

Research Article

Evaluating the interaction between gamma radiation and *Carum copticum* essential oil for the control of *Tribolium confusum* (Coleoptera: Tenebrionidae)

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Abstract: The combined effect of gamma radiation and *Carum copticum* C. B. Clarke essential oil were determined for the management of *Tribolium confusum* Du Val larvae and adults. Experiments were carried out at 27 ± 1 °C and $65 \pm 5\%$ R. H. under dark condition and three steps were designed: (A) Application of gamma radiation and essential oil at the same time. (B) Irradiation followed by essential oil. (C) Essential oil followed by irradiation. Radiation doses of 100 to 500 Gy were applied in combination with 5.97, 7.52 and 10.47 $\mu\text{l/l}$ air of essential oil for the adults and 1.91, 4.08 and 12.02 $\mu\text{l/l}$ for the larvae. The result showed that the combination of gamma radiation with *C. copticum* oil increased larval and adult mortality compared with the control, so that, the interaction of 500 Gy with 10.47 $\mu\text{l/l}$ air for adults and 12.02 $\mu\text{l/l}$ air for larvae caused 100% mortality in 13 and 8 days, respectively; although 100% of the larvae and adults mortality at 500 Gy alone caused within 22 days. It was revealed that the combination of irradiation and essential oils could be used as an effective control method and a good alternative to fumigants.

Key words: *Carum copticum*, combined effect, essential oil, gamma radiation, synergistic effect

Introduction

Insect infestation is listed as the most serious problems that plague the stored grain products such as flour because it decreases the products quality and quantity. It causes 10-40% losses of the stored product annually in developing countries where the modern storage technologies have not been introduced (Bagheri Zenouz, 1996; Shaaya *et al.*, 1997; Keita *et al.*, 2001; Chaubey, 2007). To annihilate the stored-

product pests by chemical fumigants has its own limitations because the insects develop insecticidal resistance (Rossi *et al.*, 2010). Also, extensive use of synthetic fumigants for this purpose is a cause of serious concern because of their unwanted side effects on the environment. Subsequently, alternative, environmentally friendly techniques are clearly needed for the control of these pests. Fumigation with essential oils as natural pesticides and gamma radiation are the two ecologically safe methods that could be employed for the destruction of the stored-product pests (Matsumura, 1985; Keita *et al.*, 2000; Enan, 2001; Aquino, 2012; Ahmadi *et al.*, 2013). Gamma radiation due to its great

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potential as a pesticide regardless of the insect's life-stage can be used as a safe and clean controlling method (Ahmed, 1990). It goes without saying that *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae) is one of the most harmful pests destroying the stored grain products that is demonstrated to be sensitive to irradiation and some essential oils (Bagheri Zenouz, 1986; Abd El-Aziz and El-Sayed, 2009; Khaghani *et al.*, 2010). Tuncbilek *et al.* (2003) reported that irradiation was effective at the dose of 20-200 Gy against the larvae and adults of *T. confusum* within 2 weeks. Also essential oils have been found to be effective as repellents, antifeedants and attractive in the stored products pests (Shaaya *et al.*, 1997; Park *et al.*, 2002; Koul *et al.*, 2008). Various botanical insecticides and essential oils have been reported for their toxic effects against *T. confusum* (Shaaya *et al.*, 1997; Isikber *et al.*, 2006).

On the other hand, application of gamma radiation in high doses or essential oils in high concentrations separately is expensive and time-consuming. Therefore, it is desirable to have a method that uses lower radiation doses and concentrations of the oils without compromising the effectiveness of the treatment. One of the possibilities to achieve this goal is to combine the two methods, setting our sights on a synergistic effect (Ahmadi *et al.*, 2013). There are some reports on combination of gamma radiations with other treatments by infrared, microwaves, insecticides and essential oils to name only few (Hassan & Rahman Khan, 1998; Sharma & Seth, 2005; Ahmadi *et al.*, 2008; Ahmadi *et al.*, 2013). Omar *et al.* (1988) reported that the combined treatments of gamma irradiation and carbon dioxide (CO₂) wrought a higher mortality in *T. confusum* than either of them alone. Ayvaz *et al.* (2002) investigated the combined effects of gamma radiation and malathion on the flour beetle, *Tribolium confusum* (du Val). They reported that the survival of adults after irradiation was negative affected with increasing dose of both irradiation and malathion treatment. Ahmadi *et al.*, (2013) have reported that the combination

of gamma radiation and medicinal plants may result in synergistic interactions that increase the mortality of *T. castaneum*. Interaction of these treatments is likely to be economical and effective against the stored product pests. Furthermore, it has advantage of leaving no residues in the products. In this study, we explored the combined effect of gamma radiation and *C. copticum* essential oils with the aim to reduce the cost of effective management of *T. confusum* using lower doses of gamma radiation and lower concentrations of essential oils.

Materials and Methods

Plant materials

Seeds of *C. copticum* were collected in September 2011 in Damghan, Iran. The seeds were dried naturally and stored at 24 °C at room temperature until they were needed for extracting.

Extraction of essential oils

Essential oil was extracted from the dried seeds with a Clevenger-type apparatus for hydrodistillation. Conditions of extraction were: 50g of seeds; 600mL distilled water at a temperature around 100 °C, after 4 h distillation, the extracted essential oil was collected. Anhydrous sodium sulphate was used to remove water after extraction. The extracted oil was stored in a refrigerator at 4 °C.

Insect

The confused flour beetle, *T. confusum* was reared in the laboratory of Nuclear Agricultural Research Institute, Karaj. The insects were kept in darkness at 27 ± 1 °C and $65 \pm 5\%$ R. H on wheat flour mixed with yeast (10: 1 w/w) in plastic containers. *T. confusum* larvae (10-15 days old) and adults (1-7 days old) were used in this experiment.

Irradiation

T. confusum larvae and adults were treated by Cobalt⁶⁰ in gamma cell at the Nuclear Agriculture Research Institute, Karaj, Iran. The

desired radiation doses were given by varying the time of exposure of the insects. To determine the sublethal doses of gamma radiation that wrought 1%, 5% and 25% mortality, healthy and active adult insects were subjected to irradiation doses between 100-500 Gy with three replicates. The dose range was determined by using the data obtained from the preliminary experiment and the logarithmic distance model. After irradiations, the insects were kept in the dark in the growth room, and the rate of mortality was measured 24 hour after the treatment.

Fumigation toxicity

To determine the fumigant toxicity of the *C. copticum* oil, filter papers (cut into 2 cm diameter) were impregnated with oil by using a micropipette, then the filter paper was attached to the under-surface of the cap of the glass vials (30 ml) and the caps were screwed tightly (Negahban *et al.*, 2007). Larvae and adults were exposed to the essential oil at different doses of oil 7 – 19 µl/l (7.2, 7.9, 8.7, 9.5, 10.5, 11.5, 12.6, 13.8, 15.1, 16.6 and 18.2 µl/l) for 24 h. Each concentration and control was replicated three times including 100 insects. Mortality was determined 72 h after exposure. Those insects that did not move any of their legs or antennae when lightly probed or shaken in the light and mild heat were considered dead. The data were analyzed using SPSS 16.0 software to estimate the LC₁, LC₅ and LC₂₅ values.

Interaction of gamma radiation and essential oil

The experiments were designed with different interactions of gamma irradiation and essential oils to determine their combined effects on *T. confusum* larvae and adults. The larvae and adults were irradiated with 100, 200, 300, 400 and 500 Gy, also they were exposed to 5.97, 7.52 and 10.47 µl/l of oil for adults and 1.91, 4.08 and 12.02 µl/l for larvae. The interaction of gamma irradiation and essential oils were undertaken as follows: In treatment A, irradiation and fumigation were used at the same time. In treatment B, the fumigated

insects were exposed to the irradiation after 3 days and in treatment C, the irradiated insects were exposed to the sublethal concentrations of *C. copticum* after 3 days. Each treatment was replicated three times including 100 insects per replication. Mortality was recorded 24 h after the first treatment. The insects were considered dead if no leg or antennal movements were observed. Mortality data were subjected to probit analysis (SPSS 16.0).

Calculation of synergistic effects

The synergistic effect of the combined treatment with radiation and an essential oil was measured using the following formula adapted from the paper by Berenbaum (1989):

$$S = \frac{d}{D} + \frac{c}{C}$$

Here, S is the synergistic effect ($S > 1$ at antagonism, $S = 1$ at additivity, and $S < 1$, at synergism), d and c are the radiation dose and the oil concentration used in the combined mode, while D and C are the radiation dose and oil concentration that produced the same biological effect when used separately.

Results

Irradiation of adults and larvae

The results show that the mortality of the adults and larvae increases with an increase in the gamma radiation dose, while their lifetime decreases (Figure 1 and 2). It is concluded that various doses of gamma radiation bring about different effects on the adults and larvae. According to the result, 500 Gy dose of gamma radiation is capable to kill 100% adults and larvae in 22 days. Also doses of 100, 200, 300 and 400 Gy causes 100% adult mortality in 32, 30, 25 and 23 days respectively (Figure 1) and for the larvae these periods were 32, 26, 24 and 24 days, respectively (Figure 2). Meanwhile, in the control, the mortality rate of the adult and larvae in 35 days after exposure reached 15% and 10%, respectively. The results demonstrated significant difference in the mortality of the irradiated adults at varying levels of irradiation.

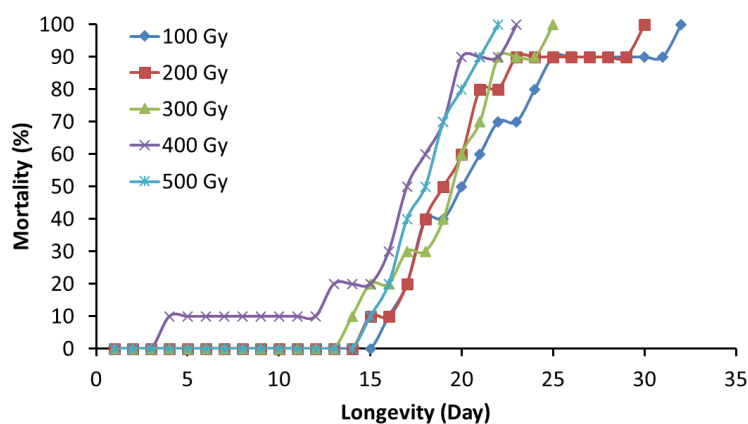


Figure 1 Effect of gamma radiation on adult longevity of *Tribolium confusum* in 35 days.

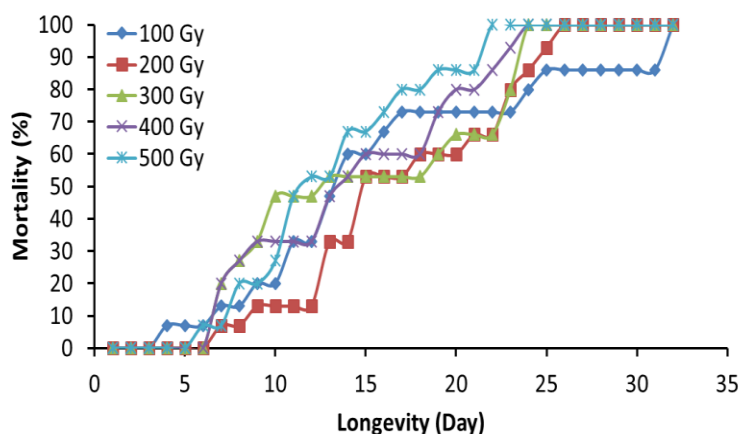


Figure 2 Effect of gamma radiation on larval longevity of *Tribolium confusum* in 35 days.

Fumigation toxicity

The results indicate that mortality rates in both adult and larvae are increased in accordance with an increase in the doses of *C. copiticum*. The observed mortality rates of the adult insects treated with essential oil at the dose of 7.2 and 18.2 $\mu\text{l/l}$ of *C. copiticum* oil, in 24 hours reached 3.33 and 73% (Figure 3) and similarly, an increase in larval mortality rate (approximately 13.33 and 43%) was recorded at the same oil doses (Figure 4). We can come to the conclusion that the low and high doses of *C. copiticum* (7.2 and 18.2 $\mu\text{l/l}$) made different effects on larvae and adult, whereas the larvae were determined to be more sensitive than the adults to *C. copiticum* oil. Mortality for each dose was investigated independently, then LC_{10} ,

LC_{25} and LC_{50} values were determined as 199.01, 250.99 and 349.26 $\mu\text{l/l}$ air for the adults and 63.82, 136.22 and 400.97 $\mu\text{l/l}$ air for larvae after 24h exposure, respectively.

Combined effect of gamma radiation and essential oil

The mortality rate of adults and larvae treated with either gamma radiation or *C. copiticum* essential oil separately was much lower than the mortality rate following the combined treatment. It was shown that the longevity of irradiated adults with 100 and 500 Gy were 32 and 22 days respectively, whereas as these adults fumigated by 10.47 $\mu\text{l/l}$ of oil simultaneously, the longevity rate decreased significantly ($P < 0.05$) to 19 and 14 days respectively (Table 1).

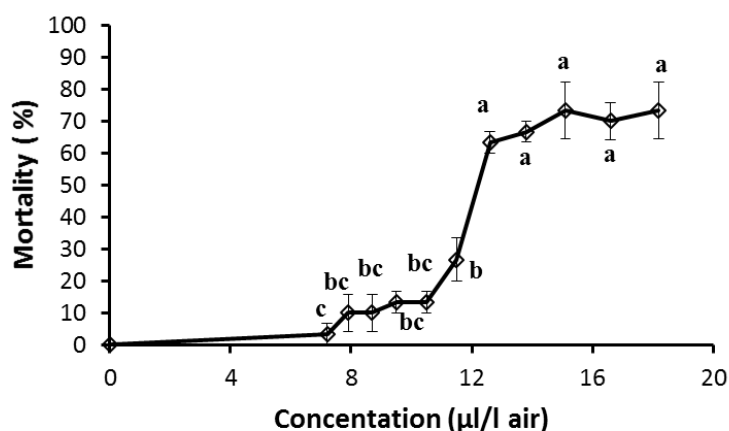


Figure 3 Effect of different concentrations of *Carum copticum* essential oil on adult mortality of *Tribolium confusum*.

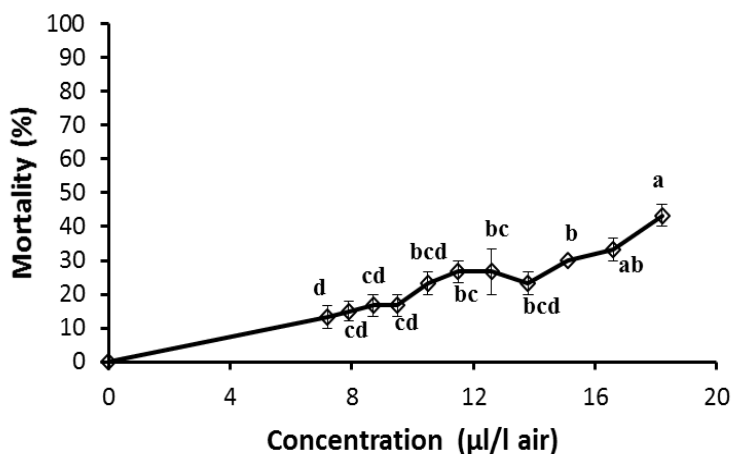


Figure 4 Effect of different concentrations of *Carum copticum* essential oil on larval mortality of *Tribolium confusum*.

Table 1 Simultaneous application of *Carum copticum* essential oil fumigation and gamma radiation on adult longevity (days) of *Tribolium confusum*.

| Concentration (µl/l) | Gamma dose (Gy) | | | | |
|----------------------|------------------------------|------------------------------|------------------------------|------------------------------|-----------------------------|
| | 100 | 200 | 300 | 400 | 500 |
| 0 | 32.00 ± 2.66 ^a | 30.00 ± 1.15 ^a | 25.33 ± 1.33 ^b | 23.00 ± 0.00 ^{bc} | 22.00 ± 0.57 ^{bcd} |
| 5.97 | 20.66 ± 0.88 ^{cde} | 20.33 ± 0.33 ^{cdef} | 21.00 ± 2.08 ^{cde} | 19.66 ± 1.33 ^{cdef} | 16.00 ± 0.00 ^{ef} |
| 7.52 | 20.00 ± 2.00 ^{cdef} | 19.66 ± 1.33 ^{cdef} | 20.33 ± 1.76 ^{cdef} | 18.00 ± 1.00 ^{defg} | 16.66 ± 0.88 ^{def} |
| 10.47 | 19.33 ± 0.88 ^{cdef} | 20.33 ± 2.18 ^{cdef} | 15.00 ± 0.00 ^g | 18.00 ± 1.00 ^{defg} | 14.66 ± 0.33 ^g |

Means followed by the same letter within a row are not significantly different using Tukey HSD at 5% level.

In interaction B in which insects were exposed to radiation 3 days after fumigation by the essential oil, it was shown that the combined doses of 100 and 500 Gy of gamma radiation with sublethal doses of *C. copticum*, had significant differences in mortality rate of

the treatments (Table 2). It was revealed that the doses of 100 and 500 Gy caused 100% mortality of irradiated adults 32 and 22 days after treatment, while in combination with 10.47 µl/l of the oil these periods decreased to 22 and 15 days, respectively (Table 2).

Through interaction C in which the adults were exposed to various concentrations of the essential oil after 3 days of irradiation, combination of 100 and 500 Gy of gamma ray with 7.52 and 10.47 $\mu\text{l/l}$ of *C. copticum* showed enough potential for killing all adults within 16 and 13 days (Table 3). Comparing with the methods A and B, interaction C produced the acceptable results because of the most effective and short-term annihilation of the adults. Table 4 shows that the synergistic effect on the adults were observed in all cases (except for combination of 500 Gy with 7.52 and 10.47 $\mu\text{l/l}$ and 300 Gy with 5.97 $\mu\text{l/l}$ of *C. copticum*) especially when the low doses of gamma radiation (100 and 200 Gy) were applied in combination with the oil. The results demonstrated that when 3 controlling methods were applied to the larvae, the combination of gamma radiation with *C. copticum* made an acceptable control

compared with either gamma radiation or essential oils separately. The combination of 100 and 500 Gy of gamma radiation with 12.02 $\mu\text{l/l}$ oil at the same time reduced the longevity of larvae from 32 and 22 days to 20 and 12 days respectively (Table 5). Furthermore, irradiation of larvae with doses of 100 and 500 Gy caused complete mortality within 32 and 22 days respectively but combination of irradiation with a dose of 12.02 $\mu\text{l/l}$ of oil decreased these periods to 16 and 12 days (Table 6). According to method C, the most acceptable control of larvae resulted in combination of 500 Gy with 12.02 $\mu\text{l/l}$ of oil demonstrating significant differences with that of the other ones (Table 7). The synergistic effects were observed when irradiation to 500 Gy was combined with *C. copticum* in all concentrations, 400 Gy with 4.08 and 12.02 $\mu\text{l/l}$ and 100 Gy with 1.91 and 4.08 $\mu\text{l/l}$ of oil (Table 8).

Table 2 Combined effects of gamma radiation three days after *Carum copticum* essential oil fumigation on adult longevity (days) of *Tribolium confusum*.

| Concentration ($\mu\text{l/l}$) | Gamma dose (Gy) | | | | |
|-----------------------------------|-----------------------------------|----------------------------------|----------------------------------|----------------------------------|---------------------------------|
| | 100 | 200 | 300 | 400 | 500 |
| 0 | 32.33 \pm 2.66 ^a | 30.00 \pm 1.15 ^a | 25.33 \pm 1.33 ^b | 23.00 \pm 0.00 ^{bc} | 22.00 \pm 0.57 ^{bcd} |
| 5.97 | 20.66 \pm 0.33 ^{cdef} | 20.66 \pm 0.33 ^{cdef} | 21.66 \pm 0.66 ^{cd} | 20.33 \pm 0.33 ^{cdef} | 18.00 \pm 1.00 ^{fg} |
| 7.52 | 20.00 \pm 0.00 ^{cdefg} | 21.00 \pm 0.57 ^{cdef} | 21.00 \pm 0.00 ^{cdef} | 19.33 \pm 0.88 ^{defg} | 17.33 \pm 0.66 ^{gh} |
| 10.47 | 22.33 \pm 0.66 ^{cd} | 19.66 \pm 1.33 ^{defg} | 21.33 \pm 0.33 ^{cde} | 18.33 \pm 1.20 ^{efg} | 15.00 \pm 0.00 ^h |

Means followed by the same letter within a row are not significantly different using Tukey HSD at 5% level.

Table 3 Combined effects of *Carum copticum* essential oil fumigation three days after gamma radiation on adult longevity (days) of *Tribolium confusum*.

| Concentration ($\mu\text{l/l}$) | Gamma dose (Gy) | | | | |
|-----------------------------------|--------------------------------|---------------------------------|---------------------------------|--------------------------------|---------------------------------|
| | 100 | 200 | 300 | 400 | 500 |
| 0 | 32.33 \pm 2.66 ^a | 30.00 \pm 1.15 ^a | 25.33 \pm 1.33 ^b | 23.00 \pm 0.00 ^{bc} | 22.00 \pm 0.57 ^{bcd} |
| 5.97 | 23.00 \pm 0.00 ^{bc} | 21.66 \pm 0.33 ^{bcd} | 18.66 \pm 2.72 ^{de} | 13.00 \pm 0.33 ^{fg} | 12.66 \pm 0.66 ^g |
| 7.52 | 13.66 \pm 0.88 ^{eg} | 22.00 \pm 0.57 ^{bcd} | 22.33 \pm 0.66 ^{bcd} | 14.66 \pm 1.00 ^{fg} | 11.66 \pm 0.66 ^g |
| 10.47 | 16.66 \pm 1.20 ^{ef} | 19.66 \pm 2.33 ^{cde} | 20.00 \pm 0.00 ^{cde} | 13.66 \pm 0.66 ^{fg} | 13.00 \pm 1.00 ^{fg} |

Means followed by the same letter within a row are not significantly different using Tukey HSD at 5% level.

Table 4 Synergistic effect of gamma radiation three days after *Carum copticum* essential oil fumigation on adults of *Tribolium confusum*.

| Concentration ($\mu\text{l/l}$) | Gamma dose (Gy) | | | | |
|-----------------------------------|-----------------|----------|----------|----------|----------|
| | 100 | 200 | 300 | 400 | 500 |
| 5.97 | S = 0.35 | S = 0.51 | S = 1.02 | S = 0.83 | S = 0.99 |
| 7.52 | S = 0.40 | S = 0.56 | S = 0.72 | S = 0.88 | S = 1.04 |
| 10.47 | S = 0.50 | S = 0.66 | S = 0.82 | S = 0.98 | S = 1.14 |

S = synergistic effect; S > 1: antagonism, S = 1: additivity, S < 1: synergy.

Table 5 Simultaneous application of *Carum copticum* essential oil fumigation and gamma radiation on larval longevity (days) of *Tribolium confusum*.

| Concentration (μl/l) | Gamma dose (Gy) | | | | |
|----------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | 100 | 200 | 300 | 400 | 500 |
| 0 | 32.00 ± 0.33 ^a | 26.00 ± 1.00 ^{bc} | 24.33 ± 0.33 ^{cd} | 24.00 ± 0.00 ^{cd} | 22.00 ± 1.00 ^d |
| 1.91 | 20.66 ± 1.33 ^{de} | 19.33 ± 0.66 ^{ef} | 17.33 ± 0.66 ^{gh} | 16.00 ± 1.00 ^{hi} | 15.00 ± 1.00 ⁱ |
| 4.08 | 21.00 ± 0.66 ^d | 18.33 ± 0.33 ^{fg} | 18.33 ± 0.33 ^{fg} | 14.33 ± 0.33 ^{ik} | 14.33 ± 0.33 ^{ik} |
| 12.02 | 20.33 ± 0.33 ^{de} | 18.33 ± 0.33 ^{fg} | 18.00 ± 0.00 ^{fg} | 14.66 ± 0.33 ⁱ | 12.66 ± 0.33 ^k |

Means followed by the same letter within a row are not significantly different using Tukey HSD at 5% level.

Table 6 Combined effects of gamma radiation three days after *Carum copticum* essential oil fumigation on larval longevity (days) of *Tribolium confusum*.

| Concentration (μl/l) | Gamma dose (Gy) | | | | |
|----------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | 100 | 200 | 300 | 400 | 500 |
| 0 | 32.00 ± 0.33 ^a | 26.00 ± 1.00 ^{bc} | 24.33 ± 0.33 ^{cd} | 24.00 ± 0.00 ^{cd} | 22.00 ± 1.00 ^d |
| 1.91 | 27.33 ± 0.33 ^b | 15.66 ± 0.33 ^{ef} | 18.66 ± 1.85 ^e | 18.33 ± 1.66 ^e | 17.00 ± 0.00 ^{ef} |
| 4.08 | 23.33 ± 0.33 ^{cd} | 14.66 ± 1.33 ^{fg} | 17.00 ± 1.00 ^{ef} | 18.66 ± 0.88 ^e | 12.33 ± 0.33 ^g |
| 12.02 | 16.33 ± 0.66 ^{ef} | 15.66 ± 0.66 ^{ef} | 16.00 ± 2.00 ^{ef} | 16.00 ± 1.00 ^{ef} | 12.33 ± 0.33 ^g |

Means followed by the same letter within a row are not significantly different using Tukey HSD at 5% level.

Table 7 Combined effects of *Carum copticum* essential oil fumigation three days after gamma radiation on larval longevity (days) of *Tribolium confusum*.

| Concentration (μl/l) | Gamma dose (Gy) | | | | |
|----------------------|----------------------------|----------------------------|----------------------------|----------------------------|---------------------------|
| | 100 | 200 | 300 | 400 | 500 |
| 0 | 32.00 ± 0.33 ^a | 26.00 ± 1.00 ^{bc} | 24.33 ± 0.33 ^{cd} | 24.00 ± 0.00 ^{cd} | 22.00 ± 1.00 ^d |
| 1.91 | 20.00 ± 0.00 ^e | 18.00 ± 1.00 ^{ef} | 15.66 ± 0.33 ^g | 16.00 ± 0.00 ^{fg} | 14.00 ± 0.00 ^g |
| 4.08 | 19.00 ± 0.00 ^e | 18.33 ± 0.33 ^e | 16.00 ± 0.00 ^{fg} | 14.33 ± 0.33 ^g | 12.00 ± 1.00 ^h |
| 12.02 | 18.00 ± 1.00 ^{ef} | 16.00 ± 0.00 ^{fg} | 16.00 ± 0.00 ^{fg} | 15.33 ± 1.33 ^g | 8.66 ± 0.88 ⁱ |

Means followed by the same letter within a row are not significantly different using Tukey HSD at 5% level.

Table 8 Synergistic effect of gamma radiation three days after *Carum copticum* essential oil fumigation on larvae of *Tribolium confusum*.

| Concentration (μl/l) | Gamma dose (Gy) | | | | |
|----------------------|-----------------|----------|----------|----------|----------|
| | 100 | 200 | 300 | 400 | 500 |
| 1.91 | S = 0.72 | S = 1.8 | S = 1.4 | S = 1.36 | S = 0.49 |
| 4.08 | S = 0.29 | S = 1.23 | S = 1.82 | S = 0.47 | S = 0.56 |
| 12.02 | S = 1.14 | S = 2.4 | S = 1.39 | S = 0.70 | S = 0.79 |

S = synergistic effect: S > 1: antagonism, S = 1: additivity, S < 1: synergy.

Discussion

The results demonstrated clearly that gamma radiation and *C. copticum* had the ability to have acceptable control over the adults and larvae of the *T. confusum*. Also with increasing the doses, the mortality rates began to follow suit while their lifetime decreased. According to the results, 500 Gy doses of gamma radiation

have resulted in all insects death (100%) of *T. confusum* within 22 days, while directly applying 100 Gy, the mortality rate is decreased to 70% at the same time. On the other hand there was no significant difference in the mortality rate induced by doses of 100 and 200 Gy as well as 300-500 Gy.

Our results showed that 300 and 400 Gy doses of gamma radiation could cause 100%

mortality of the adults in 23 and 25 days, respectively. The rate of mortality observed in this experiment was the same as that previously reported by Prabhakumary *et al.* (2011) who investigated into the effect of radiation on *Tribolium castaneum* (Herbst) which estimated that 350 Gy dose would completely control the pests within 18-21 days, and lower doses could prevent the reproduction. Moreover, the results by Guo *et al.* (2009) confirm the findings of our study that the adults are annihilated by 200 Gy in 42 days, also they have demonstrated that dose of 500 Gy could control the adults after 28 days exposure while in our study this period was reduced to 22 days. It was realized that *C. copticum* is toxic to the adults and larvae of *T. confusum*. The results demonstrated that the adults are more sensitive to the essential oil than the larva after 24h. of exposure. Also it was shown that with increasing the concentration of the oil, mortality rate was increased. Sahaf *et al.* (2007) verified the effect of *C. copticum* essential oil on *Sitophilus oryzae* (L.) and *T. castaneum* with different concentrations and different time intervals. They concluded that *T. castaneum* could become more resistant against *C. copticum* compared with *S. oryzae*. The dose of 111.1 µl/l of *C. copticum* could control 50% of the *S. oryzae* adults in 9 hours; albeit for *T. castaneum*, it was 33.14µl/l. Habashi *et al.* (2011) studying the effect of *C. copticum* on *Rhyzopertha dominica* F., *Oryzaephilus surinamensis* L. and *T. confusum*, showed that the adults of *T. confusum* have more resistance to the pesticide compared with others. They determined that LC₅₀ values of *C. copticum* for controlling the infestation of *R. dominica*, *O. surinamensis* and *T. confusum* were 1.69, 19.01, and 58.70 µl/l air, respectively. According to Sahaf and Moharamipour (2007) and Habashi *et al.* (2011) reports, with increasing the *C. copticum* dose, the mortality rate of *T. confusum* was increased.

Irradiation and fumigation with essential oils are two main methods that could be used in combination with integrated pest management. It was shown that the lifetime of the adults

exposed solely to 500 Gy of gamma ray was 22 days but when combined with 10.47 µl/l of *C. copticum*, this period decreased to 13 days. Also the larvae of *T. confusum* exposed to 500 Gy were effectively controlled within 22 days, whereas these irradiated larvae if exposed to 12.02 µl/l of *C. copticum*, the time reduced to 8 days. The results demonstrated that the combined treatments with gamma radiation and essential oil from *C. copticum* caused a higher mortality of *T. confusum* than did each of the two treatments alone. Also the treatment with the essential oils and gamma irradiation appeared to have a significant synergistic effect under some conditions of our experiments.

There are various reports on gamma irradiation combined with other items, such as chemical insecticides (Mehta *et al.*, 2004), infrared and microwave (Kirkpatrick *et al.*, 1973), microbes (Jafri, 1967), and thiodicarb (Ramesh *et al.*, 2002) or azadirachtin (Sharma & Seth, 2005). The combination effects of gamma irradiation with infrared or microwave irradiations and chemical on *Rhyzopertha dominica* in wheat, have been reported to be more effective than the corresponding means used separately. It was revealed that the average reductions in adult emergences were 54%, 55%, and 42% for gamma, infrared, and microwave rays, respectively, however the reductions were 99% for the gamma-infrared combination and 96% for the gamma-microwave combination, and the mortalities were about 20% higher than predicted. These data agree with the results by Ahmadi and Negahban (2012) who studied the combined effects of gamma radiation and *Artemisia sieberi* (Besser) for the control of *Callosobruchus maculatus* F. They showed that the combination of gamma radiation and *A. sieberi* oil increased the death rate of *C. maculatus* rather than each treatment application separately. Also they indicated that 1-3 days time intervals between radiation and fumigation had an important role in mortality rate of the insects and the insect exposed to the essential oil before radiation were annihilated faster than by using the other methods. Our

findings are also aligned with those by Ahmadi *et al.* (2013) and Ahmadi and Moharramipour (2012) who indicated that medicinal plants could be combined with gamma radiation to enhance the mortality effect of irradiation 2-4 times. In another study by Ahmadi *et al.* (2008) where the combined and synergistic effects of gamma radiation and the essential oil of *Perovskia atriplicifolia* (Benth) on *T. castaneum* were studied, it was concluded that exposure to 100 Gy of gamma ray, resulted in death rate of 12.5% while if these irradiated insects exposed to 7.66µl/l of *P.atriplicifolia*, the mortality rate reached 32.5%.

In contrast to our study Sharma and Seth (2005) demonstrated that when the irradiated larvae of *Spodoptera litura* F. by 100 Gy and then fumigated by 0.75-1µl of neem extract immediately, the growth rate was increased from 17.66 to 22 days, meanwhile our results showed that with simultaneous exposure of larvae to gamma radiation and *C. copiticum* essential oil, the larvae could not complete their life cycle and would be controlled in a short time compared with carrying out the treatments separately. The results of our study are in agreement with the findings of Mehta *et al.* (2007) showing the synergistic effects of chemical insecticides and radiation combination.

Our data suggest that a combination of treatment with essential oils and irradiation can play a main role in managing of the stored-product insects. However, the vapor pressure of the essential oils is too low to enable them to penetrate into the agricultural produce to destroy pests, and the treatment can be employed only on a small scale. In order to make practical application of the essential oils, these compounds should be formulated. The synergistic effects of gamma radiation and essential oil were demonstrated in this study; however, the effectiveness of this combination at low doses and the assessment of mortality over longer periods of time after treatments need to be evaluated thoroughly in the coming future, so as to open up a new avenue in this field of research.

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ارزیابی اثر متقابل پرتو گاما و اسانس زنیان *Carum copticum* در کنترل *Tribolium confusum* (Coleoptera: Tenebrionidae)

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چکیده: اثر متقابل پرتو گاما و اسانس گیاه زنیان *Carum copticum* به‌منظور مدیریت کنترل لارو و حشرات کامل شپشه آرد *Tribolium confusum* Du val تعیین گردید. آزمایشات در شرایط کنترل دمای 27 ± 1 درجه سلسیوس، رطوبت نسبی 65 ± 5 درصد و در شرایط تاریکی در سه حالت مختلف طراحی گردید که شامل: (الف) استفاده از پرتو گاما و اسانس گیاهی به‌طور هم‌زمان (ب) استفاده از پرتو گاما پس از کاربرد اسانس گیاهی (ج) استفاده از اسانس گیاهی پس از کاربرد پرتو گاما بود. دزهای ۱۰۰ تا ۵۰۰ گری از پرتو گاما در ترکیب با دزهای ۵/۹۷، ۷/۵۲ و ۱۰/۴۷ میکرولیتر بر لیتر هوای اسانس زنیان برای حشرات کامل و دزهای ۱/۹۱، ۴/۰۸ و ۱۲/۰۲ میکرولیتر بر لیتر هوای اسانس برای لاروها مورد استفاده قرار گرفت. نتایج نشان داد که تلفیق پرتو گاما با اسانس گیاه زنیان میزان مرگ‌ومیر لاروها و حشرات کامل شپشه آرد را در مقایسه با شاهد (تیمارها به‌صورت انفرادی) افزایش داده به‌طوری‌که تأثیر متقابل دز ۵۰۰ گری پرتو با دز ۱۰/۴۷ میکرولیتر بر لیتر هوای اسانس برای حشرات کامل و دز ۱۲/۰۲ میکرولیتر بر لیتر هوای اسانس برای لاروها موجب ایجاد اثر کشندگی ۱۰۰ درصدی به‌ترتیب در ۱۳ و ۸ روز پس از تیمار اولیه گردیده است، اگرچه دز ۵۰۰ گری از پرتو گاما به تنهایی پس از ۲۲ روز موجب مرگ‌ومیر ۱۰۰ درصدی می‌گردد. می‌توان نتیجه گرفت که کاربرد تلفیقی پرتو گاما با اسانس گیاهی می‌تواند موجب ایجاد اثر کنترلی مؤثر شده و جایگزین مناسبی برای گازهای شیمیایی باشد.

واژگان کلیدی: زنیان، اثر تلفیقی، اسانس گیاهی، پرتو گاما، اثر سینرژیستی