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Implementation of nuclear technology, Inesfly paint and some integrated pest management elements for controlling *Ephestia* spp. in date palm orchards and date warehouses in Iraq

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Abstract
Ephestia spp., *E. cautella*, *E. figulilella* and *E. calidella* are the most serious pests infest dates caused economic losses in the orchards and warehouses in Iraq which produces between 600-700 thousand tons of dates annually.In order to reduce this damage different pest control methods were selected using environmentally sound technologies such as Radiation, Egg and larva parasitoids, pheromone and mating disruption and Inesfly paint. The results of radiation indicated that the killing dose of gamma rays to all stages of the three *Ephestia* spp. was 0.7 kGy (700 Gray). This dose caused complete sterility to all date moths found 1-2 days after irradiation in comparison with 87% in the control treatment. Furthermore, examination of storage dates after 30-180 days showed no live stages of all moths. Egg and larval parasitoids *Trichogramma evanescens* and *Bracon hebetor* either alone or with pheromone and Dismate PE reduced the percentages of infestation to 1.4 after six months of storage compared to 17.9% for the control in the orchard and warehouses. Inesfly 5A IGR NG paint caused highly mortality to *Ephestia* spp. Adults, larvae and the associated arthropods in the warehouses. Based on the trial results, using one or a combination of the tested measures ensure clean dates without implementing methyl bromide.

Keywords: *Ephestia*, stored dates, Iraq, Inesfly paint, radiation, parasitoids

1. Introduction
Date palm orchards are cultivated in central and southern of Iraq mainly in Karbala, Babylon, Basra, and Diyala provinces. The official census showed that the high date's production is in Babylon, Karbala, Diyala and Basrah [1]. The date's warehouses are infested with many stored product pests belong to Lepidoptera, Coleopteran and mites, however the *Ephestia cautella*, *E. figulilella*, and E. *calidella* are the most dominant problem facing dates trade in Iraq and worldwide [1]. The infestation of dates started in the orchards and transferred to the stored dates in the warehouses where the conditions are suitable for the insects offering food, shelter and reproduction [4, 11]. Methyl Bromide was the first choice to control stored dates due to its effectiveness killing all insect stages; however this fumigant proofed to be ozone depleting agents [18]. It was prevented since 2005 in developing countries and restricted in the beginning of 2015 in the under developing counties as documented by Montreal Protocol [19]. Therefore, the scientists are searching for new alternative technologies acceptable for date disinfestation. The micro-encapsulated water based vinyl paint that enables the gradual release of the active ingredients increasing the persistence of the compounds on different types of walls have been used extensively to control disease vectors in South America, Africa and Spain [8, 9] and against agricultural pests like red palm weevil [17] and as barrier against ants on citrus [16]. The objectives of this investigation are: 1-Testing the low-dose gamma radiation as effective method for insect disinfestations purpose.2- Releasing of egg and larval parasitoids in the date palm orchards and date warehouses.3-Evaluation of pheromone traps and mating disruption system Dismate PE for monitoring and control of *Ephestia* spp 4-Testing microencapsulated insecticide paint Inesfly 5A IGR NG emulsion on walls, and floors if needed as an alternative measure.

2. Materials and Methods
2.1Testing the low-dose gamma radiation
An approximate uniform distribution of a high infestation rate of all developmental stages of *Ephestia cautella* was obtained in 2014/2015 by placing 45 Kg of dry dates, Zahdi variety of

the latest harvest, in four Plexiglas cages and were infested by releasing ten pairs of *E. cautella* every ten days for three times. After forty-five days from the start of infestation, a sample of 5 Kg of dates was taken from the 4 cages at random for inspection. The laboratory examinations of the sample counted a total number of 3241 insects at different stages among them 84% were alive. Such a percentage of alive insects were taken as a measure of the effectiveness of this method of date disinfestations. This infested date fruits were transferred to 160 small carton boxes (CBs), known commercially as window carton lunch boxes each has 250 grams of dry dates [3]. Batches of 40 out of the 160 CBs were put into one standard carton box (SCB) each. The 4 SCBs were then treated as follows:

a) SCB No. 1 was stored at 25 ^{0}C and 40-60% relative humidity (Control 1)
b) SCB No.2 was stored at 40 ^{0}C for 48h (+2h for thermal distribution), then transferred to 25 ^{0}C (Control 2)
c) SCBs No. 3 and 4, each with date packages (40 CBs) have been treated with gamma radiation by placing each two CBs at a time in a gamma cell-220/irradiation chamber with a ^{60}Co source having a dose rate of approximately 17.4 kGy.h^{-1}. The dose administered for SCB No.3 was 0.35 kGy and for SCB No.4 was 0.7 kGy at the central field of the chamber with a ^{D} max/ ^{D} min of ca. 77/61= 1.26, which is acceptable with regard to date disinfestations. These SCB were then stored at 25 ºC.

Examination of 4 CBs from each SCB took place approximately 1, 15, 30, 45, 75, 105, 135, 165, 205 and 235 days after treatment. The number and percent of live insects per CBs were recorded to assess the killing effect caused by the radiation dose used.

Development and genetic test have been conducted for measuring the fertility of *E. cautella* adults that were collected from some CBs after 24h of post-treatment storage followed the procedure of [2]. Biochemical analyses of some samples of treated CBs packed dates have been studied to measure the changes in protein and amino acid contents using the procedure described by [9].

2.2 Releasing of egg and larval parasitoids in the date palm orchards and date warehouses.

Rearing of egg parasitoid *Trichogramma evanescens* and larval parasitoid *Bracon hebetor* were carried out following the procedures described by [12, 15]. The rearing procedure includes the host *E. cautella* on artificial diet composed of 81% crashed wheat, 12% glycerin, 6% date syrup and 1% dry yeast. Several pairs of *E. cautella* adults were released on the prepared artificial diet in specific cages leaving them to establish a colony of larvae. The last larval instars were collected in thousands and exposed to the adults of *B. hebetor* for either continue rearing the parasitoids or releasing them as adult parasitoids in the date warehouses and orchards. *Ephestia cautella* eggs were prepared in big numbers exposed to UV light to kill the embryos then exposed to *T. evanescens* adults for 48 hours. The parasitized eggs were incubated and after 6-7 days they are ready to be implemented in the date warehouses or in the orchards. All the biological and paint experiments have been conducted during 2015 and 2016.

Two orchards, located in Rashydia area near Baghdad each of 7 Iraqi donums (2500 seq. meter each) were selected for this investigation. In one of them, 15-18 thousands egg parasitoid *T. evanescens* were released / donum as pupae inside their host eggs two times in autumn and spring. Samples of 125-150 dates were collected randomly from each palm (50 date palm trees) from treated and non-treated orchards at the time of date harvesting beginning of November. The date fruit samples kept in polyethylene bags and transferred to the laboratory for inspection and counting the percentage of infestation with different *Ephestia* spp, larval instars and or feces alone or both

In order to evaluate the effectiveness of the combination of parasitoids, pheromone, and mating disruption technology in the date storages, two warehouses were prepared each of 4x10x6 meters at the site of the Iraqi Manufacturing and Marketing Dates Company in Baghdad. The first store serves as a control while the second was used for conducting the treatment. An amount of 10 tons of Khistawi date variety were stored in each warehouse using specific plastic boxes (each 50x30x25 cm) its capacity was.10-12 Kg. To identify which *Ephestia* species are dominant in the warehouse three pheromone traps of each species were hung in the treated warehouse while one pheromone trap for each species was hung in the control. Dismate PE mating disruption pheromone was also hung on the wall of the treated warehouse. The distance between Dismate unit was 1-1.25 meter and about 2.5 meters from the floor of the warehouse (about 1 meter over the top of the dates) [14]. During the first week from storing the dates in the treated warehouse 120000-140000 individuals of *Trichogramma evanescens* and 4 thousands individuals of *Bracon hebetor* were released, then a second release were repeated after two weeks in using 6000-7000 individuals of *T. evanescens* and 100 individuals of *B. hebetor* / ton of date [15]. The experiment continues for six months and samples of date were randomly taken from the treated and control warehouses, and examined in the laboratory to evaluate the efficacy of this bio-agents combination.

2.3 Efficacy of Inesfly 5A IGR NG paint to control *Ephestia* spp. and associated arthropods in the date warehouses.

Three small stores each of 4x3x3 meters at the site of the Iraqi Manufacturing and Marketing Dates Company were well prepared. One of them was painted with Inesfly 5A IGR NG which is a micro-encapsulated water based vinyl paint that enables the gradual release of the active ingredients (Alphacypermethrin, D-allethrin and Pyriproxyfen),and assures long lasting efficacy of the compounds on wall surfaces like blaster, concrete and mud. In the second store, egg and larval parasitoids were released in addition to three pheromone traps for each *Ephestia* spp. and Dismate PE as a disruption technique. The third store was painted with a conventional paint as a control. Ten tons of date, Zahdi variety from the last season was stored in each warehouse. The initial percentage of infestation in the three warehouses was between 4.5% - 6.5%.The average temperature at the beginning of the trial in December /2015 was 10±2 ^{0}C and relative humidity between 50-60%. A random sample of dates was taken from warehouses periodically examined carefully in the laboratory and the results were recorded. Pheromone traps were installed inside the store for *Ephestia* moth adults monitoring as an indicator for paint efficacy.

Another experiment was conducted in Tuwaitha area by choosing a store of 1.5x1.75x3 meter. It was painted by Inesfly 5A IGR NG in a dilution rate of 15% and left for 48 hours to dry. A number of 200 *Ephestia* moth adults and 200 larvae were released in the warehouse twice in an interval of one week apart however the third release was only larvae on dates stored for one week for further effectiveness counting. The number of dead adults and larvae were counted after 72 hours.

2-4 Statistical analysis
The data for alive insect stages percentage were subjected to one-way analysis of variance; differences between treatment means were determined by Duncan's multiple range test at the 5% level of probability. Where for effect of releasing parasitoids in controlling *Ephestia* spp. T test used to compare live and dead insect in each treatment. Data were analyzed using SAS program.

3. Results and Discussion
3.1 Disinfestation of date packages by gamma radiation
Table (1) shows the percentages of live insect stages detected in every storage period in 4 CBs, examined after exposure to low ^{60}Co gamma radiation doses (0.35 and 0.7 kGy). The effect of exposure at 40 ^{0}C for 48h was obvious, especially when CBs were examined directly 1 day after treatment, when the percentage of live insects was (14.8%) which is significantly lower than that resulting from any other two type of treatments (0.35 and 0.7 kGy). However, treatment only with heat (40 ^{0}C) did not cause sterility because the examination of all live insects that were fertile and gave rise to a quite high percentage of infestation after 105 day of storage. Treatment with 0.35 or 0.7 kGy resulted in a more effective disinfestations method and left no fertile insects starting from 105 days for 0.35 kGy and from 30 days for 0.7 kGy. These results are in agreement with previously mentioned results [2].

3.2 Development and genetic test
Table (2) shows that the exposures at 40 ^{0}C for 48h did not cause any level of genetic sterility in *E. cautella*. However, using 0.35 kGy induced a complete sterility in both sexes (2 females and 1 male). This is statistically significant reduction in the egg hatch to zero in spite of these three insect mated to the opposite sex. This is again in agreement with previously mentioned results [5, 6].

3.3 Biochemical analysis of packed dates after treatment
The present results of the biochemical analysis of some packed dates show that proteins are stable at the dose of 0.7 kGy (Table 3). This is in agreement with the previously mentioned results [3, 9]. Analysis of seventeen major amino acids showed that amino acid contents (Table 4), after exposing date packages to such combination treatment, are also not significantly different from the control. Such results confirm the already stated wholesomeness of irradiated dry dates [21, 9].

Table 1: Live insect stages % found in 4 CBs of artificially infested dates when treated with gamma radiation.

Treatments	Alive insect stages percentage found in treated date package after a period of times (in days) *									
	1	15	30	45	75	105	135	165	205	235
25 ^{0}C	69.2a	25.9a	31.7a	44.9a	77.2a	-	-	-	-	-
40 ^{0}C	14.8b	8.5 b	5.2 b	20.8b	17.1b	54.2a	-	-	-	-
0.35 kGy	87.1a	3.8 b	3.3 b	1.6 c	1.3 c	1.2 b	0.0	0.0	0.0	0.0
0.7 kGy	96.8a	5.9 b	0.0 b	0.0 c	0.0 c	0.0 b	0.0	0.0	0.0	0.0

***** Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range tests.
- Not examined due to high infestation rate

Table 2: Fertility tests of *E. cautella* adults developed from pupae or larvae found in date packages exposed to different types of treatment.

Type of treatment	Male or female x Opposite sex			
	No. of adult tested	No. of eggs Laid	Hatching %*	No. spermatophore per female
25 ^{0}C	20	2348	68.4 a	1
40 ^{0}C	1	80	55.0 a	1
0.35 kGy	3	289	0.0 b	1

* Means followed by the same letter in column are not significantly different at the 5% level using Duncan's multiple range tests.

Table 3: Protein content of dates (%), fresh weight, exposed to different treatment.

Dose (kGy)	25 ^{0}C	40 ^{0}C for 48h
0.0	1.57	1.31
0.7	1.66	1.58

Table 4: Total amino acid content of dates (mg/g fresh weight) exposed to different treatment.

Dose (kGy)	25 ^{0}C	40 ^{0}C for 48h
0.0	10.27	9.53
0.7	10.51	9.28

3-4 Effect of releasing biological agents on controlling *Ephestia* spp in date palm orchards
Table 5 represents the results of releasing the egg parasitoid *Trichogramma evanescens* in the field. It is clearly seen significant differences (P<0.05) in the percentage of infested dates collected directly from date palm trees of the treated orchard in comparison with that of the control orchard. The results of releasing *T. evanescens* reduce the percentage of infesting date collected directly from palm trees from 2.8 to 0.4. It is highly recommended to start implementing the IPM means while the dates are still on the trees choosing the optimum time of maturation to transfer the dates from the field to the warehouses.

3-5 Efficacy of releasing parasitoids to control *Ephestia* spp in date warehouses
The results represented in table 6 shows also significant differences (P<0.05) in the percentage of infested dates in the date warehouses treated with *T. evanescens* and *B. hebetor* in addition to pheromone traps and mating disruption PE compared to the control warehouse. These results agreed with other studies [20,11, 7, 12, 13] Furthermore, the results of the same table showed that the average number of *Ephestia* spp. in the treated warehouse were 0.7, 0.6 and 0.4 insect/ trap/ month in comparison with 10.7, 12.3 and 2.5 insect /trap/month for the control warehouse.

Table 5: Effect of releasing the egg parasitoid *T. evanescens* on the infestation with *Ephestia* moth in date palm orchard.

orchard	No. of date fruits inspected	infested %	non infested %	Remarks
Treated	6400	0.4	99.6	Infested either with different
Non-treated	6750	2.8	97.2	larval instar or larval feces alone or both

* T-test showed significant difference between infested dates (P<0.05) collected from treated and non treated orchard. Calculated T value = 3.666 and Tabulated T value= 3.182

Table 6: Effect of releasing parasitoids in controlling *Ephestia* spp. in date warehouses.

Date of inspection	% Infested dates *				Average No. of *Ephestia* spp. captured by pheromone trap **					
	Treated		Control		Treated ***			Control ***		
	Live	Dead	Live	Dead	1	2	3	1	2	3
Oct./2014	0.7	1.2	1.2	1.6	1.8	1.1	1.3	5.8	6.7	3.4
Nov./2014	0.4	1.1	1.6	6.2	1.3	1.4	1.1	9.6	10.8	2.5
Dec./2014	0.2	1.2	1.8	14.6	1.1	1.2	0.0	7.8	11.3	1.3
Jan./2015	0.0	1.3	2.1	19.5	0.0	0.0	0.0	6.8	13.4	2.3
Feb./2015	0.0	1.4	2.8	23.8	0.0	0.0	0.0	14.6	15.2	1.2
Mar./2015	0.0	1.3	3.2	29.1	0.0	0.0	0.0	19.6	16.1	19.6
%	0.2	1.2	2.1	15.8	/	/	/	/	/	/
Average	/	/	/	/	0.7	0.6	0.4	10.7	12.3	2.5

* T-test showed significant differences (P<0.5) between the average % infested dates in the treated date warehouse in comparison with control date warehouse. Calculated T value= 3.402, Tabulated T value= 2.441

** T-test showed significant differences (P<0.5) between the average Nos. of captured *Ephestia* spp. by pheromone traps in the treated warehouse in comparison with the control date warehouse. Calculated T value= 12.590, Tabulated T value= 2.441.

*** 1= *E. cautella*, 2 = *E. figulilella*, 3 = *E. calidella*

3.6 Efficacy of Inesfly 5A IGR paint

The results of using Inesfly 5A IGR NG paint have showed a very encouraging performance in reducing the number of *Ephestia* moth and all associated arthropods. It is not only having a slow release characters however it shows an extremely knockdown effect on the *Ephestia* moth adults and the arthropods entered either with the dates or accidentally to the warehouses. Table 7 shows there were no dead insects in the first three months December/2015, January and February/2016 where the temperature was 10±2 °C however when the temperature increased in March 28±2 °C the *Ephestia* moth population is also increased. As table 7 shows the number of the fallen dead larvae on the ground were 248 whereas the associated arthropods were 46 different stages. The collected arthropods were ants, flies, centipedes, millipedes, sow bugs and spiders. The number of dead larvae in the control warehouse was only 8 while the rest are moving on the store walls searching for a corners to pupate. In the warehouse where the parasitoids, pheromones and Dismate PE were used the number of dead larvae were only 3 whereas the rest are either dead due to the parasitoids or cached by the traps as adults. Furthermore, the result of the third warehouse in which Inesfly 5A IGR NG paint was used agreed with the investigations done on vectors, cockroaches and other pests [8, 10]. These trials have been conducted for the first time on such microencapsulated insecticide in a formulation of paint to control stored product insects, and it can stay for long time on the wall performing high mortality to larvae and adults.

The second trial performed in Tuwaitha area around 40 Km far away from the first warehouse, 200 adults and the same numbers of larvae were released in a painted store and readings were taken after 48 hours. The results were unexpected that the mortality of both stages was 100%.The same results gained when the trial has been repeated for the third time after two weeks of the first trial.

Table 7: Efficacy of applying Inesfly 5A IGR NG paint and parasitoids in controlling *Ephestia* spp in date warehouses (Average of four inspection/ month).

Date of Inspection	Type of Treatment	% Infested dates with alive, dead insect stages or feces*	No. of dead insect stages of *Ephestia* spp. on the floor **	Average No. of *Ephestia* spp. captured by pheromone trap/ month ***, ****		
				1	2	3
Dec./2015	Control	6.9 (0.7)	/	/	/	/
	Inesfly 5A IGR	8.7 (0.5)	/	/	/	/
	Biological control	7.2 (0.2)	/	/	/	/
Jan./ 2016	Control	8.9 (1.4)	/	/	/	/
	Inesfly 5A IGR	9.6 (0.3)	/	/	/	/
	Biological control	7.8 (0.2)	/	/	/	/
Feb./ 2016	Control	10.9 (2.3)	/	2	1	/
	Inesfly 5A IGR	9.6 (0.4)	/	/	/	/
	Biological control	7.7 (0.2)	/	/	/	/
March 2016	Control	18.1 (6.2)	8	5	6	3
	Inesfly 5A IGR	9.0 (0.4)	294 (46)	/	/	/
	Biological control	8.7 (0.1)	3	/	/	/
April 2016	Control	32.1 (8.7)	/	16	5	4
	Inesfly 5A IGR	9.4 (0.3)	/	1	/	/
	Biological control	8.6 (0.1)	/	3.1	2.4	2.1

* Numbers between parentheses represent the alive larval instars of *Ephestia* spp.

** Numbers between parentheses represent the number of other dead Arthropods.

/ = zero capture of *Ephestia* spp. or zero number of dead stages (larvae/adult *Ephestia* spp.)* 1=*E. cautella*; 2=*E. figulilella*, 3=*E. calidella*

In conclusion, a combination of the four methods, paint, parasitoids, pheromone traps and Dismate PE and the radiation achieved complete reduction of dates moth and associated arthropods in warehouses. In case of date production and processing factories, the owner can implement the technology of radiation and paint after manufacturing and packaging's dates or releasing egg and larva parasitoids, pheromone and Dismate PE and Paint before preparing dates for manufacturing and packaging for export instead Methyl Bromide. Giving high attention to the sanitation of dates on the trees and in warehouses assures low pests infestation and high quality of products.

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