

EFFECT OF DIFFERENT MODIFIED ATMOSPHERES, AN ALTERNATIVE TO METHYL BROMIDE ON DIFFERENT STAGES OF SAW TOOTHED GRAIN BEETLES, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae)

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ABSTRACT

The present study aims to evaluate effectiveness the modified atmospheres of larvae and adults of the saw-toothed grain beetle, *Oryzaephilus surinamensis* based on either high carbon dioxide (CO₂) contents or high nitrogen (N₂) contents at optimum conditions (25°C and 65±5 % RH). The experiments were carried out using 20, 40, and 50% CO₂ as well as 97 and 98% N₂ gases in the air at different exposure periods.

The mortality was recorded at exposure periods; 3, 6, 12, 24, 48, 72, 96, 120 and 144 h. Results showed that the larvae and adults mortalities of *O. surinamensis* responded to modified atmospheres (MAs) enriched with either CO₂ or N₂ and increased significantly ($P < 0.01$) with increasing either exposure time length or gas concentration. MAs enriched with N₂ were more effective than those contained CO₂. Modified atmospheres tested had strong effects against all stages of *O. surinamensis*. Six days were adequate to kill larvae and adults completely under all tested modified atmospheres contained different concentrations of CO₂. Five days were required to kill larvae were required completely under the two modified atmospheres contained 97 and 98% N₂ whereas 72 h to kill adults under the same concentrations.

Keywords: Methyl bromide, Date palm fruits, Carbon dioxide, Nitrogen, *Oryzaephilus surinamensis*.

INTRODUCTION

Date is one of the fruits that have great economic importance in Egypt. The date is stored for months until it reaches the consumers. Sometimes the storage of date fruits extends for more than a year. During this storage, the date fruits can be attacked by numerous of insect pests that affect their marketing value (Sen *et al.*, 2010). The most common insect pests that cause great losses in date fruits are the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae) and fig moth *Cadra*, (*Ephestia cautella*) (Lepidoptera: Pyralidae) (Abbas *et al.*, 2011).

The saw-toothed grain beetle, *Oryzaephilus surinamensis*, is an important and widespread pest of stored grains and cereal products. It is usually found as a secondary pest on grain damaged by other insects, such

as the grain weevil, *Sitophilus granarius* (L.) as the larvae cannot develop on sound grains (Tuncbilek, 1997). Therefore, the important factors that contribute to it, its seriousness on dates include its ability to develop resistance to insecticides (Dakhil, 1987) and the tendency of larvae to feed inside date fruit (Al-Taweel *et al.*, 1990).

Moreover, the use of methyl bromide to fumigate food commodities and facilities must be phased out in accordance with the montreal protocol due to its effect on the ozone layer (UNEP, 2006). The development of alternative treatments for pest control is an increasing demand for food industry and has been promoted by governments through national legislations and funding the research projects. Alternatives should meet consumer demands towards reducing or eliminate the use of pesticides and maintained the same time high degree of control efficacy (Riudavets *et al.*, 2010).

Modified atmospheres have been used for disinfesting raw or semi-processed food products, such as cereal grains and dried fruits, while still in storage. Treatments based on reduced oxygen (O₂) and high carbon dioxide (CO₂) or nitrogen (N₂) contents are technically suitable alternatives for arthropod pest control in durable commodities (Riudavets *et al.*, 2010). Atmospheres rich in CO₂, with more than 40% in the air, are faster at controlling pests than those with high contents of N₂ (Navarro, 2006). Literatures on the effects of different types of CO₂ treatments and dosages on key pests are available for many species and stages of stored-product pests under particular sets of conditions (Annis & Morton, 1997). Depending on the temperature, CO₂ treatments may take from a few days to several weeks to be effective in gas-tight chambers or silos (Riudavets *et al.*, 2009). The toxicity of CO₂ to insects is known to vary among species, developmental stages and age groups. Parameters of the physical environment, such as temperature, humidity, and CO₂ levels in storage, also influence toxicity. In the majority of studies involving CO₂, much attention has been focused on determining the time required to kill insect pests (Van Epenhuijsen *et al.*, 2002).

Modified atmospheres containing high levels of N₂ were experimentally tested against some stored-products insects beside other MAs enriched with various levels of CO₂. Larvae, pupae, and adults of *Tribolium castaneum* (Herbst) were exposed to atmospheres containing high N₂ or CO₂ concentrations at about 50% R.H. and 27°C for periods up to 72 hr. Overall, 99% N₂ caused greater mortality in adults than did 58% CO₂ while 58% CO₂ was more effective against pupae. The difference in larval mortality exposed to the two atmospheres was not significant, though 99% N₂ caused greater mortalities of ten 48 h. Mortality of all life stages tested were low when the insects were exposed to an atmosphere of 97% N₂ (Jay and Cuff, 1981). The use of N₂ gas to attain low oxygen atmospheres for eradicating insect infestation of museum objects is a feasible alternative to toxic gases. All insects commonly found in museums can be eradicated in a 0.1 % oxygen atmosphere (Daniel *et al.*, 1993). Moreover, Ofuya and Reichmuth (1993) investigated the mortality of eggs, larvae, pupae and adults of the cowpea

bruchid, *Callosobruchus maculatus* (F.) and the bean bruchid, *Acanthoscelides obtectus* (Say) in 100% N₂ atmosphere at 25 and 32°C, respectively at 70±5% R.H. They found that all adults of both bruchids were killed within one day of exposure to pure N₂ atmosphere. All eggs and young larvae of *A. obtectus* were killed within 3-5 days, while complete mortality of larvae and pupae was observed within 5-9 days.

However, scanty attention has been paid on Effect of different modified atmospheres, an alternative to methyl bromide on different stages of Saw toothed grain beetles, *Oryzophilus surinamensis* (L.) (Coleoptera: Silvanidae).

Therefore, this study was designed to quantify the dosage mortality of *O. surinamensis* to CO₂ or high N₂ and more specifically to define the concentration and time combinations that give high mortality for larvae and adults stages.

MATERIALS AND METHODS

Insect culture

The insect strain tested was obtained from naturally infested dried fruits. It was reared on the dust of date-palm fruits. Cultures and test insects were kept in a darkened incubator maintained at 30±2°C and 65±5% R.H. The insects used in this study were reared on the same food medium. The dust of date-palm fruits was sterilized at 60°C for 10 h to eliminate possible contaminants (El-Kady, 1978). Adults of known age were obtained from cultures which were sieved daily. The adults were then put into jars containing black cloths. The eggs were collected daily from the black cloths.

Laboratory experiments

MAs were investigated in the laboratory of Modified atmospheres at the Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University according to the method described by Hashem *et al.* (2012). Experiments aimed to study the susceptibility of larvae and adults of *O. surinamensis* to different concentrations of CO₂ and N₂ in the air at 25°C for different exposure periods. Larvae and adults required for the MAs experiments were obtained from the stock culture as described earlier. After exposure to MAs, treated stages were maintained under the optimum constant laboratory conditions of 25°C and 65±5 % R.H. All tested MAs treatments were repeated three times and three similar replicates of every treatment were left untreated for control purpose.

Gas treatment equipment

As described by Desmarchelier (1984), treatment with gas mixtures took place inside gas-tight sealed glass bottles of 550 cm³ capacity (Dreschel flask). Every flask was tightly plugged with a special glass stopper containing two lateral valves (an inlet and outlet valve) leading to two vertical glass tubes. One of these tubes was long and reached near the bottom of the flask, while the other was short and reached the upper quarter of it. The long tube worked as a gas inlet and the short one worked as a gas outlet. Valves were opened at the beginning of the treatment and left open until the desired gas concentration inside the flask was obtained, as indicated by an oxygen

analyzer (Hashem, 1990). CO₂ or N₂ cylinders were used for gases supply and were connected to the inlet tube of the flask with a short hose. The outlet tube of the Dreshel flask was connected to the CO₂-O₂ analyzer with another short hose (Servomex 570 A).

Preparing larvae and adults for gas treatment

Third instar larvae and newly-emerged adults were used in this study. Larvae and adults were separated from the medium by camel brush. Twenty specimens for each stage were put in each glass tube which containing 2 g dust of palm-date fruits. The tubes were closed with cloths and tighten with rubber band. Then, the tubes were introduced into the dreshel flasks to be tested with MAs.

The CO₂ and N₂ concentrations in air and exposure periods tested

Using the gas treatment equipment described above, larvae and adults were exposed to three different air concentrations of CO₂ and two concentrations of N₂ under the prevailing laboratory conditions. The tested MAs containing CO₂ were: 1) 20% CO₂, 16% O₂ and 64% N₂, 2): 40% CO₂, 12% O₂ and 48% N₂ and 3) 50% CO₂, 10% O₂ and 40% N₂. The tested MAs containing N₂ were: 1) 97% N₂ & 3% O₂) and 98% N₂ & 2% O₂. The exposure periods for each of the three tested MA treatments containing CO₂ were 3, 6, 12, 24, 48, 72, 96, 120 and 144 h for each adults and larvae treatments. The exposure periods of each of the two MA treatments containing N₂ were 3, 6, 12, 24, 48 and 72 h for adult treatments and exceeded to 96 and 120 h for larvae treatments. After treatment, the Dreshel flasks were transferred to incubators adjusted to constant temperatures of 25 ± 2 °C and 65 ± 5 % R.H.

Mortality percentages of the different developmental larval instars

By the end of the tested exposure periods, dreshel flasks were aerated, and the treated stages were removed and incubated under the conditions of 25 ± 2 °C and 65 ± 5% R.H. remained inside the tubes and were examined daily to record mortality percentages.

Statistical analysis

Mortality counts were corrected using Abbott's formula (Abbott, 1925). F. and Duncan tests were adopted for calculating the corrected mortality rates of larval instars and were performed with an SPSS computing program using ANOVA, as described by Snedecor & Cochran (1967). Data on the effect of exposure periods on the mortality of the larvae and adults stages were subjected to probity analysis as described by Finney (1971). LT₅₀ and LT₉₅ values were also calculated using the computer program developed by Noack and Reichmuth (1978).

RESULTS

1- Effect of CO₂ on mortality of larvae and third instar adults of *O. surinamensis*

1-1- Larval stage

Mortality percentages of 3rd instar larvae of *O. surinamensis* exposed to three concentrations of CO₂ at 25°C were indicated in Fig (1). The mortality % increased with increasing both CO₂ concentrations and exposure period.

Mortality percentage was 100% at six days with 20% CO₂, five days with 40% CO₂ and three days exposure period with 50% CO₂. LT₅₀ and LT₉₅ values at 25 °C declined gradually from low to high concentrations of CO₂. LT₅₀ values were 1.07, 1.15 and 0.55 with 20, 40 and 50% CO₂. LT₉₅ values were 8.76, 11.37 and 2.61 with the same concentrations, respectively (Table 1).

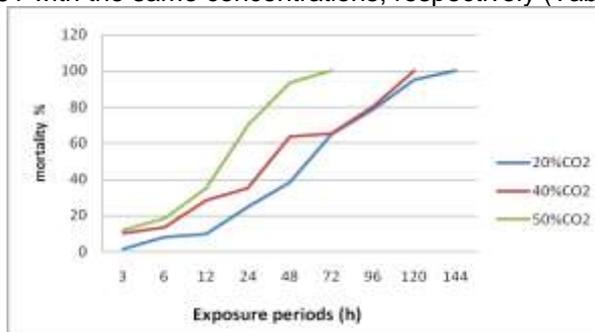


Fig. (1): Mortality percentages (Mean±SE) for Larva of *O. surinamensis* exposed to different modified atmospheres (MAs) containing different concentrations of CO₂ combined with different exposure periods at 25°C.

Table (1): LT₅₀ and LT₉₅ values, and their confidence limits, for Larva of *O. surinamensis* exposed to different modified atmospheres (MAs) combined with different exposure periods at 25°C.

Modified atmosphere	LT ₅₀ (h)	LT ₉₅ (h)	Confidence limits (h)			
			LT ₅₀		LT ₉₅	
			Lower	Upper	Lower	Upper
20 % CO ₂	40.8	210.456	27.7176	57.0912	172.133	405.5808
40 % CO ₂	27.744	273.024	18.1488	41.4696	209.508	650.9016
60 % CO ₂	13.32	62.736	9.1416	18.9288	48.8568	126.3528

1-2- Adult stage

The mortality percentage increased with increasing both CO₂ concentrations and exposure period. Mortality percentage was 100% at 6, 5 and 4 days exposure period with 20, 40 and 50% CO₂, respectively at 25°C. The high concentration (50%) of CO₂ had the highest effect on *O. surinamensis* adults at 25°C. It could cause 100% mortality after four days exposure period (Fig.2).

Table (2) showed the LT₅₀ and LT₉₅ values at 25 °C. Those declined gradually from low to high concentrations of CO₂. LT₅₀ was 1.96, 0.86 and 0.62 with 20, 40 and 50% CO₂, respectively. LT₉₅ values were 10.79, 3.41 and 2.29 with the same concentrations, respectively.

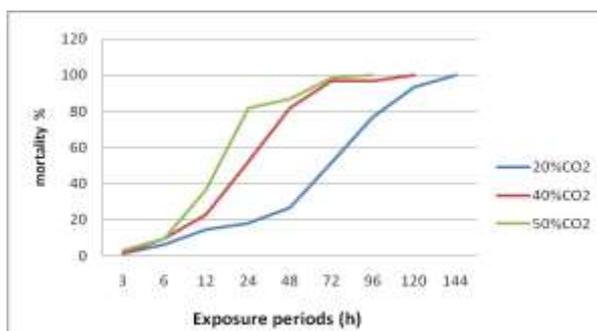


Fig. (2): Mortality percentages (Mean±SE) for adult of *O. surinamensis* exposed to different modified atmospheres (MAs) containing different concentrations of CO₂ combined with different exposure periods at 25°C.

Table (2): LT₅₀ and LT₉₅ values, and their confidence limits, for adult of *O. surinamensis* exposed to different modified atmospheres (MAs) combined with different exposure periods at 25°C.

Modified atmosphere	LT ₅₀ (h)	LT ₉₅ (h)	Confidence limits (h)			
			LT ₅₀		LT ₉₅	
			Lower	Upper	Lower	Upper
20 % CO ₂	47.08 8	259.10 4	28.382 4	73.946 4	233.685 6	718.485 6
40 % CO ₂	20.66 4	81.888	18.542 4	22.944	70.188	98.4144
50 % CO ₂	14.88	55.104	11.762 4	18.595 2	44.0664	80.6064

2- Effect of N₂ gas on for mortality of adults and third instar larvae of *O. surinamensis*

2-1- Larval stage

The mortality percentage of 3rd instar larvae of *O. surinamensis* exposed to 97% and 98% Nitrogen at 25°C. increased with increasing both N₂ concentration and exposure period (Fig 3). Mortality percentage was 100% with 98% N₂ at five days exposure period but the N₂ concentration of 97% caused 98.3 % mortality with the same exposure period. The high concentration of N₂ (98%) had the highest effects on *O. surinamensis* larvae. It could cause 100% mortality after five days exposure periods. Table (3) showed the respective findings of LT₅₀ and LT₉₅ together with their confidence limits in cases of 97% N₂ (35.684 and 225.986 h) and 98% (24.48 and 163.64 h).

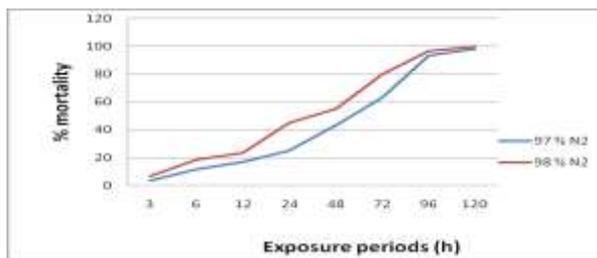


Fig. (3): Mortality percentages for larvae of *O. surinamensis* exposed to different nitrogen atmospheres combined with different exposure periods at 25°C.

Table (3): LT₅₀ and LT₉₅ values, together with their confidence limits for 3rd instar larvae of *O. surinamensis* exposed to different nitrogen atmospheres combined with different exposure periods at 25°C.

Modified atmosphere	LT ₅₀ (h)	LT ₉₅ (h)	Confidence limits (h)			
			LT ₅₀		LT ₉₅	
			Lower	Upper	Lower	Upper
97% N ₂	35.684	225.986	21.305	58.354	197.023	665.212
98% N ₂	24.482	163.647	15.222	37.102	129.701	374.087

2-2- Adult stage

The adult mortality percentage of *O. surinamensis* exposed to 97% and 98% Nitrogen increased with increasing both Nitrogen concentrations and exposure period (Fig. 4). The mortality percentage was 100% after three days exposure period. The LT₅₀ and LT₉₅ values at 25 °C declined gradually from low to high N₂ concentrations. LT₅₀ and LT₉₅ values were (11.9 and 63.4 h), (7.3 and 40.4 h) with 97 and 98% N₂ concentrations, respectively (Table 4).

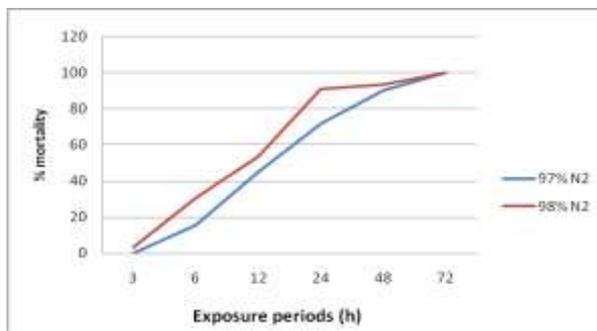


Fig. (4): Mortality percentages for adults of *O. surinamensis* exposed to different nitrogen atmospheres combined with different exposure periods at 25°C.

Table (4): LT₅₀ and LT₉₅ values, together with their confidence limits for adults of *O. surinamensis* exposed to different nitrogen atmospheres combined with different exposure periods at 25°C.

Modified atmosphere	LT ₅₀ (h)	LT ₉₅ (h)	Confidence limits (h)			
			LT ₅₀		LT ₉₅	
			Lower	Upper	Lower	Upper
97% N ₂	11.92	63.43	4.81	24.27	59.37	408.35
98% N ₂	7.31	40.35	4.23	10.99	29.60	107.12

DISCUSSION

The study conducted by Hashem *et al.* (2012) tested different modified atmospheres (MAs) enriched with CO₂ against the saw-toothed grain beetle as methyl bromide (MB) alternative. They proved that all stages of *O. surinamensis* could be killed within four days exposure. Furthermore, there is a previous study showed that no adverse affects of MAs the date fruit's quality (Dehghan Shoar *et al.*, 2010). In this research, different MAs containing 20, 40 and 50% CO₂ in air were firstly tested against larvae and adults of *O. surinamensis* at 25°C and 65±5% R.H.

Few researches applied MAs in controlling saw-toothed grain beetle, *O. surinamensis*. Riudavets *et al.* (2009) applied MAs containing 50% and 90% CO₂ against all stages of *O. surinamensis* for four and eight days at 25°C. While, Riudavets *et al.* (2010) used CO₂ at high pressure with reduced the time required to kill the insect pests. Nielsen (2001) applied atmospheres with high content of CO₂ under a pressure against the adults of *O. surinamensis*. However, Locatelli and Daolio (1993) studied the effectiveness of CO₂ under reduced pressure against life stages of *O. surinamensis*. MAs used to protect commodities throughout their storage life by using low O₂ levels (Conyers and Bell, 2007). Additionally, Leelaja *et al.* (2007) used CO₂ to enhance the toxicity of allyl acetate against the adult beetle of *O. surinamensis*.

The present study applied different MAs based on either high CO₂ or high N₂ contents at optimum conditions of 25°C and 65±5 % RH. The experiments were conducted using 20, 40, and 50% CO₂ as well as 97% and 98% N₂ gases in the air against 3rd instar larvae and newly emergence adults of saw-toothed grain beetle *O. surinamensis* at different exposure periods. The target was to identify the sensitivity of *O. surinamensis* stages to high levels of CO₂ or N₂ as well as to detect the exact time to achieve 100% mortality of each stage. This study is considered the first that used these levels of CO₂ at such exposure periods (3, 6, 12, 24, 48, 72, 96, 120 and 144 h for adults and larvae and 3, 6, 12, 24, 48, 72 h for adults and exceeded to 96 and 120 h for larvae with N₂ concentrations. Only a single previous study that conducted by Riudavet *et al.* (2009) used CO₂ against all stages of *O. surinamensis*. They applied only two levels of MAs that contained 50 and 90% CO₂ at two exposure periods, 4 and 8 days. There was a large gap between the two tested modified atmospheres and the two

exposure periods. So, some stages may be killed completely with MAs contained CO₂ less than 90% through time less than that recorded by Riudavet *et al.* (2009).

Complete mortalities for adults were recorded at exposure period of 144 h with MAs contained 20% CO₂, 120 h with MAs contained 40% CO₂ and 96 h with 50% CO₂ while complete mortalities for adults were recorded at exposure period of 72 h with modified atmospheres contained 97 and 98% N₂. The mortality % of third instar larvae were 100% at exposure period 144 h at 20% CO₂, 120 h with MAs containing 40% CO₂ and 72 h with 50% CO₂, 97 N₂ and 98% N₂. These findings agree with that recorded by Riudavets *et al.* (2009). They found that all stages (exceptionally, pupa) of *O. surinamensis* which treated with MAs contained 50% CO₂ were completely killed at 4 days exposure. They added that pupa was completely killed at the same period when it was treated with MAs contained 90% CO₂.

In conclusion, five tested MAs contained CO₂ (20, 40 and 50%) and N₂ (97 and 98%) had strong effect against larvae and adults of the saw-toothed grain beetle *O. surinamensis*. Six days were adequate to kill larvae and adults completely under all tested MAs contained different concentration of CO₂. Five days were required to kill larvae completely under the two MAs containing 97 and 98% N₂ and 72 h to kill adults completely under the same concentrations. In addition, modified atmospheres as can effectively replace to the Methyl bromide in controlling these pests that infest palm-date fruits.

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تأثير بعض الأجواء الهوائية المعدلة كبداية لبروميد الميثيل على الأطوار المختلفة لخنفساء السورينام (*Coleptera: Oryzaephilus surinamensis* (L.) Silvanidae)

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تهدف هذه الدراسة إلى تقييم التأثيرات المختلفة لبعض الأجواء الهوائية المعدلة على كل من العمر البرقي الثالث والحشرة الكاملة لخنفساء السورينام. وتم تعديل الجو الهوائي بزيادة تركيز غاز ثاني أكسيد الكربون في الجو الهوائي أو النيتروجين، وتم التقييم عند ظروف النمو المثلى للحشرة وهي 25°م و 65% رطوبة نسبية. وتم دراسة تأثير ثلاثة أجواء هوائية معدلة تحتوي على نسب مختلفة من ثاني أكسيد الكربون في الهواء (20، 40، 50 %) واثنين من الأجواء الهوائية المعدلة تحتوي على غاز النيتروجين (97، 98%) وذلك على فترات تعريض مختلفة حيث تم تسجيل النسبة المئوية للموت على فترات التعريض 3، 6، 12، 24، 48، 72، 96، 120، 144 ساعة.

أظهرت النتائج زيادة النسب المئوية لموت اليرقات والحشرات الكاملة بزيادة كل من تركيز ثاني أكسيد الكربون في الجوالهوائي أو النيتروجين، وزيادة فترة التعريض. وأظهرت النتائج أن الأجواء الهوائية المعدلة بزيادة تركيز غاز النيتروجين أكثر تأثيراً على اليرقات والحشرات الكاملة من تلك الأجواء الهوائية المعدلة بزيادة غاز ثاني أكسيد الكربون. وأوضحت النتائج أيضاً أن فترة التعريض لمدة 144 ساعة (سنة أيام) للأجواء الهوائية المعدلة باستخدام غاز ثاني أكسيد الكربون كانت كافية للقضاء على اليرقات والحشرة الكاملة لخنفساء السورينام، بينما يكفي التعريض لمدة 120 ساعة (خمسة أيام) للأجواء الهوائية المعدلة باستخدام غاز النيتروجين لوصول نسبة موت إلى 100% لليرقات، و72 ساعة فقط (ثلاثة أيام) للحشرات الكاملة.