

Irradiation as a phytosanitary treatment against *Trogoderma granarium* (Coleoptera: Dermestidae)

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Abstract

A study was conducted to determine a gamma irradiation dose that would serve as a phytosanitary treatment against the khapra beetle (*Trogoderma granarium* Everts; Coleoptera: Dermestidae). The results show that 100 Gy applied to the adult—the most tolerant stage—caused complete sterility in treated females crossed to either irradiated or normal males and reduced their fecundity by 48%. Eggs produced by normal females crossed to irradiated males exposed to the same dose (100 Gy), however, were more viable; egg hatch was 32%, and 3% of 1st instars reached the adult stage, but all failed to reproduce. In a subsequent confirmatory trial in which an estimated 147,800 insects of a mixed population of khapra beetle (69.5% adults, 22% pupae and 8.5% larvae) were irradiated with 100 Gy, no progeny were produced. This result exceeds the conventional quarantine security of probit 9 at the 95% confidence level. Our studies indicate that ionizing radiation could be used as an alternative to chemical fumigation for a phytosanitary treatment of commodities potentially infested with the khapra beetle and the required dose (100 Gy) is relatively small.

Key Words: khapra beetle; gamma irradiation; phytosanitary treatment; sterility; stored products

Resumen

Se realizó un estudio para investigar los efectos de la irradiación gamma en adultos del escarabajo khapra (*Trogoderma granarium* Everts; Coleoptera: Dermestidae). Los resultados muestran que la dosis de 100 Gy aplicada a los adultos—el estadio más tolerante—ocasionó esterilidad completa en hembras tratadas y posteriormente apareadas con machos irradiados o normales, y su fecundidad se redujo en un 48%. Los huevos producidos por hembras normales cruzadas con machos irradiados expuestos a la misma dosis (100 Gy), sin embargo, fueron más viables; la eclosión de los huevos fue de un 32% y el 3% de las larvas del primero estadio llegaron al estadio adulto, pero no todas se reprodujeron. En un ensayo de confirmación posterior en el que se estimó que 147,800 insectos de una población mixta de escarabajo khapra (69.5% adultos, 22% pupas y 8.5% larvas) se irradiaron con 100 Gy, no se produjo progenie. Este resultado supera la seguridad cuarentenaria convencional del probit 9 en el nivel de confianza del 95%. Nuestros estudios indican que la radiación ionizante se podría utilizar como una alternativa a la fumigación química para el tratamiento de cuarentena de los productos potencialmente infestados con el escarabajo khapra y la dosis requerida (100 Gy) es relativamente pequeña.

Palabras Clave: escarabajo khapra; irradiación gamma; tratamiento fitosanitario; esterilidad; productos almacenados

The khapra beetle (*Trogoderma granarium* Everts; Coleoptera: Dermestidae) is a very destructive pest of stored agricultural products (Stibick 2007). The pest is also of quarantine importance in many parts of the world and strict quarantine measures are imposed against agricultural commodities imported from infested countries (Phillips & Throne 2010; Hallman 2013). This pest species can be transferred from one country to another as eggs, larvae, pupae, and adults—adults do not fly. Consequently, for any quarantine treatment against this pest to be accepted commercially, it should be effective against any stage present in the shipped commodity.

The khapra beetle occurs in Syria and the prevailing climatic conditions in the country are conducive to serious outbreaks of this pest (Ghanem & Shamma 2007). Phosphine, a very toxic fumigant, can be effective against this pest; however, it requires a long exposure time. In addition, it loses activity at low temperatures and resistance to phosphine has been reported in this species (Borah & Schalal 1979; Hole 1981; Bell et al. 1983). Methyl bromide (MB) fumigant is highly effective against this insect and has been used as a phytosanitary treatment in exported commodities. With the phasing out of MB due to environmental concerns (Heather & Hallman 2008; Besri 2010), an urgent solution is very much needed.

Research has demonstrated that irradiation is an effective treatment against pests of stored products (Tilton & Burditt 1983; Phillips 2006; Hallman 2013), and radiotolerance of insects generally increases as they proceed through successive developmental stages (Hallman et al. 2010). Several studies on the effects of gamma radiation on different developmental stages—including the adult stage—of the khapra beetle have been done (Carney 1959; Huque 1963; Nair & Rahalkar 1963; Ahmed 1975; Younes et al. 1980; Ahmed & Iqbal 1985; Mehta & Sethi 1986; Szlendak & Davis 1989; Abdel-Kawy 1999; Gao et al. 2004). Gao et al. (2004) found that 200 Gy prevented reproduction in khapra beetle adults; a lower dose was not attempted. Szlendak & Davis (1989) found that < 22 Gy prevented reproduction in khapra beetle females. Carney (1959) reported that 50 Gy was sufficient to sterilize females, while males required about 3 times that dose to produce the same effect. Furthermore, Nair & Rahalkar (1963) reported that, while 60 Gy was required to sterilize females, sterilizing males required 160 Gy. In addition, it should be pointed out that these studies were carried for the purpose of stored product pest control, not quarantine. Therefore, the results do not meet the required phytosanitary standards of a very high level of efficacy (Heather & Hallman 2008). Consequently,

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more research is needed to determine the dose of ionizing radiation that can be adopted as a phytosanitary treatment against this pest.

The objectives of this study were to provide data on the effects of gamma radiation on khapra beetle adults and determine the required dose for treatment based on probit 9 (99.99683% efficacy) security standards.

Materials and Methods

REARING

Khapra beetle larvae were obtained from infested grains taken from a grain storage facility in Damascus in the summer of 2009. The larvae were reared on cracked wheat in polyethylene jars covered with a fine mesh inside a climatic chamber. Cultures were maintained at $35 \pm 1^\circ\text{C}$, $40 \pm 5\%$ RH and total darkness. Under these conditions, eggs hatched after 3 d, the larval stage required ~20 d, and pupation to adult emergence required an additional 5 d or more.

To obtain virgin adults for irradiation, newly formed male and female pupae (pupae formed over a 12-14 h. period) were isolated and carefully transferred into separate 9 cm Petri dishes. The dishes were incubated under the same conditions mentioned before ($35 \pm 1^\circ\text{C}$, $40 \pm 5\%$ RH and total darkness) for 6 days after which the emerged adults were irradiated at the appropriate dose.

IRRADIATION

Two Co-60 gamma radiation sources were used in this study and 2 kinds of tests (small scale laboratory tests and large scale confirmatory ones) were conducted. In the small scale laboratory tests, Petri dishes containing 1-2 d old virgin *T. granarium* adults were exposed to gamma radiation dosages in a gamma cell supplied with a Co-60 source around the cylindrical (15 × 25 cm) irradiation chamber (Issledovatel Gamma Irradiator, Techsnabexport Co. Ltd. Moscow, Russia). The average dose rate at the time of irradiation was about 8.7 Gy/min with a dose uniformity ratio (the ratio of the max. to the min. absorbed dose) of about 1.14. At each dose level, 5 Petri dishes each with 10 *T. granarium* adults (either males or females), were irradiated simultaneously ($n = 5$). The Petri dishes were placed at the center of the irradiation chamber and radiation dosages ranged between 50 and 200 Gy at 50 Gy increments. Large scale confirmatory tests were irradiated using a pilot scale gamma irradiation facility. The dose rate of the irradiation facility at the time of irradiation was approximately 23 Gy/min, the dose uniformity ratio, 1.11, and the absorbed dose were calibrated (in both cases) with the Fricke solution.

EFFECTS OF GAMMA RADIATION ON FERTILITY AND FECUNDITY

Irradiated (I) virgin adults, either males (M) or females (F), 10 of each sex in each replicate, were transferred into new clean Petri dishes, crossed with normal (N) or non-irradiated insects of the opposite sex (NF × IM and IF × NM) and allowed to lay eggs for 72 h after which the adults were discarded. In addition, the corresponding crosses were made involving irradiated males (IM) and irradiated females (IF). Each dish, after the removal of the adults, was provided with ~1 g of cracked wheat at the periphery of the dish to feed the neonate larvae, and then this preparation was incubated under the same previously mentioned conditions. After 5 d of incubation, the dishes were examined under a binocular microscope and the numbers of deposited and hatched eggs were recorded.

EFFECTS OF GAMMA IRRADIATION ON SURVIVAL TO THE ADULT STAGE

Dishes with hatched eggs were each provided with ~40 g of cracked wheat, and the dishes were incubated under the same rearing con-

ditions as the colony and examined daily. After 3 wk these cultures were examined for pupation and adult emergence, and the numbers of formed pupae and emerged adults were recorded. Percentage pupation and percentage adult emergence were calculated from these data.

LARGE SCALE CONFIRMATORY TESTS

In order to demonstrate probit 9 (99.99683%) mortality at the 95% confidence level subsequent confirmatory trials were carried out. In these trials, estimated numbers of 102,700 adults, 32,500 pupae, 12,600 larvae, and an unknown number of eggs were exposed in their rearing medium (cracked wheat) to a target dose of 100 Gy (92.9–103.45 Gy measured doses). The insects were irradiated in polyethylene jars (17 cm high × 12 cm in diam) half filled with cracked wheat covered with a fine mesh and a batch of 100 insects (50 males and 50 females) was held as a control. Following irradiation, the jars containing the insects were incubated at $35 \pm 1^\circ\text{C}$, $40 \pm 5\%$ RH in total darkness. The jars were examined for live insects at 2-wk intervals.

DATA ANALYSIS

Data from experiments on the effects of gamma irradiation on egg hatch, pupation, and adult emergence were subjected to analysis of variance. Means were separated at the 5% level of probability by Fisher's protected least significant difference (PLSD) test. In addition, female fecundity data from the 3 different crosses were subjected to regression analysis. Furthermore, percentage pupation and adult emergence data from crosses between irradiated males and normal females were subjected to probit analysis. Probit analysis, including probit transformation of percentage mortality and log 10 transformation of dose, was performed using SPSS (2008).

Results

Data on the effects of radiation on fecundity of *T. granarium* irradiated females, normal females crossed to irradiated males, or irradiated females crossed to irradiated males are presented in Table 1. The results clearly show that irradiation negatively and significantly ($P < 0.05$) affected the fecundity of the irradiated females and this effect was more severe when irradiated females were crossed to irradiated males. For instance, while fecundity of females exposed to 100 Gy and crossed to normal males was reduced to about 68% of the fecundity of control females, this percentage decreased to about 52% when both males and females were exposed to the same dose. This effect was also observed, but to lesser extent, when irradiated males were crossed to normal females; normal females crossed to irradiated males exposed to 200 Gy produced about 86% of the eggs compared with the control. Regression analysis of the data (Table 1) shows a very strong negative relationship between irradiation dose and fecundity ($R^2 > 0.94$). The analysis also shows that the estimated doses that would cause complete cessation of egg laying (zero fecundity) for crosses of irradiated males with normal females, normal males with irradiated females and irradiated males with irradiated females would be 1,381, 336 and 284 Gy, respectively.

Table 2 presents data on egg hatch and survival to the pupal and adult stages of F_1 generation insects produced from crosses between normal females and irradiated males. Results show that male fertility decreased significantly with increasing irradiation dose ($F = 1080.2$; $df = 4$; $P < 0.0001$); 50 Gy significantly reduced egg hatch and 200 Gy completely prevented it. When considering either F_1 pupation or adult emergence as a criterion for measuring efficacy, however, irradiation had more severe effects on these 2 processes than on fertility ($F =$

Table 1. Effects of gamma irradiation on fecundity of 1–2 d-old *Trogoderma granarium* adult females.

Dose (Gy)	No. of eggs/female (mean \pm SD) ^a		
	NF \times IM	IF \times NM	IF \times IM
0	37.0 \pm 4.1a	37.0 \pm 4.1a	37.0 \pm 4.1a
50	36.6 \pm 2.8ab	33.3 \pm 2.9b	26.1 \pm 3.5b
100	35.5 \pm 1.7ab	25.3 \pm 1.6c	19.3 \pm 1.6c
150	32.9 \pm 3.2bc	19.0 \pm 1.7d	16.6 \pm 1.5c
200	31.8 \pm 1.9c	16.4 \pm 1.7d	11.6 \pm 2.0d
Linear regression			
Slope \pm SE	-0.027 \pm 0.004	-0.11 \pm 0.010	-0.12 \pm 0.018
R ²	0.95	0.96	0.94
Predicted dose for 0% fecundity (Gy)	1381	336	284

^a I = irradiated, N = non-irradiated, M = male, F = female; means in a column followed by different letters are significantly different ($P \leq 0.05$; ANOVA and LSD test).

768.2 and 1327.4 for pupation and adult emergence, respectively; $df = 4$; $P < 0.0001$), i.e., complete prevention of pupation and adult eclosion were achieved with 150 Gy, while complete sterility required 200 Gy. Thus no pupae developed at 150 Gy while adult emergence was reduced to 3.2% at 100 Gy and all emerged adults (52 in total) failed to reproduce. The results of the probit analysis show that the estimated LD₅₀ values for preventing development to pupae or to adults, or to prevent egg hatch were 156, 118, and 199 Gy, respectively.

The effects of gamma irradiation on *T. granarium* female reproduction (Table 3) was more severe ($F = 4227.6$; $df = 4$; $P < 0.0001$) than on males. When irradiated females were crossed to normal males, 50 Gy dose reduced egg hatch to ~42%, but at 100 Gy no egg hatch was observed.

Table 4 presents data on the effects of gamma irradiation on *T. granarium* adult reproductive potential when both males and females were irradiated. The data clearly show that when both sexes were irradiated the effects of irradiation on fertility was much more severe ($F = 1951.5$; $df = 4$; $P < 0.0001$); 50 Gy significantly reduced egg hatch to ~32%, and at 100 Gy no egg hatch was observed.

In a large scale confirmatory trial where a mixed population of close to 148,000 khapra beetles in different stages of development (69.5% adults, 22% pupae and 8.5% larvae) were exposed to a dose of 100 Gy and given the chance to reproduce on a suitable substrate (cracked wheat), not a single living insect was observed 45 d after irradiation. At the same time 1,986 larvae, pupae, and adults were collected from the control.

Table 2. Probit analysis of the effects of gamma irradiation on percent egg hatch, percent pupation and percent adult eclosion of F₁ generation progeny of *Trogoderma granarium* obtained from crosses between normal females and irradiated males.

Dose (Gy)	Egg hatch (%)	Pupation (%)	Emergence (%)
0	91.9 \pm 2.9a	90.1 \pm 3.1a	89.0 \pm 3.3a
50	65.0 \pm 4.2b	53.8 \pm 5.0b	41.7 \pm 4.1b
100	20.2 \pm 3.5c	11.2 \pm 4.1c	3.2 \pm 1.1c
150	3.9 \pm 1.1d	0d	0d
200	0e	0d	0d
Probit analysis			
df	18	13	13
χ^2	16.4	23.4	5.8
Slope \pm SE	4.59 \pm 0.19	4.97 \pm 0.26	5.67 \pm 0.38
ED ₅₀ (95% fiducial limits)	199.5 (184.4–218.6)	156.0 (137.7–183.8)	118.4 (108.3–132.7)

Means in a column followed by different lowercase letters are significantly different ($P \leq 0.05$; ANOVA and LSD test).

Table 3. Effects of gamma irradiation on percent egg hatch, percent pupation and percent adult eclosion of F₁ generation progeny of *Trogoderma granarium* obtained in from crosses between irradiated females and normal (non-irradiated) males.

Dose (Gy)	Egg hatch (%)	Pupation (%)	Emergence (%)
0	91.9 \pm 2.9a	90.1 \pm 3.1a	89.0 \pm 3.3a
50	41.9 \pm 1.2b	28.9 \pm 3.7b	17.5 \pm 0.8b
100	0c	0c	0c
150	0c	0c	0c
200	0c	0c	0c

Means in a column followed by different lowercase letters are significantly different ($P \leq 0.05$; ANOVA and LSD test).

Discussion

Phytosanitary treatments are used to disinfest agricultural products of quarantine pests so that such products can be shipped out of quarantined areas. Among other techniques, ionizing radiation is being used increasingly as a treatment to overcome biological barriers to trade in agricultural products (Hallman 2011). This technique poses several advantages over other treatments including the applicability to packaged products, high tolerance by treated commodities, avoidance of the induction of resistance in pests and preservation of the nutritive contents of the treated product (Heather & Hallman 2008). In this report, the effects of gamma radiation on *T. granarium* adult fecundity, fertility, and reproductive potential were examined. In addition, the required irradiation dose for phytosanitary treatment against this pest was identified.

In practice, when shipping commodities potentially infested with *T. granarium*, the shipment may contain eggs, larvae, pupae, and adults. Because the radiotolerance of an insect species increases with each

Table 4. Effects of gamma irradiation on percent egg hatch, percent pupation and percent adult eclosion of F₁ generation progeny of *Trogoderma granarium* obtained in from crosses between irradiated males and irradiated females.

Dose (Gy)	Egg hatch (%)	Pupation (%)	Emergence (%)
0	91.9 \pm 2.9a	90.1 \pm 3.1a	89.0 \pm 3.3a
50	32.2 \pm 3.5b	26.0 \pm 3.1b	15.0 \pm 2.2b
100	0c	0c	0c
150	0c	0c	0c
200	0c	0c	0c

Means in a column followed by different lowercase letters are significantly different ($P \leq 0.05$; ANOVA and LSD test).

succeeding stage of develop (Hallman et al. 2010), adult insects are the most radiotolerant stage. Therefore, any irradiation treatment proven to be effective against the adult stage should be effective against any other stage.

The results of this study show that the sensitivity of 1–2 day old *T. granarium* adults to irradiation increased with increasing dose. Females were more susceptible to irradiation than males, as is often the case (Hallman 2000, 2001). Sex dependent irradiation sensitivity was also reported for 2 other species from the same genus, *T. inclusum* (LeConte) and *T. variabile* (Ballion) (Brower & Tilton 1972; Tilton et al. 1966). Cole et al. (1959) suggested that the sterilizing dose was negatively correlated with the size of the insect body; the larger the body size the smaller the sterilizing dose. This may explain the differences in sensitivity observed in this study because khapra beetle females are much larger than males and, perhaps consequently, they require a lower dose to be sterilized. Hallman (1998) offers 2 other hypotheses why females may be more radiosensitive regarding reproduction than males: (1) the egg is more complex than the sperm, thus offering more possibilities for radiation-induced damage, and (2) males contain many more gametes than females; i.e., 99.9% sterility of males leaves many gametes viable while 99.9% sterility of females may leave no viable eggs.

When survival to the adult stage was used as a criterion for measuring efficacy, the effects of irradiation were more severe. Less than 3% of the eggs that resulted from crosses between males exposed to 100 Gy and normal females were able to reach the adult stage and none of these were able to reproduce. Brower & Tilton (1972) working on 2 species from the same genus (*T. inclusum* LeConte and *T. variabile* Ballion) found that completely sterilizing males in both species required > 200 Gy dose. These results indicate that khapra beetle males are similarly sensitive or even slightly more sensitive to gamma irradiation than the males in these 2 closely related species.

Irradiation also negatively affected female fecundity and this effect was most severe when irradiated females were crossed to irradiated males. For instance, while egg production by normal females crossed to males exposed to 200 Gy was ~86% of the control, irradiated females crossed to normal males exposed to the same dose produced only about 44% of the eggs produced by the control and this decreased to less than 32% when both sexes were exposed to the same dose.

When irradiating commodities potentially infested with *T. granarium* for the purpose of phytosanitary treatment, both sexes will be irradiated. According to this study, 100 Gy dose caused completely prevented of egg hatch and, consequently, pupation and adult emergence when both sexes were irradiated. This indicates that 100 Gy is sufficient to prevent reproduction in this species.

Security levels for insect phytosanitary treatments are sometimes based on probit 9 (99.99683%) control. Although this security level was first suggested for fruit flies (Baker 1939), it was later adopted for other insects of quarantine importance (Burditt 1982). At this level of control, not more than 32 insects are expected to emerge as adults for each one million treated individuals. Couey & Chew (1986) showed that demonstration of probit 9 security at the 95% confidence level (CL) requires cumulative tests on 93,613 insects without survivors. However, the total number of insects to be treated without any survivors is determined by the level of security desired by the importing country. For instance, no survivor from a treated population of ~30,000 individuals (99.99% efficacy at the 95% CL) is considered adequate by some countries (Heather & Hallman 2008). Exposing a mixed population of > 102,700 khapra beetle adults, > 32,500 pupae, and > 12,500 larvae to a dose of 100 Gy did not result in any reproduction. Consequently, a dose of 100 Gy applied to commodities potentially infested with any stage of the khapra beetle will achieve a probit 9 level of quarantine security.

In summary, results of this study indicate that the use of ionizing radiation as a quarantine treatment for *T. granarium* infested commodities is feasible and requires a relatively low dose (100 Gy). In such a treatment, prevention of reproduction should be used as a criterion of effectiveness. This dose is lower than the suggested generic phytosanitary irradiation dose for stored product pests and much lower than the maximum allowed dose for irradiation of stored products, which is 1 k Gy for most countries that permit it (Hallman 2013).

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