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Comparative Efficacy of CO₂ and Ozone Gases Against *Ephestia cautella* (Lepidoptera: Pyralidae) Larvae Under Different Temperature Regimes

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ABSTRACT. Comparative efficacy of three different modified atmospheres: $100\% \text{ CO}_2$, $75\% \text{ CO}_2 + 25\% \text{ N}_2$, and 22 ppm ozone were examined against larval mortality of the almond moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) at temperature regimes of 25°C and $35 \pm 2^{\circ}\text{C}$ and $60 \pm 5\%$ relative humidity, and 9:15 dark and light. Wandering young larval instars, which are fast growing, large enough in size and considered as more tolerant to modified atmosphere, were collected directly from the rearing culture, placed inside pitted date fruits of vars.: "Khudri," "Ruziz," and "Saqie," were treated with aforementioned gases for 24, 48, and 72 h. The immediate and delayed larval mortality was recorded after each exposure timing. Ozone possessed the strongest fumigant toxicity causing 100% mortality with all varieties, at 25 and 35°C after 24 h exposure and was more effective than $75\% \text{ CO}_2$ that caused 83 and 100% immediate mortality with variety ruziz at 25 and 35°C , respectively. Extending the treatments exposure time to 72 h, 100% mortality was recorded by exposing larvae to any of the studied gases at $25 \text{ and } 35^{\circ}\text{C}$. These results suggest that gases and temperature used in this study can be effectively used to control *E. cautella* in dates and stored grains.

Key Words: date palm, storage, modified atmosphere, food processing, Saudi Arabia

Date palm *Phoenix dactylifera* (L.) is among the oldest crops that have been grown in arid and semiarid regions of the Arabian Peninsula (Chao and Krueger 2007). In the Middle East, the average date consumption is estimated to be 20–30 dates per day (Al-Shahib and Marshall 2003). In Saudi Arabia, there are 23 million date palm trees grown on an estimated area of 161,975 hectares, with an annual production of 1.1 million tons of date fruits, worth 2 billion Saudi Riyals/yr (MOA 2011). Prior to marketing, harvested date fruits are often stored and a variety of treatments, including fumigation, are used to control insect pests in storage (Al-Abbad et al. 2011).

Several serious pests can infest date fruits causing economic losses. These pests include almond moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) and sawtooth grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Cucujidae) (Al-Zadjali et al. 2006). *E. cautella* is an important pest in warm climate (Navarro and Gonen 1970) that damages date fruits, cereals, dried fruits, and nuts (Arbogast et al. 2005). *E. cautella* infestation occurs in both orchard and storage. Conventional control methods like methyl bromide and phosphine gases have been widely used as an effective and cheap sources of fumigation for stored products. However, excessive use of these chemicals poses some environmental concerns. Methyl bromide has been declared as an ozone depleting chemical, and is being phased out of production and use in 2015 (USEPA 2014).

The use of modified atmospheres is a logical potential alternative (Brandle et al. 1983, Soderstrom et al. 1986, Donahaye et al. 1994, Riudavets et al. 2009, Navarro 2012). The efficacy of 80% CO₂ and 20% N₂, for 12 h exposure, at 32.2°C showed 100% mortality of Indian meal moth *Plodia interpunctella*, (Hubner) (Lepidoptera: Pyralidae) pupae (Sauer and Shelton, 2002). Similarly, ozone efficacy of 5–45 ppm for 3 d exposure showed 92–100% mortality of three stored product pests in maize; red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), maize weevil, *Sitophilus zeamais* (Motsch) (Coleoptera: Curculionidae) adults; and *P. interpunctella*

larvae (Kells et al. 2001). Ozone (2.0 ppm) efficacy for 12 h exposure showed 83 and 27% mortality of *E. cautella* adults and larvae, respectively (Abo-El-Saad et al. 2011). However, there is little information on the efficacy of modified atmosphere against *E. cautella* larvae on artificially infested dates. To enhance this knowledge, this study has a potential and value but its effectiveness is notably varied according to the increase in temperature and exposure time levels.

Aim of this study was to test the efficacy of various gases such as 100% CO₂, 75% CO₂ + 25% N₂, and ozone against the *E. cautella* larvae, under different temperature regimes and exposure times. Because of their potential against stored products pest and environment friendly nature, these gases could be considered a possible alternative to methyl bromide for the fumigation of stored date fruits.

Materials and Methods

Insects. *E. cautella* culture was maintained at the Economic Entomology Research Unit (EERU), Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia. The colony was maintained in an environmental chamber at $25 \pm 2^{\circ}$ C and $65 \pm 5\%$ relative humidity, and 9:15 dark and light on an artificial diet media developed by Al-azab (2007). Larvae (13 to 15 d old; 4th instar) (Aldawood et al. 2013) were gently removed from the colony and transferred into already prepared pitted dates.

Containers Preparation and Artificial Infestation of Dates. Airtight 1.9 liter capacity (64 oz) (Lock and lock absolutely, Vietnam) plastic containers (dimensions, 8.1 by 5.3 by 4.6 inches) were used as fumigation chambers. For introduction and evacuation of gas/air valves were put in place. Date fruits of vars. "Khudri," "Ruziz," and "Saqie"were bought from the local date market in Riyadh, Saudi Arabia. All date fruits were sorted and only best quality dates were chosen for the experiment. The caps and seeds from the date fruits were removed by one longitudinal cut.

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One larva was manually placed inside the internal cavity of each pitted date fruit, and 20 dates containing larvae, were transferred to a cloth bag. A cloth bag containing infested dates was prepared for each of the three varieties and then three bags were placed inside each container. Containers were wrapped with a cling film plastic (12.5 μm thick), lids of containers were tightened, containers were transferred to an environmental chamber maintained at 25°C and 65% relative humidity, and 9:15 dark and light. Containers were incubated for 3 d for larvae to acclimate prior to being exposed to experimental treatments.

Gas Introduction. Gases were purchased locally from "Abdullah Hashim Industrial Gases & Equipment Co. Ltd." (Riyadh, Saudi Arabia, AGH). After acclimation of the larvae, the infested dates were exposed to three different modified atmospheres, namely, 100% CO₂, $75\% \text{ CO}_2 + 25\% \text{ N}_2$, 22 ppm ozone, and control (ambient air). Larvae were exposed for three durations, namely, 24, 48, and 72 h, at temperatures of $25 \pm 2^{\circ}$ C and $35 \pm 2^{\circ}$ C. There were four replicates in each treatment, each replicate having 20 pitted date fruits of each variety having a larvae inside. The relative humidity was maintained at $65 \pm 5\%$ during the experiment and 9:15 dark: light conditions. For CO₂ introduction, the outlet valve of the container was opened and a tube was connected with a gas cylinder and attached to the inlet valve. As the gas was introduced through inlet valve, air came out from outlet valve and after 10–15 s the gas concentration was measured from outlet valve with a check point (PBI- Dan sensor, Denmark). Before measuring the gas sensor was stabilized at ambient temperature and caliberated right before each measurement series. When the sensor indicated the required concentration, tube was removed and both valves were tightly closed. In the control treatment, ambient air was introduced into the containers as already described for CO2 introduction. After introduction of the gases, containers were placed in the respective environmental chambers maintained at 25 and 35°C and 65% relative humidity.

For each treatment a separate but similar type of environmental chamber was used. In the ozone treatment, containers were kept open inside the environmental chamber. An ozone generator (Air-zone ozone generator T-6000 S Xetin, Taiwan) placed inside the environmental chamber was used to generate ozone. There was an opening in the environmental chamber that was used to lead through the electric wires and an outlet pipe to measure the ozone concentration. Ozone concentration was measured with EC-P2 gas detector (Honeywell, China). Then containers were placed to their respective environmental chambers at 25 and 35°C and 65% relative humidity. Ozone generator was turned off when 22 ppm ozone concentration was recorded. After each exposure timing, the relevant treatment was taken out and mortality was recorded. After recording the immediate mortality, infested dates were maintained under normal conditions (25 \pm 2°C and 65 \pm 5% relative humidity, and 9:15 dark and light) until mortality was assessed.

After the respective exposure timings, containers were taken out and larval movement was immediately evaluated. Mortality rate was recorded daily until all the larvae in the control treatment were found to be molted into pupae. Larvae which showed no movement, changed color, and had stiff shrunken bodies were considered dead.

Statistical *Analysis.* Larval mortality was corrected by using the formula developed by Abbott (1925). Data were analyzed by using the GLM Procedure of SAS Institute (2009), with larval mortality as response variable and gases as main effects. Means were separated using Tukey-Kramer honestly significant difference (HSD) test at P < 0.05.

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In general, the ozone treatment caused 100% larval mortality in all three varieties after 24 h of exposure and above at both temperature regimes (Tables 1 and 2). After 24 h of exposure at 25°C with variety "Khudri" a significant differences were observed in the larval mortality among the three gases levels that were examined (F = 10.3; df = 5, 23; P = 0.0001). Similarly, significant results were noted in the larval mortality at 25°C with variety "Ruziz" (F = 8.55; df = 5, 23; P = 0.0003)

and "Saqie" (F = 8.58; df = 5, 23; P = 0.0003) (Table 1). Hence, significantly more larvae were dead at 35°C than at 25°C. In contrast, mortality at 25 and 35°C did not differ significantly among three date varieties.

Data showed that ozone treatment killed all the *E. cautella* larvae only after 24 h exposure. As with 24 h exposure, ozone treatment for 48 h resulted in complete mortality in all larvae at both temperature regimes. However, a significant difference was observed among all gasses in the immediate and delayed mortality at 25°C. Delayed mortality was at least as high in the 75% $\rm CO_2$ as in the 100% $\rm CO_2$ treatment after 24 h exposure. At 35°C, there was 100% mortality after 24 h exposure with all gases in variety ruziz (df = 5, 23). Although few survivors remained in khudri (F = 1.00; df = 5, 23; P = 0.4457) and saqie (F = 0.66; df = 5, 23; P = 0.6572) at 25°C (Table 1).

Higher larval mortality was recorded after 48 h of exposure as compared with 24 h treatment with 100% CO₂ and 75% CO₂ treatments at 25°C. The application of 100% CO₂ and 75% CO₂ gave a 100% *E. cautella* immediate larval mortality at 35°C, while at 25°C immediate mortality was far below (only 60–83%) with any gas even for an exposure timing of 48 h. However, 100% delayed mortality was noted at 25°C for 75% CO₂, while for 100% CO₂ a few survivors remained in all varieties at 25°C.

The comparative results of larval mortality (%) \pm SE for 48 h exposure in all three date varieties with different gases and temperatures are summarized in Table 2. In case of khudri, the the complete immediate larval mortality (100%) was recorded with ozone, followed by 94.1 and 88.1% mortality with 100% CO2 and 75% CO2 treatments, respectively. Although, the delayed mortality was not significantaly increased with 100% CO₂ treatment but with 75% CO₂ treatments 100% delayed mortality was noted (F = 11.04; df = 5, 23; P = < 0.0001). Similarly, in ruziz, the maximum immediate larval mortality of 100% was recorded with ozone, followed by 94.6 and 97.5% mortality with 100% CO₂ and 75% CO₂ treatments, respectively. Although, in ruziz for the delayed mortality there was no significant increase with 100% CO₂ treatment, but, the delayed mortality of 100% with 75% CO₂ treatments was noted (F = 1.49; df = 5, 23; P = 0.2427). Similarly, in sagie, a 100% immediate larval mortality was recorded with ozone, followed by 90.8 and 90.6% mortality with 100% CO₂ and 75% CO₂ treatments, respectively. Although, for the delayed mortality again there was no significant increase with 100% CO₂ treatment, but, the delayed mortality of 100% with 75% CO₂ treatments was noted (F = 2.03; df = 5, 23; P = 0.1223) (Table 2).

As the major objective of this study was to determine the best exposure timing, temperature, and gases concentration to achieve 100% *E. cautella* larval mortality. Table 3 summaries the *Ephestia* larval mortality (%) \pm SE when exposed for 72 h to different gases and temperatures. Almost a complete mortality (100%) was observed in both CO₂ treatments. A high larval mortality (\sim 100%) was recorded with exposure to CO₂ at 25°C for 72 h as compared with 48 h. Similarly, the immediate mortality was also observed high (almost 100%) when exposed to CO₂ for 72 h (Table 3) as compared with an exposure timing of 48 h (Table 2).

Discussion

In this study, we examined the effectiveness of different gases at different temperature regimes for various exposure timings on *E. cautella* larvae. The salient findings of this research includes treatment with 22 ppm ozone resulted 100% larval mortality in all tested date varieties, exposure timings, and temperatures. Isikber and Oztekin (2009) treated Mediterranean flour moth, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) larvae (21 d old from oviposition), with a continuous ozone flow of 13.9 mg/liter (6,482 ppm). The larvae were placed on the top of the fumigation chamber that was filled with 2 kg of wheat, and they achieved 100% larval mortality after 5 h of exposure. When larvae were placed on the bottom of the chamber, 94% larval mortality was recorded with the same treatment. It reflects that direct and continuous exposure of the larvae to ozone can affect the mortality. In our study the

Table 1. Mean (%) \pm SE mortality of *E. cautella* larvae exposed for 24 h to various gases at 25 and 35°C

Temperature	Variety	Immediate mortality			Delayed mortality		
		100% CO ₂	75% CO ₂	Ozone (22 ppm)	100% CO ₂	75% CO ₂	Ozone (22 ppm)
25°C	Khudri	31.6 ± 6.6c	62.5 ± 12.2bc	100 ± 0.0 a	60.6 ± 7.5bc	76.8 ± 12.7ab	100 ± 0.0a
	Ruziz	$40.7 \pm 3.9b$	$65.2 \pm 14.8ab$	$100 \pm 0.0a$	$66.7 \pm 6.7ab$	$83.8 \pm 9.8a$	$100 \pm 0.0a$
	Sagie	$31.9 \pm 2.8b$	44.8 ± 15.2b	$100 \pm 0.0a$	$73.5 \pm 13.1ab$	68.2 ± 11.6 ab	$100 \pm 0.0a$
35°C	Khudri	$97.3 \pm 2.6a$	$100 \pm 0.0a$	$100\pm0.0a$	$100 \pm 0.0a$	$100 \pm 0.0a$	$100 \pm 0.0a$
	Ruziz	$100 \pm 0.0a$	$100 \pm 0.0a$	$100 \pm 0.0a$	$100 \pm 0.0a$	$100 \pm 0.0a$	$100 \pm 0.0a$
	Sagie	$98.5 \pm 1.5a$	$97.1 \pm 2.9a$	$100 \pm 0.0a$	98.5 ± 1.5a	$100 \pm 0.0a$	$100 \pm 0.0a$

Table 2. Mean (%) ± SE mortality of E. cautella larvae exposed for 48 h to various gases at 25 and 35°C

Temperature	Variety	Immediate mortality			Delayed mortality		
		100% CO ₂	75% CO ₂	Ozone (22 ppm)	100% CO ₂	75% CO ₂	Ozone (22 ppm)
25°C	Khudri	94.1 ± 2.4ab	88.1 ± 2.2b	100 ± 0.0a	98.6 ± 1.4a	100 ± 0.0a	$100 \pm 0.0a$
	Ruziz	$94.6 \pm 2.2a$	$97.5 \pm 2.5a$	$100 \pm 0.0a$	$97.5 \pm 2.7a$	$100 \pm 0.0a$	$100 \pm 0.0a$
	Sagie	$90.8 \pm 5.4a$	90.6 ± 3.3a	$100 \pm 0.0a$	$93.3 \pm 4.9a$	$100 \pm 0.0a$	$100 \pm 0.0a$
35°C	Khudri	$100 \pm 0.0a$	$100 \pm 0.0a$	100 ± 0.0 a	$100 \pm 0.0a$	$100 \pm 0.0a$	$100 \pm 0.0a$
	Ruziz	$100 \pm 0.0a$	$100 \pm 0.0a$	$100 \pm 0.0a$	$100 \pm 0.0a$	$100 \pm 0.0a$	$100 \pm 0.0a$
	Sagie	$100 \pm 0.0a$	$100 \pm 0.0a$	$100 \pm 0.0a$	$100 \pm 0.0a$	$100 \pm 0.0a$	$100 \pm 0.0a$

Table 3. Mean (%) \pm SE mortality of *E. cautella* larvae exposed for 72 h to various gases at 25 and 35 $^{\circ}$ C

Temperature	Variety	Immediate/delayed mortality			
		100% CO ₂	75% CO ₂		
25°C	Khudri Ruziz	100 ± 0.0 a 100 ± 0.0 a	100 ± 0.0 a 100 ± 0.0 a		
	Saqie	$98.7 \pm 1.3 a$	$98.7 \pm 2.6a$		
35°C	Khudri	$100 \pm 0.0a$	$100 \pm 0.0a$		
	Ruziz	$100 \pm 0.0a$	$100 \pm 0.0a$		
	Sagie	100 ± 0.0 a	$100 \pm 0.0a$		

Within each row means followed by the same letter do not differ significantly (HSD test at $\it{P} < 0.05$)

larvae inside the dates were directly and continuously exposed to ozone for the period of 24 h, which resulted in 100% mortality.

Kabkab dates infested with Indian meal moth, P. interpunctella larvae were exposed to $>2,000\,\mathrm{ppm}$ ozone, Niakousari et al. (2010) recorded complete larval mortality only in 2 h. Similarly, when 5th instar larvae of P. interpunctella, placed in a cotton muslin tea bags, and exposed to 70 ppmv ozone, there was only 0.76 ± 0.07 survival ratios of the larvae after 24 h of exposure times (Bonjour et al. 2011).

Most of the work has been done with application of ozone to *P. interpunctella* larvae and coleopteran beetles. A number of studies compared the succeptibility of lepidopterans and coleopterans stored product insects to various ozone concentrations but only few closely related examples are cited here. McDonough et al. (2011) calculated that the minimum time of 90 min with 1,800 ppm ozone is required to achieve 100% mortality of *P. interpunctella* of different age larvae.

Different age larvae of *P. interpunctella* were exposed to continuous flows of ozone in a dose of \sim 33 ppm for 6 d at different temperatures (7.3, 7.9, 29.6, and 31.6°C) (Hansen et al. 2013). They observed some survivals under all studied temperatures. This study confirms our findings of similar results of *E. cautella* 100% larval mortality with 22 ppm ozone at 25 and 35°C for 24 h exposure timings.

Treatment with CO_2 resulted in 100% mortality for exposures at 35°C for 48 h or greater. Although at 25°C, the CO_2 treatment resulted in 100% immediate mortality after 72 h exposure. Hashem et al. (2014)

accessed the comparative effectiveness of modified atmosphere enriched with CO₂ and N₂ for the larval instars of E. cautella. They observed that the 1st and 2nd instars larvae of E. cautella proved more succeptible to the modified atmosphere enriched with either CO₂ or N₂ than the later instars. They further reported that the modified atmosphere enriched with 60% CO₂, 8% O₂ and 32% N₂, took almost 72 h to kill 100% 4th instars larvae of E. cautella at 30°C. The same CO₂ concentration and for an exposure of 60 h at 27°C, resulted in 95% mortality in navel orangeworm, Amyelois transitella (Walker) (Lepidoptera: Pyralidae) when 25-d-old larvae were placed into 0.95 liter jars containing 250 ml of fresh medium (Brandle et al. 1983). Soderstrom et al. (1990) achieved 95% mortality after treating the codling moth, Cydia pomonella (L.) (Lepidoptera: Torticidae) mature larvae with 60% CO₂ when exposed for 7 d at 25°C. These findings are in strong conformity with present results, as 100% mortality of E. cautella 5th instar larvae was achieved with a treatment of CO₂ 100% after an exposure of 72 h at 25°C, while a similar mortality was achieved after 48 h at 35°C. Several studies have been done by using modified atmosphere to the various developmental stages of lepidopteran stored product pests and most of them concluded that larva was the most tolerant stage, particularly the larvae of *P. interpunctella*, *Ephestia* spp. and angoumois grain moth Sitotroga cerealella (Olivier) (Lepidoptera: Gelechiidae). Ahmed and Hashem (2012) evaluated the susceptibility of different life stages of P. interpunctella and E. cautella to modified atmospheres containing 40, 60, and 80% CO₂ in air at 27°C; they found that at 80% CO₂, 6–7 d are required to achieve complete mortality of P. interpunctella and E. cautella.

In this study we used ozone, CO_2 and a blend of CO_2 and N_2 . Previous studies have confirmed the combined effects of CO_2 mixed with N_2 on larvae of various warehouse moths, for example, when larvae of *E. kuehniella* and *P. interpunctella* were exposed to a modified atmosphere with an initial content of 50% CO_2 (balanced with N_2 and 3% O_2) at 25°C, 100% mortality was achieved after 4 d exposure time (Riudayets et al. 2009).

Our results demonstrate that ozone was very fast in killing at both 25 and 35°C temperature regimes. After ozone, the action of 75% $\rm CO_2 + 25\%~N_2$ was faster than 100% $\rm CO_2$ when exposed for 24 h. It showed that use of 75% $\rm CO_2 + 25\%~N_2$ could be a good option for controlling *E. cautella* in a short exposure timing. Although, the efficacy of

 $100\% \text{ CO}_2$ was good, its action was slower than $75\% \text{ CO}_2 + 25\% \text{ N}_2$. It is true that there was no significant difference of date fruit varieties on larval mortality under CO₂ and ozone treatments. Moreover, gases used in this study were more effective at higher temperature (35°C) compared with 25°C. There is an evidence that high temperature denatures the proteins, imbalance the hemolymph pH, and adversely effect the enzyme action (Neven 2000), and the situation is exacerbated under stressful environment (ozone or CO2 stress). Many factors that determine the choice of a particular modified atmosphere, out of which time is the most important factor. Sensitivity of the S. cerealella immature stages was studied by Hashem et al. (2012) by using modified atmospheres of 30, 45, 65, and 75% CO₂ in air at 27°C. They found that a larva was the most tolerant stage, and it took 264 h to complete kill. Although, Ahmed et al. (2014) studied the same modified atmospheres against the immature stages of the same insect at 20 and 34°C. They found that 20°C delayed the response; however, at 34°C the response was rapid and it took 96 h to get 100% reduction of adult emergence from 4th instar larva.

Ozone is a toxic gas that kills insects faster than many other gases, its transportation is easy and can be produced at the application site. The cost of the equipment and labor charges are also important factors that could make ozone a priority choice as modified atmosphere. An ozone generator can also be easily moved around for different uses. Also, it is worth mentioning that this gas is quickly decomposed (halflife is 20–50 min) to molecular oxygen without any harmful threats to commodities and the environment. All these are positive attributes make ozone more attractive for use in fumigation techniques (Kells et al. 2001). Ozone also has some limitations, like poor ovicidal effect and not penetrative, reducing its ability to kill insect pests at deeper levels. Higher air velocities are desired for ozonated air to penetrate deep enough into commercial-size bins (Mendez at al. 2003). Different stages of P. interpunctella, E. kuehniella, and O. surinamensis, confused flour beetle, Tribolium confusum Du Val (Coleoptera: Tenebrionidae) were exposed to ozone for different concentrations and results indicated that there was complete mortality of larvae, pupae, and adults while, 100% mortality of eggs was not achieved (Isikber and oztekin 2009; Niakousari et al. 2010).

Our present results conclusively demonstrates that ozone atmosphere in general, may be a good candidate for E. cautella control as compared with CO_2 , but there is further need to investigate its efficacy. In our opinion, the results further suggest that a modified atmosphere, like a blend of gases and heat treatment could be the best choice for managing E. cautella, a warehouse pests of date fruits and other stored grains.

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References Cited

- **Abbott, W. S. 1925.** A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.
- Abo-El-Saad M. M., H. A. Elshafie, A. M. Al Ajlan, and I. A. Bou-Khowh. 2011. Non-chemical alternatives to methyl bromide against *Ephestia cautella* (Lepidoptera: Pyralidae): microwave and ozone. Agric. Biol. *J. North Am.* 2: 1222–1231.
- Ahmed, S. S., and M. Y. Hashem. 2012. Susceptibility of different life stages of Indian meal moth *Plodia interpunctella* (Hubner) and almond moth *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) to modified atmospheres enriched with carbon dioxide. J. Stored Prod. Res. 51: 49–55.
- **Ahmed, S. S., M. Y. Hashem, and S. I. El-Sherif. 2014.** Comparative effects of different modified atmosphere exposures at 20 and 34° C on the immature stages of angoumois grain moth *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae). J. Stored Prod. Res. 56: 54–59.

- Al-Abbad, A., M. Al-Jamal, Z. Al-Elaiw, F. Al-Shreed, and H. Belaifa. 2011. A study on the economic feasibility of date palm cultivation in the Al-Hassa Oasis of Saudi Arabia. J. Dev. Agric. Econ. 3: 463–468.
- Al-Azab, A. M. 2007. Alternative approaches to methyl bromide for controlling Ephestia cautella (Walker) (Lepidoptera:Pyralidae). M.S. thesis, King Faisal University, Saudi Arabia.
- Aldawood, S. A., K. G. Rasool, A. H. Alrukban, A. Sofan, M. Husain, K. D. Sutanto, and M. Tufail. 2013. Effects of temperature on the development of *Ephestia cautella*(Walker) (Pyralidae: Lepidoptera): a case study for its possible control under storage conditions. Pak. J. Zool. 45: 1573–1578.
- **Al-Shahib, W., and R. J. Marshall. 2003.** The fruit of the date palm: its possible use as the best food for the future? Int. J. Food Sci. Nutr. 54: 247–259.
- Al-Zadjali, T. S., F. F. Abd-Allah, and H. S. El-Haidari. 2006. Insect pests attacking date palms and dates in Sultanate of Oman. Egypt. J. Agric. Res. 84: 51–59.
- Arbogast, R. T., S. R. Chini, and P. E. Kendra. 2005. Infestation of stored saw palmetto berries by *Cadra cautella* (Lepidoptera: Pyralidae) and the host paradox in stored-product insects. Fla. Entomol. 88: 314–320.
- Bonjour, E. L., G. P. Opit, J. Hardin, C. L. Jones, M. E. Payton, and R. L. Beeby. 2011. Efficacy of ozone fumigation against the major grain pests in stored wheat. J. Econ. Entomol. 104: 308–316.
- Brandle, D. G., E. L. Soderstrom, and F. E. Schreiber. 1983. Effects of low-oxygen atmospheres containing different concentrations of carbon dioxide on mortality of the navel orangeworm, *Amyelois transitella* Walker (Lepidoptera: Pyralidae). J. Econ. Entomol. 76: 828–830.
- Chao, C. C., and R. R. Krueger. 2007. The date palm (*Phoenix dactylifera* L.): overview of biology, uses, and cultivation. HortScience 45: 1077–1082.
- Donahaye, E., S. Navarro, and M. Rindner. 1994. The influence of temperature on the sensitivity of two nitidulid beetles to low oxygen concentrations, pp. 88–90. *In Proceedings*, 6th International working Conference on Stored Product Protection, 14-17 April 1994, Canbera, CAB International, Wallingford, United Kingdom.
- Hansen, L. S., P. Hansen, and K. M., Vagn Jensen. 2013. Effect of gaseous ozone for control of stored product pests at low and high temperature. J. Stored Prod. Res. 54: 59–63.
- Hashem, M. Y., E.S.M. Risha, S. I. El-Sherif, and S. S. Ahmed. 2012. The effect of modified atmospheres, an alternative to methyl bromide, on the susceptibility of immature stages of angoumois grain moth *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae). J. Stored Prod. Res. 50: 57–61.
- Hashem, M. Y., S. S. Ahmed, M. A. El-Mohandes, A.R.E. Hussain, and S. M. Ghazy. 2014. Comparative effectiveness of different modified atmospheres enriched with carbon dioxide and nitrogen on larval instars of almond moth *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae). *J. Stored Prod. Res.* 59: 314–319.
- **Isikber, A. A., and S. Oztekin. 2009.** Comparison of susceptibility of two stored-product insects *Ephestia kuehniella* Zeller and *Tribolium confusum* du Val to gaseous ozone. J. Stored Prod. Res. 45: 159–164.
- Kells, S. A., L. J. Mason, D. E. Maier, and C. P. Woloshuk. 2001. Efficacy and furnigation characteristics of ozone in stored maize. J. Stored Prod. Res. 37: 371–382.
- McDonough, M. X., L. J. Mason, and C. P. Woloshuk. 2011. Susceptibility of stored product insects to high concentrations of ozone at different exposure intervals. J. Stored Prod. Res. 47: 306–310.
- Mendez, F., D. E. Maier, L. J. Mason, and C. P. Woloshuk. 2003. Penetration of ozone into columns of stored grains and effects on chemical composition and processing performance. J. Stored Prod. Res. 39: 33–44.
- (MOA) Ministry of Agriculture. 2011. Annual Agriculture Statistical Book. Riyadh, Saudi Arabia.
- Navarro, S. 2012. The use of modified and controlled atmospheres for the disinfestation of stored products. J. Pest Sci. 85: 301–322.
- Navarro, S., and M. Gonen. 1970. Some techniques for laboratory rearing and experimentation with *Ephestia cautella* (Walk) (Lepidoptera Phycitidae). J. Stored Prod. Res. 6: 187–189.
- Neven, L. G. 2000. Physiological responses of insects to heat. Postharvest Biol. Tec. 21: 103–111.
- Niakousari, M., Z. Erjaee, and S. Javadian. 2010. Fumigation characteristics of ozone in postharvest treatment of kabkab dates (*Phoenix dactylifera* L.) against selected insect infestation. J. Food Protect. 73: 763–768.
- Riudavets, J., C. Castane, O. Alomar, M. J. Pons, and R. Gabarra. 2009. Modified atmosphere packaging (MAP) as an alternative measure for controlling ten pests that attack processed food products. J. Stored Prod. Res. 45: 91–96.
- SAS Institute. 2009. SAS/STAT 9.2. users guide. SAS Institute, Cary, NC.

- Sauer, J. A., and M. D. Shelton. 2002. High-temperature controlled atmosphere for post-harvest control of indian meal moth (Lepidoptera: Pyralidae) on preserved flowers. *J. Econ. Entomol.* 95: 1074–1078.
- **Soderstrom, E. L., B. E. Mackey, and D. G. Brandl. 1986.** Interactive effects of low-oxygen atmospheres, relative humidity, and temperature on mortality of two stored-product moths (Lepidoptera: Pyralidae). J. Econ. Entomol. 79: 1303–1306.
- Soderstrom, E. L., D. G. Brandl, and A. Mackey. 1990. Responses of codling moth (Lepidoptera: Tortricidae) life stages to high carbon dioxide or low oxygen atmospheres. J. Econ. Entomol. 83: 472–475.
- (USEPA) U.S. Environmental protection agency. 2014. Ozone layer protection-regulatory programs. The phase out of methyl bromide. EPA-HQ-OAR-2014-0065

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