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Effects of The Allelochemical Compounds of the *Deglet Nour* Date on The Attractiveness of the Caterpillars of *Ectomyelois Ceratoniae* (Lepidoptera: Pyralidae).

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ABSTRACT

The date moth Ectomyelois ceratoniae Zeller (Lepidoptera: Pyralidae) is considered at present a permanent danger for date palm in Algeria. The polyphagia of this insect and its wide distribution on various hosts as well as the particular behavior of its neonatal caterpillars make this insect a major pest of the Algerian palm groves. In this context, the study of the attractiveness of this bio-aggressor by the extract of the Deglet Nour variety with hexane was tested on larvae in different stages. These extracts were identified and quantified by coupling gas chromatography / mass spectrometry (GC / MS).
The attractiveness tests carried out in a close chamber and in the olfactometer reveal that the older individuals are significantly attracted than the first and second larval stages by the odor of the Deglet Nour variety, this attraction is due to the presence of odorous molecules which attract this formidable pest. Chromatographic analysis has identified about fifty products that can play a very important role in the attraction of this insect.

Keywords: *Ectomyelois ceratoniae, odorous substances, attractiveness, Deglet Nour, GC / MS.*

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INTRODUCTION

Algeria, with its rich and diverse date palm heritage (around 17 million date palms, of which 14 million are productive potential), is one of the world's leading producers of dates. Moreover, it ranks first in terms of quality, thanks to the variety *Deglet Nour* which represents more than 50% of the total production (Benziouche & Cheriet 2012). In the arid and Saharan zones, the dates represent a providential fruit, one of the pillars of the oasis economy, providing the stability of the populations and ensuring a diversity of the food. Date palm is considered as the central hub around which economic life is structured (Dubost 1990 and Hadjeb et al. 2017).
Infestation of dates by the *Ectomyelois ceratononiae* moth poses a threat to the sustainability of the oasis ecosystem. According to Mehaoua et al. (2013), *Ectomyelois ceratoniae* can cause damage that can sometimes affect 80% of the harvest. Also, given the economic importance of this single exported crop, weathering of the date by infestation and desiccation is the main handicap to its long-term conservation. The market value of the date is depreciated by weight loss, cracking and browning of the fruit. It becomes fibrous and its taste and fruity odor disappear (Doumandji 1981).
The relationships between insects and plants have been established and then diversified to the point where half of the insect species are now phytophagous. Plants represent their food source, their shelter and their place of development, mating or laying. Faced with these phytophages, plants defend themselves by various physical means and chemically by synthesizing extraordinarily diversified secondary metabolites. They are often known for their toxicity to herbivores, and they profoundly affect the behavior of phytophagous insects (Masson & Brossut 1981 and Darazy-Choubaya, 2002).
In insects, the perception of volatile substances and their identification has been the subject of numerous studies (Vinson 1985 and Ouakid 2006). Particular attention was paid to volatile chemical compounds from the environment (host plant in particular). Some of these very comprehensive studies due to their interest in several biological levels (from the behavior with the molecule), made better understanding of the olfactory perception and its influence on the behaviors. For example, they established the molecular mechanisms of odor detection (Kaissling 1986; Kaissling 1987; Vogt 1987; De Kramer & Henberger 1987; Payne et al. 1987; Masson & Mustaparta 1990 and Pelosi & Garibotti 1993).
In the study of relationship between *E. ceratoniae* and the phenological stages of various host plants, Gothilf (1970) reported that females show an oviposition preference on carob pods infected with the *Phomopsis fungus*. Gothilf et al. (1975) demonstrated the impact of short alcoholic chains on female attraction of this pest at the time of oviposition. Also, in the United States, Cosse et al 1994 demonstrated that females from *E. ceratoniae*, in oviposition phase are attracted by substances emitted from some date cultivars.

Most of the work carried out on interactions of *Ectomyelois ceratoniae* - host plants, notably the date palm, is focused on the pest-host relationships mainly through describing the attack strategies developed by the pest during its life cycle in response, biological needs (nutrition and oviposition).

On the other hand, there is little information on the behavioral, physiological and / or chemical factors and mechanisms underlying this interaction between *E. ceratoniae* and the *Deglet Nour* date. For that, olfactometric tests were carried out on the spot to determine possible bioactive molecules (attractive or repulsive).

We chose working on the larvae of this lepidopteran. Although adults play a very important role in the selection of a host plant (Schoonhoven 1987 and Bernays & Weiss 1996), caterpillars are responsible for the damage (Glendinning et al. 1998).

MATERIAL AND METHODS

1. Mass rearing

Our breeding was conducted with a strain of *E. ceratoneae* which comes from the wormy dates of the year 2016 harvested from palm groves of the region of Biskra.

We placed infested dates in breeding cages in a room with controlled atmosphere (temperature: 28 ° ± 2 C, Relative humidity: 65% ± 10 and a photoperiod: 16 hours of light / 8 hours darkness). At emergence, the adults were caught, then they were put inside the mating jars without sexing.

After mating, the females laid inside the jars, the eggs were placed in a breeding medium (date flour, wheat bran and drops of water) previously prepared.

2. Preparation of extracts

All extracts were made from 4g of fruit taken randomly from the available date stock. Different pieces of fruit were taken from the surface (including skin), pulp and the area around the core. Three samples were made for the *Deglet Nour* variety. An apolar solvent was used, hexane (better extraction of apolar compounds) (4 ml per sample extracted). The extraction lasted 20 min at about 22 ° C and was vortexed for 30 sec every 5 min. After that, the obtained extracts filtered on glass wool and then concentrated to 50 µl under a stream of nitrogen. These were the extracts to test the attractiveness of caterpillars.

3. Chemical analysis

One microliter (1µl) of each sample was injected into a gas chromatograph coupled to a mass spectrometer (SHIMADZU 2010) at the Research Laboratory on Aromas of INRA Dijon FRANCE. The apparatus was equipped with a polar capillary column of fused silica type CPWax 58CB (60 m x 0.15 mm ID, phase thickness 0.15 microns, Varian). This type of column was selected following various tests on columns of polar type, apolar or intermediate polarity.

The applied temperature programming was as follows: 40 ° C to 300 ° C, 3 ° C / min, then 10 min at final temperature. The integration of the different peaks was performed automatically and the chemical nature of the different compounds determined by comparison with the mass spectra contained in the data bank of the device. Only a qualitative study of the chemical composition of the extracts was carried out.

4. Olfactory tests

The attractiveness tests were carried out according to two methods: The first tested the caterpillars in a glass aquarium (56 x 16 x 25 cm) wrapped by a glass cover. The soil was divided into three parts: a start zone (zone A: 6 cm), a path zone (zone B: 44 cm) and a zone where the odor source was deposited (zone C: 6 cm).The caterpillars were isolated one hour before starting the test. One to two drops of the test extract was deposited in C. The aquarium was closed for a few seconds and then the larvae was placed in zone A (Fig. 1.a). We then noted the latency (TL) put by the larva to leave the zone A and the time of arrival (TA), necessary to traverse zone B and to reach zone C (Runyon et al. 2006).

The second method was olfactometry; the larvae were tested in a Y-shaped glass tube with one inlet and two outlets (Fig. 1.b), the latter two being fed with a stream of compressed air. The extract was introduced at level 2 to let the odor diffuse, then the caps were placed on two outlets; next, the test individual was introduced at level 1, while level 3 was a control. We noted the time that the larva took to complete the path of the detection zone (this was the detection time), then the time that the individual took to reach the area where the source of the odor (this was the journey time). The sum of latency and journey time gave the overall time of attraction (Vet 1983).

All tests were performed at 25 ± 2 ° C and 70% -75% RH, in a dark room under low intensity red light. Each test was repeated 25 times on each larval stage. The times were compared using the Kruskall-Wallis test. Some results were refined by multiple step-by-step comparison (Scherrer 1984).

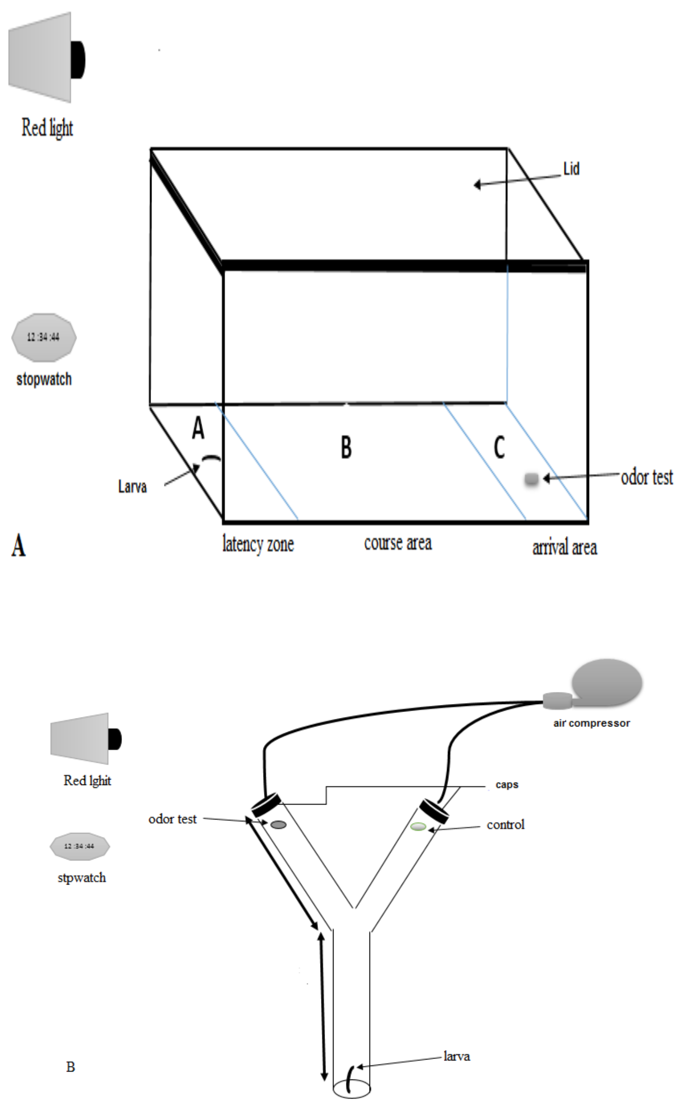

Figure 1. Olfactory test device (A. Aquarium - B. Olfactometer)

RESULTS

1. Chemical analysis

The different identified compounds (Fig.2 and Tab.1) were part of different chemical classes: hydrocarbons (saturated or unsaturated), aldehydes, acids (saturated or not), esters and terpenes. Two peaks (peaks A and B) could not be identified; it would be two unsaturated hydrocarbons. Four major compounds were identified in *Deglet Nour* dates, they were the heaviest compounds that we extracted, they were phytohormones (1,30-Triacontanediol, Olean-12-in-3-one, Lupenone, Lupeol) . A number of extracted compounds (peaks 7, 11, 12, 14, 22, 30, 32, 34, 35, 38) have already been demonstrated in core and flesh oil extracts of different varieties of date (Fayadh & Al-Showiman 1990). Others have been identified in the volatile fraction emitted by the fruits dates during their maturation (peaks 4, 6, 9, 12, 15, 16, 21, 22, 29, 31, 32, 34, 35, 38, 46) (Besbes et al. 2004; El Arem et al. 2011 and El Arem et al. 2012).

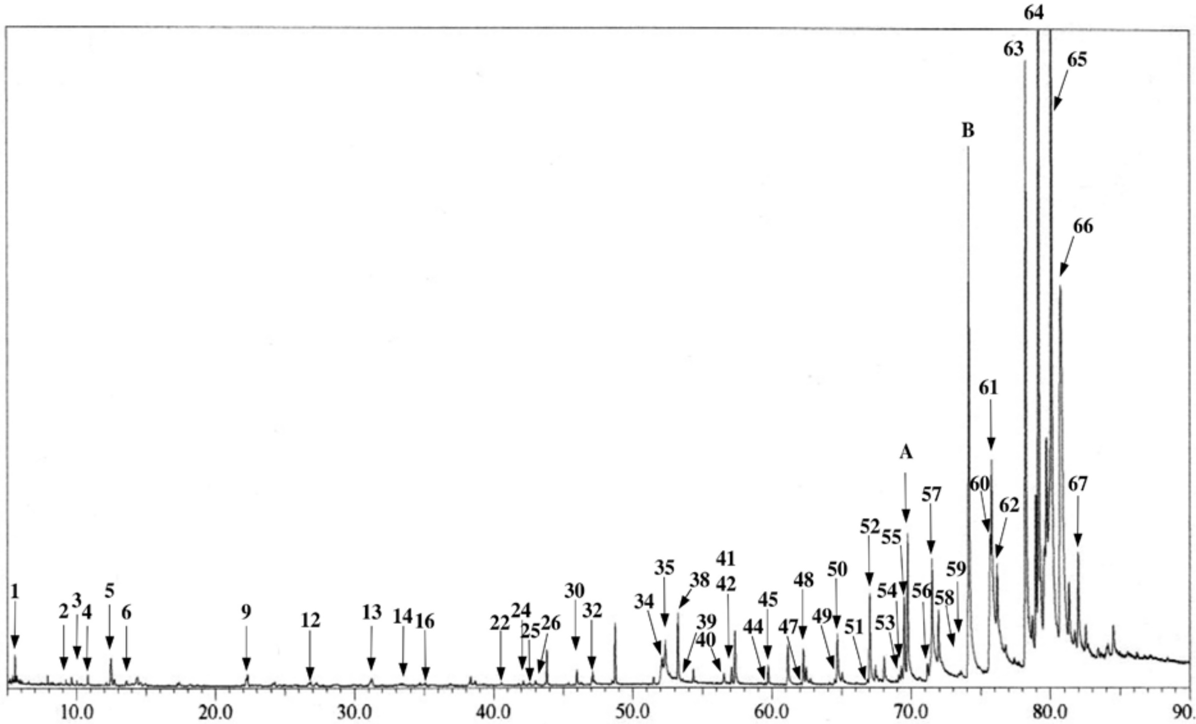

Figure 2. Chromatogram of the extract of *Deglet Nour* Variety with Hexane.

Table 1. Chemical analysis and quantification of the various compounds present in the extracts of the *Deglet Nour* variety. +++,> 200 ng / mg of fruit; ++, 5 to 20 ng / mg of fruit; +, 1-5 ng / mg of fruit; - <1 ng / mg of fruit

Peak's number	Substances	Signification	*Peak's number*	Substances	Signification
Volatile fraction			44	9-Tetracosene	-
1	4-methyloctane	-	45	Tetracosane	-
2	Hexanoicacid	-	47	9-Pentacosene	-
3	Decane	-	48	Pentacosane	-
4	Limonene	-	49	9-Hexacosene	-
5	Dodecane	-	50	Hexacosane	-
6	Nonanal	-	51	9-Heptacosene	-
9	Pentadecane	-	52	Heptacosane	-
12	Ethyldecanoate	-	53	9-Octacosene	-

13	Dimethyl-ethylphenol	-	54	Octacosane	-
14	Dodecanoicacid	-	55	Squalene	-
16	Hexadecane	-	56	9-Nonacosene	-
22	Myristicacid	-	57	Nonacosane	-
24	Tetradecanal	-	58	9-Triacontene	-
25	Isopropylmyristate	-	59	Triacontane	-
26	Pentadecanone	-	60	9-Hentriacontene	-
Heavy fraction			61	Hentriacontane	-
30	Palmitoleicacid	-	62	2-Nonacosanone	-
32	Palmiticacid	-			
34	Linoleicacid	-	**Phytohormone**		
35	Oleicacid	-	63	1,30-Triacontanediol	++
38	Stearicacid	-	64	Olean-12-en-3-one	++
40	Parsol	-	65	Lupenone	++
41	9-Tricosene	-	66	Lupeol	+
42	Tricosane	-	67	Lupenylacetate	-

2. Biological tests of attractiveness

Our results show that the older individuals (stages L4 and L5) were significantly better attracted than the first two larval stages (L2 and L3) by the smell of the *Deglet Nour* variety in olfactometer and closed enclosure, this attraction was due to the presence of odorous molecules that attract *Ectomyelois ceratoniae* (Tab.2).

Older larvae took less time to escape from the enclosed latency zone (32.87 ± 4.63 for L4 and 40.92 ± 6.20 for L5), the older individuals also spent less time to detect the source of odor in olfactometer (156.43 ± 5.81 L4 and 156.86 ± 10.44 L5).

The molecules extracted from the *Deglet Nour* variety were attractive to *E. ceratoniae*, they were essentially apolar in nature since they were extracted with hexane. The feeding behavior of the larvae began with the detection of the odor, then the attractiveness of the individual, the larva went towards the odoriferous source.

Table 2. Time of attraction of *Ectomyelois ceratoniae* by the extract of the date *Deglet Nour* in closed enclosure and in olfactometer (a, b and c: homogeneous groups).

	Aquarium		**Olfactometer**	
larval stage	**Latency time**	**Travel time**	**Detection time**	**Journey time**
L2	79,35±14,91a	238,05±31,70a	410,05±12,55a	194,41±17,94a
L3	52,04±16,04ab	110,54±19,01b	212,85±8,91b	121,00±9,17b
L4	32,87±4,63^{b}	83,75±7,69^{b}	156,43±5,81^{c}	116,56±8,60^{b}
L5	40,92±6,20^{b}	79,83±10,37^{b}	156,86±10,44^{c}	111,45±6,37^{b}

DISCUSSION

The date content of primary metabolites has been extensively studied. This fruit is rich in sugars and dietary fiber, contains significant amounts of vitamins and minerals, but is low in fat and protein (Al-Farsi et al. 2005; Baloch et al. 2006 and Farahnaky & Afshari-Jouybari 2011).

Studies on its content of secondary metabolites, especially phenolic, remain modest and do not concern Algerian varieties, these metabolites are beginning to be very extensive and their interests are emphasized day by day, given their therapeutic potential and their implications in many activities. Indeed, some studies have highlighted the role of polyphenols in the antioxidant activity of the date (Vayalil 2002; Mansouri et al. 2005 and Al-Farsi et al. 2007). Others have attributed an anti-microbial, anti-inflammatory, anti-cancer and antiviral effect to phenolic compounds in general (Hagerman 1988; Fine 2000 and Vermerris & Nicholson 2006). Recently, Chaira et al. (2009) also found that among the common date cultivars of Tunisia, the *Korkobbi* variety had the highest phenolic compound content (54.66 mg / 100 g fresh weight) while the *Mermella* variety had the lowervalue (5.73 mg / 100 g fresh weight).

However, there is very little information on the behavioral mechanisms associated with the search for nutrients at different larval stages (Etilé 2008), for which Boulard (1988) indicated that the nutritional requirements of an insect vary according to the stage of development.

The signals used by insects to locate their host can be in different types: chemical, visual, tactile and acoustic signals (Kevan & Baker 1983; Dobson 1994; Roy & Raguso 1997 and Raguso and Willis 2002). These signals will be perceived more or less long distance according to their nature. Thus, visual and tactile signals are important in the location of the host, but will be used at close range. The most common signals used by insects to locate their host remotely are of the chemical type, and therefore correspond to volatile organic compounds. The transmission of information through chemical communication between two partners is called chemical communication. It is involved in a large number of interactions between plants and insects, such as pollination or herbivory, when locating the host in a far distance (Visser 1986 & Dobson, 1994).

CONCLUSION

Our work focused on the biological analysis of interactions between *E. ceratoniae* and the *Deglet Nour* date. It is clear that it is a starting point and will be necessary to follow other attractiveness tests on several varieties of dates and with other solvents.

Although plant nutrients also play a role, it is generally considered that secondary substances are directly responsible for the diet selectivity of phytophagous insects. This selectivity is associated with metabolic adaptations but also with behavioral capacities allowing the pests to find their host plants but also to avoid those which do not suit them. These insects have developed a chemosensory system that allows them to recognize and respond to this diversity of plant secondary metabolites.

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