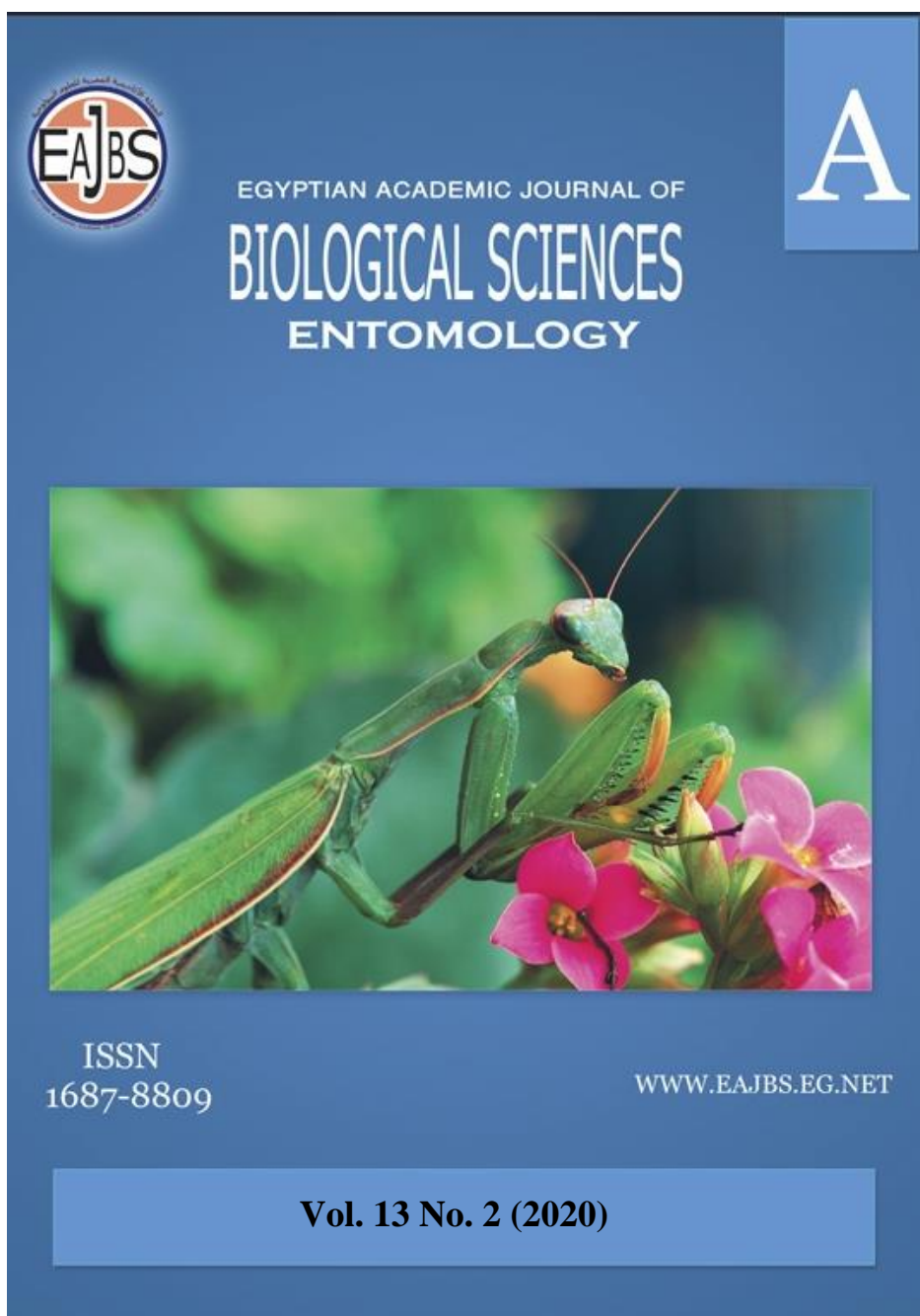


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Modified Atmosphere Enriched with Argon Gas as An Alternative Measure for Controlling Four Stored Dates Pests

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ABSTRACT

This work aimed to evaluate the efficacy of modified atmosphere (MA) enriched with Argon gas as an alternative measure for controlling four stored dates pests, two from order: Lepidoptera, *Ephestia cautella* (Walker) & *Plodia interpunctella* (Hübner) and two from order Coleoptera; *Oryzaephilus surinamensis* Linnaeus & *Stegobium paniceum* (L.) and its effect on some enzymes activity of tested insects. Argon gas concentrations of (60, 80 and 100 %) had been tested against the four mentioned insects at different exposure times ranged from 3 to 144 hrs. The results revealed that Lepidopterous were more sensitive to Argon gas than Coleopterous. Also, data indicated that not all tested insects have the same sensitivity to Argon gas. Obtained results indicated that the reduction % of adult emergence increased by increasing exposure duration and or concentration. At the concentration of 100 % Argon, data reported that the larvae of *E. cautella* were the most susceptible and reached a 100% reduction after 12 hrs. While the adults of *O. surinamensis* was the most tolerant insect stage to Argon gas where reached 74.60 % reduction after 72 hrs. Exposure of *P. interpunctella* and *O. surinamensis* to LT 50 of Argon increased the activity of two defensive enzymes within tested insects, Acid phosphatase, & Carboxylesterases. and decreased the activity of LDH enzyme.

INTRODUCTION

In many countries palm is considered as one of the most important crops, with more than 2200 species distributed throughout the tropic and subtropic areas (Johnson, 1996); representing an important part of tropical forests (Johnson 1995). The palm trees have a significant effect on the economy of millions of people around the world. Many products can be obtained from these different palm species. There is wide variation in palm products such as, fruits, oil, starch, seeds, honey, crystallized sugar and sap products. Also, palm trees which can be used in food, feed, furniture, energy, clothing and gardens Jones (1995).

The almond moth, *E. cautella* (Walker) (Lepidoptera: Pyralidae) is one of the most major date fruit pests in Egypt. The infestations begin from the field to the storehouse through infested dates which have various generations (Howard *et al.*, 2001). Beside date palm fruits, dried fig, cereal and its' products, cocoa, chocolate, raisin, dried fruit, spices,

nuts, peanut and processed foods are reported as hosts of almond moth (Hodges and Farrell, 2004; Rees, 2007).

P. interpunctella (Indianmeal moth) (Lepidoptera: Pyralidae subfam. Phycitinae) is an important insect of stored food products (Doud and PhillipS, 2000; Nansen *et al.*, 2004; Nansen and Phillips, 2004). Because of *P. interpunctella* uses a wide range of products in its diet it has a very strong economic importance (Sauer and Shelton, 2002; Rees, 2004).

The saw-toothed grain beetle, *O. surinamensis* (Coleoptera: Silvanidae), is one of the key stored grain pests in worldwide (Rossiter *et al.*, 2001; Hashem *et al.*, 2012). It is a secondary feeder which infests whole grains with minor cracks or mechanical damage (Prickett *et al.*, 1990). It can feed on different stored product goods such as date fruits, cereals, millets, flours, oilseeds, dried meat and nuts etc., (Barnes, 2002; Bowditch and Madden, 1997). Although the neem seed embryo has some pesticide properties, *O. surinamensis* beetle nourishes and grows well on it (Sarup and Srivastava, 1971).

The drugstore beetle *S. paniceum* (Coleoptera: Ptinidae) is also distributed worldwide, as a strange species (Umeya 2012). It consumes a wide variety of dried plant products and biological specimens in museum collections (Gilberg and Brokerhof 1991). Adults of both *O. surinamensis* and *S. paniceum* lay eggs on dry foods in food storage containers. After feeding of the larvae dead beetles and its' wastes remain inside food, causing huge economic damage (Ashworth 1993).

Chemical insecticides such as Malathion, methyl bromide and phosphine, have been widely used for controlling insects because they are fast-acting and cheap. On the other hand, chemical insecticides have a bad deep impact on humans, animals and useful insects (Muir and White 2001). In addition, using chemicals insecticides fumigants develop resistance. Nowadays, many markets no longer accept products with chemical insecticides residues. As a result, other control methods need to be developed and applied. The modern trend focuses on developing physical methods of insect control. These methods depend on the ecology of stored-product insects. These methods that involve manipulation of the physical environment, like the composition of atmospheric gas (Argon). This paper aime to evaluate the effect of modified atmospheres enriched with Argon gas as an alternative measure for controlling four stored dates pests.

MATERIALS AND METHODS

Experiments were conducted at date pests and diseases Dept., Central Laboratory of Date Palm, Agricultural Research Center, Giza, Egypt and the laboratory of stored product pests' control, Plant Protection Dep. Fac. of Agriculture, Benha University.

Test Insects:

1. Rearing of Insects Culture:

The four insect species were collected from infested date fruits were reared on their standard food diets. Insects culture was kept in an incubator at $27\pm2^{\circ}\text{C}$ and $65\pm5\%$ relative humidity (r.h.). The adult insects have reared on dry date fruits Frihi cultivar. The date fruits were sterilized before use by continuous freezing (-10°C) for at least two months, then kept under laboratory conditions for 12 hrs, before use (Hussain, 2008). The eggs and larvae of Lepidoptrous species; *Ephestia cautella* and *Plodia interpunctell* were separately evaluated while in the case of Coleopterous species; *Oryzaephilus surinamensis* and *Stegobium paniceum* larvae and adults were tested.

Modified Atmosphere:

1. Gases Used.

Argon was provided as pure gas in pressurized steel cylinders. The cylinder was connected to a pressure regulator. The dilution method was used to achieve the required

argon concentration. For the atmosphere of nearly pure Ar 100%, the valve of each cylinder was opened for three minutes in order to fill the gastight Dreshel exposure flask with the gas. Modified Atmosphere (MA) of Argon concentrations 60 and 80 % in air were prepared using Gas Distribution device. Determination of the concentrations of Ar was monitored using a gas Analyser model 2(10-600 Gow-Mac-Instruments Company U.S.A.).

2. Preparing the Insect Species Samples for Bioassay Tests of Modified Atmosphere:

A number of 50 *E. cautella* & *P. interpunctella* eggs were kept into small cloth bags (4×8 cm) filled with about 25 g artificial diet and closed with rubber bands. 30 fourth instar larvae were put also with 25g of artificial diet. In the case of *O. surinamensis* and *S. paniceum*, 30 larvae and 20 adults of each species were put into small cloth bags (4×8 cm) filled with about 25 g artificial diet and closed with rubber bands. Cloth bags were taken and introduced into the gastight Dreshel-flasks of 0.55L volume. Various insect stages in the gastight flasks were treated with the MA at mentioned concentration and different exposure periods ranged from 3 to 144 hrs. at $27 \pm 2^\circ\text{C}$. After the exposure periods, the flasks were aerated for 24 hrs. and the insect stages were transferred into Petri dishes and kept at $27 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH and were examined daily to record adult emergence until the emergence of adult stopped for reduction% assessment.

3. Bioassay Tests of Modified Atmospheres:

The efficacy of MA containing various Argon concentrations was investigated against four species of stored date fruits pests at tested temperature ($27 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ R.H.). Experiments were conducted inside 0.55 L gastight flasks at the laboratory. Different insect species stages were used for the bioassay to study their sensitivity to Argon concentrations. The reduction % of adult emergence was calculated according to the formula of Henderson and Tilton (1955).

$$\text{Reduction \%} = \frac{\text{Control} - \text{treated}}{\text{Control}} \times 100$$

4. Biochemical Studies:

A known weight of tested species larvae and adults (1 g) which was tested with LT_{50} and the same weight of untreated ones were kept in the deep freezer until used for certain physiological purposes as follows: each specimen was homogenized in 1ml distilled water by using chilled glass Teflon homogenizer (ST- 2 Mechanic-Preczyina, Poland). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 5°C and the supernatant was used for enzyme assay Amin (1998).

5. Determination of Enzymes Activity:

The current study was carried out to clarify the effect of Modified Atmosphere enriched with Argon gas on the activities of the following enzymes:

-Carboxlesterases determination, Carboxlesterase activity was measured according to the method described by Simpson *et.al.* (1964).

-Determination of phosphatases, Acid phosphatases was determined according to the method described by Powell and Smith (1954).

-LDH, the method described here is derived from the formulation recommended by the German Society for clinical chemistry (DGKC, 1972).

6. Statistical Analysis:

Data on the effect of MA concentrations and exposure periods on the tested four insect species were subjected to probit analysis, as described by Finney (1971). LT_{50} and LT_{90} values were calculated using the computer program developed by Noack and Reichmuth (1978). Data of analysis insect species enzymes were analyzed using Proc., ANOVA in SAS (SAS Institute 2006).

RESULTS AND DISCUSSION

1. The Effect of MA Contains Argon Gas Application on Four Tested Insects:

Results concerning the evaluation efficacy of concentration 100% of Argon on eggs and larvae stages of *E. cautella* and *P. interpunctella* and on adult and larvae stages of *O. surinamensis* and *S. paniceum* are shown in Table 1. The results revealed that reduction % in emerged adults increased gradually by increasing the exposure period. Complete reduction (100%) was observed in Lepidoptera (*E. cautella* and *P. interpunctella*) after the exposure time of 12 hrs. in the larval stage but in the case of the Egg stage after 18 hr and 24 hrs. resp., Whereas, in the case of Coleoptera (*O. surinamensis* and *S. paniceum*) complete reduction (100%) was noticed only for *O. surinamensis* larval stage after the exposure time of 18 hrs.

Table 1: Effect of MA enriched with Argon 100 % on adult emergence of the four tested insects.

Insect	stage	Reduction %							
		Exposure time (hrs.)							
		3	6	9	12	18	24	48	72
<i>Plodia interpunctella</i>	Egg	18.87	43.4	56.6	77.36	94.34	100	-	-
	Larva	24.07	59.26	72.22	100	100	100	-	-
<i>Ephestia cautella</i>	Egg	25.86	48.27	68.96	80.39	100	-	-	-
	Larva	39.22	68.63	80.39	100	100	-	-	-
<i>Oryzaephilus surinamensis</i>	adult	2.12	6.88	11.64	14.29	20.63	46.03	59.26	74.60
	Larva	33.33	46.30	70.37	92.59	100	-	-	-
<i>Stegobium paniceum</i>	adult	6.99	18.99	23.99	36.96	54.00	75	86	96
	larva	14.81	16.67	33.33	48.15	50	62.96	72.22	88.89

Results concerning the evaluation efficacy of concentration 80% of Argon on Eggs and larvae stages of *E. cautella* and *P. interpunctella* and on adult and larvae stages of *O. surinamensis* and *S. paniceum* are shown in Table 2. The maximum reduction % in adult emergence resulted from treated larvae after 6 days in the case of *P. interpunctella* and 4.5 days in *E. cautella*. Meanwhile, *O. surinamensis* complete reduction % in adult emergence in larvae after 4.5 days while it was 6 days in the adult stage. In the case of *S. paniceum* the complete reduction % in adult emergence resulted from treated adults and larvae after exposure time was 5 and 6 days %, respectively.

Table 2: Effect of MA enriched with Argon 80 % on adult emergence of different tested insects.

Insect	Stage	Reduction %								
		Exposure time (day)								
		0.5	1	2	2.5	3	4	4.5	5	6
<i>Plodia interpunctella</i>	Egg	5.66	11.32	32.08	49.06	58.49	66.04	71.70	75.47	94.34
	Larva	11.11	16.67	38.89	55.56	61.11	72.22	75.93	77.78	100
<i>Ephestia cautella</i>	Egg	8.61	24.13	39.65	53.45	65.51	72.41	81.03	89.65	100
	Larva	14.81	31.48	44.44	55.56	68.52	77.78	100	-	-
<i>Oryzaephilus surinamensis</i>	adult	8.99	17.46	36.51	51.85	60.85	62.43	74.07	86.24	100
	Larva	12.96	27.78	40.74	51.85	62.96	75.93	100	-	-
<i>Stegobium paniceum</i>	adult	32.99	46.99	53.00	57.00	74.00	83.00	87.00	100	
	larva	9.26	14.81	35.19	42.59	44.44	55.56	66.67	72.22	100

Results concerning the evaluation efficacy of concentration 60% of Argon on eggs and larvae stages of *E. cautella* and *P. interpunctella* and on adult and larvae stages of *O. surinamensis* and *S. paniceum* are shown in Table 3. The complete reduction % in adult emergence resulted only from the treated adult of *S. paniceum* after 5 days. Our results stated that grubs were more tolerant of Argon treatment than caterpillars.

Table 3: Effect of MA enriched with Argon 60 % on adult emergence of different tested insects.

Insect	Stage	Reduction %								
		Exposure time (day)								
		0.5	1	2	2.5	3	4	4.5	5	6
<i>Plodia interpunctella</i>	Egg	0.00	0.00	9.44	20.76	30.19	37.74	41.51	47.17	50.94
	Larva	3.70	9.26	16.67	24.07	35.19	44.44	50	55.56	59.26
<i>Ephestia cautella</i>	Egg	3.44	6.89	17.24	32.75	39.65	44.82	51.72	58.62	62.07
	Larva	7.41	16.67	22.22	35.19	42.59	48.15	55.56	62.96	66.67
<i>Oryzaephilus surinamensis</i>	adult	3.17	8.99	14.29	23.81	33.33	40.74	42.86	48.68	56.08
	Larva	7.41	12.96	22.22	33.33	40.74	53.70	61.11	64.81	72.22
<i>Stegobium paniceum</i>	adult	16.99	28.99	35.99	45.99	58	70	79	100	
	larva	0.00	5.56	12.96	24.07	40.74	46.30	50	57.41	64.81

2. Lethal Time LT_{50} and LT_{90} Values Per Hour and Parameters of the Mortality Regression Line for Two Stages of Different Four Insects Exposed to Argon (100%):

LT_{50} and LT_{90} (time required to kill 50 and 90% of the population at a certain concentrations) values of 100 % Argon gas against eggs and larvae stages of *E. cautella* and *P. interpunctella* and on adults and larvae stages of *O. surinamensis* and *S. paniceum* are shown in Table 4. Results showed that argon was more effective for the four species when concentration and exposure periods were increased from 60 to 100 %. LT_{90} for *P. interpunctella* was 21.73 and 15.35 hrs. for egg and larva stages, respectively; also, LT_{90} was 16.99 and 13.29 hrs. for eggs and larvae stages of *E. cautella*, respectively. But argon gas was less effective against *O. surinamensis* and *S. paniceum* where LT_{90} recorded 25.41 and 106.15 hrs. in larva stages, respectively. Whereas, at the adult stage, LT_{90} was 139.54 and 47.20 hrs. respectively. The obtained data revealed that Lepidoptera was more sensitive to Argon gas than Coleoptera. Also, data indicated that not all insects have the same sensitivity to argon gas.

Our data is in agreement with El-Shafei, *et al.* (2019) who revealed that the required exposure duration to get 100 % mortality of the adult mite females decreased by increasing of the Argon concentration from 50 to 100 % and Argon was the most efficient gas at 100%. Also, Valentin (1993) reported that to kill all life stages of *S. paniceum* by using Argon gas need 96 h at 30 C°.

Table 4: Lethal time values per hour and parameters of mortality regression line for two stages of tested insects exposed to MA enriched with Argon (100%).

insect	stage	LT ₅₀ (hr)	LT ₉₀ (hr)	Confidence limits (hr)				Slope ± SE	Chi square χ ²
				LT ₅₀		LT ₉₀			
				Lower	Upper	Lower	Upper		
<i>Plodia interpunctella</i>	Egg	6.88	21.73	6.10	7.76	17.01	31.87	2.56±0.3	2.09
	larva	5.25	15.35	4.59	5.97	11.92	23.66	2.75±0.3	0.58
<i>Ephestia cautella</i>	Egg	5.62	16.99	4.93	6.30	13.85	23.10	2.66±0.3	2.19
	larva	3.86	13.29	3.09	4.50	10.23	21.43	2.38±0.3	2.83
<i>Oryzaephilus surinamensis</i>	larva	5.55	25.41	4.51	6.59	16.12	69.48	1.91±0.3	2.94
	adult	34.94	139.54	30.86	40.30	106.38	202.75	2.13±0.18	6.68
<i>Stegobium paniceum</i>	larva	17.16	106.15	14.78	20.15	72.38	192.52	1.61±0.18	6.67
	adult	15.16	47.20	13.65	17.11	36.63	69.52	2.59±0.2	4.27

3. Determination of the Effect of MA Contains Argon Gas on Three Enzymes Activity of Two Tested Insects:

The results obtained in Table (5) indicated that acid phosphatase insignificantly increased in *Oryzaephilus surinamensis* but in the case of *P. interpunctella* significantly increased compared with control after exposure to LT_{50} MA enriched with Argon gas. On the contrary, LDH content differed insignificantly decrease in the case of *Oryzaephilus*

surinamensis and significantly decreased in *P. interpunctella* compared with control. Carboxylesterases activity significantly increased in *Oryzaephilus surinamensis* after treatment with argon gas and insignificantly increased in *P. interpunctella*. Similar findings are obtained by (Li *et al.*, 2007, 2009) mentioned that the activity of carboxylesterase increased after exposure to CO₂-enriched atmosphere in *S. paniceum* and *Lasioderma serricorne*, compared to control. Also, CO₂ stress increase the activity of Acid phosphatase with increasing of exposure time (Li *et al.*, 2008). Exposuring to CO₂-enriched MA (75% CO₂, 5% O₂ and 20% N₂) for 3 h increased significantly the activities of acid phosphatase and carboxylesterase in *Araecerus fasciculatus* (Li *et al.*, 2012). Rasha A. Zinhom and El-Shafei (2019) found that the activity of LDH enzyme insignificant decrease in treated larvae of *Plodia interpunctella* with ozone.

Table 5: Changes in Carboxylesterases, LDH and Acid phosphatase content in two insects treated with MA enriched with Argon gas

Insect	Treatment	Acid phosphatase	LDH	Carboxylesterases
<i>Plodia interpunctella</i>	Control	147.00±3.6 ^b	3666.67±25.3 ^a	117.67±3.9 ^a
	Argon	260.67±3.5 ^a	3543.33±55.5 ^a	128.00±6.3 ^a
	L.S.D.	13.91	169.20	20.49
<i>Oryzaephilus surinamensis</i>	Control	282.00±4.4 ^a	891.67±5.9 ^a	141.00±3.1 ^b
	Argon	290.00±6.7 ^a	298.33±10.1 ^b	172.67±6.5 ^a
	L.S.D.	22.10	143.97	19.91

Conclusion:

The efficacy of MA containing 60, 80 and 100% Argon at 27±2°C against four stored dates pests was varied through the different concentrations and different types of insects. The results revealed that Lepidopterous were more sensitive to Argon gas than Coleopterous. The most efficient MA is that containing 100% of Argon. So, it is recommended to use these high levels of MAs, in particular, Argon gas in controlling the four tested date pests.

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ARABIC SUMMARY

الاجواء المعدلة الغنية بغاز الأرجون كوسيلة بديلة لمكافحة اربعة من آفات التمور المخزونة

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يهدف هذا العمل الى تقييم فاعلية الاجواء المعدلة الغنية بغاز الارجون كطريقة بديلة لمكافحة اربعة من افات التمور المخزونة أثنان من رتبة حرشفية الاجنحة (*P. interpunctella* (Hübner) و *E. cautella* (Walker) وأثنان من رتبة غمدية الاجنحة (*O. surinamensis* (L.) و *S. paniceum* (L.) وتأثير غاز الارجون على نشاط بعض الانزيمات فى الحشرات المختبرة . تم اختبار تركيزات غاز الارجون (٦٠ , ٨٠ و ١٠٠ ٪) ضد الحشرات الاربعة على فترات تعريض مختلفة تراوحت بين ٣ الى ١٤٤ ساعة. اظهرت النتائج ان رتبة حرشفية الاجنحة أكثر حساسية من رتبة غمدية الاجنحة. كما اوضحت النتائج ايضا انه ليست للحشرات المختبرة نفس الحساسية لغاز الارجون. بينت النتائج المتحصل عليها ان نسبة الخفض فى اعداد الحشرات زادت بزيادة فترة التعريض او التركيز. اوضحت النتائج انه عند تركيز ١٠٠ ٪ ارجون كانت يرقات *E. cautella* الأكثر حساسية واعطت نسبة موت ١٠٠ ٪ بعد ١٢ ساعة في حين أن الحشرة الكاملة لخنفساء السورينام *O. surinamensis* كانت الأكثر مقاومة لغاز الارجون حيث بلغت نسبة الخفض فى اعداد الحشرات ٧٤,٦٠ ٪ بعد ٧٢ ساعة . تعريض *P. interpunctella* و *O. surinamensis* الى LT 50 من غاز الارجون ادى الى زيادة فى نشاط انزيمين من انزيمات المقاومة داخل الحشرات المختبرة Acid phosphatase و Carboxylesterases ونقص فى نشاط انزيم LDH .