerol"	+
Unsaturated cyclic hydrocarbons	"Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-"	+

162 + Presence

163

164 **Impact of Chemicals combination on *E. ceratoniae* Oviposition rate**

165 **Oviposition rate** of *E. ceratoniae* to certain synthetic chemicals that have been identified in  
 166 three Algerian date cultivars revealed the allelochemical tendencies of this moth (Table 3). The  
 167 GsbI stimuli group, composed mainly of 8 single substances, received 75% of the eggs laid,  
 168 followed by GsbII, with 15%, from which each stimulus is composed of 2 substances. GsbIII,  
 169 GsbIV, GsbV, and GsbVIII groups received 4%, 2%, 1%, and 3% of **eggs laid** respectively,  
 170 whereas, the GsbVI and GsbVII groups did not presented any egg-laying. The stimulus mixture  
 171 (Mix) **with** all **compounds**, **presented** 2.93% of the eggs laid **as** control to the GsbII (0.53%)  
 172 and GsbV (2.12%) stimulus, **whereas** it did not attract any females when tested alone.

173

174 **Table 3. Oviposition rate** of *E. ceratoniae* to allelochemicals compounds identified in three Algerian date cultivars  
 175 (Degla Nour, Ghars, and Degla Beidha).

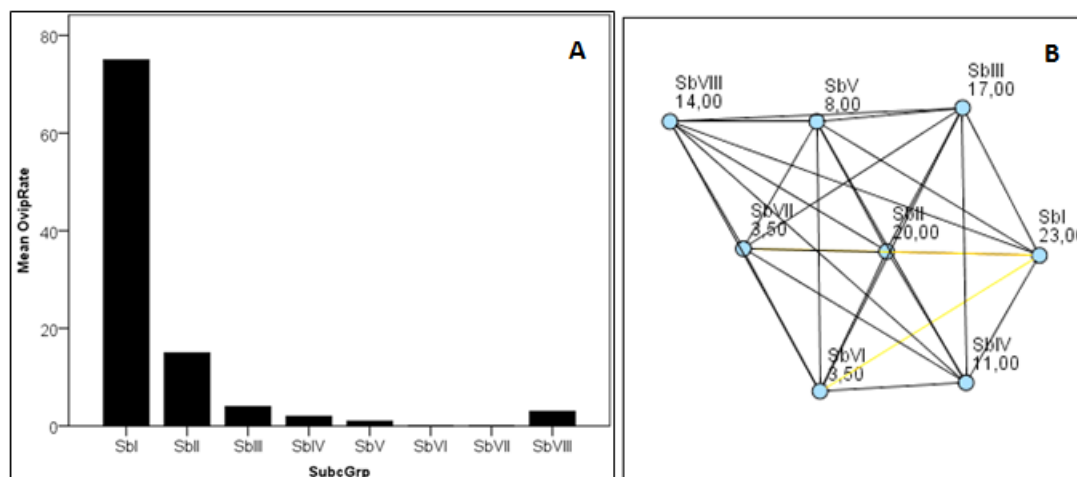
Substances	Oviposition rate (%)	Substances	Oviposition rate (%)	Substances	Oviposition rate (%)	Substances	Oviposition rate (%)
Sb1	5.06	Sb11	0.84	Sb21	0.56	Sb50	0,28
Sb2	7.87	Sb12	1.12	Sb24	1.12	Sb51	0,56
Sb3	5.90	Sb13	0.84	Sb26	0.56	Sb52	0,56
Sb4	21.63	Sb14	0.84	Sb27	1.40	Sb55	0,28
Sb5	6.74	Sb15	1.12	Sb28	0.28	Sb56	0,56
Sb6	12.36	Sb16	0.56	Sb36	1.97	Mix	3,09
Sb7	16.57	Sb17	0.56	Sb38	1.40	Ctrl	0,00
Sb8	1.12	Sb18	0.56	Sb41	0.28		
Sb9	0.84	Sb19	0.84	Sb43	0.28		
Sb10	0.56	Sb20	0.56	Sb44	0.28		

176 N.B. No oviposition was recorded on these compounds: Sb22, Sb23, Sb25, Sb29, Sb30, Sb31, Sb32, Sb33, Sb34,  
 177 Sb35, Sb37, Sb39, Sb40, Sb42, Sb45, Sb46, Sb47, Sb48, Sb49, Sb53, Sb54, Sb57, Sb58, Sb59, Sb60, Sb61, Sb62  
 178 and Ctrl.

179

180 A Kruskal-Wallis test revealed a significant difference between ranks means ( $K-W H = 23; df =$   
 181  $34; P = 0,002; (P < 0.05)$ ). The pairwise comparisons, showed that only SbI was significantly  
 182 different to SbVI and SbVII; ( $P = 0,017$ ); (Figure 1).





183

184 **Figure 1.** Impact of chemicals number forming each stimulus group on *E. ceratoniae* oviposition rate (Kruskal-  
 185 Wallis test; A: K-W  $H=23$ ;  $df=7$ ;  $P=0,002$ ; B: pairwise comparisons: SbI-SbVI and SbI-SbVII,  $P=0,017$ ; ( $P$   
 186  $<0.05$ )).

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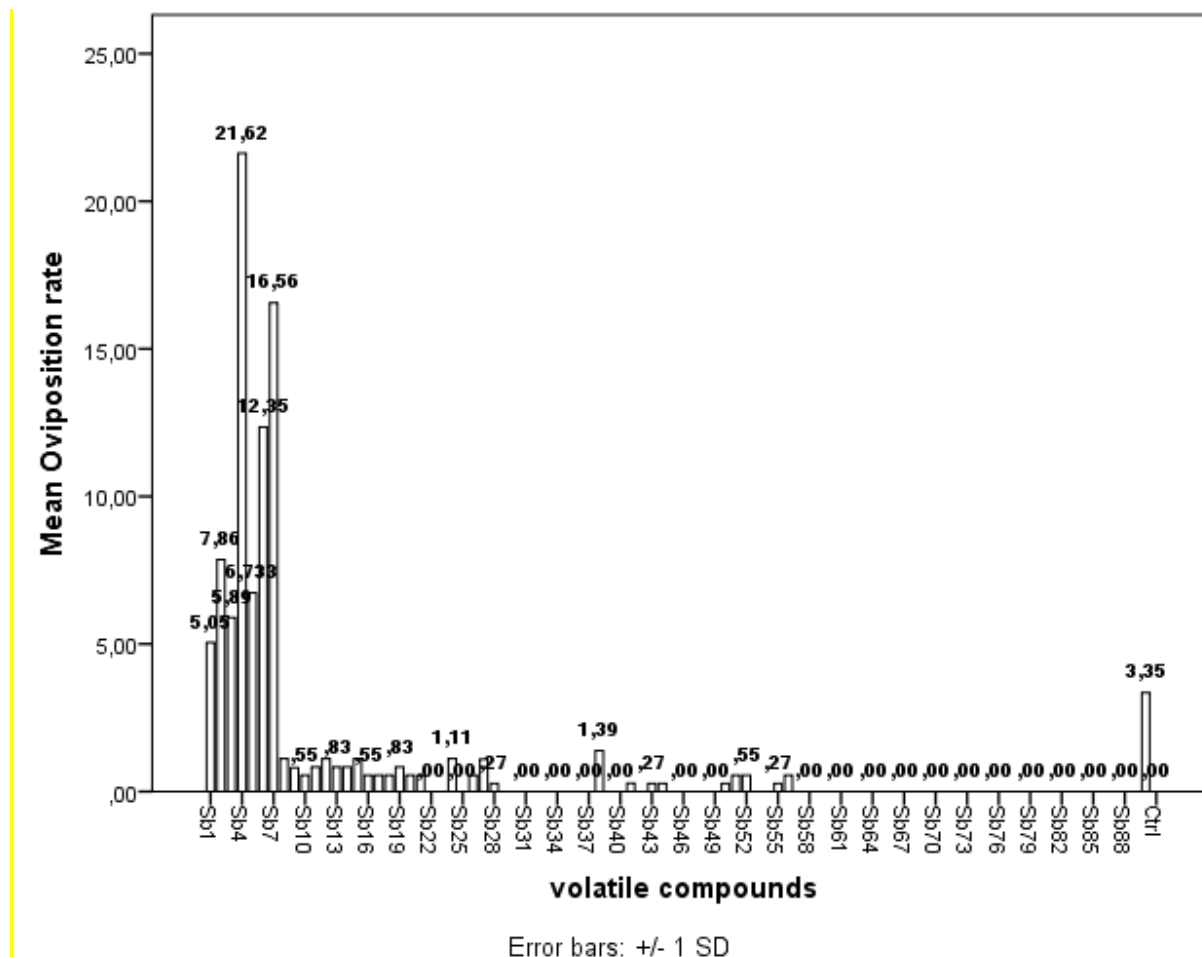
### 188 Effect of the Chemical Nature on *E. ceratoniae* Oviposition rate

189 Group I (GsbI), consisting mainly of 8 single chemical substances (mono-compound),  
 190 presented the highest oviposition rates, with Sb1 = 4.79% ( 2-Undecanone); Sb2 = 7.45%  
 191 (Limonene); Sb3 = 5.59%; (Butylated Hydroxytoluene); Sb4 = 14.89% (Linalool), Sb5 = 6.38%  
 192 (Methyl N-methyl anthranilate); Sb6 = 11.70% ( $\beta$ -thujone); Sb7 = 15.69% ( $\alpha$  -Isomethyl  
 193 ionone), and Sb8 = 8.78% ( $\alpha$ -thujone). The Mix and control presented no oviposition. For  
 194 Group II (GsbII), only 23 stimuli among the 39 combinations attracted females with low  
 195 oviposition rates compared to that of the sbI group, ranging from 0.27 to 1.33%, which  
 196 correlates to substances (Sb34 (Sb5+Sb7) and Sb36 (Sb6+Sb7), and Sb32 (Sb7+Sb8) [(Methyl  
 197 N-methyl anthranilate +  $\alpha$  -Isomethyl ionone and  $\beta$ -thujone +  $\alpha$  -Isomethyl ionone) and ( $\beta$ -  
 198 thujone +  $\alpha$  -Isomethyl ionone)], respectively. In addition, the sbIII group presented five  
 199 responses among the 30 chemical combinations, with Sb45, Sb48, Sb52, Sb54, and Sb55,  
 200 showing oviposition rates of 1.86, 1.33, 0.27, 0.53, and 0.27% respectively. For sbIV, which  
 201 consists of 22 combinations, only four responses were found with oviposition rates of 0.27%  
 202 for Sb58 and Sb65, and 0.53% for Sb67 and Sb68. However, for sbV, three responses were  
 203 found among the 15 combinations, namely: Sb74, Sb75 and Mix with oviposition rate of 0.27%,  
 204 0.53%, and 2.12% respectively. Concerning the sbVI, sbVII and sbVIII groups, no responses  
 205 were reported (zero egg-laying). There was a significant difference in oviposition rates  
 206 according to the chemical nature of different volatile compounds (K-W  $H=101.007$ ;  $df=34$ ;  
 207  $P<0.05$ ). The post hoc tests, revealed a significant difference between the independent groups;  
 208 namely Sb28- {[(Sb4, Sb6 and Sb7; ( $P=0.001$ )); (Sb2, Sb3 and Sb5; ( $P=0.001$ )); (Sb1; ( $P=$   
 209 0.002)), (Mix; ( $P=0.003$ )); (Sb38; ( $P=0.004$ )); (Sb8, Sb12, Sb15 and Sb24; ( $P=0.012$ ))];



210 (Sb27; (P = 0.014)) }; Sb43-{( Sb4, Sb6 and Sb7; (P = 0.001)); (Sb2, Sb3 and Sb5; (P = 0.001));  
 211 (Sb1; (P = 0.002)); (Mix; (P = 0.003)); (Sb38; (P = 0.004)); (Sb8, Sb12, Sb15 and Sb24; (P =  
 212 0.012)); (Sb27; (P = 0.014))} and Sb44- { (Sb4, Sb6 and Sb7; (P = 0.001)); (Sb2, Sb3 and Sb5;  
 213 (P = 0.001)) ; (Sb1; (p= 0.002)), (Mix; (P = 0.003)); (Sb38; (P = 0.004));(Sb8, Sb12, Sb15 and  
 214 Sb24; (P = 0.012)); (Sb27; (P = 0.014))]; (Figure 2).

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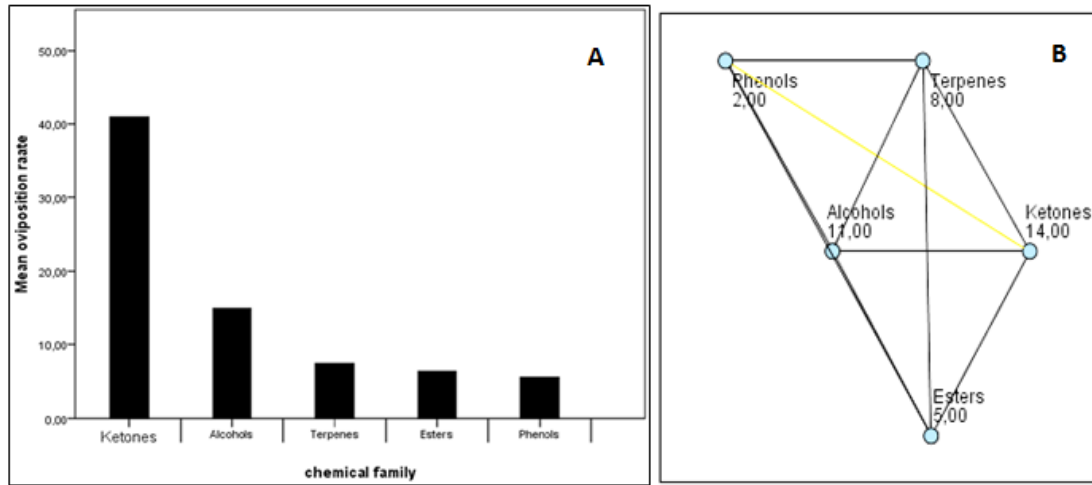
217 **Figure 2.** Variation in oviposition rate of *E. ceratoniae* females in response to synthetic chemicals (Kruskal-Wallis  
 218 test: K-W H = 101,007; df = 34; P = 0,000; (P < 0.05)).

219

### 220 *E. ceratoniae* ovipositional Tendencies According to the Stimuli Chemical Family

221 We are limited to the sbI stimulus group, to ascertain the effect of the chemical family of each  
 222 substance on *E. ceratoniae* oviposition behaviour, and to avoid confusion that may accompany  
 223 the use of mixtures of substances. It is noticed that, ketone is more attractive with about 41%  
 224 of the total eggs laid rate, followed by alcohol with about 15%, then, terpene with 7.44%. The  
 225 esters and phenols presented 6.38% and 5.58% oviposition rate, respectively. A Kruskal-Wallis  
 226 H test showed that there was a statistically significant difference in *E. ceratoniae* ovipositional  
 227 tendencies according to the chemical family; (K-W H: 13.524; df = 4; P = 0.009). Among the

228 five categories of chemical family, only phenols was significantly different from ketone ( $P =$   
 229 0.010); (Figure 3).



230  
 231 **Figure 3.** *E. ceratoniae* ovipositional tendencies according to the stimuli chemical family (Kruskal-Wallis test; A:  
 232 K-W  $H = 13.524$ ;  $df = 4$ ;  $P = 0.009$ ; ( $P < 0.05$ ); B: pairwise comparisons: phenols and ketone,  $P = 0.010$ ; ( $P <$   
 233  $0.05$ )).

234

## 235 DISCUSSION

236 The results highlighted the interspecific semiochemical effect of certain compounds and/or  
 237 **their** mixtures to *E. ceratoniae* oviposition **rate**. The substances tested individually are the most  
 238 attractive than those mixed for *E. ceratoniae*. As it was also noted, the higher the number of  
 239 substances tested, the lower the rate of eggs laid. Indeed, in its study on the chemical basis of  
 240 differential egg-laying by Lepidoptera, Honda (1995), noted that Lepidoptera appear to lead to  
 241 spawning in response to a single host-specific compound. The same results were obtained by  
 242 Wolf et al. (2012), in a study conducted on *Cassida stigmatica* oviposition behaviour **among**  
 243 several chemotypes (various combinations of mixtures of chemical substances) where, females  
 244 of *C. stigmatica* showed a clear preference for pure chemotype over mixed chemotype and no  
 245 preference when only mixed chemotype were offered in the selection tests. However, Ayelo et  
 246 al. (2021) noted that Kairomone mixtures are likely to elicit stronger olfactory responses in  
 247 natural enemies than single kairomones.

248 The high levels of eggs laid by *E. ceratoniae* females recorded in the 8 pure (individual)  
 249 compounds (sbI stimulus group), is probably due to the rapid detection of these compounds,  
 250 given their simplicity (single), which generates oviposition. Furthermore, it was found that the  
 251 formation of stimuli based on mixtures of the same compounds led to a decrease or even a total  
 252 absence of *E. ceratoniae* females' attractiveness in certain groups of stimuli. **The** kairomones  
 253 are generally involved in the insect's choice of the food source or laying site (Chapman 1974;

254 Ayelo et al. 2021). The «no choice» of the insect (case of resistant plants) is essentially due to  
255 the lack of kairomones or to the insufficient quantities to elicit a behavioural response or  
256 kairomones being inhibited by antagonistic compounds (Panda and Khush1995). Although no  
257 previous information was available on the effect of the number of volatile compounds in a given  
258 mixture (stimuli) on the oviposition behaviour of this species, it is likely that the decrease or  
259 lack of *E. ceratoniae* females response to the different stimuli groups (mixture) is due to the  
260 antagonistic effect of the substances between them or to the concentrations of these substances  
261 in the mixture. That is especially evident in the case of the stimulus Sb29 (Sb4 = 14.89%  
262 (Linalool)+ Sb7 = 15.69% ( $\alpha$ -Isomethyl ionone)), which did not attract moths at all while is  
263 composed of two most attractive single compounds. According to Vucetic et al. (2014) the  
264 insects reactions to certain individual substances differed when combined with others. Certain  
265 molecules have the ability to repel others, but other compounds have the ability to either mask  
266 or inhibit these effects (Bruce and pickett 2011). It is not always the case that a plant is attractive  
267 or repellent to insects just because it contains components that make it repellent or attractive,  
268 however, the volatile combinations affects the function of volatile compounds (Bruce et al.  
269 2005).

270 The tests carried out in the wind tunnel show that the substances tested, divided into 5 chemical  
271 families (ketone, terpene, phenol, alcohol and ester) have a strong ovipositional rate effect for  
272 *E. ceratoniae* females. According to Rutledge (1996); Tasin et al. (2007) and Schwab et al.  
273 (2008), ketones, alcohols, esters and terpenes, play important role in the choice of laying site  
274 by several insects. The studies conducted by Gothilf et al. (1975) and Cossé et al. (1994), on *E.*  
275 *ceratoniae* ovipositional stimulants, highlighted the ability of alcohol and esters to stimulate  
276 the flight of mated females of this species and the landing at the odour source (wind tunnel).  
277 This stimulation can be explained by the sensitivity and sensory selectivity faculties that allow  
278 the detection and choice of odorous molecules by *E. ceratoniae*.

279 We found that  $\alpha$ -Isomethyl ionone;  $\beta$ -thujone,  $\alpha$ -thujone and 2-Undecanone ketones had  
280 oviposition rates 15.69%, 11.70%, 8.78% and 4.79% respectively. The  $\alpha$ -Isomethyl ionone  
281 compound is the most attractive of the 8 compounds tested. This compound has been described  
282 by Ishida et al. (2008), as an effective attractant to *Bractocera latifrons*. Similarly, Cáceres et  
283 al. (2016) reported the attractive oviposition effect of  $\alpha$ -Isomethyl ionone on *Bemisia tabaci*  
284 while  $\beta$ -ionone has a deterrent effect.

285 In addition, the patent filed by Gabel et al. (1993), relating to attractive compositions of females  
286 of Tortricidae Lepidoptera, is characterized in that they include limonene,  $\alpha$ -thujone and  $\beta$ -

287 thujone as active ingredients. Moreover, the treatment of plant odours in antennal lobes of  
288 females of *Lobesia botrana* through the use of intracellular registration and colouring  
289 techniques has enabled (Masante-Roca et al. 2002), to note the involvement of  $\beta$ -thujone in the  
290 most common physiological responses in this species. Similarly, the characterization of the  
291 trichoid sensilla of *Culex quinquefasciatus* female reveals the effect of  $\alpha$ -thujone, 2-  
292 Undecanone and linalool in the activity of odorous receptor neurons. According to Ehlers and  
293 Schulz (2022), linalool is a common semiochemicals released by flowers or leaves, involved in  
294 the full spectrum of plant–pollinator interactions. In combination with other floral volatile and  
295 visual cues it elicits feeding responses in *Heliconius* butterflies (Andersson and Dobson, 2003),  
296 a complex interaction mediated by linalool between plant defense and insects attractiveness  
297 (Raguso, 2016). Female-specific responses to (S)-(+)-linalool in the silk moth *Bombyx mori*  
298 and enantio-specific responses (higher sensitivity to (R)-(-)-linalool) in the noctuid moth  
299 *Mamestra brassicae* are reported by Anderson et al. (2009) and Ulland et al. (2006). Indeed,  
300 *Manduca sexta* Females oviposited more on plants emitting (+)-linalool, either alone or in  
301 combination (mixture), whereas plants emitting (-)-linalool (alone or in mixtures) were less  
302 preferred (Reisenman, et al. 2010). These results, in conjunction with the homologous olfactory  
303 receptor neurons that exhibit linalool-specific responses (Grosse-Wilde et al. 2011). Regarding  
304 N-methyl anthranilate, kairomonal activity was observed in three species of Lepidoptera,  
305 Nymphalidae (*Argynnis paphia*; *Argyronome ruzlana* and *Damora sagana*) and two  
306 Hymenoptera (*Bombus hypocrite* and *Bombus diversus*) (Pellmyr 1986). For butylated  
307 hydroxytoluene, Yi et al. (2018) screened 19 active compounds that act on the behaviour of  
308 *Sclerodermus sp*; among these, butylated hydroxytoluene.

309

## 310 CONCLUSIONS

311 In summary, it can be concluded that the information obtained in the current study, as well as  
312 the volatile cluster on the oviposition rate (attractiveness) of a caterpillar pest. The study  
313 evaluated the effects of some volatile compounds released by three Algerian date cultivars  
314 (Deglet Nour Ghars and Degla Beidha). The compounds were identified by HS-SPME-GC-MS  
315 and tested with a wind tunnel for the oviposition rate of the insect. The compound chemical  
316 nature and their impact on the oviposition behaviour of *E. ceratoniae*, could open up other  
317 research paths to manage this pest. The reactions of the females of *E. ceratoniae* to the different  
318 stimuli expressed by the precise orientation and oviposition on the various sources of odours  
319 (synthetic substances) in the wind tunnel, clearly reflects the impact of these volatile substances  
320 on the mobility of this species, in particular, on the selection of oviposition sites. Thus, further

321 more advanced techniques such as electrophysiological are needed to clarify the electrical  
322 activity caused by *E. ceratoniae* sensilla stimulation by recording their reactions to the different  
323 compounds contained in each cultivar. These studies will not only elucidate allelochemicals  
324 and their behavioural mechanisms, but also suggest a possible role for oviposition specific  
325 compounds to be used for future monitoring of *E. ceratoniae* field populations under natural  
326 conditions, as well as for attractive lures in an Integrated Pest Management (IPM) perspective,  
327 and then it can help to developing control strategies against this pest.

328

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