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# Nitrogen-Based Modified Atmosphere Technique for Controlling Pulse Beetle, *Callosobruchus maculatus* (F) in Stored White Beans

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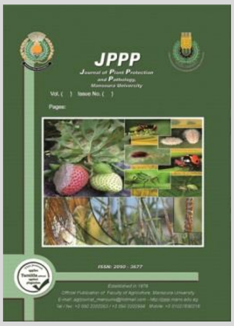


## ABSTRACT

Bruchid beetle (*Callosobruchus maculatus)* is a dangerous pest in stored legumes environment. The present study aimed at assessing the effectiveness of the nitrogen-based modified atmosphere method for combating the bruchid beetle in the managed ecosystem. Besides the control treatment, five laboratory treatments and their replicates were conducted at controlled climatic conditions in 2019 in Egypt. The different oxygen gas concentrations of 2, 5, 8, 11, 14, and 21% $O_2$ were mixed with 98, 95, 92, 89, 86, 79% $N_2$ to form different gas mixtures for the fifth, fourth, third, second, first, and control treatments, respectively, at different exposure times of three, five, and seven days. The adult and egg mortalities were enlarged either by decreasing the concentration of oxygen or by extending the exposure period. The number of newly emerged adults was nearly similar at all the exposure times for all treatments registering no significance of adult emergence variations. The treated stored grains in the fifth treatment achieved 100% egg mortality after seven days of exposure. Seven days of exposure at the rate of 2% $O_2$ were unfavorable conditions for insect growth achieving 100% mortality for the developmental stages of *C. maculatus.* The lethal concentrations of gases were 2% $O_2$ and 98% $N_2$ at seven days of exposure. The adapted atmospheres of the managed ecosystem with high nitrogen and low oxygen are environmentally friendly insect management techniques for controlling bruchid beetle in stored grains. This research may offer insights for operative stored insect management without any harm to the environment.

***Keywords**:* Pulse beetle, White beans, Modified atmosphere, Nitrogen, Egypt.

## INTRODUCTION

Legumes have major benefits for Egyptians' food as they are highly nutritive and healthy (Hashem *et al*., 2021). White beans are often stored in grain stores for a long time after the harvesting until received by Egyptian customers. During the stored time, the beans are highly susceptible to many arthropod insects (Duan *et al*., 2014; Kedia *et al*., 2015) such as *Zabrotes subfasciatus, Acanthoscelides obtectus,* and *Callosobruchus maculatus* that negatively affect their qualities and reduce marketing values (Thangarasu *et al*., 2021). *Callosobruchus maculatus* are dangerous pests in the field and stored legumes in Egypt, affecting huge losses in stored grains (Tuda *et al*., 2005; Duan *et al*., 2016). Accordingly, there is an urgent need to replace the negative impacts of insecticide residues in grains with alternative controlling technologies. The modified atmosphere eco-friendly technique is the promoting method to control the insects in storage entomology. It keeps the quality of stored grains in a good condition through managing the stored ecosystem by the in-situ fumigation (Wilkins, 2020). Furthermore, the managed atmospheres always offer a safe alternative method instead of the conventional chemical insecticides for combating insects that damage grain products in the storage ecosystem (Hashem *et al*., 2021). The dangerous insects damage the stored products as well as the field crops and horticulture during all plant stages through the entire season and can attack the stored grains and seeds as well (Cao *et al*., 2019). Techniques of managed atmospheres of the storage ecosystem with controlling the atmospheric gases concentrations provide a cost-effective method to kill target pests and protect stored products (Iturralde-García *et al*., 2016). The atmospheric environment gases can be managed by using different levels of nitric oxide (NO), ozone ($O_3$), and reducing oxygen ($O_2$) rate or increasing both nitrogen ($N_2$) and carbon dioxide ($CO_2$) forming an unsuitable gas mixture for insect growth. The modified atmosphere (MA) techniques may be attained biologically in a storage container by exclusion with $N_2$ and then sealing it hermetically, destroying pest growth (Cheng *et al*., 2013) feeding, development, and population enlargement (Yan *et al*., 2016; Wilkins, 2020). The most significant damaging bruchid is a *Callosobruchus chinensis* (pulse weevil) that has severe destruction to stored food products and mostly occupies the arid and semi-arid environments in Egypt (Hashem *et al*., 2021). Numerous studies have applied for integrated pest management through methods of modified atmosphere around the world (Mohapatra *et al*., 2015), but very few studies of modifying the atmospheric gases in the storage ecosystem were implemented in Egypt. Furthermore, while the modified atmospheric techniques have good results in the field of storage entomology, their marketable use isn't widely spread in Egypt (Wilkins, 2020; Hashem *et al*., 2021).

Chemicals, semiochemicals, and physically modified atmosphere techniques have been used to protect the stored grains from insect attacks (Cox, 2004; Wilkins, 2020; Hashem *et al*., 2021). Semiochemicals are safer and environmentally more satisfactory than chemical insecticides because semiochemicals nearly occur naturally. Modifying the composition of the atmosphere to combat the insects in grains storage has a favorable forthcoming era

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compared to traditional chemical pesticides and semiochemicals (Navarro *et al.* 2012; Wilkins, 2020). The controlled atmospheric ecosystem has numerous merits compared to chemicals and semiochemicals of pesticides (Wilkins *et al*., 2020). Modified atmosphere methods can easily remove pests from storage structures without contaminating the environment and are safer than conventional fumigant pesticides. The treated storage ecosystem with manipulating the atmospheric gases such as decreasing oxygen gas ($O_2$) and increasing nitrogen gas doesn't have harmful residues compared to chemicals and semiochemicals (Navarro *et al.* 2012).

Bean grains storage is a complicated ecosystem consisting of numerous interactions among the biotic and abiotic factors (Wong-Corral *et al*., 2013, Thangarasu *et al*., 2021). The responsible criteria affecting the pest population dynamics in the atmosphere of the storage structure are gaseous concentrations, moisture level, temperature status, broken grain particles, and relative humidity (Mason, 2019; Thangarasu *et al*., 2021) as shown in Fig. 1. The insects in the stored ecosystem have specific mobility to seek suitable conditions to eat, live, and breathe (Wilkins *et al*., 2020). Examining the insect behavior in terms of how fast it can transfer, what distance it would move, and what direction it would desire, will aid in the study of changing the suitable conditions related to oxygen and nitrogen to unsuitable for the movement of an insect within a stored structure (Wilkins, 2020).

Anoxia and hypoxia may be caused by nitrogen when used as an individual at a high concentration (Wilkins *et al*., 2020). For rapid kill of insects in stored products, the oxygen concentration should be less than three percent. The concentration below one percent oxygen has overturned effect for adult weevils in rice (Mehmood *et al*. 2018). The modification of gases amounts by keeping oxygen gas at a specific concentration important for pest development may avoid the infestation of grains in the stored bin (Mehmood *et al*. 2018; Wilkins *et al*., 2020). The managed atmospheric technique can cause hypoxia for insects by decreasing the oxygen rate (Cheng *et al.* 2012; 2013) and hypercapnia by increasing carbon dioxide (Li *et al*. 2012).

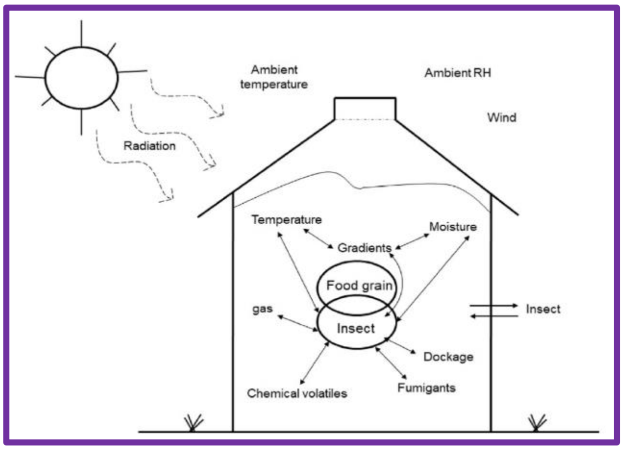


**Fig. 1. A schematic illustration of the multifarious interrelationship between insect and stored grain in the presence of abiotic and biotic aspects within the container-managed ecosystem (Thangarasu *et al*., 2021).**

The controlled atmospheric ecosystem techniques are often utilized in developed countries more than in developing countries. Therefore, introducing these methods in Egypt is highly required by modifying the stored grains ecosystem through making a different mixture of gases via manipulating the concentrations of nitrogen and oxygen rates. Hence, the main objectives of the current research were to i) evaluate the potential effectiveness of the nitrogen-based modified atmosphere, containing high levels of nitrogen in the presence of low concentrations of oxygen, against *C. maculatus*, and ii) identify the lethal concentrations of gases mixture to the developmental stages of the studied insect on stored white beans in the stored managed ecosystem under laboratory conditions in Egypt.

## MATERIALS AND METHODS

**Experimental Setup and Pest Infestation**

**1.Mass culturing of *C. maculatus***

White beans of a variety Giza 3 were used to perform this experiment in the laboratory under semi-arid conditions of Egypt in 2019. This experiment was involved in small grain masses to mimic ecological circumstances through controlling the gases of the storage atmosphere related to oxygen and nitrogen levels. Mass culturing was made by collecting the insect adults understudy from stored grains. The collected adults of studied beetles were introduced on grains of white beans. The studied grains were kept in a freezer for eight days to kill all developmental stages of the studied insect. Plastic containers were utilized in the laboratory experiment. A specific quantity (150 grams) of stored white beans was put in the container. Then, unsexed freshly emerged adults of the studied insect (Fifty pairs) were reared in the container containing the stored beans. A muslin cloth was used to cover the plastic containers. The stock containers were placed in a rearing cage and the experiments were implemented in a climatic room at 28±4 C° and relative humidity of 63±10% with 16:8 hour for light and dark. Furthermore, the emerged adults were used for the preservation of other subcultures following the same methodology as mentioned above. To get freshly matured adults of the studied insect, twenty pairs of insects were reared into the specific weight of hundred grams of fresh grains for 2 to 3 days for laying the insect eggs. The uniform matured insect adults were collected after twenty-five days from egg-laying. Numerous mass subcultures of the insect growth stages (egg and adult mortality) were produced under standard procedures and maintained under standard storage measures to perform the experiment treatments properly and achieve the objectives of the current research paper.

**Experiment Details**

The storage conditions in modified atmospheric were made by using 500 $cm^3$ glass containers and covered with metal screw caps. Standard pieces of hose, teflon coated silicon septum, and hose copper holes were used to airtightly regulate the gas into the managed ecosystem of stored products. Preliminary studies were done to check the airtightness of the container. The selected concentrations of oxygen ($O_2$) and nitrogen mixture ($N_2$) were regularly flushed into the standard containers. The standard and pressurized cylinders were used to inject the required contents of gases to form the desired gas mixture. The mixture of gases was maintained at the wanted level and the oxygen analyzer was used to check the desired gas concentration for high accuracy.

**Treatments and experiment layout**

Various concentrations of the gases ($O_2$ and $N_2$) were used in the current experiment. The gases were exposed to modify the atmosphere of the targeted insect at different periods. Factorial Completely Randomized Design (FCRD) was used to perform the treatments and their replicates. Each treatment has three replicates to get the high accuracy of the data. Five treatments, as well as the control treatment, were established as follows:

| Treatments | : | Oxygen ($O_2$, %) | plus | Nitrogen ($N_2$, %) | Exposure times (days) |
|---|---|---|---|---|---|
| T1 | : | 14 | + | 86 | 3,5 and 7 |
| T2 | : | 11 | + | 89 | 3,5 and 7 |
| T3 | : | 8 | + | 92 | 3,5 and 7 |
| T4 | : | 5 | + | 95 | 3,5 and 7 |
| T5 | : | 2 | + | 98 | 3,5 and 7 |
| T6 (Control) | : | 21 | + | 79 | 3,5 and 7 |

**Forming a gases mixture in the managed ecosystem**

To obtain a low oxygen atmosphere, releasing nitrogen gas ($N_2$) into the airtight glass container was done at various levels ranging from 86 to 98%. The experimental glass containers were stored for diverse times at three, five, and seven days. A quantity of 100 grams of white beans was transferred to a 500 $cm^3$ glass bottle. Then, unsexed adults of the studied insect were reared into the experimental container, and a nitrogen gas cylinder was used to introduce the nitrogen gas in the container at selected concentrations. To get high accuracy, the experimental containers were airtight and designed with a specific inlet hole in the lid to control the gas.

For evacuating $N_2$ gas, the nozzle port of the container was opened to clear away the undesirable gases from the container. The needed time to obtain wanted contents of oxygen was two minutes for 14% $O_2$, four minutes for 11% $O_2$, five minutes for 8% $O_2$, five minutes for 5% $O_2$, and eight minutes for 2% $O_2$. To avoid the outflow of the gases from the container, the ports of both inlet and outlet were directly locked after injecting the desired gases. The oxygen analyzer was used to measure and monitor the oxygen level in the container.

**Calculation of percent mortality**

The observation of mortality of insect adults was recorded for each treatment. The treated adults were collected and observed to determine their mortality percentage (Hashem *et al.*, 2021). The percentage of the insect's adult mortality was determined based on equation No. 1 and corrected concerning control treatment using Abbott's equation which is No. 2.

$$\textbf{Percent adult mortality} = \frac{Number\ of\ dead\ adults}{Number\ of\ injected\ adults} \times \mathbf{100}.\,(\mathbf{1})$$

**Corrected adult mortality (%)**

$$= \frac{(Percent\ treated\ mortality - Percent\ mortality\ in\ control)}{(\mathbf{100} - percent\ mortality\ in\ control\ treatment)} \times \mathbf{100} \ldots (\mathbf{2})$$

Eggs of the studied insect were collected from the same sample after computing percent adult mortality. The laid eggs were calculated by counting using the microscope at each of the exposure times. The percent survival of insect egg and mortality percentage was estimated based on the equations Nos. 3 and 4 as per the standard procedures given by Howe (1971).

$$\textbf{Percent survival of insect's egg} = \frac{\textbf{Number of emerged adults}}{\textbf{Number of laid eggs}} \times \mathbf{100}.\,(\mathbf{3})$$

$$\boldsymbol{Mortalty}\ (\%) = \mathbf{100} - \boldsymbol{Survival\ of\ insect's\ egg}\ (\%) \ldots\ldots (\mathbf{4})$$

**Data processing and analyses**

The arithmetic mean was calculated for the obtained values of the replicates for each treatment and presented in the tables. Furthermore, the arithmetic mean was again determined for the mean values of treatments at different exposure times. The ANOVA examination was utilized to statistically examine the significance of the data collected from each treatment using the SPSS program as per the standard descriptions of Snedecor and Cochran (1967). Standard procedures given by Finney (1971) were used to study the collected data of the exposure times related to the adults' mortalities. The percent values of mortalities for both eggs and adults were converted using the arcsine method. Furthermore, the registered values of both egg-laying and emerged adults were altered using the square root tool. The least significance difference (LSD) test was followed to separate the registered mean values.

## RESULTS AND DISCUSSION

### 1.The adult mortality of pulse beetle

The findings of percentage means of adult mortality across different exposure times are presented in Table (1). A significant difference in adult mortalities of the pulse beetle, *C. maculatus*, was found in the treatments after seven, five, three days of exposure at different concentrations of $N_2$ and $O_2$. Adults were highly sensitive to the fifth treatment (T5), with mean mortality (92.5%) occurring after seven days of exposure with 2% oxygen and 98% nitrogen (Table 1). The adults of the studied insect, *C. maculatus*, were highly tolerant (42.8%) at 3 days of exposure. To get 100% mortality, seven days of treatment with 98% $N_2$ were needed. The mean percentages of adult mortality were 51.2, 63.5, 73.4, 83.0, and 92.5 for the first, second, third, fourth, and fifth treatments, respectively. The exposure time was found to be significantly impacted the mortality of pulse beetle adults. The insect mortality increased by increasing the $N_2$ level and decreasing the $O_2$ concentration by increasing the exposure time. Stored pests consume less $O_2$ under the treatment with different levels of nitrogen to escape the impact of anoxia (Wong-Corral *et al.*, 2013). The growth of pests under the impact of anoxia nearly terminates and survival depends only on the ability to reduce metabolism requirements and increase glycolytic products (Hashem *et al.*, 2021). Wong-Corral *et al.* (2013) registered a rise in mortality of bruchid species (*C. maculatus, Z. subfasciatus,* and *A. obtectus)* with increasing exposure days.

**Table 1. Percentage values of adult mortality of pulse beetle after the used exposure times.**

| Treatments | Adult mortality of insect per exposure time (%) | | | |
|---|---|---|---|---|
| | **Three days** | **Five days** | **Seven days** | **Mean** |
| T1 | 42.8 | 47.5 | 63.2 | 51.2[e] |
| T2 | 51.3 | 62.4 | 76.8 | 63.5[d] |
| T3 | 63.5 | 74.2 | 82.7 | 73.4[c] |
| T4 | 72.0 | 84.8 | 92.2 | 83.0[b] |
| T5 | 85.8 | 91.8 | 100.0 | 92.5[a] |
| T6 | 4.4 | 8.9 | 13.4 | 8.9[f] |
| Mean | 53.3[c] | 61.6[b] | 71.4[a] | |
| | Content of gas | Time | Relationship | |
| CD at 0.05 | 4.3 | 3.0 | Not significant | |
| SE (d) | 2.1 | 1.5 | 7 | |
| CV (%) | 8.0 | | | |

**Explanations: The values for each column at the time of exposure are expressed as the mean of different replicates for each treatment; Values of the mean column (last column) were calculated as percentages from the mean values of the treatment at different exposure times. They followed by different letters for every kind are significantly different (P = 0.05, least significance difference, LSD).**

**2.Development of progeny**

**i. Laying of eggs**

The significant variation in the selected treatments and eggs susceptibility at different times is presented in Table 2. The lowest number of eggs was registered in the fifth treatment (T5) at different exposure days. All treatments at three days exposures were significantly more tolerant to oxygen shortage than those at five to seven days. The eggs number of three-day exposures varied from 1.6 at the fifth treatment to 9 at the first treatment compared to the control check (17.5 eggs). The highest numbers were registered in the seven-day exposures eggs (2.3-13.7) compared to control treatment (22.3). Data in Table 2 showed that the number of the egg was laid in an increasing pattern with increasing both of exposed days and O2 level. Wong-Corral *et al*. (2013) studied the effects of CO2-modified atmospheres on the well-known pest species (*Z. subfasciatus, C. maculatus, and A. obtectus*). He found that the eggs laid stage were the most tolerant stages to hypercarbia than adult stages for the studied insects. The results found by the current research paper were in line with the findings of Elisabetta *et al*. (2009) and Wilkins (2020).

**Table 2. Egg-laying of pulse beetle treated with nitrogen modified atmosphere within the laboratory treatments.**

| Treatments | The mean number of eggs laid at various studied exposure days | | | |
|---|---|---|---|---|
| | **Three days** | **Five days** | **Seven days** | **Mean** |
| T1 | 9.0 | 13.0 | 13.7 | $11.9^{b}$ |
| T2 | 5.5 | 8.0 | 9.7 | $7.7^{c}$ |
| T3 | 5.1 | 6.2 | 7.9 | $6.4^{d}$ |
| T4 | 2.8 | 3.1 | 3.3 | $3.1^{e}$ |
| T5 | 1.6 | 2.2 | 2.4 | $2.1^{f}$ |
| T6 (control) | 17.5 | 21.1 | 22.3 | $20.3^{a}$ |
| Mean | $6.9^{b}$ | $8.9^{a}$ | $9.9^{a}$ | |
| | Content of gas | Time | Relationship | |
| CD at 0.05 | 0.2 | 0.1 | Not significant | |
| SE (d) | 0.1 | 0.0 | 0.2 | |
| CV (%) | 7.9 | | | |

**Explanations: The values for each column at the time of exposure are expressed as the mean of different replicates for each treatment; Values of the mean column were calculated as percentages from the mean values of the treatment at different exposure times. They followed by different letters for every kind are significantly different (P = 0.05, LSD).**

**The emerged adults**

The emerged adult numbers of *C. maculatus* in the managed ecosystem within the studied treatments at different exposure levels are shown in Table 3. The adult emergence was significantly impacted by the level of oxygen. There is a direct relationship between the new adults and oxygen concentration, where the new population of insect adults was decreased with decreasing the oxygen level (Table 3). No adults of *C. maculatus* emerged in the fifth treatment at seven exposure days. By contrast, the highest emergence number of adults (23.5 adults) was found in the control check replicates at seven days managed environment. The number of new adults was nearly similar at all the exposure times for all treatments and their replicates (Table 3). Accordingly; the significance of adult emergence variations among treatments was none at all exposure times.

**Table 3. The emerged adults of pulse beetle across the studied treatments under different exposure times in the managed environment.**

| Treatments | Emerged adult number per exposure level | | | |
|---|---|---|---|---|
| | **Three days** | **Five days** | **Seven days** | **Mean** |
| T1 | 7.1 | 8.2 | 10.5 | $8.6^{b}$ |
| T2 | 4.3 | 4.6 | 5.3 | $4.7^{c}$ |
| T3 | 2.9 | 3.1 | 3.4 | $3.1^{d}$ |
| T4 | 1.0 | 1.0 | 1.0 | $1.0^{e}$ |
| T5 | 0.8 | 0.7 | 0.0 | $0.5^{f}$ |
| T6 (Check) | 17.8 | 19.9 | 23.5 | $20.4^{a}$ |
| Mean | $5.7^{b}$ | $6.25^{a}$ | $7.3^{a}$ | |
| | Concentration of gas | Time | Relationship | |
| CD at 0.05 level | 0.2 | Not significant | Not significant | |
| SE (d) | 0.1 | 1.0 | 0.2 | |
| CV (%) | 9.9 | | | |

**Explanations: The values for each column at the time of exposure are expressed as the mean of different replicates values for each treatment; Values of the mean column were calculated as percentages from the mean values of the treatment at different exposure times. They followed by different letters for every group. Mean values are significantly different (P = 0.05, LSD).**

**Percent egg mortalities**

The impacts of the controlled ecosystem of stored white been grains and the percentages of pulse beetles' eggs mortality under various exposure days are presented in Table 4. The values of percent egg mortalities are expressed as mean values for the replicates of each treatment. The 100% egg mortality was recorded in the fifth treatment after seven days of exposure. After 3 days of exposure, the values of egg mortality percentages varied ascendingly from the first treatment (22.6%) to the fifth treatment (67.6%). The same trend was found for the other exposure times. The statistical analysis showed that the relationship among egg mortalities across studied treatments was a highly significant difference due to the difference in $O_2$ levels at different exposure times. At different exposure times within the studied treatments, the mean values of egg mortality were 32.3% in the first treatment (T1) to 80.4% in the fifth treatment compared to the control check treatment which registered a 3.5% egg mortality.

**Table 4. Percentages of pulse beetle eggs mortality inside the treated white beans under different exposure times in the controlled ecosystem.**

| Treatments | Egg mortality percentage of studied insect at specific exposure times (%) | | | |
|---|---|---|---|---|
| | **Three days** | **Five days** | **Seven days** | **Mean** |
| T1 | 22.6 | 29.1 | 45.3 | $32.3^{d}$ |
| T2 | 23.1 | 33.3 | 52.5 | $36.3^{d}$ |
| T3 | 44.9 | 54.8 | 56.3 | $52.0^{c}$ |
| T4 | 61.5 | 67.6 | 73.2 | $67.4^{b}$ |
| T5 | 67.6 | 73.5 | 100 | $80.4^{a}$ |
| T6 (Control) | 2.5 | 3.5 | 4.5 | $3.5^{e}$ |
| Mean | $37.0^{b}$ | $42.5^{b}$ | $55.3^{a}$ | |
| | Level of gas | Time | | Relationship |
| CD at 0.05 level | 8.87 | 6.25 | | Not significant |
| SE (d) | 4.35 | 3.13 | | 7.57 |
| CV (%) | 22.53 | | | |

**Explanations: The values for each column at the time of exposure are expressed as the mean of different replicates for each treatment; Values of the mean column (last column) were calculated from the mean values of the treatment at different exposure times. Mean values followed by different letters for every kind are significantly different (P = 0.05, LSD); All values are expressed as percentages of egg mortality for each exposure period.**

In the current experiment, the adults of pulse beetle were reduced significantly by reducing oxygen level to 2%. It concluded that the eggs were more tolerant to the minimum concentrations of oxygen than the adults of the studied insect. This may be attributed to the negative impact of the high level of nitrogen on egg hatching and the minimum survived eggs as well. Similar findings were identified by Wilkins *et al*., (2020).

## CONCLUSION

Agricultural insects are a type of major biotic criteria of a storage ecosystem besides the abiotic factors. The interaction among abiotic factors and biotic aspects seriously impact the insect population. Under the storage ecosystem, physical methods to combat insects in stored products for a long time were introduced in this research paper. Besides control treatment, five treatments were conducted in 2019 under laboratory conditions in Egypt to combat the infestation of *C. maculatus* in the storage ecosystem. $N_2$-modified atmosphere technique with a 2% content of $O_2$ as in the fifth treatment exhibited highly effect on the pest's progeny growth. Among all treatments, the highest percentage of adult mortality reached 100% after seven days of exposure in treatment No. 5 at a very low rate of oxygen. The findings showed that the number of eggs laid was reduced under a modified atmosphere (2-14% $O_2$ plus 98-86% $N_2$) compared with the atmosphere of the control treatment (21% $O_2$ plus 79% $N_2$). Furthermore, the emerged adults after seven days of exposure were absent. The egg stage of *C. maculatus* in stored white beans was the most resistant stage to the managed atmosphere of the storage structure because they breathe at a very low concentration of $O_2$ than the adult stage of the same insect. The results of this research confirmed that the concentrations of 2% $O_2$ and 98% $N_2$ in the mixture of the atmosphere at seven days of exposure were the lethal rates of gases to effectively kill adults and eggs or reduce egg-laying of the insect in the controlled ecosystem. Accordingly, this paper explored the difficulty of studying insect populations in stored white beans and offered an eco-friendly technique to reduce the damage during storage. Eventually, understanding the optimum growth requirements of insects from atmospheric gases aids to establish a standard procedure in combating infestations by stored insects. This would provide high quality and quantity of beans after the long-time of stored grains for Egyptian consumers.

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## تقنية الأجواء المٌعدلة المعتمدة على النيتروجين لمكافحة خنفساء البقوليات في الفاصوليا البيضاء المُخزنة

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تتعرض بذور البقوليات لأضرار كبيرة أثناء فترة التخزين بسبب الإصابة بالعديد من الآفات الحشرية والتي من أهمها خنفساء البقوليات Pulse Beetle ، فهي تعتبر آفة رئيسية وخطرة إقتصادياً بالنسبة للبقوليات المخزنة والتي من أبرزها الفاصوليا البيضاء التي تسبب خسارة كبيرة في الحبوب المُخزنة كماً ونوعاً. بالإضافة إلى أن إستخدام المُبيدات الحشرية الكيميائية سببت مشاكل عديدة على صحة الانسان والبيئة، لذا كان لابد من التفكير في وسيلة فعالة وصديقة للبيئة لمكافحة آفات الحبوب المخزونة. إستهدفت الدراسة تأثير إستخدام تقنية الأجواء المُعدلة من خلال التحكم في نسبة النيتروچين في بيئة نمو الحشرة Nitrogen-based Modified Atmosphere Technique على مكافحة أطوار خنفساء البقوليات في الفاصوليا البيضاء المُخزنة تحت ظروف المعامل المصرية، وكذلك لمعرفة التركيز المميت لأطوار الحشرة من الغازات المستخدمة لتكوين خليط النظام البيئي أثناء فترة تخزين الحبوب. تم تقييم دراسة الأجواء المعدلة باستخدام غاز النيتروچين بالمخزن بحيث تصبح الظروف غير ملائمة لحياة الآفة بأطوارها وبالتالي تتعرض للهلاك، حيث تم تصميم التجربة عام ٢٠١٩ بعدد خمس معاملات رئيسية مُختلفة في تركيز الغازات المكونة للخليط بالإضافة إلى معاملة الكنترول Control treatment عن طريق حقن النظام البيئي المحكم لتخزين الحبوب تحت الدراسة بخليط معين من تركيزات مختلفة من غاز الاوكسُچين مع إستكمال النسبة المتبقية من غاز النيتروچين، حيث صُممت التجربة بالمعاملات التالية: (١): المعاملة الأولى كانت بنسبة خليط ١٤% أوكسُچين + ٨٦% نيتروچين، (٢): المعاملة الثانية كانت بنسبة خليط ١١% أوكسُچين + ٨٩% نيتروچين، (٣): المعاملة الثالثة كانت بنسبة خليط ٨% أوكسُچين + ٩٢% نيتروچين، (٤): المعاملة الرابعة كانت بنسبة خليط ٥% أوكسُچين + ٩٥% نيتروچين، (٥): المعاملة الرابعة كانت بنسبة خليط ٢% أوكسجين + ٩٨% نيتروچين، بالإضافة إلى معاملة المقارنة Control treatment والتي كانت بنسبة خليط ٢١% أوكسُچين + ٧٩% نيتروچين، بالإضافة إلى إستخدام عدد من المكررات لكل معامل على حدة، وتم تعرض كافة المعاملات والمكررات لفترات تعرض متفاوتة Exposure days وهي: ٣ ، ٥ و ٧ أيام. أوضحت النتائج أن نسبة موت الآفة Adult mortality محل الدراسة تزداد كلما انخفض تركيز الاكسُچين أي بزيادة نسبة غاز النيتروچين مع إطالة فترة التعرض إلى التركيزات المنخفضة من غاز الاوكسُچين. كما أوضحت النتائج أن الحبوب المعاملة بخليط من الغاز بنسبة الاكسُچين ٢% ونيتروچين ٩٨% حققت نسبة ١٠٠% موت للبيض Egg mortality بعد التعرض لسبعة أيام، كما لم يظهر طور الحشرة الكاملة Adult emergence عند نفس هذه من غازات الخليط بالمقارنة بالكنترول التي وصلت إلى ٢٣,٥ حشرة كاملة (كمتوسط لقيم مكررات معاملة المقارنة)، في حين أنها كانت بالمعاملة الأولى (خليط الغاز بنسبة ١٤% اوكسُچين + ٨٦% نتيروجين) ١٠,٥ حشرة كاملة (كمتوسط لقيم مكررات المعاملة الأولى)، كما تشابهت كافة نتائج المعاملات في خروج الحشرة الكاملة Adults emergence حيث لا يوجد إختلاف بصورة معنوية بين كافة المعاملات، بينما تأثر وضع البيض Egg-laying بنسبة الأكسُچين في خليط الغاز في النظام البيئي المُحكم Controlled ecosystem فقد تراوح متوسط عدد البيض عند التعرض لسبعة أيام خلال المكررات لكل معاملة ما بين ٢,٤ في المعاملة الخامسة إلى ١٣,٧ في المعاملة الأولى وذلك بالمقارنة بمعاملة الكنترول، حيث وصل متوسط مكررات معاملة الكنترول Control إلى ٢٢,٣ بيضة. وبناءاً على نتائج تأثير الأجواء المعدلة بتركيزات مختلفة من الاوكسُچين و النيتروچين، فقد وجد أن خليط الغازات المكون لجو النظام البيئي المحكم لتخزين الحبوب هو ٢% أكسُچين + ٩٨% نيتروچين بفترة تعرض Exposure time لا تقل على سبعة أيام هو التركيز المميت Lethal concentration لأطوار الحشرة تحت الدراسة. وخُلصت الدراسة إلى قدرة تقنية الأجواء المُعدلة بإستخدام خليط النيتروچين والأكسُچين على تغيير وتعديل الجو الملائم لنمو وتكاثر خنفساء اللوبيا البيضاء ليصبح غير ملائم لكلاً من: خروج طور الحشرة الكاملة Adult emergence، وضع البيض بالصورة المناسبة Egg-laying ، موت الحشرة الكاملة Adult mortality ، موت بيض الحشرة Egg mortality ؛ ومن ثم القضاء الفعال عليها بصورة آمنة بيئياً.