Gamma irradiation used on adult *Tetranychus urticae* Koch as a quarantine treatment

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Abstract: The effect of gamma radiation with 0, 200, 250, 300, 350, 400, and 450 Gy intensities on the longevity, total number of eggs, and the percent of hatched eggs laid by irradiated females of *Tetranychus urticae* Koch (Tetranychidae) was evaluated. Two different groups (0–24 h old and 48–72 h old) of adult females were irradiated. The results showed that 350 and 300 Gy doses significantly reduced the longevity of the 0–24 h old females and the 48–72 h old females. The younger females were more tolerant at lower dose rates than the older females. There was a quadratic relationship between dose rates and young females, while it was linear in older females. The total number of eggs laid by females of both ages was significantly reduced with a linear trend by 250 Gy irradiation. The eggs laid by females of both the 0–24 h olds and the 48–72 h olds lost their hatchability when the dose rate was 350 Gy. It was concluded, that applying a dose rate of 320 Gy on one of the mates (male or female) before mating, or a 300 Gy on both of them, would be sufficient to cause sterility in adult mites.

Key words: adult, gamma ray, irradiation, quarantine, sterility, *Tetranychus urticae*

Introduction

Throughout the world, the trade of agricultural products is growing at a rapid rate and this increases the risk of transferring exotic plant pests to new areas. Such transfers can have enormous costs due to crop damage, control programs, and quarantine restrictions (Follett and Neven 2006).

The two spotted spider mite, *Tetranychus urticae* Koch, is one of the most damaging polyphagous pests of various important agricultural crops and their products. This mite was reported on more than 1,000 hosts in more than 100 plant families (Migeon and Dorkeld 2012). It is also one of the most damaging pests in forests, grasslands, and greenhouses. Almost all the broad-leaved plants are susceptible to this mite (Mayer 1987; Migeon and Dorkeld 2012) Iran, because of its diverse and suitable climate, produces and exports cut flowers and ornamentals which are most favorable hosts of the two spotted spider mite. This mite could cause considerable damage on the cut flowers and ornamentals. Pesticides such as methyl bromide and ethylene dibromide are generally used against quarantine pests. Pesticides have toxic residues and ethylene dibromide which may have possible carcinogenic effects. For this reason, application of most pesticides is prohibited. Methyl bromide has a depleting effect on the ozone layer and will be limited by 2015 based on international agreements (Montreal protocol) (Hallman 1998; Sulaiman *et al*. 2004). Alternative disinfestation methods have been studied worldwide to find a more environmentally friendly substitute for quarantines purposes. Irradiation has been under consideration in recent decades as a quarantine treatment for many agricultural products, such as cut flowers, ornamentals, fruits, and storage products. Irradiation has desirable characteristics, such as a short treatment time, lack of undesirable residues, environmental compatibility, applicability to packaged commodities, and a lower economical cost that make it a desirable treatment for fresh products (Sulaiman *et al*. 2004; Follett and Grifin 2006; Hallman *et al*. 2010). Irradiation differs from other commercial treatments in that the end point of the treatment is not an acute mortality but prevention of further biological development and reproduction (Hallman 2011). The studies on cut flowers and ornamentals like chrysanthemums, carnations, gladiolus, lilium and some tropical flowers have shown that these products have a good resistance to irradiation of up to 500 Gy. Also, the use of such preservative solutions as a sucrose solution would increase the tolerance of flowers remarkably (Bhuiya *et al.* 1999; Hayashi *et al.* 1999; Kikuchi *et al.* 1999; Kikuchi 2003; Sangwanangkul *et al*. 2008; Osouli *et al*. 2011).

There are only a limited number of investigations which show that mites are most likely more tolerant to radiation than insects (Follett *et al*. 2007; Follett 2009). The dose rates which cause immediate mortality in mites (1–3 KGy) could also seriously damage the products and create phytotoxicity effects and hence, may not be used as a quarantine treatment (Sulaiman *et al*. 2004). It is neces-

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sary to find lower dose rates which are in the tolerance range of fresh commodities and meanwhile could prevent the mites from reproducing new generations.

In this research, the effects of gamma dose rates of up to 450 Gy (which is in the tolerance range of most cut flowers) on the biological reaction of adult mites *T. urticae*, was studied to determine an effective quarantine treatment dose rate which causes sterility in adults.

Materials and Methods

Collecting and identification

The first group of mites was collected from the colony reared in the Faculty of Agriculture, University of Tehran, Karaj, Iran and transferred to the entomology laboratory of Agricultural, Medical and Industrial Research School, Tehran. A fertile female which was observed mating with a male under the binocular, was used to produce the preliminary colony. Mites were identified in the acarology laboratory at the University of Tabriz.

Mass production

Up to five generation of mites were reared on detached leaves of beans (*Phaseolus vulgaris* L.) Sunray cv., in an incubator [27±1°C temperature, relative humidity of 60±5% and a photoperiod of 16 : 8 (L : D) h] to diminish any effects of the previous hosts. A culture consisted of glass boxes (20 × 14 × 16 cm) containing cotton wool saturated in water and the bean leaves which were pressed on top of the cotton wool.

To produce the required number for a mite population, the preliminary colony was transferred to the beans, Sunray cv. in the greenhouse. They were kept at a temperature range of 23–27°C during the day and at 18–25°C during the nights, at a relative humidity of 45–50%.

Producing mites of similar age for irradiation

To study the radiation effect on different stages of mites, it was important to irradiate them at the same age and size. Hence to develop adults mites of similar age for irradiation, eggs 0–12 h old were kept in the incubator at 27±1°C with a relative humidity of 60±5% and a photoperiod of 16 : 8 (L : D) h.

Irradiation of mites

From each group (0–24 h old and 48–72 h old), 50 adult mites were put on 10 leaf squares (1 × 1 cm, each hosting 5 mites) in a 7.5 cm diameter Petri dish, on wet cotton. Each Petri dish was used for one replication of each dose rate. The Petri dishes had a 2.5 cm hole on their tops for ventilation. Irradiation was carried out using ^{60}Co PX γ-ray source facility of PX-30, made in Russia, and its calibration was carried out by the Fricke dosimeter. After irradiation, the Petri dishes were moved to the incubator and mite activity was observed and recorded daily (every 24 h) using a stereo microscope (Bhuiya *et al*. 1999).

Biological reactions

Experiment 1

In this experiment the effect of 0, 200, 250, 300, 350, 400, and 450 Gy gamma doses on the following biological aspects of adult two-spotted spider mites was studied:
- longevity of adult mites irradiated when the mites were 0–24 and 48–72 h old,
- total number of laid eggs by 50 irradiated females (in each replication) when the female mites were 0–24 and 48–72 h old,
- the percentage of hatched eggs laid by females irradiated when the females were 0–24 and 48–72 h old.

Experiment 2

The effect of 0, 200, 250, 300, 320, 340, 360, 380, and 400 Gy gamma doses on the fecundity and fertility of adult females was studied in the following cases:
- the fecundity and fertility of irradiated adult females after mating with un-irradiated males,
- the fecundity and fertility of un-irradiated adult females after mating with irradiated males,
- the fecundity and fertility of irradiated adult females after mating with irradiated males.

Statistical analysis

A randomized complete block design with three replications was used to statically analyze the data. The means were compared with the Duncan's multiple range test. Regression analyses of the data on dose rates were also made regarding the longevity of adult females and the total number of eggs. Statistical analyses were carried out using MSTAT-C and SPSS 18.5 software.

Results

Experiment 1

Longevity of adult females irradiated when the females were 0–24 and 48–72 h old

The analysis of variance of adult female longevity after irradiation with different doses of gamma radiation when the females were 0–24 and 48–72 h old, showed that applied dose rates had a significant effect ($p < 0.001$) on the longevity of the adult females in both age groups. The mean longevity of irradiated females decreased with increasing dose rates, and at 350 Gy, the longevity of females was significantly lower than the control in both age groups (6.247 and 3,893 days for the 0–24 h olds and the 48–72 h olds, compared with 6.887 and 4.347 days of the control, respectively) ($p < 0.05$; Table 1).

Considering the fitted regression functions of the irradiation effect on the longevity of females and their coefficients of determination, it was revealed that around 97% and 90% of the variation in the longevity of females at 0–24 and 48–72 h old, respectively, related to dose effects (Figs. 1 and 2). The longevity of both female types was

Table 1. Different biological reactions (mean ±SEM) of adult two-spotted spider mites after irradiation with different gamma ray doses. Least Significant Difference (LSD) (5%) is for all comparisons within the same column

Dose [Gy]	Longevity of adult female after irradiation (day)		Total number of eggs		Hatchability [%]	
	0–24 h old females	48–72 h old females	0–24 h old females	48–72 h old females	0–24 h old females	48–72 h old females
0	6.887 (0.059)	4.347 (0.024)	1,171.00 (51.647)	965.00 (21.377)	94.450 (1.964)	96.30 (0.888)
200	6.727 (0.029)	4.407 (0.018)	1,001.00 (196.505)	961.67 (14.099)	6.58 (0.971)	13.06 (2.032)
250	6.467 (0.035)	4.233 (0.020)	910.30 (16.756)	769.67 (13.691)	2.81 (0.378)	2.07 (0.160)
300	6.533 (0.0437)	4.213 (0.020)	917.70 (16.334)	753.667 (4.256)	0.163 (0.083)	1.02 (0.089)
350	6.247 (0.264)	3.893 (0,0857)	640.70 (21.957)	680.33 (11.465)	0.00	0.00
400	4.980 (0.170)	3.167 (0.104)	539.30 (11.140)	514.33 (2.906)	0.00	0.00
450	4.107 (0.074)	3.040 (0.0503)	493.30 (5.487)	346.33 (11.977)	0.00	0.00
LSD (5%)	0.4134	0.1779	226.6	36.67	2.730	3.417

reduced when the dose rate was increased. The reduction in mean longevity was linear for mites 48–72 h old (Fig. 2) while it was curvelinear for mites 0–24 h old. In mites 0–24 h old, the reduction in longevity was initially slow but it was rapid at the higher dose rates of above 350 Gy (Fig. 1).

Total number of laid eggs by adult females irradiated when the females were 0–24 and 48–72 h old

Analysis of variance of total laid eggs by the females irradiated with different dose rates in both groups (0–24 and 48–72 h old) showed the significant effect ($p < 0.001$) of gamma ray treatments on the egg laying capability of mites (Table 1). In both age groups, the radiation doses of 250 Gy and above, significantly reduced total laid eggs (910.39 and 769.67 for 0–24 and 48–72 h old females, respectively) compared with the control (1,171.00 and 965.00 for 0–24 and 48–72 h old females, respectively) ($p < 0.05$; Table 1). The reduction was linear for both ages (Figs. 3 and 4). There were no significant differences ($p < 0.01$) between the slopes of the two regressions, indicating that both groups have similar sensitivities to the irradiation for this characteristic.

Percentage of hatched eggs laid by adult females irradiated when the females were 0–24 and 48–72 h old

The mean hatchability of eggs laid by females of both groups was reduced when the dose was increased ($p < 0.05$). There was a significant difference in the hatchability of eggs laid by 0–24 and 48–72 h old females irradiated with doses of 200 Gy and higher (6.58% and 13.06% for 200 Gy, compared with 94.45% and 96.30% of the control, respectively). The laid eggs of both mite groups lost their hatchability when doses were 350 Gy and above. Hence, these females were not able to produce future generations (Table 1).

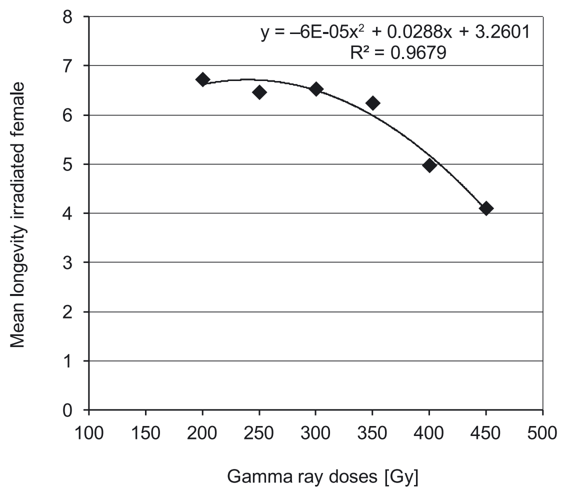

Fig. 1. Dose response curve for the longevity of 0–24 h old irradiated females of the two spotted spider mites

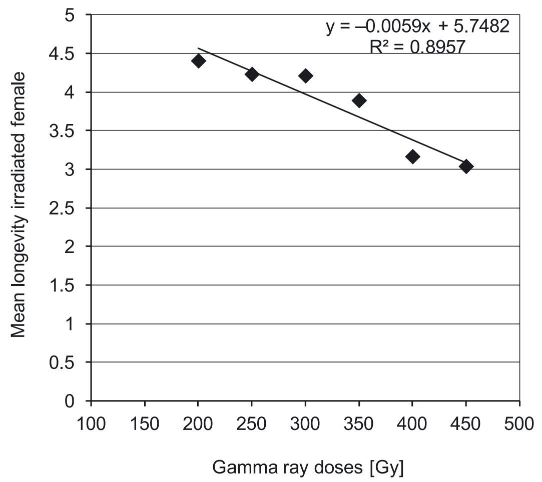

Fig. 2. Dose response line for the longevity of 48–72 h old irradiated females of the two spotted spider mites

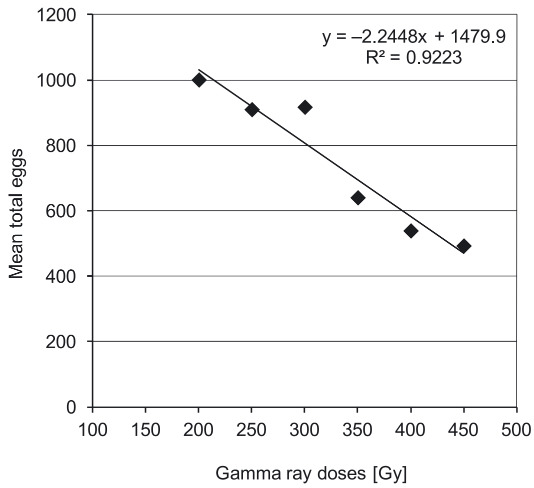

Fig. 3. Dose response line for the total eggs laid by 0–24 h old irradiated females of the two spotted spider mites

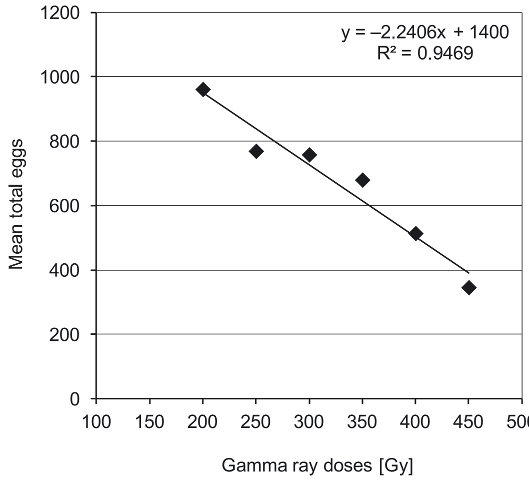

Fig. 4. Dose response line for the total eggs laid by 48–72 h old irradiated females of the two spotted spider mites

Experiment 2

Fecundity and fertility of adult two-spotted spider mites irradiated with different gamma dose rates

Based on the results of Experiment 1, the sterility dose rate of TSSM seems to be higher than 300 Gy. For this reason, those irradiating dose rates of higher than 300 Gy were considered with smaller intervals of 20 Gy (300, 320, 340, 360, 380, and 400).

The laid eggs of irradiated females mated with un-irradiated males lost their survival completely from a dose rate of 320 Gy and egg hatchability was reduced 93.59% as compared to the control. The mean total number of eggs laid was reduced from 999 for the control to 750.67 at 320 Gy. A significant reduction ($p < 0.05$) was observed in the mean total number of laid eggs when the dose rate was 200 Gy and higher (Table 2).

The dose rate of 320 Gy caused complete sterility of male two-spotted spider mites. All the progeny resulting

Table 2. Fecundity and fertility (mean±SEM) of two spotted spider mites after irradiation with different gamma ray doses. Least Significant Difference (LSD) (5%) is for all comparisons within the same column

Gamma ray doses [Gy]	Irradiated female × Unirradiated male		Unirradiated female × Irradiated male					Irradiated female × Irradiated male	
	total eggs	hatch-ability [%]	total eggs	hatch-ability [%]	resultant female number	resultant male number	sex ratio	total eggs	hatch-ability [%]
0	999.00 (7.211)	93.59 (0.444)	1,043.00 (28.000)	91.84 (1.565)	707.33 (31.072)	218.33 (3.930)	3.240 (0.130)	1052.00 (50.540)	94.91 (1.545)
200	788.33 (4.702)	5.83 (0.701)	791.00 (2.517)	49.60 (0.404)	14.67 (1.202)	264.67 (6.741)	0.056 (0.006)	977.67 (9.493)	11.59 (0.218)
250	758.67 (3.983)	2.54 (0.116)	855.00 (19.313)	31.41 (0.904)	7.33 (0.334)	177.00 (6.807)	0.041 (0.001)	730.33 (1.453)	1.46 (0.358)
300	750.67 (2.835)	0.29 (0.286)	927.33 (62.653)	24.69 (1.949)	5.33 (0.882)	141.33 (2.186)	0.038 (0.005)	720.00 (12.741)	0.00
320	695.33 (9.135)	0.00	837.33 (2.404)	23.60 (0.545)	0.00	130.67 (4.667)	all male	719.00 (2.517)	0.00
340	616.00 (16.773)	0.00	830.33 (10.478)	13.31 (0.555)	0.00	71.33 (2.186)	all male	669.67 (2.404)	0.00
360	620.67 (5.175)	0.00	826.67 (37.671)	12.66 (0.584)	0.00	67.67 (9.135)	all male	651.00 (5.773)	0.00
380	594.67 (6.360)	0.00	740.67 (5.044)	18.05 (0.268)	0.00	62.67 (0.882)	all male	512.67 (7.535)	0.00
400	443.00 (4.932)	0.00	672.67 (17.130)	18.75 (3.130)	0.00	53.00 (4.163)	all male	48.00 (1.155)	0.00
LSD (5%)	55.36		74.97	4.033				51.98	

from the mating of sterile males with non-sterile females were male mites because of parthenogenesis. The sex ratio was more male biased as the dose rate increased. Sex ratio in the control was 3.240 (female to male) (Table 2). It was also observed that the mean total number of laid eggs decreased as the dose rate increased, and at 200 Gy (891.00 for 200 Gy compared with 1,043.00 of the control), the control showed a significant difference ($p < 0.05$). Also, the percentage of the mean hatchability reduced significantly ($p < 0.05$) at 200 Gy (49.60% compared with 91.84% for the control).

Furthermore, when both males and females were irradiated, the percentage of egg hatchability decreased following the increase in the dose rate and reached 0 at 300 Gy (compared with 94.91% in the control). The mean total number of laid eggs also decreased when the dose was increased, and at 200 Gy (974.67) a significant reduction ($p < 0.05$) for the control (1,052.00) was observed (Table 2).

Discussion

As expected, gamma irradiation had a deteriorative effect on the biological aspects of *T. urticae*, including the longevity of adult female mites as well as the total number and viability of eggs. The irradiation effects intensified as the dose rate was increased. Also, irradiation could cause sterility of adults at certain dose rates.

In the present research (Experiment 1), the effect of different gamma radiation doses on the longevity, fecundity and fertility of adult TSSM, irradiated when the mites were 0–24 and 48–72 h old, was studied. With regard to longevity, comparing the regressions showed that the younger females (0–24 h old) were more tolerant to gamma radiation when irradiation was done with certain lower doses, as compared to the 48–72 h old females. The reason could be due to their higher ability to repair irradiated tissues. Also, the total of the eggs laid by the irradiated females of both groups was significantly reduced from an irradiation dose of 250 Gy when compared with the control. But even at the highest irradiation dose of 450 Gy, there were still considerable eggs laid by both female groups (439.3 for the 0–24 h old group and 346.33 for the 48–72 h old group). Based on the regressions, there were no significant differences between the 0–24 h old group and the 48–72 h old group regarding this characteristic. On the other hand, the hatchability and survival of eggs laid by the irradiated females of both the 0–24 h old group and the 48–72 h old group were zero at a dose rate of 350 Gy. This means that with a dose rate of 350 Gy, the ability of females to produce offspring of the next generation was prevented. Ignatowicz and Banasik-Solgala (1999) also reported a significant reduction in the longevity of older females from a dose of 300 Gy but they did not observe a significant change in the longevity of younger females at doses of 100, 200, and 300 Gy. In the studies of Bhuiya *et al.* (1999) the longevity of irradiated males of *Oligonychus biharensis* Hirst (Tetranychidae), was reduced to less than half that of the control mites, with doses of 400 and 500 Gy. Goodwin and Wellham (1990) did not report any considerable reduction in the longevity of young and old females of irradiated two-spotted spider mites, and they found no difference in the egg laying ability of younger and older irradiated females. Ignatowicz and Banasik-Solgala (1999) did observe a reduction in the egg laying ability in older females at 300 Gy. Furthermore, these researchers found a 90% reduction in the survival of eggs laid by the irradiated females which had received 300 Gy (the highest dose applied). Hayashi *et al.* (1999) found the sterility dose rate to be above 400 Gy for adult *T. urticae* mites.

The results of Experiment 2 in this investigation, suggested a sterility dose of 320 Gy when either the male or female is irradiated. When both the male and female are irradiated, a lower dose of 300 Gy was suggested as causing sterility. Ignatowicz and Banasik-Solgala (1999) and Henneberry (1964) also reported that a 320 Gy dose rate is appropriate for the sterility of both sexes. However, other researchers reported different dose rates for the sterility of *T. urticae*, which ranged from 80 to 400 Gy for females, 240 to 300 Gy for males, and 300 to 320 Gy when both sexes were irradiated (Ignatowicz and Banasik-Solgala 1999).

According to Hallman (1998), in most insects, the females are sensitive to irradiation equally or more so than the males and require a lower dose rate to be sterilized. Bakri *et al.* (2005) explained that the sensitivity of mitotically active reproductive cells (MARC) in female insects is further complicated by the presence of nurse cells that are subject to injury. Nurse cells are extremely radiosensitive when they are undergoing endomitosis. Thus, females are, in general, more radiosensitive. However, the results of this investigation and some other studies (Ignatowicz and Banasik-Solgala 1999; Hallman 2012) on Tetranychidae have shown a relatively equal sensitivity of adult males and females to sterilizing dose rates.

Conclusions

Based on the results of the current study, dose rates of above 300 Gy could cause sterility in adult male and female mites of *T. urticae*. The use of gamma irradiation seems to have good potential as a quarantine treatment of cut flowers, ornamentals, and perhaps other fresh commodities having much higher tolerance than this dose rate, against the family of studied pests.

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