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Ozone gas greatly reduced the survival of carob moth larvae in stored date palm fruit

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Abstract - Among the vast insect pest attacks suffered by date palm, the carob moth (*Ectomyelois ceratoniae*) is the most destructive causing the largest damages every season. Methyl bromide is the most effective insecticide for date palm but its use will be shortly forbidden and sustainable alternatives must be found. The current work firstly evaluated the efficacy of the eco-friendly ozone gas treatment against *E. ceratoniae* at larvae stage on intentionally infected Deglet Nour dates. Results showed that the mortality of *E. ceratoniae* depended on the ozone level and the exposure time. In fact, with 12.2 mg L^{-1} for 80 min the carob moth mortality was ten-fold higher (82 ± 3%) than in control samples (8 ± 3%). This allows advancing in knowing the effect of ozone gas as an emergent alternative to methyl bromide on larvae of *E. ceratoniae* and on its possible application at commercial scale by the handling industry of fresh date palm. But, further studies are required to reach a 100% of carob moth mortality.

Keywords: Postharvest sustainable fumigant / *Ectomyelois ceratoniae* / Alternative to methyl bromide / Deglet Nour cv.

1. Introduction

Ectomyelois ceratoniae (Lepidoptera: Pyralidae) Zeller, commonly known as carob moth or moth of pyrale due to its ubiquity, polyphagia and polychromy, has received several names such as *E. ceratoniella*, *pryerella*, *oporedestella*, *zelleriella* or *phoenicis* and *Spectrobates ceratoniae Z.* (Dhouibi 1982). The carob moth attacks fruit on the tree in many areas of the Mediterranean basin (mostly in North Africa and Middle East), Southern Russia, South West Asia, America and Western Australia (Dhouibi 1982). The moth also infests stored products, especially dried fruits, nuts, and seeds (Abo-El-Saad et al. 2011), reducing its quality and leading to important economic losses. In Tunisia the presence of *E. ceratoniae* has been reported everywhere from north to south (Dhouibi 1982), being the most important and destructive insect pest attacking date palm (*Phoenix dactylifera*) mainly Deglet Nour, the most produced cv. (Mediouni Ben-Jemâa et al. 2004). In fact, that pest causes in Tunisia up to 20 % of date palm damage during cropping season and throughout postharvest life (GIFruits 2009), while date yield losses increase up to 30% in Morocco (Bouka et al. 2001). It has been reported that in the USA *E. ceratoniae* infests 10–40% of the harvestable dates annually (Farrar 2000).

Methyl bromide (CH_3Br) is the most widely used postharvest fumigant as quarantine treatment against *E. ceratoniae* and other insects of Tunisian dates at harvest time (Hassouna et al. 1994). But, due to its harmful effects on human health, as well as on the environment, the use of CH_3Br is scheduled for worldwide withdrawal application in 2015, under the directive of the Montreal Protocol on ozone-depleting substances. For that reason, the search of commercial alternatives is needed. Among them, to protect plant and animal health and commodities trading (fruit, vegetables and grains) from different insects, phosphine -PH_3-, sulfur dioxide -SO_2-, carbon sulfate -CS_2-, carbon dioxide -CO_2- alone or mixed with ethylene oxide -C_2H_4O-, microwaving, freezing, irradiation, heat treatment, ultraviolet (UV-C) radiation, and ozone (O_3) among others, have been reported (Aegerter and Folwell 2000; Bell 2000; Leesch et al. 2003; Erjaee et al. 2006; Abo-El-Saad et al. 2011; Ben-Lalli et al. 2013).
The insecticidal effect of O_3 is due to a combination of its high oxidation potential and its ability to diffuse through biological cell membranes. Upon released, O_3 is very efficient in destroying microorganisms and avoiding their growth by the progressive oxidation of vital cell components, it has a potential use as an insecticide mainly for food-stored products (Whangchai et al. 2006; Abo-El-Saad et al. 2011; Niakousari et al. 2010). The O_3 can be generated on-site, being able to extend the shelf-life of many intact and minimally processed fruit and vegetables, while leaving no residues since it decomposes to O_2 quickly (Restaino et al. 1995; Parish et al. 2003; Guzel-Seydim et al. 2004; Artés et al. 2007). That degradation constitutes both a challenge for practical application and a benefit for the environment (Hansen et al. 2013). It has a Generally Recognized as Safe status as a food-processing aid agent for the treatment, storage and processing of foods, approved for use in direct contact with them under Good Manufacturing Practices (FDA 2001; Suslow 2004; Artés et al. 2007). Consequently, the O_3 could be used in post-harvest Integrated Pest Management programs. In that way, in a preliminary study we found that dipping for 2 min in ozonated (0.6 ppm O_3) water at 15ºC induced a *E. ceratoniae* mortality in naturally infected Deglet Nour dates of about 15% compared to about 26% in control fruits after 30 days at 20ºC (Jemni et al. 2014).
The aim of this work was to evaluate the effect of O_3 gas at different concentrations and different exposure times on the survival of *E. ceratoniae* at larvae stage in intentionally infested Deglet Nour dates. To the best of our knowledge, this is the first report about the use of O_3 gas to control *E. ceratoniae* on date palm.

2. Material and methods

2.1. Origin of dates and carob moth

Dates of Deglet Nour cv. were selected because it is the most produced date palm cv. in Tunisia. The bunches of dates were hand harvested by professional pickers at the end of October at fully mature ('Tamar' stage) from a commercial farm located in the Oasis of the Governorate of Kebili (South of Tunisia). The bunches were placed on the ground to avoid crushing and the abscission of dates. Each bunch was then cut into spikelets and about 20 kg were placed in polystyrene boxes and transported around 500 km by car at ambient temperature to the Laboratory of Entomology of the National Institute of Agronomic of Tunisia. Dates were manually detached from the spikelets and carefully inspected, being only sound dates intentionally infested by one carob moth at larvae stage (Stage 5). About 15 kg of infested dates were then placed in ventilated polystyrene box and transported then by plane to Madrid (Spain), and finally by car around 400 km to the Pilot Plant of the Technical University of Cartagena. Total transport duration was about 7 days.

2.2. Ozone generation

An ozonated air-flow was obtained from an industrial O_3 generator producing 0.6 mg s^{-1} (AMBICON, Murcia, Spain). The O_3 concentrations were monitored with a sensor (EcoSensors, Inc., A-21ZX model, Santa Fe, NM, USA) that was sited inside a hermetically sealed glass jar (Artés-Hernandez et al., 2003). Twenty carob moth infested dates were placed into each glass jar of 1000 mL, which were exposed to a continuous O_3 enriched-air flow as described in Table 1. One glass jar was considered as a replicate, and for each experiment three replicates were used.

Table 1. Central composite design arrangement and results of percentage of carob moth mortality. Data are means (n = 60) ± SD

Trial n°	Factors		Y= % carob moth mortality
	Z_1 (ppm)	Z_2 (min)	
1	1	30	23.34±2.8
2	5	30	31.67±2.6
3	1	60	28.34±2.8
4	5	60	45±4.5
5	3	45	30±3.76
6	0.57	45	20±5
7	5.43	45	33.34±3.6
8	3	26.775	25±5
9	3	63.225	35±4.7

All treated samples were then incubated at 28°C and 75% RH in air for 9 days. As control, untreated samples were used. The carob moth mortality, expressed in percentage, was calculated by the equation (1).

The carob moth mortality = $(1- N_d/N_s) \times 100$ (%) (1)

Where N_d was the number of carob moth dead after incubation, and N_s was the total number of carob moth (N_s = 20).

2.3. Experimental design

An orthogonal central composite design (CCD) for two factors was performed in order to determine the optimal conditions of moth mortality. The variables investigated were the O_3 flow concentration and the exposure time to O_3. Each variable at five coded levels (indicated as -1.215, -1, 0, 1, +1.215 as shown in Table 2) was designed as experimental runs using the Analysis Tool Pack 'linear Regression' in Excel 2007. The real values of the independent variable (Z) were coded according to equation (2).

Table 2 Experimental domain and distribution of variables used for the evaluation of the carob moth mortality

Factors	Symbols		Level*				
	Coded	Uncoded	-1,215	-1	0	+1	+1,215
$[O_3]$ ppm	X_1	Z_1	0.57	1	3	5	5.43
Time (min)	X_2	Z_2	26.775	30	45	60	63.225

*$X_1 = (Z_1 - 3)/2$, $X_2 = (Z_2 - 45)/15$

$$X_i = (Z_i - Z_i^{\circ}) / \delta Z_i \quad (2)$$

Where Z_i is the real value of the independent variable; Z_i° is the central value; X_i is the coded value of the independent variable given by the experimental matrix. Z and X values are shown in Table (2).

The experimental response was measured as the percentage of moth mortality, which was estimated taking into account the influence of the experimental factors. A full quadratic model containing 6 coefficients was used to describe the responses observed and to fit the equation (3).

$$Y_{\text{moth mortality}} = a_0 + a_1 \times X_1 + a_2 \times X_2 + a_{12} \times X_1 \times X_2 + a_{11} \times X_1 \times X_1 + a_{22} \times X_2 \times X_2 \quad (3)$$

Where a_0 is constant, a_1 and a_2 are linear coefficients of O_3 flow (X_1) and time of exposition to O_3 gas (X_2), a_{12} was the cross product coefficients and a_{11} and a_{22} were the quadratic coefficients. The attained fit of second order equation was checked by the coefficient of determination R^2. The analysis of variance and the estimation of response surface by multiple linear regressions were performed using the Analysis Tool Pack 'Linear Regression' in Excel 2007.

2.4. Physicochemical parameters and sensory evaluation

Physical and chemical quality of dates were evaluated by analyzing firmness (N), pH, titratable acidity (TA), expressed as g of citric acid on kg^{-1} fresh weight, moisture (g kg^{-1} fw), water activity (aw), and color, based on CIELab* scale.

Measurement of mentioned quality parameters was accomplished as previously described by Jemni et al. (2014). Analyses were performed on dates treated with the optimum concentration of ozone and on a control sample without treatment.

Visual appearance, color, texture, flavor and overall quality were evaluated based on a five-point hedonic scale (1: extremely poor, 2: poor, 3: acceptable and limit of usability, 4: good and 5: excellent). These sensory attributes were evaluated by a trained panel (6 members ranging between 25 and 65 years) over a representative sample coming from dates treated by O_3 and control dates.

All parameters were determined on three samples. Average of these samples determinations is presented. Statistical analysis was performed with Info Stat (version 1). Analysis of variance (ANOVA) and LSD test were applied in order to evaluate the influence of ozone treatment. A least significant difference (LSD) multiple range test at 5% probability level was used to determine significant differences between means.

3. Results

3.1. Predictive response model

The matrix of the effects, which shows the tests and the responses of composite design of experiments, is given in Table 1. A carob moth mortality for control sample of 8 ± 3% was found.

The Analysis Tool Pak 'Linear Regression' provides statistical regression and analysis of variance (P= 0.04). The coefficient of determination R^2 was 0.95, being close to 1, corresponding to a model of good quality. The coefficients values were calculated and tested for their significance. The P values are commonly used as a tool to check the significance of each coefficient. The model was represented by equation (4).

$$Y_{\text{moth mortality}} = 28.15 + 5.93 \times X_1 + 4.38 \times X_2 + 2.08 \times X_1 \times X_2 + 2.44 \times X_2 \times X_2 \quad (4)$$

As expected, it was found that the O_3 concentration (X_1) ($P=0.0116$) and the duration of the treatment (X_2) ($P=0.0264$) were the most important factors (a_1 = 5.93; a_2 = 4.38) on carob moth mortality, while the interaction between O_3 concentration and time ($X_1 \times X_2$) (P = 0.023)and the interaction only with time ($X_2 \times X_2$) ($P=0.027$) were also relevant factors (a_{12} = 2.08 and a_{22} = 2.44). However, the interaction with O_3 concentration only ($X_1 \times X_1$) was not significant (P = 0.926).

3.2. Trace of the response surface

The response was a hyperbolic parabolic surface which is designated in a more imaged by 'horse saddle' (Fig. 1). The response surfaces of the regression equations were obtained by using the Analysis Tool Pak 'Linear Regression'. The region where the response changed little despite significant variations in the factors was located around a stationary point which the coordinates were found by differentiation.

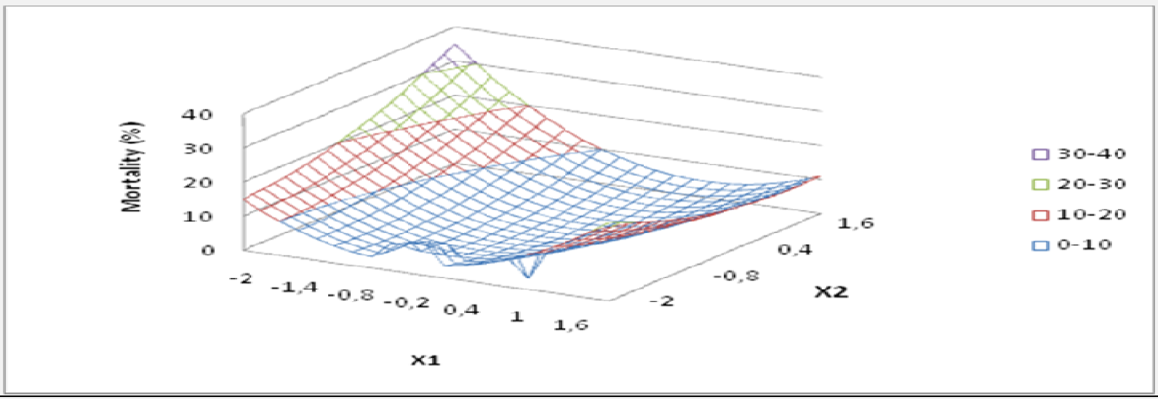

Figure 1. Isoresponse curves showing the zone of stability of carob moth mortality.

The solver provided an optimum dose of 12.2 mg L^{-1} for 80 min. The theoretical response optimized by the solver (99.995% mortality) and the target responses (100% mortality) does not show a significant difference.

3.3. Validation of the optimum

Before recommending these results for commercial application, it was necessary to perform several new experiments for validation. In this way, the application of a continuous flow of 12.2 mg L^{-1} for 80 min on 20 dates infested by carob moth, replicated three times, gave a moth mortality of 82 ± 3 %.

3.4. Physicochemical parameters and sensory evaluation

As shown by table 3, the treatment by ozone did not affect the physicochemical parameters of dates. In fact, they maintained the pH, acidity, moisture, firmness and aw. But, there is a decrease in values of color parameters (L*, Chroma and Hue°) as compared with the control. This later could be explained by browning reaction caused by O_3.

Table 3 Effect of ozone on skin color parameters, pH, acidity (g citric acid/100g FW), firmness (Newton), moisture (%) and aw of Deglet Nour date treated by a continuous flow of 12.2 mg L^{-1} of ozone for 80 min.

		Control	**O_3**
Color	L*	32.07±2.4 a	30.54±2.8 b
	Chroma	20.04±2.72 a	17.97±4.6 b
	Hue°	62.3±4.8 a	60.92±7.6 b
Acidity		0.113±0.107 a	0.117±0.001 a
pH		5.53±0.33 a	5.51±0.27 a
Moisture		20.29±0.001 a	21.27±0.82 a
Firmness		6.001±0.80 a	5.58±0.54 a
aw		0.64±0.003 a	0.63±0.011 a

Data are means (n = 3) ± SD. Means followed by different letters in the same line are significantly different ($p \leq 0.05$) according to LSD test.

In addition, overall quality, texture and flavor were maintained after treatment by a continuous flow of 12.2 mg L^{-1} of ozone for 80 min. The color was appreciated by tasters in spite of the brown color (Figure 2).

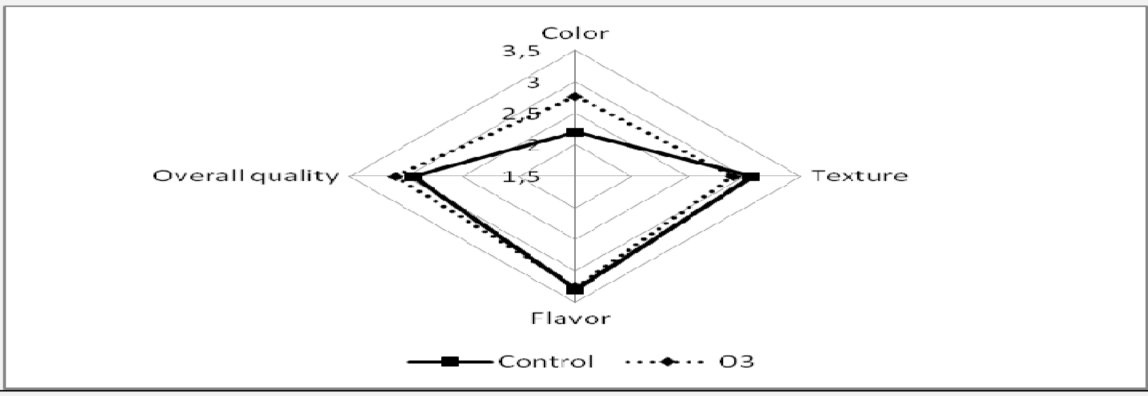

Figure 2 Changes in flavor, texture, color and overall quality of *Deglet Nour* date treated by a continuous flow of 12.2 mg L^{-1} of ozone for 80 min. Data are means (n = 3) ± SD.

4. Discussion

The current study showed that O_3 greatly disturbs the survival of *E. ceratoniae* larvae. In control dates a low natural carob moth mortality of 8 ± 3% was found while the rise in O_3 concentration in the air flow and in the exposure time increased mortality. This could be explained by the O_3 effect on lowering the respiration rate of insects (Baoqian et al. 2009) as well as by the fact that O_3 acts as a toxic which can cause oxidative damage on tissues even at low concentrations (Liu et al. 2007). According to our results both the O_3 concentration and the exposure time were very important factors influencing the *E. ceratoniae* mortality rate.
The achievement of 100% mortality derived from the theoretical responses optimized by the solver, *E. ceratoniae* requires an O_3 concentration of 12.2 mg $L^{-1}$for 80 min. However, the application of this result in experimental gave a moth mortality of 82 ± 3%. Abo-El-Saad et al. (2011) have proved that 2 mg L^{-1} O_3 for 12 h was effective against the different developmental stages of *Ephestia cautella* reaching near 90% of adults' mortality, being larvae less sensitive than adults, where mortality was about 30%**.** Erjaee et al. (2006) showed that an exposure of Kabkab dates to 50 g O_3/day for 30 min at 20ºC killed almost 90% of the adults and larvae of *P. interpunctella*. More recently, Niakousari et al. (2010) have proved that exposing samples to higher than 2000 mg L^{-1} O_3 for 120 min resulted in complete mortality of larvae and adults of Indian meal moth *P. interpunctella* in Kabkab dates. However, the mortality obtained in wheat for *T. castaneum* and *P. interpunctella* under a continuous O_3 flow of 13.9 mg L^{-1} for 120 min as higher than in the experiments presented here (Isikber and Oztekin 2009). This could be explained by the different species of insects and by the concentration and contact time between O_3 and the insect.
The physicochemical and sensorial qualities of dates treated by a continuous flow of 12.2 mg L^{-1} of ozone for 80 min were maintained and the values reached for each parameter agree with those found by Jemni et al. (2014), El Arem et al. (2011), Besbes et al. (2009) and Achour et al. (2003).
As in fact 100% of mortality was not achieved in the current work, complementary experimental studies, must be conducted. It should be also studied the combination of O_3 gas with other techniques, like high CO_2, within the frame of the hurdle technology, as early showed by Leesch et al. (2003).

5. Conclusion

As main conclusion, the O_3 gas greatly lowered the survival of carob moth larvae in Deglet Nour date palm fruit during commercial storage. The O_3 gas showed a positive effect on E. ceratoniae mortality despite the presence of pulp dates as a border isolating the direct action of O_3 on the moth. In fact, with 12.2 mg L^{-1} for 80 min the carob moth larvae mortality was about ten-fold higher than in control samples. Further studies should be conducted to optimize these preliminary results, to examine the O_3 gas effects on E. ceratoniae at different stages of growth, and on its application at commercial scale. The current results provide encouragement in finding an emergent alternative to CH_3Br useful for the industry of fresh date palm around the world.

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