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Efficacy of CO and CO_2 Treatment to Control the Stored-Date Insects, *Ephestia kuehniella* and *Ectomyelois ceratoniae* for Organic Dates Production

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Abstract* – This study evaluates the efficacy of CO_2 and CO treatment against *Ectomyelois ceratoniae* and *Ephestia kuehniella.* Dates infested with *E. ceratoniae* and different stages of *E. kuehniella* were both fumigated with carbon monoxide (CO) and carbon dioxide (CO_2). This experiment showed that the mortality of the two species increases with the increase of treatment time. In fact, for 6 hours exposure to 1624 g/m^3 of CO, *E. ceratoniae* and *E. kuehniella* mortality rates were much higher (between 75% and 100%) than 4 hours and 30 minutes exposure to the same dose. Complete mortality was reached after 6 hours treatment with 1624 g/m^3 of CO for *E. kuehniella* and after 6hours and 30 minutes treatment for *E. ceratoniae.

Carbon dioxide (CO_2) laboratory results showed that the mortality rate of *E. kuehniella* and *E. ceratoniae.* depends on the gaz level and the treatment duration. After only 20 hours exposure to 8,5kg/m^{3}of CO_2, 100% mortality was recorded for all the stages of the two tested insects. Following the promising results, fields trials were carried out. 100% of the two insect species mortality was achieved with CO_2 dose of 2800g/m^3 for 48 hours. This result can be improved if the airtightness of the fumigation chamber was more suitable for application of higher doses of CO_2.

***Keywords* – Efficacy, CO, CO_2, Stored-Date Insects, *Ephestia kuehniella*, *Ectomyelois ceratoniae,* Organic Dates Production.**

I. INTRODUCTION

Dates are an important export commodity in Tunisia. In fact, Tunisian dates are ranked 3rd in the world in terms of exported quantities and the first in terms of foreign exchange earnings [12]. Nevertheless, the Tunisian date industry is faced with many challenges due to the technical and social conditions of production. The most economically important constraints to this sector is insect infestation, especially the carob moth, *Ectomyelois ceratoniae* and the Mediterranean flour moth *Ephestia kuehniella*. These two insect species are reported as the most widespread and abundant moth-pests of stored dates in Tunisia [9]-[13]. Typical infestation rates of the carob moth are about 20%

of the harvestable crop annually [12]. The variety 'Deglet Noor' is heavily attacked by carob moth, although most other varieties are also attacked [9]. Stored dates can contain these insects despite of protective procedures taken during the cropping stage. Therefore, a rapid disinfestation treatment time is required to fulfill phytosanitary regulations at the time of export or deal with infestation problems diagnosed during trading [2]-[3]. For many years, methyl bromide has been relied upon for this purpose.

It acts rapidly, controlling insects in less than 48h, and it has a wide spectrum of activity, controlling not only insects but also nematodes and plant-pathogenic microbes [2]-[11]. However, due to the toxicity and negative environmental impacts of methyl bromide, this chemical product can no longer be produced or used [3]. Moreover, the use of methyl bromide is phased out in developing countries in accordance with the Montreal Protocol due to its effect on the ozone layer [25].

However, due to the toxicity and negative environmental impacts of methyl bromide, this chemical product can no longer be produced or used [3]. Moreover, the use of methyl bromide is phased out in developing countries in accordance with the Montreal Protocol due to its effect on the ozone layer [25]. The development of alternative treatments for pest control is in increasing demand from the food industry and has been promoted by governments through legislation and the funding of research projects [22].

Additionally, a public awareness has arisen with the negative impacts of chemicals on human health and their harmful influence on the environment. Consequently, there is now a high demand for non-chemical disinfestation treatments while at the same time maintaining a high degree of control efficacy to satisfy International regulations and standards necessary for export [19]-[22]-[3]. For that reason, many physical methods including heat [10]-[15]-[19]-[1], cold [28]-[10], microwaving [21]-[30] and gamma radiation [16] have been studied for years. However, in Tunisia, the only non-chemical alternative to methyl bromide used for treating organic dates, is freezing at –18°C or lower for at least 48 hours. During freezing, inter-cellular ice crystals are formed. These ice crystals may cause mechanical damage to the fruit. In addition, freezing may lead to membrane injury and accelerate ripening [14]-[23]-[27]-[24]. It is also a highly energy consuming technique [7]-[23]-[4] [5]-[6].

The only technology that retains the special capacity of fumigation for in-situ treatment of stored commodities, as well as offering a similar diversity of application technologies, and being safe and environmentally benign is the modified atmosphere (MA) method [20]. Modified atmospheres have the potential to kill all pests but require supplementary measures to increase their speed of action. However, raised temperature can shorten treatment times [2]. In cases where rapid disinfestation of commodities is

required, the possibility of using CO_2 at raised temperatures should be considered [20].

Carbon dioxide (CO_2) is regarded as an environmentally friendly technique for control of insects without residues in stored products [29]-[26]. Additionally, insecticide treatments affecting the respiratory system are more pronounced at higher temperatures [17]. Many researchers studied the combined effects of high CO_2 and optimal elevated temperatures against insects have shown a corresponding increase in insect mortality with increase in temperature [8]-[2]-[18].

Carbon monoxide is a poisonous gas to mammals, has most of the merits of CO_2, except for its flammability at high concentration [26]. Carbon monoxide (CO) is a simple diatomic gas molecule, being odorless, tasteless, colorless and with low water solubility [33]-[32]. It is produced from incomplete combustion of organic matter [31]. CO has been used in storage primarily for its beneficial effects on the commodity, but, disease suppression has been reported [34]. In fact, carbon monoxide can be used as a fungistatic component at low oxygen level on stored fruits [36]. CO might be also applied to preserve postharvest fruits quality [35] and it is toxic to some insects [26]. [37] studied the response of *Tribolium castaneum* to exposure of 30% and 40% CO of carbon monoxide and 100% mortality was recorded at the highest dose.

This present study aims to evaluate the effect of CO2 treatment at raised temperatures on stored-dates insects (*Ectomyelois ceratoniae* and *Ephestia kuehniella*), try to explore a novel method based only on CO gaz and determine whether these postharvest treatments could be used as a substitute for methyle bromide.

To the best of our knowledge, this is the first report about the use of CO gaz to control *E. ceratoniae* and *E. kuehniella* on dates.

II. Materials and Methods

***Fruits.** The dates used for the experiments were infested by the carob moth *Ectomyelois ceratoniae*.

***Insects.** Laboratory cultures of the test insect *Ephestia kuehniella* were reared under standard conditions of 25±1°C temperature, 60±5% relative humidity and 16/8 photoperiod on an artificial diet.

Temperature (°C) and relative humidity (%) inside the chamber were measured using a programmable thermo-hygrometer (Testo: hobo).

2.1 Laboratory experiments of carbon monoxide and carbon dioxide

For each treatment, 50 insects of each stage of *E. kuhniella* (eggs, young larvae, old larvae, nymphs and adults) were selected and placed into plastic boxes.

Matched samples of 50 fruits per replicate were also selected, and three replicates per treatment were used for each experiment. Reported results are the average of the three trials.

Dates and insects samples were placed inside an hermetically sealed fumigation chamber of 0.0282 m^3. It has a vacuum gauge, a vacuum pressure recorder and a temperature recorder. After the vacuum was drawn, the gaz (CO or CO2) was injected into the chamber.

In the first experiment, insects and dates samples were fumigated with CO gaz at the same dose of 1624g/m^3 of, for different treatment times (4,5 hours, 5 hours, 5,5 hours, 6 hours, 6,5 hours and 7 hours) and under the temperature of 35±1°C.

In a second series of tests, we used CO2 gaz at different doses of (7kg/m^3, 8kg/m^3 and 8,5kg/m^3) for 16 hours and 20 hours, and at the same temperature of 35±1°C.

2.2 Field trials of carbon dioxide

Field trials were carried out in the commercial drying company: Boudjebel SA VACPA, located at Nabeul Governorate in the North of Tunisia. The different tests were conducted in a specially designed fumigation chamber with a volume of 140 m^3, that was used for methyl bromide treatment. This chamber is provided with a system for applying, distributing the fumigant and removing it at the end of treatment. It is also fitted with a vacuum gauge, a vacuum pressure recorder and also a temperature recorder. The fumigation gas-tightness of the enclosure is poor compared with the chamber used for the laboratory experiments, so we had to use low doses of CO2 gaz (2,4 kg/m^3, 3,2 kg/m^3 and 3,6kg/m^3) or else the concentration of fumigant would not maintain sufficient to kill all stages of insects. This will also cause the waste of money and would result in the development of resistance. The different tests were conducted for 24 hours, 36 hours and 48 hours. Details were as follows. Dates were thoroughly mixed and divided into samples of about 300 dates per replicate, and for each experiment three replicates per treatment were used. The dates samples and the living stages of *Ephestia kuehniella* were placed inside the chamber. After the vacuum was drawn, the samples were exposed to CO2 enriched-air flow. For each bioassay, about 50 insects of each stage were used to test for mortality. Three subsequent trials were carried out. In each trial, five crates (containing the insects of the five different stages: eggs, young instars, old instars, nymphs and adults) were placed in different points inside the fumigation chamber. Reported results are the average of the three trials.

After each experiment, the infested fruits were analyzed in the laboratory for survival insects.

2.3 Laboratory analysis

After each treatment, insects were returned to the rearing conditions (temperature: 25±1°C, humidity: 60±5% and photoperiod 16/8) for incubation and a weekly assessment of survival based on egg hatch and adult emergence.

As to the fruit samples, they are examined to determine the effect of the gaz treatment on insect mortality rate. Date infestation percentage and the population structure of the *E. ceratoniae* during treatment are also determined before and after treatment.

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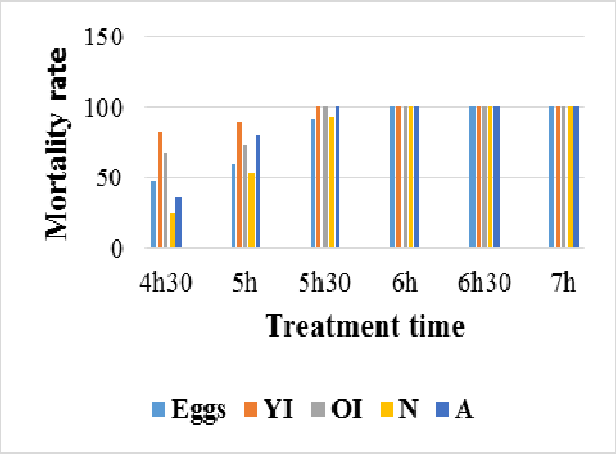
III. Results and Discussion

3.1 Laboratory experiments

- *Carbon monoxyde*

Results show that all the stages of *E. kuehniella* can be controlled within 6 hours at a temperature of ±35°C and CO dose of 1624 kg/m$^{3.}$

The most tolerant stages of *E. kuehniella* were the nymphs and the eggs, for which 6 hours were required for complete control. However, complete mortality of larvae and adult was reached within 5 hours and 30 minutes exposure.



Yl: Young larvae; Ol: Old larvae; N: Nymphs; A: Adults

Fig.1. Mortality rate of the different stages of Ephestia kuehniella at a dose of 1624 kg/m^3 of CO for different treatment time (temperature= 35±1°C).

As expected, *E. ceratoniae* mortality rate was also highly dependent on treatment time. *E. ceratoniae* proved to be more tolerant to CO treatment than *E. kuehniella*. In fact, 6 hours and 30 minutes exposure was required to achieve complete mortality.

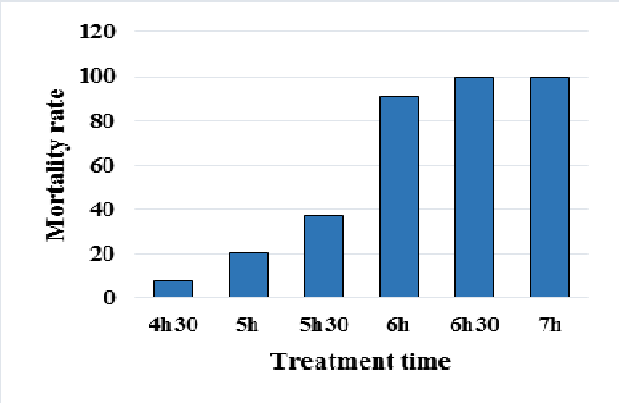


Fig.2. Mortality rate of the different stages of Ectomyelois ceratoniae at a dose of 1624 kg/m^3 of CO for different treatment time (temperature= 35±1°C

Table 1. Mortality rate of various stages of Ephestia kuehniella after treatment tested at 35±1°C (average of 3 replicates) and different treatment time (4h30, 5h, 5h30, 6h, 6h30 and 7h) at the same dose of CO (1624g/m^3)

Treatment time (hours)	**Stages of *Ephestia kuehniella***	**Number of insects per stage**				
		Replicates				
		R1	**R2**	**R3**	**MR (%)**	**CMR (%)**
4h30	Eggs	50	50	50	46,67	100
	YL	50	50	50	82	100
	OL	50	50	50	68	100
	N	50	50	50	24,67	100
	A	50	50	50	36	100
5h	Eggs	50	50	50	60	100
	YL	50	50	50	89,34	100
	OL	50	50	50	73,34	100
	N	50	50	50	53,34	100
	A	50	50	50	80	100
5h30	Eggs	50	50	50	92	100
	YL	50	50	50	100	100
	OL	50	50	50	100	100
	N	50	50	50	93,34	100
	A	50	50	50	100	100
6h	Eggs	50	50	50	100	100
	YL	50	50	50	100	100
	OL	50	50	50	100	100
	N	50	50	50	100	100
	A	50	50	50	100	100
6h30	Eggs	50	50	50	100	100
	YL	50	50	50	100	100
	OL	50	50	50	100	100
	N	50	50	50	100	100
	A	50	50	50	100	100
7h	Eggs	50	50	50	100	100
	YL	50	50	50	100	100
	OL	50	50	50	100	100
	N	50	50	50	100	100
	A	50	50	50	100	100

MR: Mortality Rate, CMR: Corrected Mortality Rate Yl : Young larvae ; Ol: Old larvae ; N : Nymphs ; A : Adults

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Table 2. Mortality rate of various stages of Ectomyelois ceratoniae after treatment tested at 35±1°C (average of 3 replicates) and different treatment time (4h30, 5h, 5h30, 6h, 6h30 and 7h) at the same dose of CO (1624g/m^3)

Treatment time (hours)	Replicates	Examine ddates	Dead stages	MR (%)	CMR (%)
4h30	R1	50	2	9,1	46,34
	R2	50	1	4,33	32,35
	R3	50	3	10,71	100
5h	R1	50	5	21,74	100
	R2	50	5	20,83	85,45
	R3	50	6	19,35	74,8
5h30	R1	50	11	36,67	100
	R2	50	12	36,36	91,91
	R3	50	12	37,5	92,98
6h	R1	50	24	75	100
	R2	50	22	100	96,55
	R3	50	38	97,44	100
6h30	R1	50	21	100	100
	R2	50	23	100	100
	R3	50	25	100	97,44
7h	R1	50	30	100	97,14
	R2	50	33	100	97,37
	R3	50	36	100	100

MR: Mortality Rate,
CMR: Corrected Mortality Rate

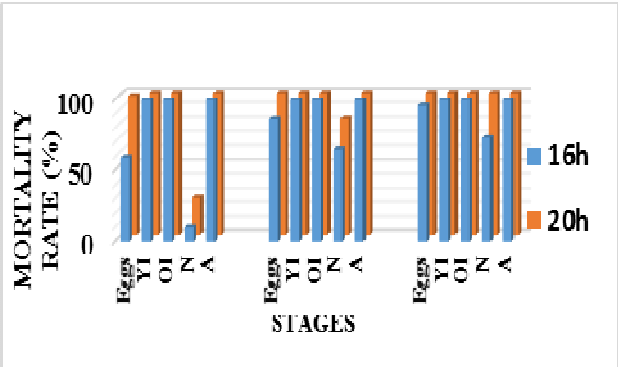
- *Carbon dioxide*

The results of laboratory tests of CO_2 are given in the table 4 and 5, and displayed graphically fig. 3 and 4.

Table 3: Quantity and doses of CO_2 used in the laboratory experiments for room 0.0282m^3

Dose	CO_2 (g/m^3)	CO_2 (L/min)
D1	7000	20
D2	8000	20
D3	8500	20

Young and old instars of *E. kuehniella* are more sensitive than other stages. In fact, total mortality for larvae stages was recorded for all doses and for both 16 hours and 20 hours CO_2 treatment. Mortality rate of eggs and nymphs increased with increasing CO_2 dose. Nevertheless, results proved these two stages are the most tolerant to CO_2 treatment.



Yl: Young larvae; Ol: Old larvae; N: Nymphs; A: Adults
Fig.3. Mortality rate of the different stages of *Ephestia kuehniella* at several doses of CO_2 for 16 hours treatment time (temperature= 35±1°C).

An important suppression of the different stages of *E. kuhniella* was achieved after 16 hours with CO_2 dose of 8500g/m^3 (D3).

As presented in Fig.3. 20 hours of CO_2 treatment was more effective than 16 hours treatment. Complete kill of all development stages of *E. kuehniella* was only achieved after 20 hours of CO_2 treatment with the dose D3 (8500g/m^3).

E. ceratoniae mortality rate was also also dependent on CO2 dose and on treatment time. In fact, 20 hours exposure to the highest dose (D3=8500g/m$^{3)}$ was required to achieve complete mortality. This is supportded by [5] who found that short duration CO_2 treatments (16 hours) are not effective against different stages of *E. ceratoniae*. This leads to the conclusion that short periods CO_2 treatment limits it effect.

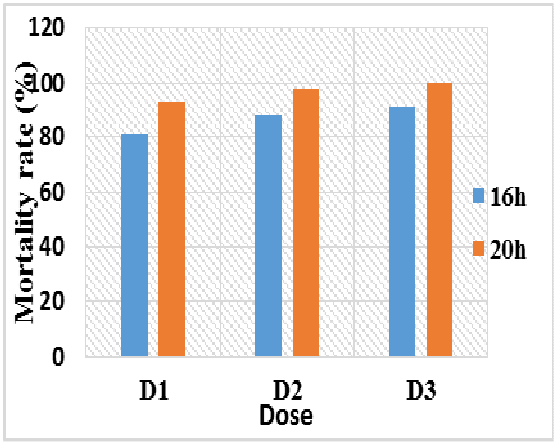


Fig.4. Mortality rate of Ectomyelois ceratoniae at several doses of CO2 for 16 hours and 20 hours treatment time (temperature= 35±1°C).

Table 4. Mortality rate of various stages of Ephestia kuehniella after treatment tested at 35±1°C (average of 3 replicates) and different doses of CO2 (D1=7kg/m^3, D2=8kg/m^3 and D3=8,5kg/m^3).

Dose	Treatment time (hours)	Stages of *E. kuehniella*	Number of insects per stage				
			Replicates				
			R1	R2	R3	MR (%)	CMR (%)
7kg/m^3	16h	Eggs	50	50	50	60	100
		YI	50	50	50	100	100
		OI	50	50	50	100	100
		N	50	50	50	10	100
		A	50	50	50	100	100
	20h	Eggs	50	50	50	98	100
		YI	50	50	50	100	100
		OI	50	50	50	100	100
		N	50	50	50	26	100
		A	50	50	50	100	100

8kg/m^3	16h	Eggs	50	50	50	86.6	100
		YI	50	50	50	100	100
		OI	50	50	50	100	100
		N	50	50	50	48	100
		A	50	50	50	100	100
	20h	Eggs	50	50	50	100	100
		YI	50	50	50	100	100
		OI	50	50	50	100	100
		N	50	50	50	82	100
		A	50	50	50	100	100
8.5kg/m^3	16h	Eggs	50	50	50	96	100
		YI	50	50	50	100	100
		OI	50	50	50	100	100
		N	50	50	50	84	100
		A	50	50	50	100	100
	20h	Eggs	50	50	50	100	100
		YI	50	50	50	100	100
		OI	50	50	50	100	100
		N	50	50	50	100	100
		A	50	50	50	100	100

MR: Mortality Rate; CMR: Corrected Mortality Rate; Yl: Young larvae; Ol: Old larvae; N: Nymphs; A: Adults

Table 5. Mortality rate of various stages of Ectomyelois certoniae after treatment tested at 35±1°C (average of 3 replicates) and different doses of CO2 (7kg/m^3, 8kg/m^3 and 8,5kg/m^3)

Dose (kg/ m^3)	Treat-ment time (hours)	Replic ates	Exa-mine d dates	Dead stages	MR (%)	CMR (%)
D1=7	16h	R1	50	22	78,57	92,73
		R2	50	25	83,33	92
		R3	50	26	81,25	96,67
	20h	R1	50	28	96,55	100
		R2	50	30	90,91	97,71
		R3	50	37	92,5	92,79
D2=8	16h	R1	50	30	83,33	97,14
		R2	50	24	88,88	97,26
		R3	50	29	93,55	94,65
	20h	R1	50	42	100	100
		R2	50	32	96,97	95,2
		R3	50	24	96	95,15
8.5 kg/m^3	16h	R1	50	18	90	100
		R2	50	35	87,5	94,92
		R3	50	33	97,06	97,36
	20h	R1	50	31	100	97,96
		R2	50	41	100	95,12
		R3	50	29	100	95,24

MR: Mortality Rate, CMR: Corrected Mortality Rate

Field trials of CO_2

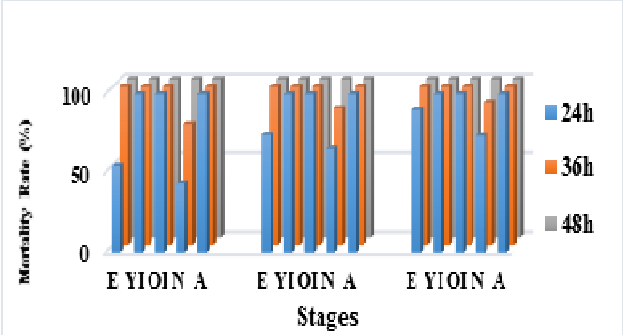
Results of field experiments are given in the table 7 and 8 and are displayed graphically in fig. 5 and 6.

Table.6: Quantity and doses of CO_2 used in the fields trials for room of 140 m^3

Dose	CO_2 (g/m^3)	CO_2 (L/min)
D1	2400	20
D2	2800	20
D3	3200	20

The fig.5 indicates that mortality rate of the different stages of *E. kuehniella* increases with the increase of CO_2 dose.

Comparing 24 hours with 36 hours treatment results, we find that the mortality rate of *E. kuehniella* increases also with the increase of exposure time at the same dose of CO_2. Therefore, for a complete control of all stages, 48 hours exposure time is needed.



(E: Eggs; Yl: Young larvae; Ol: Old larvae; N: Nymphs; A: Adults)

Fig.5. Mortality rate of the different stages of Ephestia kuehniella at several doses of CO_2 for 24 hours, 36 hours and 48 hours treatment time (temperature= 35±1°C).

The first dose of CO_2 (D1= 2400g/m^3) didn't cause significant mortality to the different stages of *Ectomyelois ceratoniae*, but further increased CO_2 levels (2800g/m^3 and 3200g/m3) caused important mortality rates (Fig.6).

Treatment time required to kill all stages of *E. ceratoniae* was 48 hours at two doses D2 (2800g/m^3) and D3 (3200g/m^3).

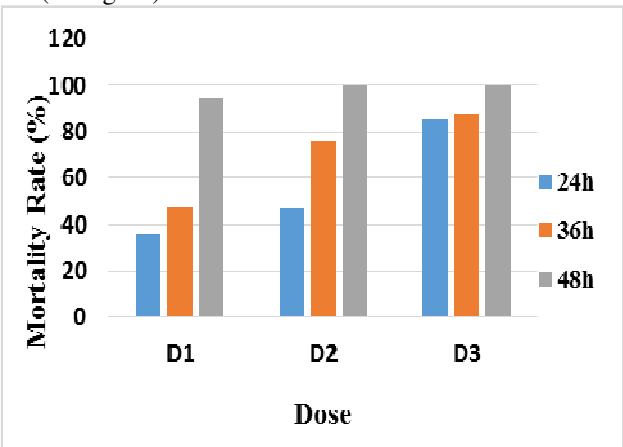


Fig.6. Mortality rate of Ectomyelois ceratoniae at several doses of CO_2 for 24 hours, 36 hours, and 48 hours treatment time (temperature= 35±1°C).

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Table 7: Mortality rate of various stages of Ephestia kuehniella after treatment tested at 35±1°C (average of 3 replicates) and different doses of CO2 (2,4kg/m^3, 2,8kg/m^3 and 3,2kg/m^3)

Treatment time (hours)	Stages of *Ephestia kuehniella*	Number of insects per stage				
		Replicates				
		R1	R2	R3	MR (%)	CMR (%)
4h30	Eggs	50	50	50	54	100
	YL	50	50	50	100	100
	OL	50	50	50	100	100
	N	50	50	50	42.66	100
	A	50	50	50	100	100
5h	Eggs	50	50	50	100	100
	YL	50	50	50	100	100
	OL	50	50	50	100	100
	N	50	50	50	76	100
	A	50	50	50	100	100
5h30	Eggs	50	50	50	100	100
	YL	50	50	50	100	100
	OL	50	50	50	100	100
	N	50	50	50	100	100
	A	50	50	50	100	100
6h	Eggs	50	50	50	74	100
	YL	50	50	50	100	100
	OL	50	50	50	100	100
	N	50	50	50	65.33	100
	A	50	50	50	100	100
6h30	Eggs	50	50	50	100	100
	YL	50	50	50	100	100
	OL	50	50	50	100	100
	N	50	50	50	86	100
	A	50	50	50	100	100
7h	Eggs	50	50	50	100	100
	YL	50	50	50	100	100
	OL	50	50	50	100	100
	N	50	50	50	100	100
	A	50	50	50	100	100

MR: Mortality Rate; CMR: Corrected Mortality Rate; Yl : Young larvae ; Ol : Old larvae ; N : Nymphs ; A : Adults

Table 8: Mortality rate of various stages of Ectomyelois certoniae after treatment tested at 35±1°C (average of 3 replicates) and different doses of CO_2 (2,4kg/m^3, 2,8kg/m^3 and 3,2kg/m^3)

Dose (kg/ m^3)	Treatment time (hours)	Replicates	Examined dates	Dead stages	MR (%)	CRM
2,4	24	R1	300	45	37,5	85,71
		R2	300	48	35,82	89,46
		R3	300	36	36	94,44
	36	R1	300	65	46,1	85,14
		R2	300	74	45,12	89,45
		R3	300	88	51,46	91,03
	48	R1	300	96	100	95,29
		R2	300	38	88,37	95,18
		R3	300	51	94,44	94,52
2,8	24	R1	300	84	44,44	100
		R2	300	99	53,8	88,24
		R3	300	65	43,05	100
	36	R1	300	87	88,77	100
		R2	300	54	65,06	100
		R3	300	78	73,58	100
	48	R1	300	121	100	100
		R2	300	132	100	98,33
		R3	300	112	100	100
3,2	24	R1	300	101	91,82	98,05
		R2	300	140	85,89	99,21
		R3	300	134	77,9	100
	36	R1	300	151	89,88	100
		R2	300	131	93,57	98,61
		R3	300	142	79,33	97,68
	48	R1	300	101	100	100
		R2	300	160	100	99,3
		R3	300	145	100	98,72

IV. Conclusion

Laboratory experiments of CO treatment proved that it could be used as a substitute for methyle bromide and mainly for organic dates rather than freezing. In fact, for 6 hours and 30 minutes exposure to 1624 kg/ m^3, complete mortality of all stages of *Ephestia kuehniella* as well as larvae and nymphs of *Ectomyelois ceratoniae* was achieved.

Treating postharvest dates with CO2 was found to be effective against all stages of *Ephestia kuehniella* and *Ectomyelois ceratoniae*. The final field results of CO_2 are: at a dose of 2800g/m3, treatment duration should be 48 hours to achieve 100% mortality rate of all stages of *E.*

kuehniella and *E. ceratoniae*. However, laboratory experiments showed that only 20 hours exposure to the highest dose (D3=8500g/m^3) was required to achieve complete mortality. Consequently, fields result can be improved if the airtightness of the fumigation chamber was more suitable for application of higher doses of CO_2.

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