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Evaluation of The Use of ECO_2-Fume Gas in Controlling Five Insect Pests of Stored Dates of the Egyptian Siwi Cultivar

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ABSTRACT

The purpose of this study was to examine the effectiveness of ECO_2-Fume fumigant gas (2% PH_3 + 98% CO_2 w/w) at three concentrations against five stored date fruits insect pest: *Ephestia cautella, Plodia interpunctella*, *Oryzaephilus surinamensis*, *Stegobium paniceum* and *Tribolium castaneum*. Also, to evaluate the effect of using this gas on some biochemical characteristics of treated date fruits. Three concentrations of ECO_2-Fume gas (30, 40 and 50 g/m^3) were tested against the five mentioned insects for three days inside four piles of Siwi dates cultivar inside in Shounat Qalyub, affiliated with the Agricultural Bank of Egypt in Qalyubia Governorate. The results showed that with increasing the concentration of the gas, the mortality percentage of the tested insects increased, and a concentration of 50 g/m^3 was sufficient to reach a 100% mortality percentage for all stages of the tested insects. The results also showed that the lepidopterous tested insects (*E. cautella and, P. interpunctella*) were more sensitive to ECO_2-Fume gas compared to the coleopterous tested insects (*O. surinamensis*, *S. paniceum* and *T. castaneum*), which were more resistant to the gas. In addition, the results showed that treating the semi-dry stored date fruits Siwi cultivar with ECO_2-Fume gas at a concentration of 50 g/m^3 did not affect the biochemical characteristics of the tested dates compared to the control. Based on the results of this study, ECO_2-Fume gas can be an effective replacement for insecticides in protecting stored dates from insect pests for marketing and exporting dates free from insect pests and pesticide residues.

Keywords: Date palm pests, Siwi, ECO_2-Fume**,** *Ephestia cautella , Plodia interpunctella , Oryzaephilus surinamensis,Stegobium paniceum* and *Tribolium castaneum*.

1. Introduction

Date palms are highly valued as food sources and cultural icons, particularly in the Arab world, where their wide distribution and varied climate have contributed to the development of date palm farming throughout the region (El-Lakwah *et al.,* 2011; El-Shafei 2011; El-Shafei and Batt 2024). Egypt has been the world's top producer of dates since 2001, making up 20% of the total date production worldwide (FAO, 2022). Numerous pests harm every component of the date palm, resulting in notable reductions in both the amount and quality of the fruits produced (Darwish *et al*., 2014; El-Shafei 2015; El-Shafei 2018; Zinhoum and El-Shafei 2019; Mahmoud *et al.,* 2022; El-Shafei and Attia 2023). Date fruits are heavily infested with several insect species from various orders (El-Shafei 2020; Assous *et al.,* 2022).One of the most significant orders, Lepidoptera, has numerous economically significant insect pests of date fruits, including the Amri date moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) and the Indian meal moth , *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae (El-Shafei *et al.,* 2018 ; El-Shafei *et al.,* 2019). According to Howard *et al.* (2001); El-Shafei *et al.* (2020); El-Shafei *et al.* (2022), infestations spread from the field to the storage via infested dates that have undergone multiple generations. Because they have a wide range and many stored products and fruits,

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their economic importance is high and very important (Nansen *et al.,* 2004; Rees, 2004). The Coleoptera order of date fruit insects are the second significant one. Numerous species of coleopterous insects heavily infest date palm fruits, with the saw-toothed grain beetle, *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae), the drugstore beetle *Stegobium paniceum* (L.) (Coleoptera: Ptinidae) and *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae) are being particularly problematic pests to stored date. These three insects are very widespread globally on many stored fruits, spices, grains and dates (Rossiter *et al.,* 2001). These insects feed on dates and products, and after their death, their various stages and products remain in the form of feces, and the rest of the dates they fed on inside the stored date and materials (Hashem *et al.*, 2012; Umeya 2012). These insects feed on the stored dates from the inside and leave their dead stages and excrement, which reduces the value of the dates. Because of them, 50% of the semi-dry and dry dates are lost annually after six months of storage, and the semi-dried dates are more damaged (Gough, 1917; Ali, *et al.,* 2001; Ali, *et al.,* 2003; Mohamed and Sayed 2017).

Chemical control of these insects in date fruits using pesticides in the past was easier to use, such as malathion and actellic pesticides, due to their cheap price and quick effect, but they are useless due to their harmful effect on humans, animals, beneficial insects and their residues that remain on the dates (Muir and White 2001).

In the past, methyl bromide gas, which has a quick and strong effect, was used in fumigating stored dates, but it has been banned and prohibited internationally because of its harmful effect on the environment in the ozone layer and its cumulative effect on human health (Hansen and Jensen, 2002). Recently, in recent decades, phosphine gas has been used as an alternative to methyl bromide in fumigating stored dates, but resistance to it has emerged in some insects, and its harmful effect has also been discovered on copper electrical wires, its presence in concentrations higher than 0.04 next to water causes fires (Lorini *et al.,* 2007).

It was also found that all of the previous methods cause the death of insects and their stages, but they remain dead inside the dates, causing the consumer to reject them and export rejection, which makes us search for a safer alternative and also reduce the presence of insects inside the dates. This solution was found in adding carbon dioxide gas to the phosphine, so the largest percentage for it is 98% and only 2% for the phosphine, which works to suffocate the insects inside the dates and make them leave the dates in search of oxygen, so they die on the ground and on the walls by the effect of both phosphine and carbon dioxide gas, away from the dates (El-Shafei 2020).

Therefore, this work aims to evaluate the effectiveness of ECO_2-Fume gas at three concentrations in controlling these insects on stored dates. Also, to evaluate the effect of using this gas on some biochemical characteristics of treated date fruits.

2. Materials and Methods

The experiment was carried out in both the Date Palm Pests and Diseases Department at the Central Laboratory for Date Palm and the Stored Products Pests Department at the Plant Protection Institute of the Agricultural Research Center in Egypt. An exposure experiment to ECO_2-Fume gas was conducted in Shounat Qalyub, affiliated with the Agricultural Bank of Egypt in Qalyubia Governorate (30°11'01.8"N 31°13'21.3"E).

2.1. Tested insects Culture and host date cultivars

The five insect species (*E. cautella, P. interpunctella*, *O. surinamensis, S. paniceum* and *T. castaneum*) were gathered from date fruits that were afflicted and raised on diets consisting of their regular foods (Fig. 1). In an incubator maintained at 27±2°C and 65±5% relative humidity (RH), insects were cultured. The Siwi cultivar of semi-dry date fruits has been the larval insect's food source. In order to prevent any contamination with other pests, the date fruits used in the rearing culture were preserved in the freezer for a period of two weeks prior to use.

Fig. 1 a: *E. cautella* larva, **b**: *P. interpunctella* larva, **c**: *O. surinamensis* adult, **d** : *O. surinamensis* larva, **e:** *S. paniceum* adult, **f**: *S. paniceum* larva, **g**: *T. castaneum* adult and **h**: *T. castaneum* larva .

2.2. Fumigation technique

One ton of Siwi cultivar date fruits were divided into four equal volumes, or heaps, with a different concentration of ECO_2-Fume in each. Fourteen plastic boxes each boxe measuring fifty by thirty-by-thirty centimeters and weighing 17.86 Kg. each were cut from each pile. Within each pile, there were three tiers of boxes set out on a concrete surface.

250 g of date fruits were placed inside a cotton bag that was firmly closed to represent a replicate, and 30 of the 4^{th} larvae of the Lepidopterous tested insects and 30 of the 4^{th} larvae + adults (2–3 days) of the Coleopterous tested insects were placed on each species' stage. Each of the five studied insect species had three bags (replicas) positioned beneath it, dispersed over all layers and directions, for a total of eighteen bags.

Three different concentrations (treatments) of ECO_2-Fume gas (30, 40, and 50 g/m^3) were applied to a pile. The fourth pile did not have gas or a single treatment applied as a control. To find the appropriate dosage of ECO_2-Fume gas, the volume of each pile was measured. Sandbags were placed on the pile's edges to seal it after a layer of reinforced plastic had been applied. After completely sealing the fumigation area, weigh the ECO_2-Fume gas cylinder on the platform balance. Switch on the valve on the cylinder. Weigh the cylinder and close the valve once we've reached the required dosage of 30, 40, and $50g/m^3$.

After being fumigated for three days, the pile was allowed to air out with the plastic cover sheet removed. To investigate and document the mortality percentages of the examined insect species, the treated date samples were carefully transferred into suitable glass jars and covered with muslin. They were then kept in incubators maintained at 27 ±2 C° and 60 ± 5% RH. Every jar was watched in accordance with Abbott's formula to document the adult emergence and calculate the mortality rate as a percentage:

2.3. Concentration of ECO_2-Fume Measured

Using a Silo check detector (Fig.2) inside the fumigated plastic sheet, the gas concentration was monitored for the whole three-day exposure period to ECO_2-Fume. Via ten-meter polyethylene tubes with a 5 mm diameter that were positioned beneath plastic sheets in the middle of the store levels within

the date boxes (which are plastic boxes that are 50 x 30 x 30 cm in size), or at the top and bottom of the Siwi cultivar date fruits storage cotton bags. These tubes were fastened to the gas detector.

Fig. 2: Silo-Check device for measuring the concentration of ECO_2-Fume gas inside a pile

2.4. Biochemical components of treated Siwi date fruits cultivar by ECO_2-Fume gas concentration of 50 g/m^3 compared to the control (untreated date fruits).

Known number of Siwi dates fruits treated with ECO_2-Fume gas concentration of 50 g/m^3 and the same number of untreated dates fruits were prepared as a control and analyzed in the analysis laboratory of the Dates Manufacturing and Marketing Department at the Central Laboratory for date palm. Total Amino Acid, Total Indols and Total Phenol Contents were determined according to McGrath (1972), Selim *et al.* (1978) and Elizabeth and Kelly (2007), respectively. Estimation of reducing sugars and total soluble sugars were determined according to Schales and Schales (1945).

2.5. Statistical analysis

The results of the mortality percentage of the five tested insects after exposure to the three concentrations of gas as well as the biochemical characteristics of the treated dates compared to the control were analyzed using Proc., ANOVA in SAS (Statistical Analysis System 2003).

3. Results and Discussion

3.1. Effect of ECO_2-Fume gas concentrations (30, 40 and 50 g/m^3) on five stored date fruits insect pest species

At Shounat Qalyub, affiliated with the Agricultural Bank of Egypt in Qalyubia Governorate (30°11'01.8"N 31°13'21.3"E), Egypt, Siwi cultivar date fruits piles under gas-proof cover at 30°C were used to test various ECO_2-Fume gas concentrations (30, 40 and 50 g/m^3) against five stored date fruits insect pest species (*E. cautella, P. interpunctella, O. surinamensis, S. paniceum and T. castaneum*). Data in Table (1) and Fig. (3) showed the impact of varying ECO_2-Fume gas concentrations on the mortality percentage of the five tested insects.

The findings showed that the mortality percentage of various date fruit insects exposed to varying concentrations of ECO_2-Fume gas increased with increasing gas concentrations. This is because the mortality percentages of these insects are directly proportional to the concentrations of the gas. It was observed that the mortality percentage of all tested insects of *E. cautella, P. interpunctella* larvae and *O. surinamensis, S. paniceum and T. castaneum* larvae and adults in the glass jars, exposure to 50 g/m^3 for 3 days reached 100% after fumigation, proving that this concentration worked well to control all the five tested insects.

In the case of exposing the five insects to a concentration of 40 g/m^3, it was found that both the larvae of the *E. cautella*, larvae and adult of *O. surinamensis* reached a mortality percentages of 100%

with a significant difference with the rest of the insects. Both (*P. interpunctella* larvae, adults of *S. paniceum*, and *S. paniceum* larvae) recorded mortality percentages of (97.78, 96.67 and 92.22 %) respectively. The least affected by this concentration were the larvae and insects of *T. castaneum*, which recorded mortality percentages (81.11 and 80.00 %) with a significant difference from the rest of the insects. While none of the five tested insect stages reached 100% mortality when exposed to a concentration of 30 g/m^3 of ECO_2-Fume gas.

These results were found to be in agreement with those of Mohamed and Sayed (2013) indicated that there was a clear correlation between the amounts of ECO_2-Fume gas and the hatchability of eggs, the mortality of larvae and pupae, and the decrease in F1 progeny. 500 ppm (36 g/m3) of ECO_2-Fume gas was the convenient dose for three days of exposure against all stages of *E. cautella* growth. Mohamed and Sayed (2017) mentioned that when using ECO_2-Fume gas at a dose of 50 g/m^3 for three days, it led to the death of all stages of the Amri date moth (*E. cautella*,) the Oasis date moth (*Ephestia calidella*), and the Surinam(*O. surinamensis*)beetle in dry and semi-dry dates. According to El Shafei (2020), utilizing a mixture including (CO_2 + phosphine) boosted the mortality percentages of *P. interpunctella* and O. *surinamensis*, which reached a maximum of 100%. At these quantities, CO_2 or phosphine alone could never produce such a level of mortality. Ahmed *et al.* (2022) pointed out that the use of carbon dioxide gas mixed with phosphine gas increased the efficiency of the fumigation process and the control of *T. castaneum and O. surinamensis* insects in stored dates and reduced the time required for fumigation. Amin *et al.* (2022) evaluated ECO_2-Fume for three days at varying doses of 25, 30, 40, and 50 g/m^3.it was found that ECO_2-Fume was administered at a rate of 50 g/m^3, all stages of both insect species, *Callosobruchus maculatus and Callosobruchus chinensis* in packed cowpea stacks were fully controlled after three days. Baume *et al.* (2024) they treated stored wheat grains infested with different stages of the Tribolium beetle (*T. castaneum)* with different concentrations of ECO_2-Fume gas 30, 35, 40 and 50 g/m^3 for a period of three days and found that the mortality percentages reached 100% for all stages of the insect after exposure to a concentration of 50 g/m^3 for three days and that treatment with this gas is an important method for protecting stored wheat.

Table 1: The mean mortality % of five species of stored date insect pests after exposure to three concentrations of ECO_2-Fume gas.

Insect species	**Insect stages**	**ECO_2-Fume gas concentrations g/m^3**		
		30	**40**	**50**
Ephestia cautella	**Larva**	80.00 ±1.92a	100.00± 0.00 a	100.00 a
Plodia interpunctella	**Larva**	75.56 ± 2.22ab	97.78 ±1.11a	100.00 a
Tribolium castaneum	**Larva**	35.56 ± 4.01e	81.11 ±2.94c	100.00 a
	Adult	31.11±1.11 e	80.00 ± 1.92 c	100.00 a
Oryzaephilus surinamensis	**Larva**	73.33 ±1.92abc	100.00 a	100.00 a
	Adult	70.00±1.92 bcd	100.00 a	100.00 a
Stegobium paniceum	**Larva**	67.78 ±1.11cd	96.67 ab	100.00 a
	Adult	65.56±2.94 d	92.22 b	100.00 a
Pr.		<.0001	<.0001	-
L.S.D. $_{0.05}$		6.9675	4.5613	-

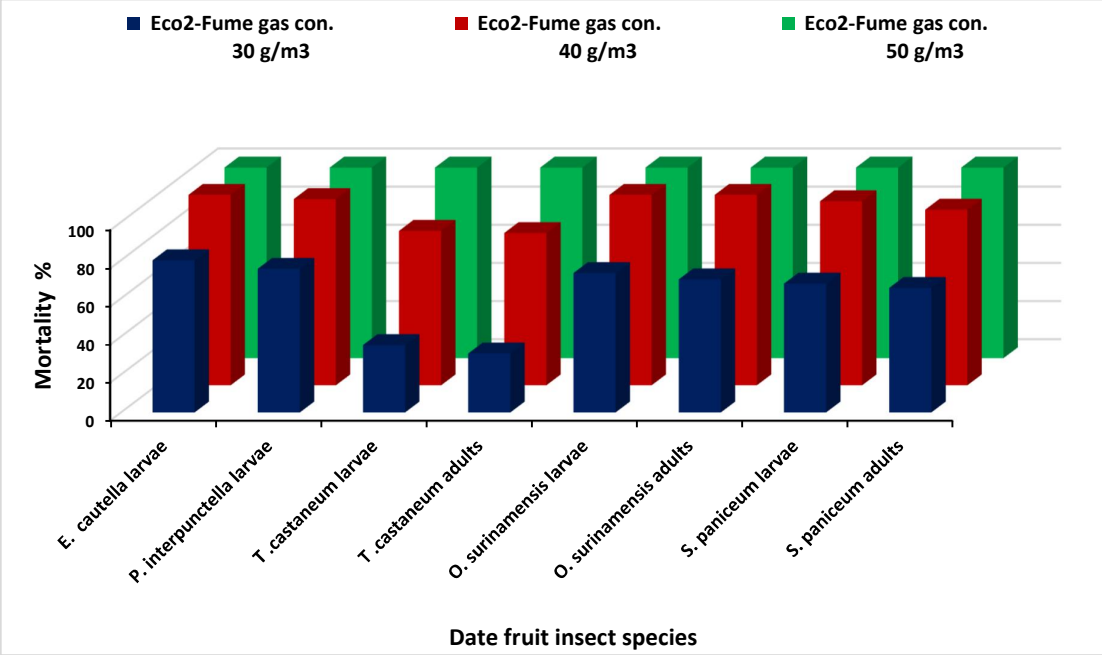

Fig. 2: Mortality % of stored date fruit insect pest after exposure to ECO_2-Fume gas.

3.2. Actual concentration of ECO_2-Fume gas inside date fruits piles

Obtained results in Table (2) indicated that the gas concentration inside each of the three piles of the three concentrations (30 ,40 and 50 g/m^3) throughout the exposure period was determined to be (1022, 1229 and1381 ppm), (879, 1080 and1228 ppm) and (712, 909 and1047 ppm) for the three tested concentrations (30 ,40 and 50 g /m^3) on the first, second, and third days, respectively. After three days, the average concentrations of ECO_2-Fume were (937, 1141 and1292 ppm) for the three tested concentrations (30 ,40 and 50 g /m^3) respectively. After three days, the average of ECO_2-Fume Reduction % in the tested piles were (17.44, 15.23 and14.59 %) for the three tested concentrations (30, 40 and 50 g/m^3) respectively. Depending on the pile's quality of sealing and suitability for ECO_2-Fume gas fumigation, this figure is thought to be highly concentrated at the end of the exposure period.

Table 2: ECO_2-Fume gas concentration (ppm) of date fruits piles under the plastic sheet at Shouna Qalyub, in Qalyubia Governorate.

Location	Concentration g./m^3	Concentration (ppm)				
		Initial time	1st day	2nd day	3rd day	Mean
Shounat Qalyub, affiliated with the Agricultural Bank of Egypt in Qalyubia Governorate	30 g./m3	1135	1022	879	712	937
	40 g./m3	1346	1229	1080	909	1141
	50 g./m3	1513	1381	1228	1047	1292.25
		Reduction % in gas concentrations				
	30 g./m3	100%	9.96 %	22.56%	37.27%	17.44%
	40 g./m3	100%	8.69%	19.76%	32.47%	15.23%
	50 g./m3	100%	8.72%	18.84%	30.80%	14.59%

3.3. Impact of 50 g/m^3 ECO_2-Fume gas on the quality and biochemical composition of treated Siwi date fruits.

The impact ECO_2-Fume gas high concentration, 50 g/m^3 on the quality and biochemical composition of the Siwi date fruits that have been treated is summarized in Table (3). The obtained results showed that total amino acids and total indoles recorded (0.0305 ,0.074 and 1.202) mg/g f.w. respectively after treated with ECO_2-Fume gas,50 g/m^3 compared to control (0.308, 0.075 and 1.201) mg/g f.w. Without any significant differences. The same pattern might be used to total sugars, reducing and non- reducing sugars which were (0.817, 0.763 and 54) mg/g f.w after treated with ECO_2-Fume gas,50 g/m^3 compared to control (0.817, 0.764 and 53) mg/g f.w, also, there were no significant differences between the treatment and the control.

Our findings are consistent with those of El-Shafei (2015), who found that there were negligible variations in the chemical fruit content, including total proteins, total sugars, reducing and non-reducing sugars, and control, under various controlling treatments of *E. cautella* by CO_2 + phosphine. And Mohamed and Sayed (2017) mentioned that no change in the color of the dates was observed as a result of the fumigation process at the tested dose, which does not affect the general appearance of the dates. Also, El Shafei (2020) mentioned that according to the experiment's results, the gases used in the treatment of the date fruits had no effect on their chemical properties when compared to the control, particularly the mixture of gases. Ahmed *et al.,* (2022) The use of carbon dioxide gas mixed with phosphine gas in fumigating dry dates improved some chemical properties of dates such as reducing free phenols and increasing both total and reducing sugars in date fruits compared to the control. Amin *et al.,* (2022) found that exposing the packed cowpea seeds to a concentration of 50 g/m^3 ECO_2-Fume did not affect their quality characteristics, whether in terms of germination or other chemical properties of the seeds

Table 3: Effect of ECO_2-Fume gas,50 g/m^3 on biochemical composition and quality of treated Siwi cultivar date fruits.

Biochemical contents Treatments	Amino acides (mg/g f.w.)	Indoles (mg/g f.w.)	Phenols (mg/g f.w.)	Total sugar (mg/g f.w.)	Reducing sugar (mg/g f.w.)	Non reducing sugar (mg/g f.w.)
Control	0.308±0.007^a	0.075±0.003^a	1.201±0.031^a	0.817±0.003^a	0.764±0.003^a	0.053±0.00^{ab}
ECO_2-Fume	0.305±0.002a	0.074±.002a	1.202±0.045a	0.817±0.002a	0.763±0.002a	0.054±0.001a
P. value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
L.S.D.$_{0.05}$	0.012	0.0052	0.0871	0.0052	0.0063	0.002

4. Conclusion

This research proved the efficiency of fumigation by ECO_2-Fume fumigant in controlling of stored date fruits insect pests. The mortality percentage of all tested insect stages increased with increasing gas concentration from 30 to 50 g/m^3 which was sufficient to kill all tested insect stages. The Lepidopterous tested insects were more sensitive to the gas compared to the resistant Coleopterous insects. Also, the treatment of Siwi dates fruits cultivar with this gas did not affect the biochemical characteristics of the exposed date fruits. It is possible to recommend the use of ECO_2-Fume gas during the storage of semi-dry and dry dates from the harvest season until the selling and export season to keep them free from insect infestations, as it is good at removing all insect stages outside the infested date fruits treated with it, thus allowing the production of dates free from insect pests, as well as free from pesticide residues and suitable for local marketing and international export.

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