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EFFECT OF DIFFERENT MODIFIED ATMOSPHERES, AN ALTERNATIVE TO METHYL BROMIDE ON DIFFERENT STAGES OF SAW TOOTHED GRAIN BEETLES, *Oryzaphilus surinamiensis* (L.) (Coleptera: 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 (CO_2) contents or high nitrogen (N_2) contents at optimum conditions (25°C and 65±5 % RH). The experiments were carried out using 20, 40, and 50% CO_2 as well as 97 and 98% N_2 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 CO_2 or N_2 and increased significantly ($P < 0.01$) with increasing either exposure time length or gas concentration. MAs enriched with N_2 were more effective than those contained CO_2. 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 CO_2. Five days were required to kill larvae were required completely under the two modified atmospheres contained 97 and 98% N_2 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 (O_2) and high carbon dioxide (CO_2) or nitrogen (N_2) contents are technically suitable alternatives for arthropod pest control in durable commodities (Riudavets *et al*., 2010). Atmospheres rich in CO_2, with more than 40% in the air, are faster at controlling pests than those with high contents of N_2 (Navarro, 2006). Literatures on the effects of different types of CO_2 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, CO_2 treatments may take from a few days to several weeks to be effective in gas-tight chambers or silos (Riudavets *et al*., 20**09**). The toxicity of CO_2 to insects is known to vary among species, developmental stages and age groups. Parameters of the physical environment, such as temperature, humidity, and CO_2 levels in storage, also influence toxicity. In the majority of studies involving CO_2, 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 N_2 were experimentally tested against some stored-products insects beside other MAs enriched with various levels of CO_2. Larvae, pupae, and adults of *Tribolium castaneum* (Herbst) were exposed to atmospheres containing high N_2 or CO_2 concentrations at about 50% R.H. and 27°C for periods up to 72 hr. Overall, 99% N_2 caused greater mortality in adults than did 58% CO_2 while 58% CO_2 was more effective against pupae. The difference in larval mortality exposed to the two atmospheres was not significant, though 99% N_2 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% N_2 (Jay and Cuff, 1981). The use of N_2 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% N_2 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 N_2 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, scantly attention has been paid on Effect of different modified atmospheres, an alternative to methyl bromide on different stages of Saw toothed grain beetles, *Oryzaphilus surinamiensis* (L.) (Coleptera: Silvanidae).

Therefore, this study was designed to quantify the dosage mortality of *O. surinamensis* to CO_2 or high N_2 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 CO_2 and N_2 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 cm^3 capacity (Dreshel 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). CO_2 or N_2 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 CO_2-O_2 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 CO_2 and N_2 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 CO_2 and two concentrations of N_2 under the prevailing laboratory conditions. The tested MAs containing CO_2 were: 1) 20% CO_2, 16% O_2 and 64% N_2, 2): 40% CO_2, 12% O_2 and 48% N_2 and 3) 50% CO_2, 10% O_2 and 40% N_2. The tested MAs containing N_2 were: 1) 97% N_2 & 3% O_2) and 98% N2 & 2% O2. The exposure periods for each of the three tested MA treatments containing CO_2 were 3, 6, 12, 24, 48, 72, 96, 120 and144 h for each adults and larvae treatments. The exposure periods of each of the two MA treatments containing N_2 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 $\pm$ 2 ºC and 65 $\pm$ 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 $\pm$ 2 ºC and 65 $\pm$ 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). LT_{50} and LT_{95} values were also calculated using the computer program developed by Noack and Reichmuth (1978).

RESULTS

1- Effect of CO_2 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 CO_2 at 25°C were indicated in Fig (1). The mortality % increased with increasing both CO_2 concentrations and exposure

period. Mortality percentage was 100% at six days with 20% CO_2, five days with 40% CO_2 and three days exposure period with 50% CO_2 . LT_{50} and LT_{95} values at 25 °C declined gradually from low to high concentrations of CO_2. LT_{50} values were 1.07, 1.15 and 0.55 with 20, 40 and 50% CO_2. LT_{95} values were 8.76, 11.37 and 2.61 with the same concentrations, respectively (Table 1).

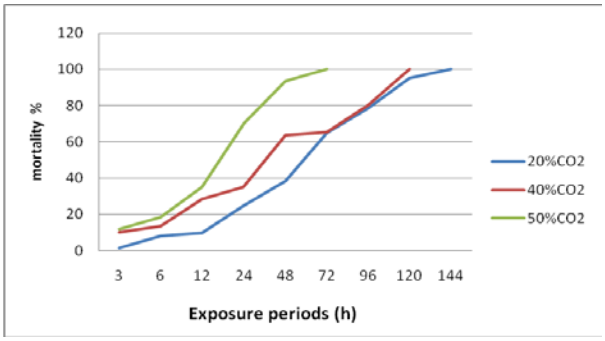

Fig. (1): Mortality percentages (Mean±SE) for Larva of *O. surinamensis* exposed to different modified atmospheres (MAs) containing different concentrations of CO_2 combined with different exposure periods at 25°C.

Table (1): LT_{50} and LT_{95} 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 CO_2 concentrations and exposure period. Mortality percentage was 100% at 6, 5 and 4 days exposure period with 20, 40 and 50% CO_2, respectively at 25°C. The high concentration (50%) of CO_2 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 LT_{50} and LT_{95} values at 25 °C. Those declined gradually from low to high concentrations of CO_2. LT_{50} was 1.96, 0.86 and 0.62 with 20, 40 and 50% CO_2, respectively. LT_{95} values were 10.79, 3.41 and 2.29 with the same concentrations, respectively.

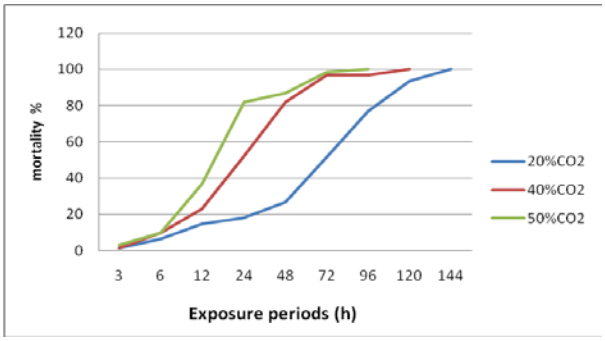

Fig. (2): Mortality percentages (Mean±SE) for adult of *O. surinamensis* exposed to different modified atmospheres (MAs) containing different concentrations of CO_2 combined with different exposure periods at 25°C.

Table (2): LT_{50} and LT_{95} 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	47.088	259.104	28.3824	73.9464	233.6856	718.4856
40 % CO2	20.664	81.888	18.5424	22.944	70.188	98.4144
50 % CO_2	14.88	55.104	11.7624	18.5952	44.0664	80.6064

2- Effect of N_2 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 N_2 concentration and exposure period (Fig 3). Mortality percentage was 100% with 98% N_2 at five days exposure period but the N_2 concentration of 97% caused 98.3 % mortality with the same exposure period. The high concentration of N_2 (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 LT_{50} and LT_{95} together with their confidence limits in cases of 97% N_2 (35.684 and 225.986 h) and 98% (24.48 and 163.64 h).

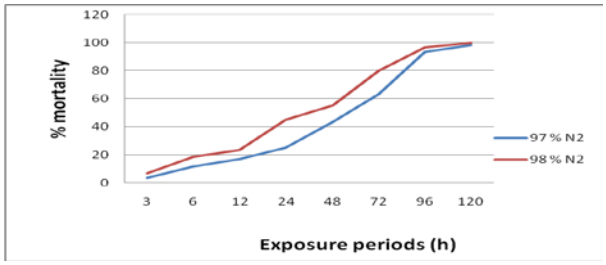

Fig. (3): Mortality percentages for larvae of *O. surinamensis* exposed to different nitrogen atmospheres combined with different exposure periods at 25°C.

Table (3): LT_{50} and LT_{95} 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 LT_{50} and LT_{95} values at 25 °C declined gradually from low to high N_2 concentrations. LT_{50} and LT_{95} values were (11.9 and 63.4 h), (7.3 and 40.4 h) with 97 and 98% N_2 concentrations, respectively (Table 4).

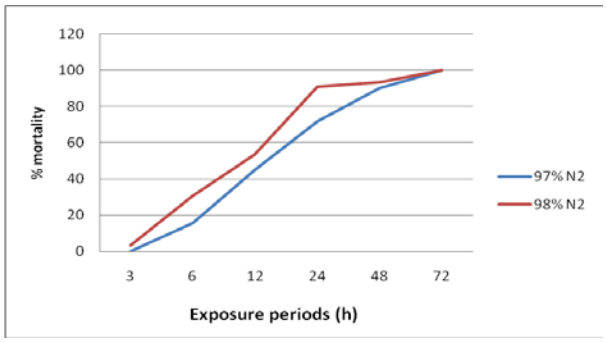

Fig. (4): Mortality percentages for adults of *O. surinamensis* exposed to different nitrogen atmospheres combined with different exposure periods at 25°C.

Table (4): LT_{50} and LT_{95} 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 CO_2 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% CO_2 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% CO_2 against all stages of *O. surinamensis* for four and eight days at 25°C. While, Riudavets *et al*. (2010) used CO_2 at high pressure with reduced the time required to kill the insect pests. Nielsen (2001) applied atmospheres with high content of CO_2 under a pressure against the adults of *O. surinamensis*. However, Locatelli and Daolio (1993) studied the effectiveness of CO_2 under reduced pressure against life stages of *O. surinamensis*. MAs used to protect commodities throughout their storage life by using low O_2 levels (Conyers and Bell, 2007). Additionally, Leelaja *et al*. (2007) used CO_2 to enhance the toxicity of allyl acetate against the adult beetle of *O. surinamensis*.

The present study applied different MAs based on either high CO_2 or high N_2 contents at optimum conditions of 25°C and 65±5 % RH. The experiments were conducted using 20, 40, and 50% CO_2 as well as 97% and 98% N_2 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 CO_2 or N_2 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 CO_2 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 and120 h for larvae with N_2 concentrations. Only a single previous study that conducted by Riudavet *et al.* (2009) used CO_2 against all stages of *O. surinamensis*. They applied only two levels of MAs that contained 50 and 90% CO_2 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 CO_2 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% CO_2, 120 h with MAs contained 40% CO_2 and 96 h with 50% CO_2 while complete mortalities for adults were recorded at exposure period of 72 h with modified atmospheres contained 97 and 98% N_2. The mortality % of third instar larvae were 100% at exposure period144 h at 20% CO_2, 120 h with MAs containing 40% CO_2 and 72 h with 50% CO_2 , 97 N_2 and 98% N_2. 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% CO_2 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% CO_2.

In conclusion, five tested MAs contained CO_2 (20, 40 and 50%) and N_2 (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 contented different concentration of CO_2. Five days were required to kill larvae completely under the two MAs containing 97 and 98% N_2 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 كبدائل لبروميد الميثيل على الأطوارالمختلفة لخنفساء السورينام (Coleptera: *Oryzaphilus surinamiensis* (L.) Silvanidae)

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تهدف هذه الدراسة إلى تقييم التأثيرات المختلفة لبعض الأجواء الهوائية المعدلة على كل من العمر اليرقى الثالث والحشرة الكاملة لخنفساء السورينام. وتم تعديل الجو الهوائى بزيادة تركيز غاز ثاني أكسيد الكربون فى الجو الهوئى أوالنيتروجين، وتم التقييم عند ظروف النمو المثلى للحشرة وهى ٢٥°م و ٦٥% رطوبة نسبية. وتم دراسة تأثير ثلاثة أجواء هوائية معدلة تحتوى على نسب مختلفة من ثاني أكسيد الكربون في الهواء (٢٠، و٤٠، ٥٠ %) واثنين من الأجواء الهوائية المعدلة تحتوى على غاز النيتروجين (٩٧، ٩٨%) وذلك على فترات تعريض مختلفة حيث تم تسجيل النسبة المئوية للموت على فترات التعريض ٣، ٦، ١٢، ٢٤، ٤٨، ٧٢، ٩٦، ١٢٠، ١٤٤ ساعة.

أظهرت النتائج زيادة النسب المئوية لموت اليرقات والحشرات الكاملة بزيادة كل من تركيز ثاني أكسيد الكربون في الجوالهوائى أو النيتروجين، وزيادة فترة التعريض. وأظهرت النتائج أن الأجواء الهوائية المعدلة بزيادة تركيزغاز النيتروجين أكثر تأثيراً على اليرقات والحشرات الكاملة من تلك الأجواء الهوائية المعدلة بزيادة غاز ثاني أكسيد الكربون. وأوضحت النتائج أيضاً أن فترة التعريض لمدة ١٤٤ ساعة (ستة أيام) للأجواء الهوائية المعدلة باستخدام غاز ثاني أكسيد الكربون كانت كافية للقضاء على اليرقات والحشرة الكاملة لخنفساء السورينام، بينما يكفى التعريض لمدة ١٢٠ ساعة (خمسة أيام) للأجواء الهوائية المعدلة باستخدام غاز النيتروجين لوصول نسبة لموت الى ١٠٠% لليرقات، و٧٢ ساعة فقط (ثلاثة أيام) للحشرات الكاملة.