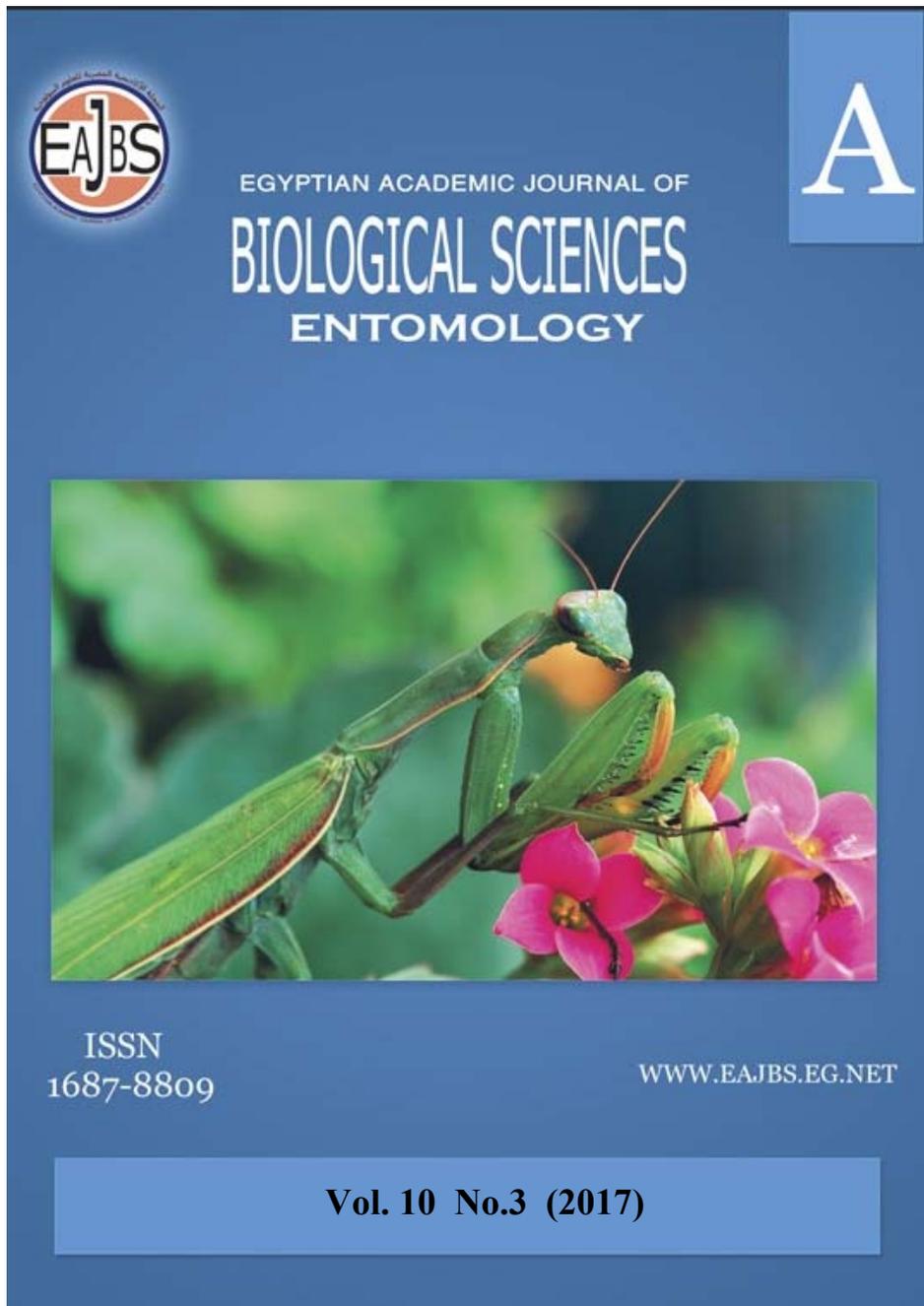


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**Efficacy of Modified Atmospheres in Controlling Museum Insect Pests,
Anthrenus verbasci (Coleoptera: Dermestidae) and *Tinea pellionella*
(Lepidoptera: Tineidae)**

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ABSTRACT

This report presents the results of the first laboratory investigation for the control of insect pests of museums, using the modified atmosphere (MA). The efficacy of using (MA); including argon or nitrogen gases against the larvae and adults of *Anthrenus verbasci* and *Tinea pellionella* was evaluated. The lethal time (LT₅₀ and LT₉₅) was determined for different exposure periods at 20°C and 30°C. Argon atmosphere achieved higher mortality than nitrogen for both insect species. The adults were more sensitive than larvae and the mortality was generally higher at elevated temperature. The univariant factorial ANOVA was used to clarify the relationship between the types of gases, the time of exposure and temperature to achieve higher mortality percentages of adults and larvae of both pests. The exposure to an atmosphere of approximately 0.01% oxygen and 99.9% argon or nitrogen was successful in limiting survival of the tested pests among the developmental stages and this condition is accelerated by warmer temperature (30°C), with the exposure time required to give complete kill, being shorter for argon.

INTRODUCTION

Archaeological materials, particularly organic materials, such as leather, wood, natural fiber textiles and paper are vulnerable to damage and deterioration by biological organisms such as vertebrate pests, insects and even microorganisms, such as mold. The varied carpet beetle, *Anthrenus verbasci*, is one of the most common species of carpet beetles found in museum settings as well as homes (Zhang, 2013). Adults lay eggs on a larval food source, such as carpets, furs, or woolen fabric. Once the eggs hatch, the larvae feed heartily on these materials and cause damage. *Tinea pellionella* is worldwide in distribution. The common name of casemaking clothes moth comes from the fact that the larva will carry a silken case with it throughout the entire larval stage until it finally uses the same case to pupate in. The case consists of silken material produced by the larva intertwined with fibers from the material it is feeding on. The food source of this pest could be any feather material, woollens, rugs and furs. The larva will drag the case with it as it feeds. If the case is removed from larva when it is near pupation it will die.

Immediately prior to pupation, the larvae will often seek a protected site such as a crevice, wall, or often the ceiling of the infested room. The emerged adults do not feed and lay the eggs in cluster (Name and Bumroongsook, 2015). To avoid biodeterioration of woolen fabric, it is recommended to store the fabrics in a suitable environment, ideally with a relative humidity of 40-55 % and a constant temperature of 10-15 °C, without use of biocide, fungicide or insecticide (David, 2001). However if deterioration has already occurred and the damage cannot be eradicated through environmental control alone, it is necessary to apply other methods to arrest the damage. Control of insect pests in conservation institutions was based on chemical pesticide fumigation and the high use of chemicals has led to accumulation of residues in the environment (Glastrup, 1987). Recently, due to the worldwide ban on the use of methyl bromide (MB) due to its detrimental effects on the Ozone layer (UNEP, 2002); it was important to focus attention on alternative methods of insect control. The advantage of low health risks combined with the residue-free benefits of the modified atmosphere (MA) technique appears to offer a suitable alternative to chemical fumigation treatments (Daniel, 1995). Uses of carbon dioxide, low oxygen atmospheres and inert gases (nitrogen, helium and argon) have been suggested for the control of several insect pests in museums (Gilberg, 1990; Daniel et al., 1993; Valentin, 1993). However, considering that the survival parameters for many insect species have been studied and that their ability to live in hypoxia is stage-specific (Hoback and Stanley, 2001), a systematic study is necessary in which the museum insect mortality of various species is determined following exposure to a range of oxygen concentrations for varying periods of time at different temperatures and relative humidity. Furthermore, most of the tests on the mortality of the insects in cultural property in anoxic environment have been done not on a laboratory reared insects, but on natural population in objects to be treated, not knowing their precise age or even their health (Berzolla et al., 2011). In this study, larvae and adults of *Anthrenus verbasci* and *Tinea pellionella* from laboratory maintained cultures were exposed to low-oxygen atmosphere under different temperatures for different periods of exposure to assess the efficacy of MA of argon or nitrogen against these pests of museums in Egypt.

MATERIALS AND METHODS

Rearing Technique of Stock Cultures:

A. verbasci was reared according to the technique described by Rust and Kennedy (1993). Larvae were reared in 3.8-liter glass jars, half filled with chicken feathers. Feathers were added to each jar once every two weeks or whenever needed. Adults were fed on honey. *T. pellionella* was reared according to Name and Bumroongsook (2015). Larvae were reared on fish food in 500 ml jars and adults do not feed. All the stock cultures were maintained under controlled conditions of 27-30°C and 55 ± 5 % RH. Both insects were maintained in the Pest Control Laboratory, Center of Research and Conservation of Antiquities Cairo, Egypt.

Gases Used:

Argon or nitrogen was used as pure gas in pressurized steel cylinders. Each cylinder was connected with a pressure regulator.

Exposure Procedure:

A circulatory multi-flask apparatus was established to provide an exposure room suitable for gas concentration applied. The Dreshel flasks (Laboclean, Germany) with a volume of 0.55 liters were connected to each other with Polyvinyl

chloride (PVC) tubing and joints were greased. Argon or nitrogen concentration was determined inside the Dreshel flasks using Oxygen Analyzer 572 (Servomex, England).

Bioassay Tests:

Fourth instar larvae and newly emerged adults of *A. verbasci* and late larvae (inside the case) and newly emerged adults of *T. pellionella* were used to investigate their sensitivity to the modified atmospheres of 99.9% argon or nitrogen. Larvae and adults of each insect species were placed separately in wire gauze cages with chicken feathers for *A. verbasci* and about 10gm of fish food for *T. pellionella*. The cages were closed with rubber stoppers. The cages were then introduced into the gas tight Dreshel exposure flasks. Insects in the flasks were exposed to argon or nitrogen and the mortality percentages were monitored at 4, 8, 12, 18, and 24 hr post exposure at 20°C and 30°C and RH of 50 %. Groups of control insects were maintained under identical conditions but held in glass jars at the ambient atmosphere. The experimental stages as well as the control were run in four replicates each containing 25 insects. After the desired exposure periods, the flasks were aerated and the insects were transferred into petri-dishes for inspection and mortality was recorded.

Statistical Analysis of the Data:

Probit regression analysis (Finney, 1971) was used to determine the lethal times for each gas. The univariant factorial ANOVA was used to test the effect of argon and nitrogen at 20°C and 30°C for different exposure times on the mortality of adults and larvae of both pests. Data were considered significant at $P \leq 0.05$.

RESULTS

Sensitivity of *A. verbasci* to modified atmosphere of argon and nitrogen gases

The efficacy of 99.9% of argon or nitrogen against larvae and adults of *A. verbasci* was tested after different exposure periods and at two different temperatures (20°C and 30°C) and 50% RH. The results illustrated in Fig.1, revealed that, adults of *A. verbasci* were more sensitive to both gases than larvae. At 20°C, The recorded LT_{50} and LT_{95} values of argon for *A. verbasci* larvae were 8.08 hr and 25.48 hr, respectively. While the LT_{50} and LT_{95} for adults were 7.73 hr and 24.59 hr, respectively.

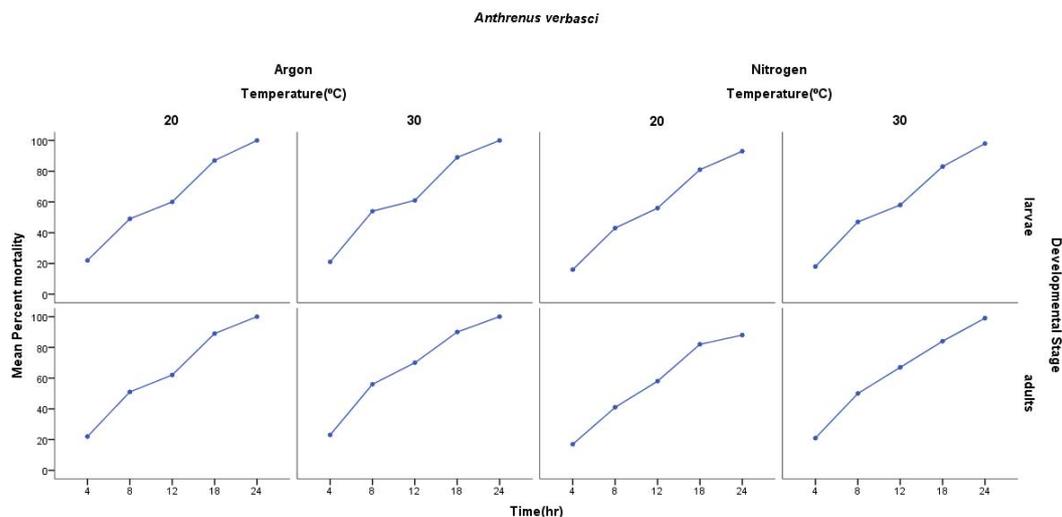


Fig. 1: Sensitivity of *Anthrenus verbasci* to modified atmosphere of 99.9% argon and nitrogen gases.

At 30°C the LT₅₀ value for larvae was 7.70 hr and the LT₉₅ was 24.42 hr. For adults, the LT₅₀ value was 7.16 hr and the LT₉₅ was 22.39 hr. The results of the efficacy of modified atmosphere of 99.9% nitrogen against *A. verbasci* are presented in Fig.1. The LT₅₀ for larvae was 9.23 hr and the LT₉₅ was 32.49 hr at 20°C while at 30°C, the LT₅₀ for larvae was 8.57 hr and the LT₉₅ was 28.38 hr. At 20°C, the LT₅₀ value for the adult was 9.30 hr and the LT₉₅ was 35.82 hr and at 30°C the LT₅₀ value for the adult was 7.82 hr and the LT₉₅ was 26.06 hr.

Sensitivity of *T. pellionella* to modified atmosphere of argon and nitrogen gases:

Susceptibility of larvae and adults of *T. pellionella* to 99.9% of argon or nitrogen gases was tested after different exposure periods and different temperatures (20°C and 30°C) at 50% RH. Adults of *T. pellionella* were more susceptible to tested gases than larvae (Fig. 2). At 20°C, the LT₅₀ and LT₉₅ values for larvae were 7.25 hr and 22.97 hr, respectively. For adults, the LT₅₀ and LT₉₅ values were 6.51 hr and 21.09 hr, respectively. At 30°C, the LT₅₀ and LT₉₅ values for larvae were 6.80 hr and 21.30 hr while for adults they were 6.32 hr and 20.01 hr, respectively. Results also indicated the efficacy of modified atmosphere of 99.9% nitrogen against *T. pellionella*.

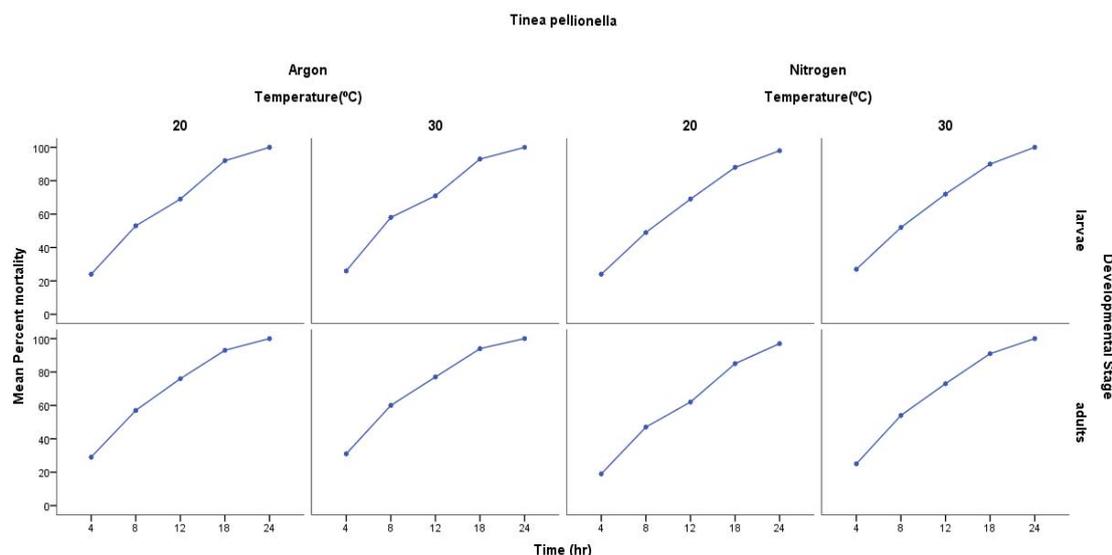


Fig. 2: Sensitivity of *Tinea pellionella* to modified atmosphere of 99.9% argon and nitrogen gases.

At 20°C, the LT₅₀ and LT₉₅ for larvae were 7.67 hr and 25.05 hr, respectively. For adults, the LT₅₀ and LT₉₅ were 8.30 hr and 27.58 hr, respectively. At 30°C, the LT₅₀ and LT₉₅ for larvae were 7.09 hr and 23.57 hr, while for adults; they were 7.00 hr and 21.81 hr, respectively.

Statistical analysis of data indicated that adults of both insects were more sensitive to argon and nitrogen than larvae ($F_{(4,240)}=3.326$, $P=0.011$). The presented results show that argon gas was generally more effective against larvae and adults of both insects, than nitrogen gas ($F_{(1,240)}=7.298$, $P=0.007$). The mortality increased as the exposure time and the temperature increased ($F_{(4,240)}=2.692$, $P=0.023$). Larvae and adults of *A. verbasci* were more tolerant to MA than larvae and adults of *T. pellionella* ($F_{(4,240)}=10.055$, $P<0.001$).

DISCUSSION

The use of modified atmospheres to control pests of museums has developed due to demands to produce methods of control that are non-toxic and residue free in order to overcome the disadvantages associated with the use of chemicals in museums. The efficacy of modified atmosphere of (99.9%) argon or nitrogen against *A. verbasci* and *T. pellionella* was tested at 20°C and 30°C for different exposure times.

In this report we present the results of the first laboratory investigation on the sensitivity of both pests to low oxygen atmosphere. Argon and nitrogen were effective against the adults and larvae of the two insect species. Nitrogen has the advantage of causing no colour change in polychrome cellulose samples exposed to its action (Koestler *et al.*, 1991) and argon causes no physical or chemical alterations in historic objects when used against museum pests (Valentin, 1993).

In the present study, argon was more effective against both insects compared to nitrogen. This finding corroborates the result obtained by Valentin (1993). This was also reported by Charles and Shin (1998) who found that the time required to achieve 100% mortality of museum pests with argon caused only 65-70% mortality when nitrogen was used. Air replacement with argon produced higher weight loss than with nitrogen suggesting that the higher molecular weight, density and solubility in water of argon may be involved in a higher water loss in the treated insects (Valentin, 1993).

Anthrenus verbasci was more tolerant to low oxygen atmosphere, of argon or nitrogen than *T. pellionella* probably due to species differences. The variability in response of different museum pests to different concentrations of oxygen deprivation is reported by Beauchamp *et al.* (1981) who pointed out that the response of one species cannot be used as an indicator of the response of other species.

The present investigation also indicated that, for both species the adult stage was more sensitive to low oxygen atmosphere compared to the larval stage. This may be due to the differences in respiration rates among the developmental stages of holometabolous insect species and/or water loss which is a significant additional factor including insect death in a shorter time (Valentin, 1993; Rust *et al.*, 1996) and the authors reported that the time required to kill 100% of the insects varied between species and even between the developmental stages of a given species, and that certain stages such as the eggs of some beetles were the most tolerant. Numerous studies on the efficacy of MAs against stored-product insects have shown that the lethal exposure times vary with temperature, moisture content, gas composition, species and developmental stage (Adler, 2001; Emekci *et al.* 2002; Emekci *et al.*, 2004; Azab *et al.*, 2013) *Tribolium castaneum* and *Rizopertha domimica* adults undergo metabolic stress at low O₂ levels showing high CO₂ production and/or increased respiration quotients value. Further biochemical research is needed to clarify the mechanism of insect mortality (Emekci *et al.*, 2002 and 2004).

High mortality of *A. verbasci* and *T. pellionella* together with a shorter exposure time was achieved at 30°C compared to 20°C. This positive effect of elevated temperature on the mortality rate is in concordance with the finding of Valentin (1993) who reported that complete eradication of *Anobium punctatum* was achieved by rising the temperature to 30°C at low oxygen percentage (0.03%) at a shorter exposure time. Our results also coincides with the work of Chapman (1998) who stated that anoxia treatment is temperature-dependant and that the effect of high temperature can be useful for speeding up the treatment time.

The results of this study indicated that the use of MA for the control of *A. verbasci* and *T. pellionella* in museums is promising. The exposure to an atmosphere of approximately 0.01% oxygen and 99.9% argon or nitrogen was successful in limiting survival of the tested pests among the developmental stages and this condition is accelerated by warmer temperature (30°C), with the exposure time required to give complete kill, being shorter for argon.

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ARABIC SUMMERY

فعالية الأجواء المعدلة في مكافحة أفتين من آفات المتاحف/أنثرينيس فيريباتشي (غمدية الأجنحة: درميستيدي) وتنيا بيليونيلا (حرفشية الأجنحة: تنيدي).

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تم تقييم كفاءة استخدام أجواء معدلة تحتوي علي ٩٩.٩٪ من غاز النيتروجين وغاز الأرجون ضد اليرقات والطور البالغ لكل من حشرة/أنثرينيس فيريباتشي وحشرة تنيا بيليونيلا وهما من آفات المتاحف. وتم رصد الوقت اللازم للحصول علي نسبة إيماته تعادل 50% و95% (LT₅₀ وLT₉₅) في فترات تعريض مختلفة وعند درجات حرارة ٢٠ و ٣٠ درجة مئوية. وأشارت النتائج إلى أن غاز الأرجون كان أكثر سمية من غاز النيتروجين في نفس الظروف لكلتا الحشرتين. وكان الطور البالغ لكلتا الحشرتين أكثر عرضة للإماتة من اليرقات. وكانت نسبة الإماتة مرتفعة لكلتا الحشرتين عند درجة الحرارة الأعلى. تدل نتائج هذه الدراسة علي إمكانية استخدام الأجواء المعدلة لمكافحة آفات المتاحف كما توضح العلاقة بين نوع الغاز وفترات التعرض ودرجات الحرارة للحصول علي أعلى نسبة إماتة للأطوار المختلفة.