

TIME NEEDED FOR POPULATION EXTINCTION OF CERTAIN STORED PRODUCT INSECTS USING MODIFIED ATMOSPHERE OF HIGH NITROGEN CONTENT INSIDE STEEL BINS.

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Abstract

Modified atmosphere (MA) of 99% N₂ at grain temperatures of 18 and 28± 2°C was used as control method for the adults and immature stages of *Sitophilus oryzae*, *Rhizopertha dominica* and *Triblium castaneum* as well as the active and diapausing larvae of *Trogoderma granarium* inside gas-tight steel bins. The obtained results indicated that efficacy of MA 99% N₂ was differed according to the tested insect, treated stage, temperature, and exposure period. The lethal times required to achieve population extinction of the different stages of *S. oryzae* at 18±2°C were 10, 35, 28 and 35 days for adults, eggs, larvae and pupae, respectively. While these values decreased to 7, 28, 21 and 28 days at 28±2°C, respectively. For *R. dominica* they were 14, 35, 28 & 35 days at the first temperature and 10, 28, 21 and 28 days at the second one, respectively. As for *T. castaneum*, these values were 14, 21, 10 & 14 days and 10, 14, 7 and 10 days, respectively. Active larvae of *T. granarium* were more susceptible (28 days) than diapaused ones (35 days) at 18±2°C, while the susceptibility of these insects were not differed (28 days) at 28±2°C. In general, results revealed that the pupa and egg stages of the different insects were more tolerant to MA of 99% N₂ than other stages.

INTRODUCTION

Pest control in stored grain and their products has been achieved generally by methyl bromide (MB). MB cause about 5-10% of total ozone depletion in the earth's stratosphere, increasing the level of UV radiation which has been linked to skin cancer, eye cataracts and degradation of immune system. Thus, the Montreal Protocol (1997) declared the phase-out of MB in industrialized countries by the year 2005. A need for effective control method became obligatory. This brought out the modified atmospheres (MAs) or the controlled atmospheres as new method. This new method was investigated by many workers as Lindgren and Vincent (1970), Reichmuth and Ofuya (1992), Hashem *et al.* (1993), El-Lakwah and Halawa (1998) and El-Lakwah *et*

al. (2002). Susceptibility of *Sitophilus granarius* (L.), and the rice weevil *S. oryzae* (L.) (Curculionidae, Coleoptera) adults in CO₂, N₂ or He atmospheres at 80, 70 and 60°F showed that, *S. granarius* adults, were the most susceptible stage to these atmospheres followed by larvae, eggs and pupae (Lindgreen and Vincent, 1970). Reichmuth and Ofuya (1992) studied the influence of controlled atmospheres containing low oxygen and high nitrogen contents (1% O₂ + 99% N₂; 2% O₂ + 98% N₂ and 3% O₂ + 97% N₂) at 25°C and 32°C, and at 70% R.H. against two bruchid species (*Callosobruchus maculatus* F. and *Acanthoscelides obtectus* Say). Complete mortality of all eggs, young larvae, old larvae, pupae, and adults of both bruchid pests was obtained at both temperatures within 1-4, 3-9, 5-13, 5-15, and 1-5 days, respectively. Hashem *et al.* (1993) investigated susceptibility of the eggs stage of three moths (i.e. *Sitotroga cerealella*, *Ephestia elutella* and *Corcyra cephalonica*) treated with O₂ deficient in pure nitrogen atmospheres at 15 and 25°C. At both temperatures, LT₉₅ values showed that eggs of *S. cerealella* were more tolerant to 1, 2, 3, 4% O₂, respectively, than eggs of the other two moth species over tested exposure periods, which ranged from 1-7 days. Eggs of *C. cephalonica* were the most sensitive at 15°C and eggs of *E. elutella* at 25°C. At 25°C, all O₂ concentrations were generally more effective than at 15°C. El-Lakwah *et al.* (1997) determined the efficacy of modified atmosphere of approximately 1% O₂ plus 99% N₂ against *S. oryzae*, the lesser grain borer *Rhizopertha dominica* (F.) (Bostrychidae, Coleoptera) and *Tribolium castaneum* at 26 and 6 °C. Time required to achieve certain kill for the various insect stages was considerably longer at the lower temperature than at the higher one. Marked difference in susceptibilities of the various stages of the insect species were obtained, whereas the adult stage was the sensitive one. *T. castaneum* was the most susceptible species. El-Lakwah and Halawa (1998) investigated the efficacy of MA containing 99% N₂ plus 1% O₂ against two species of stored products pests (*C. maculatus* and the mite *Tyrophagus putrescentiae*) at 26 and 6°C. Time required to obtain mortality for the various stages of the both species was obviously longer at the lower temperature than at the higher one. Adults and larvae of *C. maculatus* were more susceptible to the modified atmosphere at the two temperatures than pupae and eggs, while in case of *T. putrescentiae* adults, nymphs and larvae were more sensitive than eggs. El-Lakwah *et al.* (2002) investigated the efficacy of MA of 99% N₂ at grain temperatures of 15 and 26 ± 2°C against the adults and immature stages of *S. oryzae*, *R. dominica* and *T. castaneum* as well as the active and diapausing larvae of

the red flour beetle, *Trogoderma granarium* Everts (Dermestidae, Coleoptera). Results indicated that MA of 99% N₂ was temperature and exposure period dependent. Insect mortality increased with increasing of the period of exposure and it was also greater at the higher grain temperature than at the lower one. Susceptibility of the insects varied according to insect species and stage. Pupae of the various tested insects were more tolerant to MA of 99% N₂ than the other stages. Also, the diapausing larvae of *T. granarium* were less susceptible to the MA of 99% N₂ than active ones. The objective of this work was to investigate the effectiveness of MA of high nitrogen content (99% N₂) against some stored product insects for determining the time needed for their population extinction inside gas-tight steel bins.

MATERIALS AND METHODS

a. Insects

Laboratory strains of *S. oryzae*, *R. dominica*, *T. granarium* and *T. castaneum* were used. They were maintained at the Stored Product Pests Laboratory, Plant Protection Dept., Faculty of Agric., Benha University. The experiments were conducted from October until February 2003. The insects were reared in glass jars of approx. 250 ml. Each jar contained about 200 g wheat kernels for the first three insects or wheat flour in case of the fourth one and covered with muslin cloth fixed with rubber band. Insect cultures were kept under controlled conditions of $26 \pm 2^{\circ}\text{C}$ and $60 \pm 5\%$ R.H. at the laboratory rearing room. Wheat grains were treated by freezing at -18°C for 2 weeks before application to eliminate any possible infestation by any insect species. The moisture content of the grains was about 14%. Around 1000 adults (1-2 weeks-old) of *S. oryzae*, *R. dominica* and *T. castaneum* were introduced to 500 g wheat kernels and wheat flour of 14% moisture content, in the glass jars and covered with muslin cloth. A roll of paper was placed on the top of the grains to serve as a suitable premise for the diapaused larvae. In case of *T. granarium*, about 600 adults insect (0-24 hrs old) were introduced to 500 g wheat grains of around 11% moisture content in glass jars of 500 ml volumes.

b. Methods of obtaining the developmental stages of the various insect species:

For the two insect species *S. oryzae* and *R. dominica*, 24-48 hrs-old eggs, 10-11 days-old larvae and 2-4 days-old pupae were selected for the experiments. Glass jars containing 500 g foods were prepared. Into each jar, approx. 1000 adult insects (1-2

weeks-old) were added to the food and left for two days in the media for laying eggs. After this, the insects were separated using a sieve (2.5 mm.) and the food was thoroughly mixed for uniformity. Obtained media portions were assumed to contain 0-48 hrs-old eggs, which considered the standard age for ovicidal action. Similar technique was used for obtaining the larvae and pupae. Portions of media containing eggs were kept for two weeks at the rearing condition after the removal of the adults to obtain larvae. To obtain the pupal stages these cultures were maintained for 24 days after the removal of the adult insects.

In case of *T. castaneum*, 24-48 hrs old eggs, 4th instar larvae and 2-3 days-old pupae were selected for the experiments. These developmental stages are easy recognizable in the media. Adults (7-14 days-old) of *S. oryzae*; *R. dominica* and *T. castaneum* were used in the tests. Active (3rd and 4th larval instars) and diapausing larvae of a weight of 1.3 ± 0.2 mg and 3.1 ± 0.2 mg, respectively, were selected for the tests.

c. Preparation of the test-insects for various treatments

Batches of 30 adult insects or 30 active and diapausing Larvae of *T. granarium* were confined in cloth bags (10 × 16 cm.) each containing 50 g wheat kernels for *S. oryzae*, *R. dominica* and *T. granarium* or 50 g wheat flour in case of *T. castaneum*. For the immature stages of the first two insect species, fifty grams of the cultures of eggs, larvae and pupae were placed in the cloth bags. For *T. castaneum*, twenty grams eggs, thirty 4th larval instar and thirty pupae were taken from the culture and put on 50 g wheat flour in each cloth bag. Each bag was well closed by a rubber band. Three replicates were conducted for each insect stage in the various treatments.

d. Exposure procedure of the insects inside the airtight steel bins:

Nitrogen (N₂) was provided as pure gas of about 99% in pressure steel cylinder. Cylinder was connected with a pressure regulator. Airtight steel bins: Each of 0.5 m³ volumes. Each bin was filled with about 450 Kg wheat grains. The bins were designed and constructed by the Military Factory for Airplanes at Helwan, Cairo, Egypt. These bins were situated on the roof of the building of the Plant Protection Department of the Fac. of Agric., Benha Univ., Qualubia, Egypt. Insect bags were inserted in the grains inside the steel bins just before each treatment. The cover of the bin was closed tightly. MA of high nitrogen content was tested at 99% N₂ concentration. Another identical steel bins was left without insect treatment to measure the temperature and relative humidity of wheat grains inside the steel bins during the

experimental period. After the desired exposure time, the steel bins were opened and aerated. Insect samples were then taken and transferred to the laboratory for mortality assessment.

For achieving (MA) of high nitrogen content (99% N₂), the upper valve of the steel bin was opened for 5 minutes after the connection of N₂ cylinder with the bin, while the bottom bin valve was opened for two minutes, after which it closed tightly. A bin without treatment was used as control. Nitrogen concentration was determined inside the bins using Oxygen Analyzer 572, SERVOMEX, England.

e. Bioassay tests

Insect samples were exposed to the various treatments for varying length of time. After the desired exposure period mortality assessment was performed. Mortality percentages were corrected by Abbott's formula (1925). Adult mortalities of *S. oryzae*; *R. dominica* and *T. castaneum* as well as active and diapausing larvae of *T. granarium* were determined after 48 hrs from aeration of the storage facility. The mortality of the immature stages of *S. oryzae*, *R. dominica* and *T. castaneum* were recorded as reduction rate of the F1 progeny which was inspected after 60 days from treatment. The inhibition rate of progeny was calculated as follows: -

% Inhibition =

$$\frac{\text{No. of emerged adults in control} - \text{No. of emerged adults in treatment}}{\text{No. of emerged adults in control}} \times 100$$

RESULTS AND DISCUSSION

A. Effect of MA of 99% N₂ against the tested insects at 18 ± 2°C, 55 ± 5% R.H.

After the varying exposures periods inside the gas-tight steel bins, the obtained results are listed in Table 1. The data revealed that mortalities of the various insect species and their developmental stages raised gradually with the increase of the exposure period of the insects to N₂ inside the bins. In case of *S. oryzae*, at 3 day-exposure, mortalities of the adult, egg, larva and pupa of *S. oryzae* were 50.2 ± 6.1, 25.2 ± 3.2, 31.2 ± 6.1 and 20.2 ± 4.1%, respectively. These values increased with the increase of the exposure period to reach complete kill at 10, 35, 28 and 35 days-exposure for the above mentioned immature stages, respectively. The exposure time

required for achieving complete kill for the various stages of *S. oryzae* using MA of 99% N₂ at 18 ± 2°C inside the bins was found to be 5 weeks. Also, egg and pupal stages were more tolerant to tested MA than adult and larval stages of *S. oryzae*.

In case of *R. dominica*, adult mortality of *R. dominica* was 40.2 ± 3.1% at 3 days-exposure and this value increased to 71.2 ± 6.1% at 7 days exposure and reached complete kill after 14 days post-treatment. Egg percentage mortality was 20.2 ± 6.1% at 3 days exposure and reached 100% after 35 days exposure. Larval percentage mortality was 33.2 ± 6.2% at 3 days-exposure and complete kill at 35 days-exposure. Under this condition to obtain 100% mortalities for the various stages of *R. dominica*, the exposure time needed was at least 5 weeks and the pupal & egg stages were less susceptible than the other stages.

Adult mortality of *T. castaneum* was 36.2 ± 3.1% at 3 days exposure and this value increased to 100% at 14 days exposure. Egg mortality was 36.2 ± 2.1% at 3 days exposure. And reached complete kill after 21 days exposure. Larval mortality was 40.2 ± 3.1% after 3 days of exposure and complete kill was achieved at 10 days post-treatment. Pupal percentage mortality was 30.2 ± 6.1% after 3 days exposure and this value reached 100% at 14 days-exposure. Time for achieving 100% kill for the various stages of *T. castaneum* using the tested conditions was at least 3 weeks. Larvae were the most susceptible stage.

In case of *T. granarium*, the recorded mortalities for the active and diapausing larvae at 3 days exposure were 3.3 ± 2.1% and 0.0, respectively. These values reached complete mortality at 28 and 35 days post-treatment for the active and diapausing larvae, respectively. The results showed also that the exposure time needed to achieve complete mortalities for active and diapausing larvae of *T. granarium* using the tested conditions was found to be at least 5 weeks.

In summary, the results showed clearly, that an exposure period of at least 5 weeks is necessary to achieve total extinction of populations of the adults and immature stages of *S. oryzae*, *R. dominica*, *T. castaneum* and *T. granarium* by using MA of 99% N₂ inside the gas-tight steel bins at grain temperature of 18 ± 2°C.

B. Effect of MA of 99% N₂ against the tested insect species at 28 ± 2°C, 55 ± 5% R.H.

Results of varying exposure periods of the tested insects with MA of 99% N₂ inside the gas-tight steel bins are given in Table (2). The results showed clearly that,

mortalities of the various insects and their immature stages increased by increasing both the exposure period and the grain temperature.

In case of *S. oryzae*, mortalities of adult, egg, larva and pupa were 60.2 ± 2.1 , 35.2 ± 2.1 , 40.3 ± 4.1 and $30.2 \pm 6.1\%$, after 3 days of exposure, respectively. These values increased to reach complete kill at 7, 28, 21 and 28 days-exposure for the aforementioned stages, respectively. The exposure time required for achieving complete kill for these stages inside the bins was 4 weeks. It was also clear, that the pupal and egg stages of *S. oryzae* were less susceptible to N_2 than the larval and adult stages.

In case of *R. dominica*, adult mortality was $50.4 \pm 2.1\%$ after 3 days of exposure and increased to complete kill at 10 day-exposure. Egg percentage mortality after 3 days of exposure was $33.2 \pm 6.1\%$ and reached 100% kill after 28 days of exposure. Larval mortality was $43.2 \pm 6.1\%$ after 3 days of exposure and this figure reached complete kill after 21 days post-treatment. Pupal mortality was $28.1 \pm 4.1\%$ after 3 days of exposure, and increased gradually to reach 100% after 28 days of exposure. Under this condition, 4 weeks of exposure time were required to obtain 100% mortalities to the various stages. Also, the pupal and egg stages of this insect were less susceptible to N_2 than the larval and adult stages.

In case of *T. castaneum*, adult, egg, larval and pupal mortality was $46.2 \pm 3.4\%$, $40.2 \pm 7.1\%$, $50.2 \pm 6.1\%$ and $43.2 \pm 1.2\%$ at 3 days of exposure, respectively. While, complete mortality was achieved after 10, 14, 7, and 10 days of exposure. For this insect, the larval stage of was the most susceptible. Mortalities of active and diapausing larvae of *T. granarium* at 3 days of exposure were 6.3 ± 2.1 and $3.3 \pm 3.2\%$, respectively. These values increased to 100% at 28 day-exposure for both larvae.

Briefly, the results showed clearly, that an exposure period of 4 weeks is needed to obtain total extinction of the populations of adults and immature stages of the four aforementioned insects after exposure to MA of 99% N_2 inside the gas-tight steel bins at grain temperature of $28 \pm 2^\circ\text{C}$.

In conclusion, using modified atmosphere (MA) of high nitrogen content (99% N_2) for controlling the various stages of the tested insects stored inside gas-tight steel bins, revealed that the exposure times for effective control have to be shortened to one month during summer conditions and extended to 5 weeks during winter times. These results are in agreement with the findings of other studies (El-Lakwah *et al.*, 1997 and 2002).

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Table 1. Response of the tested stages of various insects to modified atmosphere (MA) of 99% N₂ inside gas-tight steel bins at 18 ± 2°C and 55 ± 5% R. H. after varying exposure periods.

Insect species	Stage	Mortality (%) after exposure period (days)							
		3	5	7	10	14	21	28	35
<i>S. oryzae</i>	Adult	50.2 ± 6.1	70.4 ± 3.2	88.2 ± 3.1	100	100	100	100	100
	Egg	25.2 ± 3.2	30.2 ± 3.1	40.2 ± 6.1	51.2 ± 6.1	73.2 ± 6.2	83.2 ± 4.2	91.2 ± 3.1	100
	Larva	31.2 ± 6.1	36.2 ± 2.1	44.2 ± 3.1	60.2 ± 2.1	80.2 ± 3.1	91.2 ± 6.1	100	100
	Pupa	20.2 ± 4.1	25.2 ± 6.2	36.4 ± 3.1	46.2 ± 2.6	58.2 ± 7.1	73.2 ± 6.2	83.2 ± 6.1	100
<i>R. dominica</i>	Adult	40.2 ± 3.1	60.2 ± 3.1	71.2 ± 6.1	90.2 ± 3.1	100	100	100	100
	Egg	20.2 ± 6.1	28.1 ± 2.1	36.2 ± 4.2	46.2 ± 3.2	70.2 ± 3.1	80.4 ± 3.1	90.2 ± 6.2	100
	Larva	33.2 ± 6.2	36.5 ± 4.2	40.2 ± 3.1	55.2 ± 8.1	77.2 ± 6.1	85.5 ± 2.1	100	100
	Pupa	20.2 ± 6.2	25.2 ± 6.3	32.4 ± 3.1	43.2 ± 2.1	53.2 ± 4.2	70.2 ± 6.1	80.2 ± 2.1	100
<i>T. castaneum</i>	Adult	36.2 ± 3.1	50.2 ± 4.1	66.2 ± 3.1	83.4 ± 2.1	100	100	100	100
	Egg	36.2 ± 2.1	43.2 ± 4.1	60.2 ± 3.1	71.2 ± 6.1	88.2 ± 2.1	100	100	100
	Larva	40.2 ± 3.1	70.2 ± 6.1	90.2 ± 3.1	100	100	100	100	100
	Pupa	30.2 ± 6.1	60.2 ± 4.2	77.3 ± 2.1	90.2 ± 6.1	100	100	100	100
<i>T. granarium</i> larvae	Active	3.3 ± 2.1	16.2 ± 4.1	28.2 ± 6.3	50.2 ± 4.2	70.4 ± 2.1	83.2 ± 6.1	100	100
	Diapause	0.0	11.3 ± 6.2	23.4 ± 6.1	43.2 ± 2.1	62.4 ± 3.1	77.6 ± 4.1	89.2 ± 3.1	100

Reduction in F₁ progeny after 60 days was 100%

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Table 2. Response of various insects of tested stages to modified atmosphere (MA) of 99% N₂ inside gas-tight steel bins at 28 ± 2°C, 55 ± 5% R. H. after different exposure periods.

Insect species	Stage	Mortality (%) after exposure period (days)						
		3	5	7	10	14	21	28
<i>S. oryzae</i>	Adult	60.2 ± 2.1	83.1 ± 3.1	100	100	100	100	100
	Egg	35.2 ± 2.1	41.2 ± 3.2	50.2 ± 6.1	63.2 ± 4.1	85.2 ± 4.1	98.2 ± 3.1	100
	Larva	40.3 ± 4.1	50.2 ± 3.1	63.2 ± 6.1	80.4 ± 4.1	95.2 ± 6.1	100	100
	Pupa	30.2 ± 6.1	44.2 ± 3.1	60.2 ± 3.1	66.2 ± 3.1	80.4 ± 2.1	93.2 ± 4.1	100
<i>R. dominica</i>	Adult	50.2 ± 2.1	70.2 ± 6.1	93.2 ± 4.1	100	100	100	100
	Egg	33.2 ± 6.1	40.2 ± 3.1	46.4 ± 3.1	60.2 ± 5.1	80.2 ± 4.2	95.4 ± 2.1	100
	Larva	43.2 ± 6.1	52.4 ± 3.2	65.3 ± 2.1	83.2 ± 6.1	93.2 ± 6.1	100	100
	Pupa	28.2 ± 4.1	40.2 ± 3.2	53.2 ± 6.1	63.2 ± 4.2	75.4 ± 3.2	90.2 ± 6.1	100
<i>T. castaneum</i>	Adult	46.2 ± 3.4	66.3 ± 4.1	90.2 ± 5.1	100	100	100	100
	Egg	40.2 ± 7.1	50.2 ± 3.2	70.4 ± 3.1	85.4 ± 3.1	100	100	100
	Larva	50.2 ± 6.1	83.2 ± 6.1	100	100	100	100	100
	Pupa	43.2 ± 1.2	70.2 ± 6.1	85.4 ± 3.1	100	100	100	100
<i>T. granarium</i> larvae	Active	6.3 ± 2.1	26.2 ± 3.1	38.4 ± 2.1	60.2 ± 3.1	80.2 ± 4.1	99.2 ± 3.1	100
	Diapause	3.3 ± 3.2	16.2 ± 4.1	26.8 ± 3.1	50.2 ± 3.1	70.6 ± 2.1	85.4 ± 3.3	100

Reduction in F₁ progeny after 60 days was 100%

"الزمن اللازم لفناء تعداد بعض حشرات المواد المخزونة باستعمال جو معدل من النيتروجين داخل صوامع من الصلب"

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لقد أجريت التجارب بقسم وقاية النبات (بمعمل آفات الحبوب والمواد المخزونة) بكلية الزراعة جامعة بنها وذلك بدعم من المشروع القومي للمكافحة المتكاملة لآفات ما بعد الحصاد وهذا المشروع ينفذ بالكلية بالتعاون مع معهد بحوث وقاية النباتات - قسم آفات الحبوب والمواد المخزونة - بمركز البحوث الزراعية بوزارة الزراعة واستصلاح الأراضي. ولقد أجريت التجارب داخل صوامع محكمة من الصلب باستعمال جو معدل يحتوى على ٩٩% من غاز النيتروجين.

ولقد أوضحت النتائج المتحصل عليها من تأثير الجوال المعدل الذي يحتوى على ٩٩% من غاز النيتروجين وذلك على درجة حرارة حبوب مقدارها 28 ± 2 °م داخل الصوامع الصلب ضد الحشرات الكاملة والأطوار الغير كاملة لكل من سوسة الأرز ، ثاقبة الحبوب الصغرى ، خنفساء الدقيق الكستنائية وكذلك اليرقات النشطة والساكنة لخنفساء الصعيد أن تأثير الغاز يتوقف على درجة الحرارة وفترة التعريض كما تبين أن النسبة المئوية للموت تزداد بزيادة فترة التعريض حيث كانت هذه النسب عالية على درجات حرارة الحبوب المرتفعة مقارنة بدرجة الحرارة المنخفضة ، وتباينت حساسية الحشرات مع الجو المعدل الذي يحتوى على ٩٩% من غاز النيتروجين تبعاً للنوع والطور الحشري فعلى سبيل المثال فقد وجد أن الزمن اللازم لفناء التعداد الحشري على درجة حرارة الحبوب 28 ± 2 °م هو ١٠ ، ٣٥ ، ٢٨ ، و ٣٥ يوم للحشرات الكاملة ، البيض ، اليرقات والعذارى على التوالي لحشرة سوسة الأرز ، وكان لحشرة ثاقبة الحبوب الصغرى ١٤ ، ٣٥ ، ٢٨ ، و ٣٥ يوم لكل من الحشرة الكاملة ، البيض، اليرقات والعذارى على التوالي، أما بالنسبة لحشرة خنفساء الدقيق الكستنائية فكان ١٤ ، ٢١ ، ١٠ ، و ١٤ يوم لكل من الحشرة الكاملة ، البيض، اليرقات والعذارى على التوالي وكذلك لليرقات النشطة والساكنة لحشرة خنفساء الصعيد فوجد أنها ٢٨ و ٣٥ يوم لكلا الطورين على التوالي.

وقد وجد أن الزمن اللازم لفناء التعداد الحشري على درجة حرارة 28 ± 2 °م هو ٧ ، ٢٨ ، و ٢١ يوم للحشرات الكاملة، البيض، اليرقات والعذارى على التوالي لحشرة سوسة الأرز، وكان لحشرة ثاقبة الحبوب الصغرى ١٠ ، ٢٨ ، ٢١ ، و ٢٨ يوم لكل من الحشرة الكاملة، البيض، اليرقات والعذارى على التوالي، أما بالنسبة لحشرة خنفساء الدقيق الكستنائية فكان ١٠ ، ١٤ ، ٧ ، و ١٠ أيام

للحشرة الكاملة، البيض، اليرقات والعدارى على التوالي وكذلك لليرقات النشطة والساكنة لحشرة خنفساء الصعيد فوجد أنها ٢٨ يوم لكلا الطورين. وقد أشارت النتائج أن طور البيض والعدراء للحشرات المختبرة كان أكثر مقاومة للجو المعدل المكون من ٩٩% من غاز النيتروجين مقارنة بباقي الأطوار الحشرية الأخرى. وأظهرت النتائج أن الصوامع الصلب كانت مناسبة لاستخدام الجو المعدل الذي يحتوى على تركيز ٩٩% من غاز النيتروجين للقضاء على تعداد الحشرات الكاملة والأطوار الغير كاملة للحشرات تحت الدراسة. كما تبين أن فعالية الجو المعدل من النيتروجين كانت مرتفعة على درجات حرارة الحبوب العالية مقارنة بدرجة الحرارة المنخفضة.