International Journal of Agriculture Innovations and Research
Volume 3, Issue 6, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received : 17/04/2015 | Accepted on : 24/04/2015 | Published : 13/06/2015

Palm Dates Fumigation in Tunisia: Efficiency of Phosphine and CO_2 Mixtures, at Different Temperatures, as an Alternative to Methyl Bromide*

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Abstract – **Several methods have been used for insect post harvest of Date Palm fruits control. Fumigation with methyl bromide (Br CH_3) was the best way to control pests of stored dates in Tunisia and other countries, for its effectiveness against different stages of insect development.**

Due to its harmful effects on humans and/or on the environment, its use is now very limited and it is forbidden and removed since 2005 in the developing countries and in 2015 in under developing countries (Montreal Protocol, is an international agreement ratified by over 160 countries including Tunisia). The aim of this current work is to present a new more efficient and economical alternative that replaces methyl bromide. It is the use of a generator that combines the phosphine (PH_3) at 2% and carbon dioxide (CO_2) at 98%. For a dose of 3 g/m^3 of phosphine, we tested the efficacy of four different temperatures (35±1°C, 30 ± 1°C, 25±1°C and 20±1°C) for four different durations of treatment (8, 12, 16 and 24 hours) on mortality of several development stages of two species of Lepidoptera (*Ectomyelois ceratoniae* and *Ephestia kuehniella*) causing damages on date palm dates. As control, untreated samples (infested dates and different moth stages) were used.

The best result is observed at 35±1°C with of a processing time of 8 hours. When the temperature is 30±1°C the processing time is 12 hours, for a temperature of 25±1°C the processing time is 16 hours and if the temperature is reduced to 20±1°C , the processing time increased to 24 hours. Organoleptic analysis of treated and untreated dates showed that these treatments do not affect the quality of dates treated compared to those not treated.

Keywords – **Disinfestation, Dates, Methyl Bromide, Generator, Phosphine, Carbon Dioxide, Temperature, Treatment Duration.**

I. History Alternatives of Treatment with Methyl Bromide

Dates sector occupies a prominent place in the national economy. Indeed, a large amount is exported, hence the importance of going through the conditioning to keep a good quality of dates. The application of methyl bromide is then limited to the treatment of dates in the packing stations (against pests, particularly *Ectomyelois ceratoniae*).

The control of insects is the first step. Dates are appreciated by many insect species especially beetles and moths that affect the quality and quantity of dates on the field and in the packing stations.

Fumigation with methyl bromide (Br CH_3) was the best way to control pests of stored dates. In the 90s, it is considered the ideal treatment.

This fumigant gas is harmful to human health (carcinogenic) and to the environment (destruction of the ozone layer). Therefore, its use is now very limited and it is forbidden and removed compulsory since 2005 in the industrialized countries and by 2015 in developing countries (Montreal Protocol). An international agreement ratified by over 160 countries including Tunisia). Several alternatives to methyl bromide have been proposed to get products free of insects. The heat treatment is among the physical methods: Temperature is raised to a level that would destroy most insects attacking dates, two hours of exposure to a temperature ranging from 60°C to 70°C allow to eliminate all of these insects ([1]; [2] ; [3]).

In this regard, [2] reported that treatment with heat or cold (freezing) can disinfect dates against the worm *Ectomyelois ceratoniae*. However, these authors reported that this method can cause deterioration of the quality of processed fruits that become caramelized. [4] showed that the batch processing of dates in the microwave for 55-90 seconds allowed to reach temperatures above 52°C and resulted in total mortality of larvae of the moth dates. In addition, these researchers reported that the quality parameters of treated dates such as color and weight are not significantly different from those of untreated dates.

More recently, [5] reported that the hot air treatment at 65°C for about 40 min of artificially infested dates, increases the internal and external fruit temperature beyond 55°C and results in the total of dates disinfestation (100%) of the larvae, nymphs and eggs of the moth. Freezing has been used for many years in controlling populations of stored product insects. The majority of stored product pests die after exposure to a temperature of from -18°C to 12°C [3]. At a temperature of 4°C, the adults of many species can survive but immature stages are exterminated [6]. In Tunisia, it is the only method used in the treatment of organic dates. Similarly, it has been suggested lowering the temperature of the storage down to -18°C for 96 hours to control insects of stored dates [7]. However, this method is very costly for the industry and result in a significant alteration of the texture and color of

*This work has been partially sponsored by the Montreal Protocol multilateral fund (MLF) and the United Nations Industrial Development Organization (UNIDO)

dates [9]. Soaking in hot water especially for dry dates will kill all stages of insects developing at the expense of fruit.

Gamma radiation is used for food preservation successfully and without any apparent adverse effect for the treated products. Indeed, the cost of this method and the application of these radiation causes chemical changes and free radicals are formed within the treated fruit, which limits its use [2].

The use of micro-waves has the following advantages: no toxic residue after treatment and the destruction of all stages of insects and food stored in a time of limited exposure which is summarized in a few seconds. However, these waves may damage the stored products.

Treatment with carbon dioxide (CO_2) is a biological alternative. It is one of the techniques of both natural and effective for the control of insects of stored dates [10].

Insects are generally killed by CO_2 faster than by the lack of oxygen [11]. In Tunisia, it was used for the treatment of organic dates in some packing stations at a rate of 2.8kg / m^3 [2], but its use was not widespread due to the use of chemical fumigants with a high efficiency.

On the other hand, the combined use of phosphine and carbon dioxide at temperature (30°C) is an alternative fumigant used for disinfestation of warehouses in the USA. 100% of the deaths were obtained from several species of pest *Tribolium castaneum* including and following a 24 hours exposure [12]. Moreover, the combined treatment (heat / phosphine / CO_2) was considered a possible alternative to methyl bromide. Phosphine or hydrogen phosphide (PH_3) is a highly effective fumigant in tablets against all stored product pests, hence its use in several countries [11].

In an enclosed space, phosphine diffuses in all directions a homogeneous way, when it is more pure, odor is undetectable, hence the need to add a signal gas [7], [8]. It is emphasized that the main advantage of phosphine is the ease of its use and the speed of its insecticidal action. The phosphine fumigation is the method commonly used for the treatment of stored foodstuffs [12]. PH_3 is very toxic, even at low doses it is effective against pests. The dose and duration of fumigation vary with temperature and the target insect [14]. It should be noted that hydrogen phosphide does not affect the germination and quality of the commodity, even at relatively high doses. On the other hand, no or little residue are left on the commodity. An important drawback of the phosphine, however, is the long period of exposure required, 5 days or more [6] and its corrosive effect on the structure. This product is widely used mainly for fumigation of products for export during transit in boats and stored products [15].

Treatment with carbonyl sulfide (COS) is a naturally occurring gas in the air that can control all stages of development of stored product insects. Indeed, an increase in the dose or duration of exposure will have an adverse effect on other pests more resistant or difficult to control [7].

The sulfonyl-fluoride is a gas that is very promising for the replacement of CH_3Br. It provides quick control of stored product insects. Methyl isothiocyanate (MITC °) is used for a long time, it is known by its proper distribution of power. Within 24 hours under the effect of this will be enough to exterminate several species of stored product pests such as *Sitophilus* spp. and *Tribolium* spp.. Hydrocyanic acid (HCN) is a gas that is able to effectively control certain pests, leaving little residue. However, it is highly toxic to humans and react with foods with sugar concentration drills in addition it is highly soluble in water [7].

There are other methods: -IPM (Integrated Pest Management)

The concept of IPM is defined as a decision-making process to assess the populations of pests and bring the level below the economic threshold of tolerance of a satisfactory and economically while preserving the environment [9]. This significantly reduces the use of CH_3 Br.

-Treatment with oil of eucalyptus: Essential oils of the plants are part of the last few years the most explored pathways in control of pests.

Eucalyptus oil can be used as a perfect smoke tested against three species of Lepidoptera storage pests: *Ephestia kuehniella*, *Ephestia cautella* and *Ectomyelois ceratoniae*.

II. Introduction

The combined use of heat, CO_2 and phosphine is much more effective than the use of each of these methods. Heat and CO_2 activate breathing insects; such phenomenons allow a minimum of PH_3 and have a maximum efficiency. This method also minimizes the amount of toxic residues. The proportions used are 3 to 7% CO_2 with 80 to 100 ppm of phosphine at a temperature of 30-36°C for 24 to 36 hours [10]. Under these conditions one can save up to 100% mortality in several species of stored product. Without CO_2 and heat, the amount of PH_3 used to have the same results is 900 ppm. This dose causes a large corrosion of the instruments. The combination of heat, CO_2 and phosphine is much cheaper than fumigation by methyl bromide alone (20 to 50% less) [13].

The objective of this work is to determine a cost-effective alternative that replaces methyl bromide for the control of post-harvest insect dates while respecting the environment and human health. In our case, we will consider the use of a generator that produces phosphine (PH_3) 2% and carbon dioxide (CO_2) 98% for the fumigation of dates at different temperatures.

III. Materials and Methods

A- Biological material

This involved, first collection, in the palm trees heavily infested by the moth *dates Ectomyelois ceratoniae* dates infestation which the rate may exceed 50%. For the realization of the various treatments, samples of 3 kg of dates (severely attacked) in bulk are used. Because of the unavailability of different stages of *Ectomyelois ceratoniae* in these fruits, samples of eggs, young and old larvae, nymphs and adults of *Ephestia kuehniella* (reared in the laboratory) are also used to determine the effect of

this combination of gas on the various stages of insect development subservient to dates.

These tests were conducted in a fumigation room with a volume of 67 m^3 at various temperatures of 35±1°C, 30±1°C, 25±1°C and 20±1°C. We tested the effect of different doses of phosphine in samples of dates infested by *Ectomyelois ceratoniae* as well as living stages of *Ephestia kuehniella* for one day (24 hours) and then for 16 hours and 12 hours and 8 hours .

The temperature (°C) and relative humidity (%) inside the chamber are measured using a programmable thermo-hygrometer (Testo). Once programmed with appropriate software, this unit is installed in the room. The pressure was monitored by a pressure gauge.

A dose of 3 g/m3 is choosen and five tests for each temperature:

- Five trials at 35±1°C for a treatment period of 8 hours.
- Five trials at 30±1°C for a treatment period of 12 hours.
- Five trials at 25±1°C for a 16 hours treatment period.
- Five trials at 20±1°C for a 24 hours treatment period.

After treatment, the samples *Ephestia kuehniella* are placed in favorable conditions (temperature: 25±1°C, humidity: 60±5% and photoperiod 16/8) for the development of nymphs hatching, about a week. As for the samples infested dates, they will be examined to determine the effect of treatment on larval mortality rate of *Ectomyelois ceratoniae*.

B- Characteristics of PH3 generator

The fumigation process needs aluminum phosphide (tablets), and water 180 kg, 5 cylinders of carbon dioxide (Aligal 2) of 30 kg of each gas, and a power source of 220Volts.

The operation of the phosphine generator is controlled by a microcomputer inside the controller.

Firstly, the two tanks of the generator are filled with water to be heated to 45°C. The generator and the five resistors CO_2 cylinders are subsequently connected to the electric current. The operator must set the amount of phosphine to use before starting operation. The generator runs automatically until the amount of phosphine is discharged into the room. To ensure the proportion of 2% phosphine and 98% carbon dioxide, phosphine generation rate is set to 72 g/min and the maximum CO_2 supply capacity is 120 l/min per cylinder is needed and five cylinders so make 600 liters of CO_2 per minute to the specified speed. The phosphine concentration is controlled by two sensors, one measuring the high concentration of phosphine within the treatment room and the other detects the low phosphine concentration outside at the areas of leakage.

The measured parameters are:

- The fruit infestation percentage before and after treatment and determining the population structure of the insect during treatment.
- The percentage of mortality (or mortality corrected) of the various stages of the insect in the different treatments.
- Some sensory parameters processed dates.

C- Physicochemical Analyses of treated dates

1- Determination of water: samples of the dates are grinded twice minimizing time, taking care to expose them as little as possible to the air. Then we take a certain amount and we spread it over the bottom of a crystallizer to obtain good contact with the heating surface of the oven. After maintaining the sample in an oven at a temperature of 105°C for 24 hours and cooled box in a dessicator and reweigh it. The percentage of water is obtained by the total weight is the weight difference before and after drying multiplied by one hundred, divided by the initial weight of the sample.

2- Determination of total sugars: We take 5g pitted dates and cut into pieces and then the hot dissolved with distilled water in a beaker to obtain a syrupy substance is then added to the distilled water and transferred the contents into a 200 ml flask. After we defecate with lead acetate (5cc) allowing rests for 30 minutes and then made up to 200 ml with distilled water and filtered in 250 ml Erlenmeyer flask (solution S). For the determination of total sugars is removed from S in a 50ml flask and 100ml of 5 ml of HCl (3N) and then hydrolyzed in a water bath for 20 minutes at 60-70°C then allowed to cool and neutralized by the same volume of 5ml NaOH (3N) and complete up to 100ml with distilled water

Dosage: in the burette is placed the sugar solution in a 10ml beaker is placed Fehling A liqueur 10ml Fehling B, 10ml of distilled water, potassium ferrocyanide 6ml and dosage is hot (95°C).

IV. Results and Discussion

Initially, attempts were made ten doses (1 g/m^3 to 10 g/m^3) for a 24 hours period of treatment at 30±1°C in the fumigation room (67 m^3). The number of PH_3 tablets to be used for each dose and the necessary time for the tablets hydrolysis and release of PH_3 then mixed with CO_2 is given in the table below (Tables 1 and 2).

Table 1: PH_3 doses used for room 67 m^3

PH3 dose (g/m3)	ALP dose (g/m3)	Total ALP for room 67m3 (g)	Total ALP (pieces)	Number of tube of ALP	
				(tube)	(pieces)
1	3	201	67	2	7
2	6	402	134	4	14
3	9	603	201	6	21
4	12	804	268	8	28
5	15	1005	335	11	5
6	18	1206	402	13	12
7	21	1407	469	15	19
8	24	1608	536	17	26
9	27	1809	603	20	3
10	30	2010	670	22	10

International Journal of Agriculture Innovations and Research
Volume 3, Issue 6, ISSN (Online) 2319-1473

Table 2: Quantity and work time CO_2 for room 67 m^3

PH3 dose (g/m3)	CO2 quantity (L)	ALP work time (min)	CO2 work time (min)
1	36600	3	63
2	37200	6	66
3	37800	8	68
4	38400	11	71
5	39000	14	74
6	39600	17	77
7	40200	20	80
8	40800	22	82
9	41400	25	85
10	42000	28	88

All the results were satisfactory except the last dose which is the lowest (1 g/m^3) did not give a good result in the treated dates but total mortality was recorded for various stages of *Ephestia kuehniella* (Figure 1).

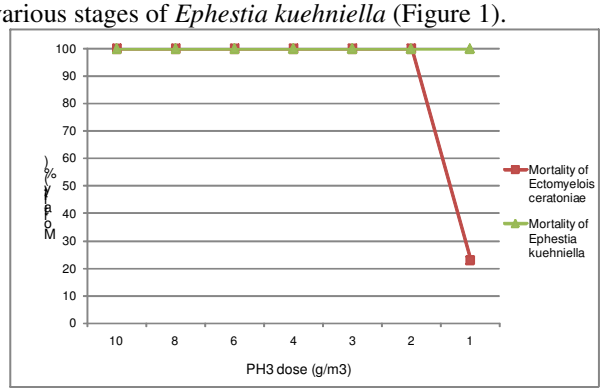

Fig.1. Larval mortality rate of *Ectomyelois ceratoniae* and different stages of *Ephestia kuehniella* according to different phosphine doses used for a 24 hours treatment time at 30±1°C.

Then we started decreasing the treatment duration to 16 hours. At a dose of 3 g/m^3 , it is observed a total mortality of the various stages of *Ectomyelois ceratoniae* and all stages of *Ephestia kuehniella*. The same effect happened for the 12-hours treatment period (Figure 2).

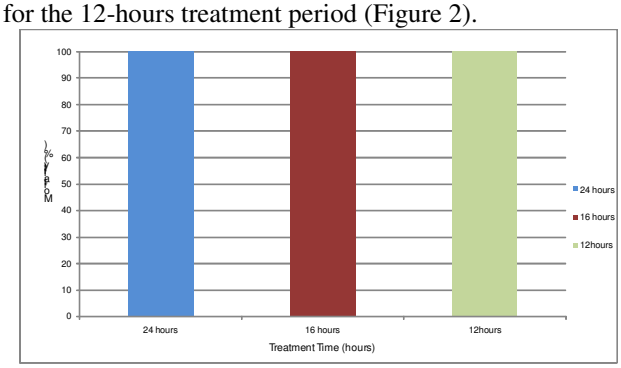

Fig.2. Mortality rate of larvae of fifth instar of *Ectomyelois ceratoniae* for the dose of 3 g/m^3 depending on the different durations of treatment at 30±1°C

The room is filled to 30% of infested dates, the air volume is therefore of 47m^3 (Table 3).

Table 3: PH_3 doses used for room 47 m^3

PH3 dose (g/m3)	PH3 dose (mg/m3)	Total PH3 for 47m3 (mg)	Room space (m3)	PH3 space concentration (PPM)	ALP (g/m3)	ALP for 47 m3 (g)	PH3 (pieces)	Number of tube of ALP		Generator work speed(72g/min)		
								(tube)	(pieces)	PH3 work time (min)	CO2 work time (min)	Need CO2 (L)
3	3000	141000	47	1920	9	423	141	4	21	6	66	39525

For all treatments (35±1°C, 30±1°C, 25±1°C and 20±1°C), there was an efficiency which translates to the total mortality of all stages of *Ephestia kuehniella* (Tables 4a and 4b).

Table 4a : Mortality rate of various stages of *Ephestia kuehniella* before treatment tested at 35±1°C, 30±1°C, 25±1°C and 20±1°C (average of 5 replicates)

			Controls (number of stages)					
			Replicates					
Temperature (°C)	Treatment time (hours)	Stages of *Ephestia Kuehniella*	T1	T2	T3	T4	T5	%LS
35±1	8h	Eggs	50	50	50	50	50	100%
		Yl	50	50	50	50	50	100%
		Ol	50	50	50	50	50	100%
		N	50	50	50	50	50	100%
		A	50	50	50	50	50	100%
30±1	12h	Eggs	50	50	50	50	50	100%
		Yl	50	50	50	50	50	100%
		Ol	50	50	50	50	50	100%
		N	50	50	50	50	50	100%
		A	50	50	50	50	50	100%
25±1	16h	Eggs	50	50	50	50	50	100%
		Yl	50	50	50	50	50	100%
		Ol	50	50	50	50	50	100%
		N	50	50	50	50	50	100%
		A	50	50	50	50	50	100%
20±1	24h	Eggs	50	50	50	50	50	100%
		Yl	50	50	50	50	50	100%
		Ol	50	50	50	50	50	100%
		N	50	50	50	50	50	100%
		A	50	50	50	50	50	100%

Yl : Young larvae ; Ol : Old larvae ; N : Nymphs ; A : Adults ; % LS : Living stages rate ;

Table 4b : Mortality rate of various stages of *Ephestia kuehniella* after treatment tested at 35±1°C, 30±1°C, 25±1°C and 20±1°C (average of 5 replicates)

			After treatment (number of stages)					
			Replicates					
Temperature (°C)	Treatment time (hours)	Stages of *Ephestia Kuehniella*	R1	R2	R3	R4	R5	%DS
35±1	8h	Eggs	50	50	50	50	50	100%
		Yl	50	50	50	50	50	100%
		Ol	50	50	50	50	50	100%
		N	50	50	50	50	50	100%
		A	50	50	50	50	50	100%
30±1	12h	Eggs	50	50	50	50	50	100%
		Yl	50	50	50	50	50	100%
		Ol	50	50	50	50	50	100%
		N	50	50	50	50	50	100%
		A	50	50	50	50	50	100%
25±1	16h	Eggs	50	50	50	50	50	100%
		Yl	50	50	50	50	50	100%
		Ol	50	50	50	50	50	100%
		N	50	50	50	50	50	100%
		A	50	50	50	50	50	100%
20±1	24h	Eggs	50	50	50	50	50	100%
		Yl	50	50	50	50	50	100%
		Ol	50	50	50	50	50	100%
		N	50	50	50	50	50	100%
		A	50	50	50	50	50	100%

Yl : Young larvae ; Ol : Old larvae ; N : Nymphs ; A : Adults ; % DS : Dead stages rate

The four treatments have generated a total mortality of larvae and nymphs *Ectomyelois ceratoniae* (Tables 5a and 5b).

Table 5a: Larval mortality rate and nymphs of *Ectomyelois ceratoniae* before treatment for testing at 35±1°C, 30±1°C, 25±1°C and 20±1°C (average of 5 replicates)

Temperature (°C)	Treatment time (hours)	Before treatment (control) Replicates	Number of dates examined	Number of living stages	Infestation rate
35±1	8h	T1	500	246	49,2
		T2	500	262	52,4
		T3	500	253	50,6
		T4	500	261	52,2
		T5	500	215	43
30±1	12h	T1	500	195	39
		T2	500	212	42,4
		T3	500	250	50
		T4	500	243	42,6
		T5	500	297	59,4
25±1	16h	T1	500	212	41,55
		T2	500	266	53,2
		T3	500	270	54
		T4	500	284	56,27
		T5	500	367	73,4
20±1	24h	T1	500	297	59,4
		T2	500	264	53
		T3	500	274	54,8
		T4	500	339	67,8
		T5	500	397	79,4

Table 5b: Larval mortality rate and nymphs of *Ectomyelois ceratoniae* after treatment for testing at 35±1°C, 30±1°C, 25±1°C and 20±1°C (average of 5 replicates)

Temperature (°C)	Treatment time (hours)	Post treatment Replicates	Number of dates examined	Number of dead stages	Corrected mortality rate (%)
35±1	8h	R1	500	259	100%
		R2	500	177	100%
		R3	500	249	100%
		R4	500	195	100%
		R5	500	219	100%
30±1	12h	R1	500	112	100%
		R2	500	210	100%
		R3	500	302	100%
		R4	500	379	100%
		R5	500	272	100%
25±1	16h	R1	500	222	100%
		R2	500	266	100%
		R3	500	260	100%
		R4	500	276	100%
		R5	500	302	100%
20±1	24h	R1	500	282	100%
		R2	500	227	100%
		R3	500	315	100%
		R4	500	315	100%
		R5	500	359	100%

Organoleptic analysis of Treated date palm fruits

At a dose of 3 g/m^3 for a 12 hours period of treatment at 30±1°C in the fumigation room (67 m^3). Five samples of treated dates and also of untreated dates are analyzed to determine quantity of sugar (g of sugar/100g of dates) and water content (% Humidity) (Table 6).

Table 6: Quantity of sugar (g of sugar/100g of dates) and water content (% Humidity) for treated and untreated dates at a dose of 3 g/m^3 for a 12 hours period of treatment at 30±1°C

Treated dates		**Untreated dates**	
Replicates	Humidity (%)	Replicates	Humidity (%)
1	17,95	1	19,28
2	18,43	2	19,61
3	20,4	3	19,73
4	19,37	4	19,62
5	20,44	5	19,85
Mean	**19,31**	**Mean**	**19,61**
Replicates	Quantity of sugar (g) /100g of dates	Replicates	Quantity of sugar (g) /100g of dates
1	26,28	1	25,02
2	25,2	2	26,42
3	25,28	3	25,8
4	27,97	4	27,54
5	23,17	5	25,33
Mean	**25,58**	**Mean**	**26,02**

Table 6 showed that there is no difference between treated and untreated dates for the quantity of sugar and the percentage of humidity.

According to statistical analysis by the khi 2 test (Statistical Software: SPSS 18), organoleptic analysis (quantity of sugar and % Humidity) of samples of treated and untreated dates did not show a significant difference in terms of quality. It is concluded that treatment with phosphine and carbon dioxide does not affect the quality of dates.

V. Conclusion

Following this work, it can be concluded that we have been able to determine the best dose of phosphine which is 3 g/m^3 and the proper treatment duration at each temperature. The final results are:

At a dose of 3 g/m^3, the combination of CO_2, Phosphine and temperature, fumigation of dates can be concluded:

- 35 ± 1 ° C treatment duration should be 8hours
- 30 ± 1 ° C treatment duration should be 12hours
- 25 ± 1 ° C treatment duration should be 16 hours
- 20 ± 1 ° C treatment duration should be 24 hours

These treatments have no effect on the quality of treated dates. The higher the temperature of the boiler water, the higher the release of phosphine is important and rapid and there will be less waste in the generator at the end of the operation. The user of this new alternative has to adapt its generator operating conditions according to the conditions of the fumigation chambers before it (heating, sealing ...).

Acknowledgments

Thanks to:

- Central of agricultural products packaging (CCPA) which facilitated the conduction of trials.
- L'Air Liquide to have provided 30 bottles of CO2 charges (Aligal2) during the beginning of trials.
- INAT: National Agronomic Institute of Tunisia for the laboratory entomology and some logistics.

- The IRESA: Institution for Research and Higher Education Agricultural: Assigning a project to the mass rearing of corn borer, date moth, these two species were used for the various tests.

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