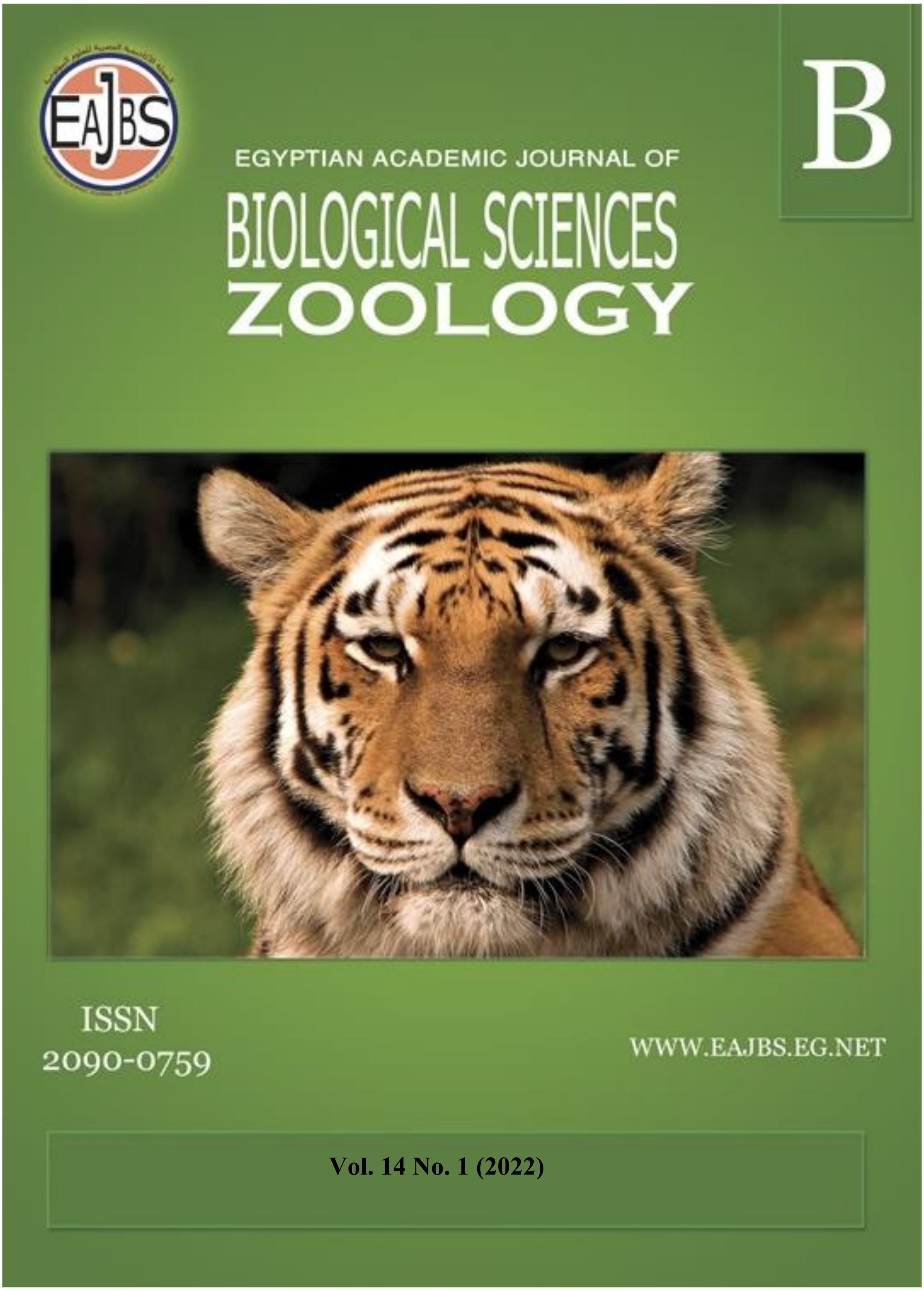

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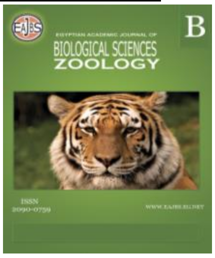

Comparison Between Two Physical Methods to Control the Stored Dates Fruit Mites, *Tyrophagus putrescentiae* (Schrank) and *Rhizoglyphus robini* Claparede (Astigmata: Acaridae)

Rania H. Mahmoud [1]; Asmaa R. Abdel-Khalik[1] and El-Shafei, W. K. M. [2]
1-Plant Protection Research Institute, Agricultural Research Centre (ARC), Giza, Egypt.
2- Department of Date Palm Pests and Diseases, Central Laboratory for Date Palm, Agricultural Research Centre (ARC), Giza, Egypt.
E-mail*: waelkamal27@yahoo.com

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ABSTRACT

Date fruit is considered one of the most important fruits crop, that helps in the increment of national income. During storage, date fruits face a lot of problems such as infestation with insects, diseases and mites. The most damaging mite species are *Tyrophagus putrescentiae* (Schrank) (Astigmata: Acaridae) and *Rhizoglyphus robini* Claparede (Astigmata: Acaridae). Therefore, this work aims to evaluate two physical control methods, gamma radiation and ozone gas treatment in controlling these two mites. The two mites were irradiated with doses of 0 (control), 100, 200, 300 and 400 Gy in a source of Cobalt-60 type Indian Gamma cell with a dose rate of 0.7967 k Gy /hr. to control eggs and mobile stages. Obtained results concluded that the mites' mortality percentage increases by increasing irradiation doses and the dose for controlling 100% of the two tested mites' species was 400 Gy. While exposing the mites stages to concentrations of 0 (control), 100, 200, 300 and 400ppm of ozone gas, the results showed that the mites mortality percentage increases by increasing ozone concentrations and/or exposure period. The results indicated that ozone has the potential to control the tested mites, particularly at 200 ppm in just 4 hrs.exposure period and 99% at 400 ppm at the same exposure time. The results clearly showed that the mobile stages were more susceptible to radiation and ozone than the egg stage. Also, although radiation and ozone are safe and environmentally friendly methods of pest control, and effective to control the two mites' species with low risk but ozone control was faster and more efficient.

INTRODUCTION

Date palm is considered a national crop around the world. In providing food, wooden products and the whole palm can be used in many ways. Due to pests and disease problems date palm production has been in decline for the past few years (Erskine *et al.*,2004). Many pest species infest the date palm around the world like the acarid mites *T. putrescentiae* and *R. robini* which infest foodstuffs widely in suitable conditions (Howard *et al.*,2001; Lepesme 1947). *T putrescentiae* and *R. robini* were found associated with date palm fruits in different regions covered 9 Egyptian

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governorates differed in their ecological conditions during the survey from 2013 to 2016(Mostafa *et al.* 2017; Bibars *et al.* 2018).

Mites in the family Acaridae are the most important acarine pests infesting agricultural and stored products. *T.putrescentiae* is a cosmopolitan mite found in the cereals, date fruits, stored foods, home dust, nests of birds and bees (Allee and Davis1996 ;García 2004; Papadopoulou 2006). This mite is found in the foods stored rich in fat and protein such as nuts, date fruits, cheese, ham, oats, barley and flour (García 2004; Aygun *et al.* 2007; Kheradm and *et al.,* 2007; Hubert *et al.* 2014). However, *T. putrescentiae* can cause enteritis, diarrhea and damages to the urinary tract besides allergic reactions when products with this mite are ingested, manipulated, or inhaled (Li *et al.,*.2003 ;Matsumoto and Satoh, 2004; Hubert *et al.,* 2007; Aygun *et al.* 2007). *T. putrescentiae* infest date fruits at stores in date palm production areas (El-Shafei *et al*.,2019). Methyl bromide was the only known fumigant that was effective at controlling these arthropod infestations of dried cured ham in the United States for many years (Marriott and Schilling 2004). Methyl bromide is not currently allowed for fumigation of dry-cured hams in the United States as requests to the international Methyl Bromide Technical Options Committee for yearly continuing use exemptions have stopped (US EPA 2019). Hydrogen phosphide, commonly known as phosphine, is the most common fumigant used for bulk-stored cereal grains (Thoms and Phillips 2004). The biggest problem for fumigating buildings like ham facilities with phosphine is to avoid the damaging corrosion that can occur from phosphine to electrical and other metallic fixtures (Zhao *et al.,* 2015). Phosphine is an acute mammalian poison, killing hamsters at 8ppm (inhalation). Potential symptoms of overexposure in humans are nausea, abdominal pain and diarrhea, thirst, chest pressure, muscle pain, chills and stupor, skin irritation, or burns (Jones *et al*., 1964). Hence the need for alternative control methods for these pests that are safe for stored materials and fruits. One of these alternatives is gamma radiation can eliminate mite pests of stored grains and fruits as well as field crops more efficiently. Regressive changes in the cells of pests occur due to the discharge of energy in the water molecules and other bio-molecules, especially to genetic materials. It is applied to targeted pests, hence it is eco-friendly technology for pest management, without causing any induced radioactivity and residual effect .The second method is ozone which finds wide application as a powerful sterilizer in water treatment, food processing and preservation and various other environmental applications. Ozone as an oxidant has numerous potential applications in the food industry because of its advantages over traditional food preservation techniques. The application of ozone either in gaseous or liquid form in fruit and vegetable processing is often employed in controlling pathogen and spoilage micro-organisms (Cullen *et al.,* 2009). Apart from the wide spectrum of microbial control, ozone also has the potential to kill storage pests and reduce mycotoxins. One of the advantages of ozone is that excess ozone auto decomposes quickly to produce oxygen and thus leaves no residues in food. Its efficacy against a lot of micro-organisms including bacteria, fungi, viruses, protozoa, and bacterial fungal spores has been reported (Cullen *et al.,* 2009; Khadre *et al.,* 2001; Restaino *et al.,* 1995). Such advantages make ozone attractive to the food industry and consequently it has been affirmed as generally recognized as safe (GRAS) for use in food processing (Graham, 1997).

The possible application of ozone in food grain and fruits preservation would address the growing concern over the use of harmful pesticides to kill storage pests. The Montreal Protocol on substances that consume the ozone layer (Fields and White, 2002). The aim of this work was to study the effect of using two physical methods: Gamma radiation and Ozone gas in controlling mites, *Tyrophagus putrescentiae* (Schrank) and *Rhizoglyphus robini* Claparede in stored date's fruit.

MATERIALS AND METHODS

Mite Cultures:

T. putrescentiae and *R, robini* were reared in the Acarology laboratory at Plant Protection Research Institute, A.R.C., Dokki. To get the pure culture of the two mites, plastic cups (1.5 cm high x 2.5 cm diameter) were filled to 0.5 cm with a substrate of (plaster of Paris and activated carbon at the rate of 8: 2, respectively). One adult female and male of each mite *T. putrescentiae* and *R, robini* was fed on semi-dry date fruits Siwi variety and suitable relative humidity after that incubated at 27±2°C and 70±5 % R.H. For mites rearing, ten newly eggs of each mite were transferred to plastic cells. Each newly larva was fed and kept till reaching adulthood. Mites were examined two times during 24 hrs. according to (Abdel-Khalik, 2018)

Radiation Source:

Indian Gamma cell radiation Unit in a source of Cobalt-60 with a dose rate of 0.7967 k Gy /hr. located at the National Center for Radiation Research and Technology, Atomic Energy Authority, Nasr City, Cairo, Egypt.

Production of Ozone Gas:

Ozone gas was produced from the air using an ozone generator Model OZO 6 VTTL OZO Max Ltd, Shefford, Quebec Canada (OZO Max Ltd, Shefford, Quebec, Canada) from purified extra dry oxygen feed gas at the laboratory of Food. Toxicology & Contaminants, National Research Center. The amount of ozone output was controlled by a monitor- controller having a plug-in sensor onboard which is changed for different ranges of ozone concentration and a belt pan in the monitor controller allows controlling the concentration in a selected range.

Efficacy of Gamma Radiation and Ozone Application against *T. putrescentiae* and *R.robini*:

Mobile stages of *T. putrescentiae* and *R. robini* were used for the bioassay to evaluate their susceptibility to gamma radiation. Mobile stages prepared on a piece of date fruit weight 1 g. was put in a small plastic tube (5ml) and covered with a bored plastic lid. All tubes were transferred to glass flasks of 0.5 L. volume mite larvae were reared separately on each feeding source in Petri dishes (9 cm diam.) and observed every 12 hrs. until reaching adulthood. As soon as females emerged, males were introduced for mating. Eggs were collected for 12 hrs. post oviposition.

Mobile stages and eggs were irradiated with a Co-60 source. The irradiator was operated at 27±2 °C and 70±5 % R.H. Mites were treated with different doses of irradiation (0 (control), 100, 200, 300, and 400 Gy) and (0, 100, 200, 300 and 400) ppm of ozone gas. Mites belonging to both species were simultaneously treated with the same dose. Each treatment consisted of three repetitions containing 30 mites each, amounting to 90 mites for treatment, and 90 mites for the control replicates. The evaluations were daily, being counted the number of mites that died, putting eggs and emerged larvae. Three untreated tubes were kept as control. After treatment, mites were held under the rearing conditions described above. Ozone toxicity was evaluated following time-response bioassays within the exposure flasks. The time-mortality curves were established using different exposure periods to ozone concentrations from 100 to 400 ppm. Tests were carried out to evaluate the maximum and minimum exposure periods for the time-mortality bioassays and exposure intervals of 0(control),1, 2 and 4 hrs. Ozone treatment was done under room temperature and humidity (27±2 °C and 70±5%

RH), while the control rooms were transferred to atmospheric air under the same conditions. Each treatment and control was replicated three times.

The mortality % of mobile stages and the eggs hatchability % of the tested mites were recorded, calculated and corrected according to the formula of Abbott (1925) as follows:

$$\text{Corrected mortality \%} = \frac{\%\ mortality\ in\ treated - \%\ motality\ in\ control}{100 - \%\ mortality\ in\ control} \times 100$$

Statistical Analyses:

The results were analyzed by one-way analysis of variance (ANOVA) using SAS for regression analysis (SAS Institute, 2006). When the ANOVA statistics were significant ($P < 0.01$), means were compared by Duncan's multiple range test. , LC_{50}, LC_{90} and slope values were calculated according to (Finney 1971) for each evaluation to predict the lethal concentrations using the computer program developed by (Noack and Reichmuth1978).

RESULTS AND DISCUSSION

Effects of Gamma Radiation on Eggs and Mobile Stages of *T. putrescentiae:*

Data represented in Table (1) showed that the effects of gamma radiation to control *T. putrescentiae* increased by increasing the irradiation dose. The least does (100Gy) showed 67% mortality percentage of mobile stages and 98.89 % in case of treatment with (400 GY). While the mean percentages of inviable eggs increased by increasing irradiation dose. The treated eggs with an irradiation dose of 100 GY induced 57.87 % inviable eggs and increased to reach 100% inviable eggs after being treated with 300 GY.

Table 1: Mean of mortality percentages and eggs percentages of *Tyrophagus putrescentiae* after 7 days of the irradiation with doses of radiation gamma of the Cobalt-60.

Dose (GY)	Mean mortality percentages of *T. putrescentiae*		Mean egg percentages %	
	Live	Died	Viable	inviable
0(control)	97.77±1.11a	2.22±1.11d	96.55±1.99a	3.45±1.99d
100	32.22±1.11b	67.77±1.11c	42.13±2.31b	57.87±2.32c
200	33.33±3.33c	86.67±3.33b	26.11±3.89c	73.89±3.89b
300	4.44±1.11d	95.55±1.11a	0.00±0.00d	100.00±0.00a
400	1.11±1.11d	98.89±1.11a	0.00±0.00d	0.00±0.00d
P. value	<.0001	<.0001	<.0001	<.0001
L.S.D.	5.645	5.646	6.965	6.971

- L.S.D.= Least Significant Difference

- Pr. = probability Level

-Mean in the same column followed by the same letters is not significant.

Effects of Gamma Radiation on Eggs and Adults of *Rhizoglyphus robini*:

Results represented in Table (2) reported that the effects of gamma radiation to control *R. robini* increased by increasing the irradiation dose. The least does (100Gy) showed 55.55% mortality percentage of mobile stages and 98.89 % in case of treatment with (400 GY). While the mean percentage of inviable eggs was 50 % after being treated with 100 Gy but in the case of 300 GY it recorded 100 % of inviable eggs.

Table 2: Mean of mortality percentages and eggs percentages of *Rhizoglyphus robini* after 7 days of the irradiation with doses of radiation gamma of the Cobalt-60.

Dose (GY)	Mean mortality percentages of *R. robini*		Mean egg percentages %	
	Live	Died	Viable	inviable
0(control)	95.55±1.11a	4.44±1.11e	95.36±1.13a	4.64±.1.13b
100	44.44±2.94b	55.55±2.94d	50.00±1.52b	50.00±.1.52c
200	16.67±3.33c	83.33±3.33c	30.49±3.10c	69.51±.3.10b
300	10.00±0.00d	90.00±0.00b	0.00±0.00d	100.00±.0.00a
400	1.11±1.11e	98.89±1.11a	0.00±0.00d	0.00±0.00d
P. value	<.0001	<.0001	<.0001	<.0001
L.S.D.	6.642	6.644	5.113	5.114

- L.S.D.= Least Significant Difference
- Pr. = probability Level
-Mean in the same column followed by the same letters is not significant.

Lethal doses of gamma radiation to eggs and mobile stages of *T. putrescentiae* presented in Table (3) showed that irradiation was more effective in mobile stages than eggs. LD_{90} values on eggs and mobile stages were 354.88 and 247.26 Gy respectively. The data obtained in the case of *R. robini* showed that the LD_{90} values on eggs and mobile stages were 362.08 and 291.43Gy respectively. Our data agree with the results of (Arthur *et al.* 2009; Castro *et al.* 2004) reported that mobile stages treated with gamma radiation were more susceptible than eggs. Boczek, *et al.* (1985) doses more than40 kilo rad completely inhibited the eggs of *Rhizoglyphus echinopus* ,and the adult mites were more sensitive to gamma radiation than its egg. Exposure to 5-kilo rad reduced the tested mite fertility by 50%. Ignatowicz & Sysiak (1990) irradiated adult mites produced eggs longer than mites treated as inactive female deutonymphs of the bulb mite. The sterilizing dose of gamma radiation was found to be 0.4-0.5 kGy for females, but for the males, it ranged between 0.5 and 0.6 kGy.

Table 3: LD_{50} and LD_{90} values with their confidence limits for eggs & mobile stages of *Tyrophagus putrescentia* and *Rhizoglyphus robini*.

Stages	Species	LD_{50} (Gy)	LD_{90} (Gy)	Confidence limits (Gy)				Slope ± SD	r
				LD_{50}		LD_{90}			
				Lower	Upper	Lower	Upper		
Eggs	*Rhizoglyphus robini*	111.82	362.08	86.83	131.55	289.97	534.89	2.512±0.404	0.962
	Tyrophagus putrescentia	88.28	354.88	56.8105	111.1	275.18	587.27	2.121±0.407	0.968
Mobile stages	*Rhizoglyphus robini*	90.76	291.43	64.92	110.87	239.49	408.83	2.529±0.424	0.994
	Tyrophagus putrescentia	62.83	247.26	33.27	85.74	205.87	333.83	2.154±0.409	0.993

- r: Correlation coefficient of regression line
- SD: Standard deviation of the mortality regression line.
-

Effects of Ozone Gas on Eggs and Mobile Stages of *T. putrescentiae* & *R. robini*

In the case of ozone gas, the results reported that mortality % increased with the increasing exposure time and or ozone concentrations. Results showed that mobile stages were more susceptible to ozone gas than eggs. Mortality percentages of the two tested stages of *T. putrescentiae & R. robini* exposed to different O_3 concentrations are reported in Tables (4) & (5) showed that treated egg stage of *T.putrescentiae* with ozone gas 100 ppm for 4 hours induced 48.67%of inviable eggs, while treated it with 400 ppm for the same exposure period recorded 99.67% of inviable eggs. In case of the treated *T.putrescentiae* mobile stages with 100 ppm the mortality percentage was 78.67%, and 99.33% after using 400 ppm ozone gas at the same exposure time. In the case of *R. Robini* treated mobile stages with 100 ppm ozone gas for 4 hours resulted in the mortality percentage 77.33%while it was 100% in the case of exposure to 400 ppm for the same exposure period. The inviable percentage of egg stage treated with 100 ppm ozone gas for 4 hours was 47.67% then it reached 99.33% when 400 ppm were used for the same exposure period. Similar results agree with Hasan, *et al.* (2016) demonstrated that 24 hrs. of exposure to a concentration of 166 ppm of ozone gas was sufficient to eliminate ham beetles and ham mites.

Table 4: Mean % mortality (±SD) of *Tyrophagus putrescentia* mobile stages and eggs exposed to four concentrations of ozone with four different exposure times**.**

Stages of *T. putrescentia*	**Exposure (hrs.)**	**Ozone concentration**			
		100	**200**	**300**	**400**
Eggs	**0**	5.33±0.33c	5.33±0.33d	5.33±0.33d	5.67±0.33d
	1	33.00±3.46b	42.33±1.45c	60.67±0.88c	76.33±2.19c
	2	42.67±1.76a	54.00±2.08b	79.00±1.00b	83.33±2.40b
	4	48.67±2.40a	73.67±1.45a	87.00±1.15a	99.67±0.33a
P. value		<.0001	<.0001	<.0001	<.0001
L.S.D.		7.472	4.800	2.927	5.353
Mobile stages	**0**	2.67±0.33d	5.00±0.00d	5.00±0.00d	4.33±0.33d
	1	39.67±1.45c	55.67±0.33c	58.67±2.03c	74.00±1.73c
	2	69.33±2.33b	75.67±2.03b	80.67±3.84b	84.33±2.60b
	4	78.67±2.03a	85.00±2.31a	90.67±1.76a	99.33±0.67a
P. value		<.0001	<.0001	<.0001	<.0001
L.S.D.		5.596	5.041	7.648	5.242

- L.S.D.= Least Significant Difference
- Pr. = probability Level
-Mean in the same column followed by the same letters is not significant.

Table 5: Mean % mortality (±SD) of *Rhizoglyphus robini* mobile stages and eggs treated using four ozone concentrations with four different exposure periods.

Stages of R. robini	**Exposure (hrs.)**	**Ozone concentration**			
		100	**200**	**300**	**400**
Eggs	**0**	5.33±0.33c	5.33±0.33c	5.33±0.33d	5.33±0.33d
	1	31.66±2.40b	41.33±2.33b	60.67±3.18c	72.33±1.45c
	2	42.33±1.15a	48.004.04b	69.00±2.08b	80.67±2.33b
	4	47.67±1.45a	58.67±2.03a	81.67±1.76a	99.33±0.33a
P. value		<.0001	<.0001	<.0001	<.0001
L.S.D.		5.979	8.314	6.854	4.548
Mobile stage	**0**	3.67±0.33d	5.00±0.00d	5.67±0.33d	4.33±0.33d
	1	38.67±2.03c	51.67±2.60c	60.67±2.03c	77.00±2.89c
	2	71.00±1.15b	75.00±2.65b	74.33±2.91b	88.67±2.03b
	4	77.33±1.45a	83.67±2.19a	88.67±2.03a	100.00±0.00a
P. value		<.0001	<.0001	<.0001	<.0001
L.S.D.		4.515	7.024	6.679	5.778

- L.S.D.= Least Significant Difference
- Pr. = probability Level
-Mean in the same column followed by the same letters is not significant.

Data summarized in Table (6) reported that the sensitivity to ozone treatments differs between eggs and mobile stages of *T. putrescentiae* based on LC_{50} values. Probit analysis of ozone concentration-response assessments indicated that *T. putrescentiae* eggs needed 1 h of treatment for 90% inviability at 1132.01 ppm on the other hand mobile stages needed 985.19 ppm respectively. In the case of *R. robini* the LC_{90} needed to get 90% of the inviable egg was 1463.65 ppm and 1101.69 ppm for mobile stages.

Ozone causes high damage to cell membranes of mites by prompting a severe increase in oxygen level (Zelac *et al.,* 1971; Jaffe *et al.,* 1967)**.** Treating stored dates with ozone gas could reduce pest populations with very low risk. (Leesch 2003; Mendez *et al.*, 2003).

Table 6: LC_{50} and LC_{90} values with their confidence limits for egg and mobile stages of *Tyrophagus putrescentia* and *Rhizoglyphus robini* exposed to ozone gas.

Stages	Species	LC_{50} (ppm)	LC_{90} (ppm)	Confidence limits (ppm)				Slope ± SD	r
				LC_{50}		LC_{90}			
				Lower	Upper	Lower	Upper		
Eggs	***R. robini***	231.79	1463.65	197.55	273.35	833.00	5179.71	1.893±0.296	0.965
	T. putrescentia	219.23	1132.01	187.04	255.72	722.84	2770.10	1.964±0.296	0.951
Mobile stages	***R. robini***	185.69	1101.69	148.33	221.90	742.25	2267.41	1.633±0.289	0.959
	T. putrescentia	182.45	985.19	138.79	223.73	686.28	1874.90	1.417±0.286	0.960

- r: Correlation coefficient of regression line
- SD: Standard deviation of the mortality regression line.

Conclusion

Due to the problems caused by infestation of *T. Putrescentiae* and *R. Robini* in stored palm date fruits. This study used two physical control methods using four concentrations for each method, The results revealed that the mortality percentage of mite stages increases by increasing either concentration or exposure period in both methods where 400 grays, 400 ppm were sufficient to kill 100 % of the mite's stages, although both methods (irradiation and ozone) were effective in controlling the two types of mite, the faster method was the ozone treatment.

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ARABIC SUMMARY

مقارنه بين طريقتين فيزيائيتين لمكافحة اكاروسات التمور المخزونه
***Tyrophagus putrescentiae* (Schrank) and *Rhizoglyphus robini* (Claparede)**
(Astigmata: Acaridae)

رانيا حسن محمود [1] , اسماء رضا عبد الخالق[1] ووائل كمال محمد الشافعى[2]
1- معهد بحوث وقاية النباتات –مركز البحوث الزراعية – مصر.
2- قسم افات وامراض النخيل – المعمل المركزى للنخيل – مركز البحوث الزراعية – مصر.

يعتبر التمر من اهم محاصيل الفاكهة والذي يساعد على زيادة الدخل القومى. اثناء التخزين يواجه التمر العديد من المشاكل مثل الاصابه بالحشرات، الامراض والاكاروسات. ومن اكثر انواع الاكاروسات ضررا ***Tyrophagus putrescentiae*** و ***Rhizoglyphus robini*** . لذلك يهدف هذا البحث الى تقييم استخدام طريقتين للمكافحة الفيزيائية و هما الاشعاع الجامي و التعريض لغاز الاوزون في مكافحة هذين الاكاروسين.تم تعريض نوعي الاكاروس لجرعات مختلفة من الاشعاع الجامى وهى صفر(كونترول) , 100 ,200 ,300و 400 جاما راي من خلال جهاز Cobalt-60 type Indian Gamma cell و الذي كان معدله 0.7967 كيلو جراى/ساعة لمكافحة البيض و الاطوار المتحركة لنوعي الاكاروس .و قد اظهرت النتائج المتحصل عليها ان نسبة الموت لنوعي الاكاروس تزيد بزيادة جرعات الاشعاع المستخدمة وان جرعة الاشعاع اللازمة للقضاء على 100% من تعداد نوعى الاكاروس المختبرين كانت 400 جاما راي. وفي حالة تعريض اطوار الاكاروسين الى تركيزات: صفر (كونترول) , 100 ,200 ,300و 400 جزء فى المليون من غاز الاوزون، اظهرت النتائج زيادة نسبة موت الاكاروسات بزيادة تركيز غاز الاوزون او زيادة مدة التعريض لنفس التركيز. اشارت النتائج الى ان الاوزون لديه القدرة على مكافحة نوعي الاكاروس بشكل جزئى عند تركيز 200 جزء فى المليون من غاز الاوزون لمدة أربع ساعات بينما قضى على 99 % من تعداد نوعي الاكاروس المختبرين عند المعاملة بتركيز 400 جزء فى المليون من غاز الاوزون لنفس مدة التعريض. واثبتت النتائج بوضوح ان الاطوار المتحركة لنوعى الاكاروس كانت أكثر حساسية من طور البيض. كما اوضحت النتائج انه بالرغم من ان طريقتي الاشعاع والاوزون فعالتين في مكافحة نوعي الاكاروس وهي طرق صديقة للبيئة واقل خطورة من الطرق الاخرى الا ان طريقة الاوزون كانت أسرع واكثركفائة من طريقة الاشعاع الجامي في مكافحة اطوار نوعي الاكاروس المختبرين.