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

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ABSTRACT

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

Keywords: Degla Beidha, Deglet Nour, Date palm, Ghars varieties, Solid phase micro-extraction.

INTRODUCTION

Plant chemistry plays a major role in plant-insect ecological interactions. The recognition of plants by herbivores mostly rely on chemoreception and frequently depends on plant allelochemicals compounds released into air and detected by insects before landing, or tasted after contact or during feeding, to locate suitable food sources, mating partners, oviposition sites and/or social interaction (Schoonhoven *et al*., 1998 and Bernays and Chapman, 1994). Allelochimicals are substances that transmit chemical messages between different species, known as interspecific communication (Vilela and Della Lucia, 2001). They are produced by individuals of one species, modify the behavior of individuals of a different species (El-Ghany, 2019). They have been divided into the following five categories (Vilela and Della Lucia, 2001):

- Allomones (The response is beneficial to the emitter),
- Kairomones (The response is beneficial to the recipient),
- Synomones (Beneficial to both the releaser and receiver),
- Antimonies (Maladaptive for both the releaser and receiver),
- Apneumones (Causing a favorable behavioural or physiological reaction to a receiving organism, but harmful to other species that may be found either in or on the non-living material).

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The response of insects to plant volatiles differs: they can be attracted to them (adapted herbivores), or repellent (non-adapted herbivore). The categorization of plant volatiles as attractants and repellents is not standardized due to fluctuation of insect behaviour responses to such biotic or abiotic factors (El-Ghany, 2019). For most species of insects and for moths in particular, olfactory cues provide information about biologically relevant resources such as food, mates, and oviposition sites (Mechaber *et al*., 2002). This is especially evident in the case of *Ectomyelois ceratoniae* , when the volatile compounds emitted by dates or carobs infested with the fungus *Phomopsis* sp., stimulate the oviposition of this species (Gothilf, 1975; Cossé *et al*., 1994).

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

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

MATERIALS AND METHODS

Chemical Analysis of Date Allelochemicals

Headspace date allelochemicals collection by Solid Phase Micro-Extraction followed by Gas Chromatography-Mass Spectrometry method (HS-SPME-GC-MS), As in El Arem *et al*. (2011), the date allelochemicals were sampled statically by the exposure of the SPME fibre for 50 min, to the headspace above the fresh date, consisted of three varieties (Degla-Beidha, Deglet-Nour, andGhars) collected in the palm grove of National Agricultural Research Institute station in Touggourt, Southern Algeria (33CW+M4W), during the 2018-2019 crop-year, and stored at –20°C until analysis. A sample of five fresh dates from each cultivar was inserted into a glass vial each time. The weight of each was, 40 g for D. Nour, 35 g for Ghars and 30 g for D. Beidha. The samples were heated at 50°C for 30 minutes. SPME extraction of empty vials was also performed as control for any volatile organic compound contaminants.

Analyses of the headspace date allelochemicals were performed using 100 μm Polydimethylsiloxane (PDMS) fibre (PROCHIMA-SIGMA Tlemcen; Algeria). Analyses were carried out on a Gas Chromatography-Mass Spectrometry (GC-MS) system, SPME fibres were thermal desorbed in a Gas Chromatograph (GC) injection chamber, (Hp 6890, HP-5Ms capillary column (30 m×0.25 mm×0.25 μm); Helium at 2 mL min^{-1}; split less injection). The oven temperature gradient was programmed at 50°C for 2 minutes, 3°C every minute up to 240°C, isothermal for 3 minutes. The analysis took 68 minutes. As for the mass spectrometer (MS), Agilent quadrupole model, functioning at a 70eV electronic impact, 230°C, and quadrupole mass analyzer. The data bank NIST002, and the data analysis collection program MSD ChemStation G1701DA D-02.00.275 were used to identify the allelochemicals.

Insects

E. ceratoniae females were obtained from a laboratory rearing, started from individuals collected during the 2018/2019 crop-year, from infested dates in Touggourt Experiment Station palm grove; then, identified via the identification key developed by Dhouibi (1991) and Gilligan and Passoa (2014). They were reared at the National Institute of Agronomic Research of Algeria (INRAA), entomology laboratory station, Touggourt, Algeria. The larvae rearing conditions were described by Mediouni and Dhouibi (2007) and Arif (2011). To obtain mated females for the experiments, the rearing was carried out in an incubator set at 28 ± 1°C, 15: 09 (Light: Dark) photoperiod and 75±5% relative humidity.

Allelochemicals

The volatile compounds used in this study as stimuli were highly purified synthetic chemical compounds obtained from PROCHIMA-SIGMA Tlemcen, (Algeria). These compounds were identified in the dates studied and it was indeed a α -thujone ($C_{10}H_{16}O$); ≥ 96%, was identified in Ghars cultivar, while β-thujone ($C_{10}H_{16}O$) ; ≥ 96%, Limonene ($C_{10}H_{16}$) ; ≥ 95%,Linalool ($C_{10}H_{18}O$); ≥ 97%, Methyl N-methyl anthranilate ($C_9H_{11}NO_2$); ≥ 97, Phenol,2,6-bis(1,1-dimethylethyl)-4-methyl ($C_{15}H_{24}O$); ≥ 99%, 2-Undecanone ($C_{11}H_{22}O$); ≥ 98%, and α-Isomethyl ionone ($C_{14}H_{22}O$); ≥ 95%, were identified in D. Beidha.

Oviposition rate

Behavioral experiments were conducted in a horizontal wind glass gallery tunnel (L x W x H: 180×50×50 cm), according to Kuenen and Baker (1982), Cossé *et al.* (1994), and Arif (2011). An air pump ensured the air flow circulation at a constant speed (0.5 m s^{-1}). This air flow passed through a plastic pipe (Ø: 9.6 mm) to a flow meter (rotameter), equipped with an active charcoal filter to control its flow and purify it. Then, the air through the vacuum flask filled 2/3 of its volume with distilled water, for humidification. The pipe coming out of the flask was connected to a cylindrical box (ventilation box) (H×Ø: 7×3.7 cm,) allowed the distribution of air by diffusing it into 10 pipes (Ø: 0.37 mm), which in turn transport the air to the 10 jars containing the stimuli placed in the wind tunnel. To ensure a good circulation of the air flow, an air extractor was placed at the other end of the wind tunnel.

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

8 groups in each test (3 repetitions were performed) according to the following arrangement (Table 1).

In addition, as proposed by Cossé *et al*.(1994), a group of 10 females aged 4–6 days was transferred from the rearing incubator in a cylindrical box (H×Ø: 7×5 cm) to the wind tunnel, 2 hours before starting the experiment. Later, the females could acclimatize to the wind tunnel conditions (24-26°C, 30 Lux, 60-70% relative humidity and 0.5 m s^{-1} air speed). The moths were placed on a high metal platform 15 cm above the bottom of the

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 Combinations	30 Combinations	22 Combinations	15 Combinations	9 Combinations	3 Combinations	2 Combinations
Total	130 combinations						

Stimulus	Substances combinations	Stimulus	Substances combinations	Stimulus	Substances combinations
Sb1	2-Undecanone	Sb32	Sb5+Sb7	Sb63	Sb2+Sb3 +Sb4+Sb5
Sb2	Limonene	Sb33	Sb5+Sb8	Sb64	Sb2+Sb3 +Sb4+Sb6
Sb3	Butylated Hydroxytoluene	Sb34	Sb6+Sb7	Sb65	Sb2+Sb3 +Sb4+Sb7
Sb4	Linalool	Sb35	Sb6+Sb8	Sb66	Sb2+Sb3 +Sb4+Sb8
Sb5	Methyl N-methyl anthranilate	Sb36	Sb7+Sb8	Sb67	Sb3+Sb4 +Sb5+Sb6
Sb6	β-thujone	Sb37	Sb1+Sb2 +Sb3	Sb68	Sb3+Sb4 +Sb5+Sb7
Sb7	α -Isomethyl Ionone	Sb38	Sb1+Sb2 +Sb4	Sb69	Sb3+Sb4 +Sb5+Sb8
Sb8	α-Thujone	Sb39	Sb1+Sb2 +Sb5	Sb70	Sb4+Sb5 +Sb6+Sb7
Sb9	Sb1+Sb2	Sb40	Sb1+Sb2+Sb6	Sb71	Sb4+Sb5 +Sb6+Sb8
Sb10	Sb1+Sb3	Sb41	Sb1+Sb2 +Sb7	Sb72	Sb5 +Sb6+ Sb7+Sb8
Sb11	Sb1+Sb4	Sb42	Sb1+Sb2 +Sb8	Sb73	Sb1+Sb2 +Sb3+Sb4+Sb5
Sb12	Sb1+Sb5	Sb43	Sb2+Sb3 +Sb4	Sb74	Sb1+Sb2 +Sb3+Sb4+Sb6
Sb13	Sb1+Sb6	Sb44	Sb2+Sb3 +Sb5	Sb75	Sb1+Sb2 +Sb3+Sb4+Sb7
Sb14	Sb1+Sb7	Sb45	Sb2+Sb3+Sb6	Sb76	Sb1+Sb2 +Sb3+Sb4+Sb8
Sb15	Sb1+Sb8	Sb46	Sb2 +Sb3+Sb7	Sb77	Sb2+Sb3 +Sb4+Sb5+Sb6
Sb16	Sb2+Sb3	Sb47	Sb2 +Sb3+Sb8	Sb78	Sb2+Sb3 +Sb4+Sb5+Sb7
Sb17	Sb2+Sb4	Sb48	Sb3 +Sb4+Sb5	Sb79	Sb2+Sb3 +Sb4+Sb5+Sb8
Sb18	Sb2+Sb5	Sb49	Sb3 + Sb4+Sb6	Sb80	Sb3+Sb4 +Sb5+Sb6+Sb7
Sb19	Sb2+Sb6	Sb50	Sb3 +Sb4+Sb7	Sb81	Sb3+Sb4 +Sb5+Sb6+Sb8
Sb20	Sb2+Sb7	Sb51	Sb3 +Sb4+Sb8	Sb82	Sb4 +Sb5+Sb6+Sb7+Sb8
Sb21	Sb2+Sb8	Sb52	Sb4 +Sb5+Sb6	Sb83	Sb1+Sb2 +Sb3+Sb4+Sb5+Sb6
Sb22	Sb3+Sb4	Sb53	Sb4 +Sb5+Sb7	Sb84	Sb1+Sb2 +Sb3+Sb4+Sb5+Sb7
Sb23	Sb3+Sb5	Sb54	Sb4 +Sb5+Sb8	Sb85	Sb1+Sb2 +Sb3+Sb4+Sb5+Sb8
Sb24	Sb3+Sb6	Sb55	Sb5 +Sb6+Sb7	Sb86	Sb2 +Sb3+Sb4+Sb5+Sb6+Sb7
Sb25	Sb3+Sb7	Sb56	Sb5 +Sb6+Sb8	Sb87	Sb2 +Sb3+Sb4+Sb5+Sb6+Sb8
Sb26	Sb3+Sb8	Sb57	Sb6+Sb7+Sb8	Sb88	Sb3+Sb4 +Sb5+Sb6+Sb7+Sb8
Sb27	Sb4+Sb5	Sb58	Sb1+Sb2 +Sb3+Sb4	Sb89	Sb1+Sb2 +Sb3+Sb4+Sb5+Sb6+Sb7
Sb28	Sb4+Sb6	Sb59	Sb1+Sb2 +Sb3+Sb5	Mix	Sb1+Sb2 +Sb3+Sb4+Sb5+Sb6+Sb7+Sb8
Sb29	Sb4+Sb7	Sb60	Sb1+Sb2 +Sb3+Sb6	Ctrl	Control
Sb30	Sb4+Sb8	Sb61	Sb1+Sb2 +Sb3+Sb7		
Sb31	Sb5+Sb6	Sb62	Sb1+Sb2 +Sb3+Sb8		

wind tunnel and 120 cm from the nearest stimulus. The bio-tests were conducted during scotophase; optimal oviposition period for *E. ceratoniae* (Cossé *et al*., 1994). After the acclimatization period, 10 females were released at the same time into the wind tunnel. The duration of each experiment was one night. The next morning of each test, the jars were removed to count the number of eggs laid on the perforated tissue covering the jar. The wind tunnel must be cleaned with 70% ethanol before each test to avoid any kind of pollution by undesirable compounds.

Statistics

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

RESULTS

The date samples presented 68 compounds, with 22 volatile compounds identified (Table

Table 2. Date allelochemicals identified in three Algerian varieties (Degla Beidha, Degla Nour and Ghars) via 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)-"	+		

\+ Presence.

2). D. Beidha was the cultivar with the highest number of identified compounds (20), whereas D. Nour and Ghars cultivars had only one compound each, against 16 and 14 unidentified, respectively. Furthermore, the identified compounds were classified into 9 chemical classes, including amine, aromatic hydrocarbons, ester, ketones, phenols, saturated aliphatic hydrocarbons, terpenic alcohols, terpenic hydrocarbons, and unsaturated cyclic hydrocarbons.

Impact of Chemicals Combination on *E. ceratoniae* Oviposition Rate

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

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

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

Group I (GSbI), consisting mainly of 8 single chemical substances (mono-compound), presented the highest oviposition rates, with Sb1= 4.79% (2-Undecanone); Sb2= 7.45% (Limonene); Sb3= 5.59%; (Butylated hydroxytoluene); Sb4= 14.89% (Linalool), Sb5= 6.38% (Methyl N-methyl anthranilate); Sb6= 11.70% (β-Thujone); Sb7 = 15.69% (α-Isomethyl ionone), and Sb8= 8.78% (α-Thujone). The Mix and the control presented no oviposition. For Group II (GsbII), only 23 stimuli among the 39 combinations attracted females with low oviposition rates compared to that of the SbI group, ranging from 0.27 to1.33%, which correlates to substances [Sb34 (Sb5+Sb7) and Sb36

Table 3. Oviposition rate of *E. ceratoniae* to allelochemicals compounds identified in three Algerian date cultivars (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		

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

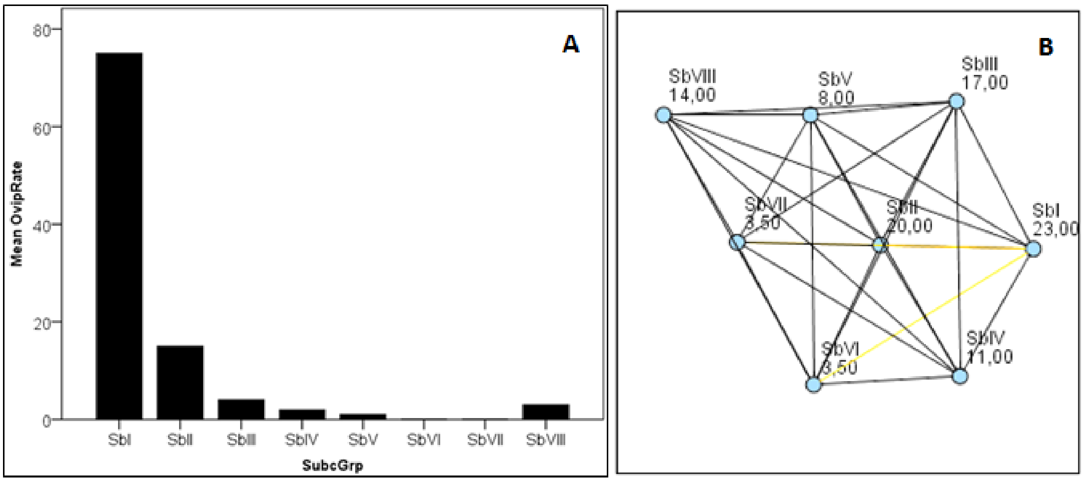

Figure 1. Impact of chemicals number forming each stimulus group on *E. ceratoniae* oviposition rate [Kruskal-Wallis test; A: K-W H=23; df= 7; P= 0.002; B: Pairwise comparisons: SbI-SbVI and SbI-SbVII, P= 0.017; (P< 0.05)].

(Sb6+Sb7), and Sb32 (Sb7+Sb8) [(Methyl N-methyl anthranilate+α–Isomethyl ionone and β-Thujone+α-Isomethyl ionone) and (β-Thujone+ α-Isomethyl ionone)], respectively. In addition, the SbIII group presented five responses among the 30 chemical combinations, with Sb45, Sb48, Sb52, Sb54, and Sb55, showing oviposition rates of 1.86, 1.33, 0.27, 0.53, and 0.27%, respectively. For SbIV, which consisted of 22 combinations, only four responses were found with oviposition rates of 0.27% for Sb58 and Sb65, and 0.53% for Sb67 and Sb68. However, for SbV, three responses were found among the 15 combinations, namely: Sb74, Sb75 and Mix with oviposition rate of 0.27, 0.53, and 2.12%, respectively. Concerning the SbVI, SbVII and SbVIII groups, no responses were reported (zero egg-laying). There was a significant difference in oviposition rates according to the chemical nature of different volatile compounds (K-W H= 101.007; df= 34; P< 0.05). The post hoc tests, revealed a significant difference between the independent groups; namely Sb28-{[(Sb4, Sb6 and Sb7; (P= 0.001)); (Sb2, Sb3 and Sb5; (P= 0.001)); (Sb1; (P= 0.002)), (Mix; (P= 0.003)); (Sb38; (P= 0.004)); (Sb8, Sb12, Sb15 and Sb24; (P= 0.012)); (Sb27; (P= 0.014))}; Sb43-{(Sb4, Sb6 and Sb7; (P= 0.001)); (Sb2, Sb3 and Sb5; (P= 0.001)); (Sb1; (P= 0.002)); (Mix; (P= 0.003)); (Sb38; (P= 0.004)); (Sb8, Sb12, Sb15 and Sb24; (P= 0.012)); (Sb27; (P= 0.014))} and Sb44-{(Sb4, Sb6 and Sb7; (P= 0.001)); (Sb2, Sb3 and Sb5; (P= 0.001)) ; (Sb1; (P= 0.002)), (Mix; (P= 0.003)); (Sb38; (P= 0.004));(Sb8, Sb12, Sb15 and Sb24; (P= 0.012)); (Sb27; (P= 0.014)]}; (Figure 2).

E. ceratoniae Ovipositional Tendencies According to the Stimuli Chemical Family

We are limited to the SbI stimulus group, to ascertain the effect of the chemical family of each substance on *E. ceratoniae* oviposition behavior, and to avoid confusion that may accompany the use of mixtures of substances. It is noticed that, ketone was more attractive with about 41% of the total eggs laid rate, followed by alcohol with about 15%, then, terpene with 7.44%. The esters and phenols presented 6.38 and 5.58% oviposition rate, respectively. A Kruskal-Wallis H test showed that there was a statistically significant difference in *E. ceratoniae* ovipositional tendencies according to the chemical family; (K-W H: 13.524; df= 4; P= 0.009). Among the five categories of chemical family, only phenols was significantly different from ketone (P= 0.010); (Figure 3).

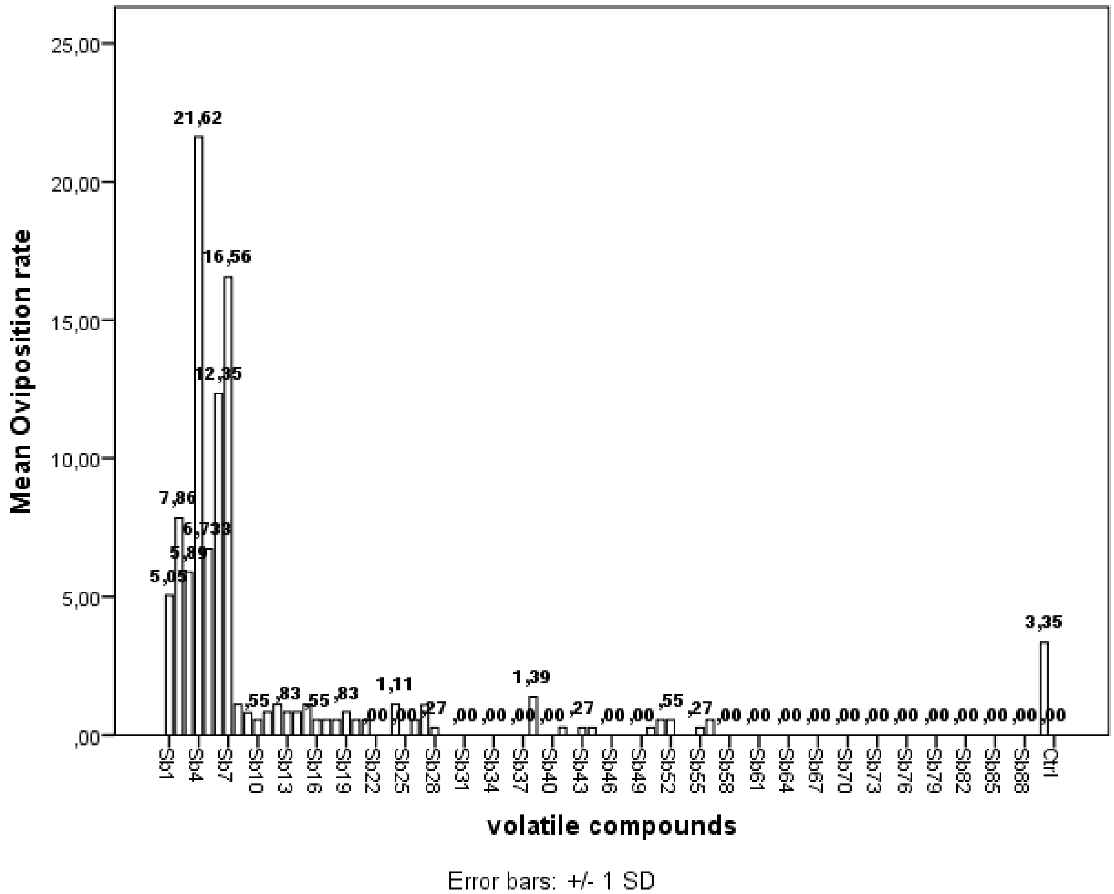

Figure 2. Variation in oviposition rate of *E. ceratoniae* females in response to synthetic chemicals [Kruskal-Wallis test: K-W H= 101.007; df= 34; P= 0.000; (P< 0.05)].

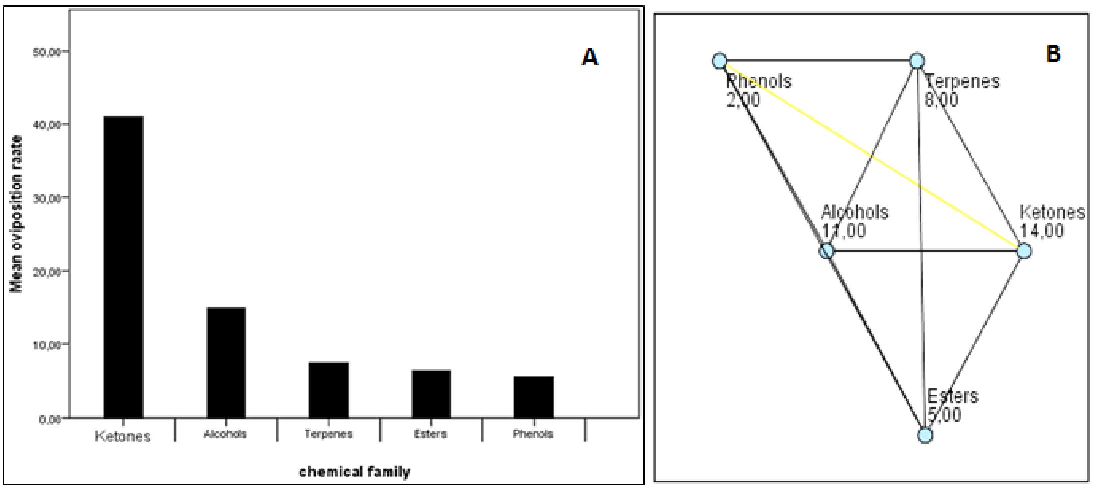

Figure 3. *E. ceratoniae* ovipositional tendencies according to the stimuli chemical family [Kruskal-Wallis test; A: K-W H=13.524; df= 4; P= 0.009; (P< 0.05); B: Pairwise comparisons: Phenols and ketone, P= 0.010; (P< 0.05)].

DISCUSSION

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

The high levels of eggs laid by *E. ceratoniae* females recorded in the 8 pure (individual) compounds (SbI stimulus group), was probably due to the rapid detection of these compounds, given their simplicity (single), which generated oviposition. Furthermore, it was found that the formation of stimuli based on mixtures of the same compounds led to a decrease or even a total absence of *E. ceratoniae* females' attractiveness in certain groups of stimuli. The kairomones are generally involved in the insect's choice of the food source or laying site (Chapman, 1974; Ayelo *et al*., 2021). The «no choice» of the insect (case of resistant plants) is essentially due to the lack of kairomones or to the insufficient quantities to elicit a behavioral response or kairomones being inhibited by antagonistic compounds (Panda and Khush, 1995). Although no previous information was available on the effect of the number of volatile compounds in a given mixture (stimuli) on the oviposition behavior of this species, it is likely that the decrease or lack of *E. ceratoniae* females' response to the different stimuli groups (mixture) is due to the antagonistic effect of the substances between them, or to the concentrations of these substances in the mixture. That is especially evident in the case of the stimulus Sb29 [Sb4= 14.89% (Linalool)+Sb7= 15.69% (α-Isomethyl ionone)], which did not attract moths at all, while was composed of two most attractive single compounds. According to Vucetic *et al*. (2014) the insects' reactions to certain individual substances differed when combined with others. Certain molecules have the ability to repel others, but other compounds have the ability to either mask or inhibit these effects (Bruce and Pickett, 2011). It is not always the case that a plant is attractive or repellent to insects just because it contains components that make it repellent or attractive, however, the volatile combinations affects the function of volatile compounds (Bruce *et al*., 2005).

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

We found that α-Isomethyl ionone; β-Thujone, α-Thujone and 2-Undecanone ketones had oviposition rates of15.69%, 11.70, 8.78 and 4.79%, respectively. The α-Isomethyl ionone compound is the most attractive of the 8 compounds tested. This compound has been described by Ishida *et al*. (2008) as an effective attractant to *Bractocera latifrons*. Similarly, Cáceres *et al*. (2016) reported the attractive oviposition effect of α-Isomethyl ionone on *Bemisia tabaci* while β-ionone has a deterrent effect.

In addition, the patent filed by Gabel *et al*. (1993), relating to attractive compositions of females of Tortricidae Lepidoptera, is characterized in that they include limonene, α-thujone and β-thujone as active ingredients. Moreover, the treatment of plant odors in antennal lobes of females of *Lobesia botrana* through the use of intracellular registration and coloring techniques has enabled (Masante-Roca *et al*. 2002), to note the involvement of β-thujone in the most common physiological responses in this species. Similarly, the characterization of the trichoid sensilla of *Culex quinquefasciatus* female reveals the effect of α-thujone, 2-Undecanone and linalool in the activity of odorous receptor neurons. According to Ehlers and Schulz (2022), linalool is a common semiochemicals released by flowers or leaves, involved in the full spectrum of plant–pollinator interactions. In combination with other floral volatile and visual cues, it elicits feeding responses in *Heliconius* butterflies (Andersson and Dobson, 2003), a complex interaction mediated by linalool between plant defense and insects' attractiveness (Raguso, 2016). Female-specific responses to (S)-(+)-linalool in the silk moth *Bombyx mori* and enantio-specific responses [higher sensitivity to (R)-(-)-linalool] in the noctuid moth *Mamestra brassicae* are reported by Anderson *et al*. (2009) and Ulland *et al*. (2006). Indeed, *Manduca. sexta* females oviposited more on plants emitting (+)-linalool, either alone or in combination (mixture), whereas plants emitting (−)-linalool (alone or in mixtures) were less preferred (Reisenman, *et al*. 2010). These results, in conjunction with the homologous olfactory receptor neurons that exhibit linalool-specific responses (Grosse-Wilde *et al*., 2011). Regarding N-methyl anthranilate, kairomonal activity was observed in three species of Lepidoptera, Nymphalidae (*Argynnis paphia*; *Argyronome ruslana* and *Damora sagana*) and two Hymenoptera (*Bombus hypocrite* and *Bombus diversus*) (Pellmyr, 1986). For butylated hydroxytoluene, Yi *et al*. (2018) screened 19 active compounds that act on the behavior of *Sclerodermus sp*; among these, butylated hydroxytoluene.

CONCLUSIONS

In summary, the information obtained in the current study, as well as the volatile cluster influences the oviposition rate (attractiveness) of a caterpillar pest. The study evaluated the effects of some volatile compounds released by three Algerian date cultivars (Deglet Nour Ghars and Degla Beidha). The compounds were identified by HS-SPME-GC-MS and tested with a wind tunnel for the oviposition rate of the insect. The compound chemical nature and their impact on the oviposition behavior of *E. ceratoniae* could open up other research paths to manage this pest. The reactions of the females of *E. ceratoniae* to the different stimuli expressed by the precise orientation and oviposition on the various sources of odors (synthetic substances) in the wind tunnel clearly reflects the impact of these volatile substances on the mobility of this species, in particular, on the selection of oviposition sites. Thus, advanced techniques, such as electrophysiological methods, are needed to clarify the electrical activity caused by *E. ceratoniae* sensilla stimulation by recording their reactions to the different compounds contained in each cultivar. These studies do not only elucidate allelochemicals and their behavioral mechanisms. Indeed, they also suggest a possible role for oviposition specific compounds to be used for future monitoring of *E. ceratoniae* field populations under natural conditions, and for attractive lures in an Integrated Pest Management (IPM) perspective, which can help to develop control strategies against this pest.

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ترکیبات آللوکمیکال میوه خرما و تأثیر آنها بر رفتار تخم‌ریزی پروانه برگ‌خوار Ectomyelois ceratoniae Zeller (Lepidoptera: Pyralidae)

یعقوب عارف، و نادیا لومبارکیا

چکیده

مقدار بروز مواد آللوشیمیایی بر رفتار تخم‌گذاری پروانه Ectomyelois *ceratoniae* Zeller (Lepidoptera: Pyralidae) بررسی شد تا محل مناسب برای تخم‌گذاری و منابع غذایی برای فرزندان آینده آن انتخاب شود. سه رقم خرمای الجزایری (غارسGhars ، دگلت-نور Deglet-Nour و دگلا-بیدها Degla-Beidha) بررسی شد. استفاده از Solid Phase Micro-Extraction از طریق میکرواستخراج فاز جامد و به دنبال آن روش کروماتوگرافی گازی-طیف‌سنجی جرمی، امکان شناسایی ۶۸ ترکیب را فراهم کرد که از این تعداد، تنها ۲۲ ترکیب در ارقام مورد مطالعه شناسایی شد. آزمایش رفتاری (behavioral test) با تونل باد، واکنش ماده‌های بالغ E. *ceratoniae* را به منابع مختلف بو، که از ترکیبات مختلف مواد مصنوعی شناسایی شده در سه نوع خرما ناشی می‌شد، بررسی کرد. مواد خالص (تک‌ترکیبی) در مقایسه با مواد موجود در مخلوط، بالاترین میزان تخم‌گذاری را نشان دادند. محرک‌های کتون با حدود ۴۱٪ از کل میزان تخم‌گذاری، جذاب‌ترین محرک بودند و پس از آن الکل‌ها (۱۵٪) و ترپن‌ها (7/44٪) قرار داشتند. استرها و فنول‌ها به ترتیب 6/38٪ و 5٪/58 تخم‌گذاری را نشان دادند. این نتایج می‌تواند مسیرهای تحقیقاتی دیگری را برای مدیریت این آفت و تأثیر آن بر گیاهان میزبان باز کند.