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Research Article

Antifeedant effect of gamma radiation and *Perovskia atriplicifolia* essential oil combination against *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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Abstract: Control of stored-product insect by gamma radiation could be enhanced by other possible techniques such as essential oils as effective alternatives to chemical insecticides. In this study, the efficiency of gamma radiation combined with *Perovskia atriplicifolia* (Benth) was verified to assess their enhanced antifeedant effect against the *Tribolium castaneum* (Herbst). Flour disc bioassay was employed to assess the nutritional indices, such as relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion ingested food (ECI) and feeding deterrence index (FDI). The results showed that irradiation, essential oil and combination of both of them significantly reduced RGR, RCR and ECI in treated larvae and adults. As irradiation at 100 Gy reduced RGR of the larvae and adults from 0.0884 and 0.0366 to 0.0596 and 0.0332 mg/mg/day respectively, when combined with doses of *P. atriplicifolia* essential oil, the reduction reached to 0.051 - 0.388 and 0.01- 0.224 mg/mg/day respectively. The reduction rates in larvae were even greater when combination of irradiation and EO treatments were used. The results showed a significant increase in the feeding deterrence due to irradiation, essential oil and specially their combination. Therefore, findings led to a conclusion that irradiation can enhance antifeedant activity of essential oils.

Keyword: *Perovskia atriplicifolia*, relative growth rate, relative consumption rate, efficiency of conversion of ingested food, feeding deterrence index

Introduction

The storage pest *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a major economic pest in flour mills and storage facilities because of its cosmopolitan distribution and affinity for stored grain, especially in tropical and sub-tropical climates (Mills and White, 1994). Under optimum conditions of 25-35°C and 60-80% R.H., the adults and larvae cause serious damage to infested grains. The insects can be found on the surface or at the depth of grain stores (Bagheri Zenouz, 1997). Members of genus *Tribolium* are known to produce toxic quinones which contaminate flour and flour products (Gorham, 1987). Damage is inflicted by both larvae and adults with a reduction in quality and quantity of stored products (Bagheri Zenouz, 1997). To control this pest, fumigants and other chemical insecticides such as methyl bromide and phosphine are widely used. The extensive use of these treatments leads to undesirable residues

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(Mensah *et al.*, 1979) in addition to development of resistance in certain insect species (Rossi *et al.*, 2010). However, methyl bromide was classified as an ozone depleter, and due to environmentally unfavorable effects, its use has recently been banned in many countries (Carpenter *et al.*, 2000). Due to these restrictions new alternative strategies should be investigated. Irradiation and essential oils have become approved and feasible alternatives to the conventional method for controlling stored product pests; because of residue free advantages over chemical fumigation. Irradiation can also extend the shelf life of various products and maintain the quality of the product over a longer period of time (Pszczola, 1997) however depending on the dose of ionizing energy applied; high doses of radiation may affect the quality of foods (Urbain, 1986; Bothaina *et al.*, 2002).

Insecticidal, repellency, anti-nutritional and genotoxic properties are very important in choosing the method to control stored product pests. Insecticidal effect of gamma radiation and also essential oil from *Perovskia atriplicifolia* (Benth) on the red flour beetle *T. castaneum* has been previously described (Brower and Tilton, 1973a, b; Brower *et al.*, 1973; Misra and Paravathy, 1998; Ayvaz *et al.*, 2002; Tuncbilek *et al.*, 2003; Ahmadi *et al.*, 2013). Moreover, genotoxic effect of gamma radiation on genital cells of *T. castaneum* has been reported by Ahmadi *et al.* (2011).

On the other hand, the increasing efforts to develop environmentally friendly pest control methods have attracted the attention of many researchers towards the use of antifeedants (Jermy, 1990). Insect gonads and midgut contain mitotically active tissues, and irradiated insects are often sterile and stop feeding soon after treatment (Follett and Griffin, 2006). Doses of 0.2 to 1.0 kGy of gamma radiation are not immediately lethal, and irradiated insects can survive for several weeks, but they feed less and are usually infertile (Katz and Weaver, 2003). According to Brower and Tilton (1973b), the amount of damage inflicted by live but sterile *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.) adults treated with gamma radiation in bulk grain would probably not be a serious problem because of the great reduction in feeding caused by radiation effects. A wide variety of plants may supply new sources of natural pesticides, antifungals, antifeedants, and repellents (Grainge and Ahmed, 1988; Arnason *et al.*, 1989; Ananthakrishnan, 1992; Cetinsoy *et al.*, 1998; Abbasipour *et al.*, 2011). In search for alternatives to conventional pesticides, the essential oil of *Perovskia* spp has been widely investigated, and showed toxic, repellent and antifeedant effects against stored product pest, red flour beetle (Arabi *et al.*, 2008).

In addition, the application of irradiation has been recommended not only as a possible alternative but also as a supplement for other control methods (Cornwell, 1964). The combination of gamma radiation with other treatments like microwave, infra-red radiation, insecticides and essential oils has also been evaluated by various researchers (Mehta *et al.*, 2004; Tilton *et al.*, 1972; Cogburn and Spiers, 1972; Cogburn *et al.*, 1971; Ahmadi *et al.*, 2013). Ahmadi and Moharramipour, (2011) have reported that combination of gamma radiation and *Rosmarinus officinalis* L. essential oil may result in synergistic interactions that reduce the feeding of *T. castaneum* larvae. Therefore, it seems likely that low doses of gamma radiation combined with essential oil treatment would have antiffedant effect in *T. castaneum* population. This work was undertaken to study the antifeedant action of gamma radiation and *P. atriplicifolia* essential oil combination as a safe control method against different life stages of *T. castaneum*.

Materials and Methods

Insect

Tribolium castaneum was reared on wheat flour mixed with yeast (10:1 w/w). The cultures were maintained in the dark in the growth chamber set at 27 ± 1 ºC and 50 ± 5% R.H. All experiments were conducted under these conditions.

Plant materials

Aerial parts of *P. atriplicifolia* were collected in full bloom stage in Tehran in April 2011. The collected plant materials were dried on laboratory benches at room temperature (23-24 ºC) for 5 days (or until crisp). The dried materials were stored at -24 ºC until processed by hydrodistillation to extract its essential oil.

Extraction of essential oils

Essential oils were extracted from the plant samples according to the method previously described by Negahban and Moharramipour (2007). In brief, using a Clevenger-type apparatus, in which the plant materials were hydrodistilled. Portions of the material (40 g of an air-dried sample, 1: 10 plant / water ratio) were distilled for 4 h. Anhydrous sodium sulfate was used to remove water after the extraction. Extracted oil was stored in refrigerator at 4 ºC.

Irradiation

Irradiation of the tested insects was administered using ^{60}cobalt gamma sources at the Nuclear Science and Technology Research Institute, Nuclear Agriculture Research School, Karaj, at a dose rate of 0.4 Gy/sec. The required dose rate was obtained by exposing treated insects for variable times. To determine lethal doses of gamma radiation, healthy and active adult (1-3 days old) and larvae (5-10 days old) were irradiated by doses of 0, 100, 200, 300, 400 and 500 Gy with five replicates for each dose. All stages were then maintained in the dark in growth chamber.

Combination of gamma radiation and essential oil treatments

Experiments were designed to combine gamma irradiation and essential oil treatments to investigate their combined effects on adults and larvae of *T. castaneum* feeding. Insects were exposed to five doses (100, 200, 300, 400 and 500 Gy) of radiation and flour discs treated with three concentrations of the diluted *P. atriplicifolia* oils with acetone (5.27, 7.56 and 13.11 µl/l air). Each experiment was replicated five times with twenty insects per replicate.

Antifeedant effect of combined irradiation and essential oil treatment

Flour discs were prepared according to the method of Xie *et al.* (1996), in brief 10 g of flour was mixed with 50 ml of distilled water. Using a micropipette, 200 ml of the prepared suspension was poured on a nylon sheet to convert the suspension to spherical discs. The discs were placed in normal room conditions for 4 h. and then transferred to sterile Petri dishes with the help of fine forceps. The flour discs were stored for 12 h. inside the hood to dry completely. In each container 2 discs covered by different doses of oil were placed (acetone used as control), and 20 irradiated insect (larvae or adults) starved for 48 h before exposure. Five replicates of each treatment were used. The weight of the flour discs and insects were accurately measured and recorded at the beginning and at 3 days post start of the experiment.

Nutritional indices were calculated according to the formula of Manuwoto and Scriber, (1992); Farrar *et al.* (1989) and Isman *et al.* (1990), (Cited by Huang *et al.*, 1997) with modifications as follows:

Relative Growth Rate (RGR) = (A - B) / (B × DAY)

Where A weight of live insects on the third day (mg) / number of insects on the third day

B = initial weight of insects (mg)/initial number of insects

Relative Consumption Rate (RCR) = D/(B × day),

Where D = biomass ingested (mg)/number of live insects on the third day

Efficiency of Conversion of Ingested Food (ECI%) = RGR/RCR × 100

Feeding Deterrence Index (FDI%) = (C-T)/C × 100,

Where C is consumption of control discs, and T is consumption of treated discs.

Statistical analysis

The dosage mortality response was determined by probit analysis (Finney, 1971). Before the statistical analysis, ECI and FDI were normalized. ANOVA and Tukey's HSD test were employed using the SPSS software to compare the means.

Results

Irradiation

The RGR, RCR, ECI and FDI values of irradiated adults and larvae of *T. castaneum* fed on flour discs for 72 h are given in Fig. 1. The results of the antifeedant test indicate that the rates of RGR and RCR indices in irradiated larvae and adults were significantly lower than those of non-irradiated ones (P < 0.05).

At a dose of 100 Gy, RGR of adults was reduced from 0.0366 to 0.0332 mg for each mg of insect weight comparing to the control (9.2% reduction rate), whereas administering the same dose for the larvae caused, 32.57% reduction rate (reduction from 0.0884 to 0.0596 mg mg-1, daily). There were 13.11, 12.02, 18.30 and 21.03% reduction rates in the RGR in adults and 32.35, 37.44, 40.72 and 44.34% reduction in larvae exposed to 200, 300, 400 and 500 Gy of gamma ray respectively. There was no significant difference in the RGR reduction rate in adults between the exposure to 200 and 300 Gy doses. Also the exposure of larvae to 100 and 200 Gy doses did not show significant difference in the RGR reduction rate (Fig. 1). The results show that 2.32, 2.26, 0.97, 3.46 and 5.01% reductions in the RCR of the adults were caused by the exposure to 100, 200, 300, 400 and 500 Gy of gamma ray respectively while these reduction rates in larvae were 38.62, 38.97, 46.90, 48.03 and 53.68% respectively. In addition, it was shown that comparative effects of 100 and 200 Gy doses on the RCR of the adults and larvae were not significant.

Moreover, with the exception of 500 Gy dose of gamma ray treatment which caused 1.85% reduction in the ECI of the exposed larvae, the other doses increased the ECI rate. On the other hand in adults, the ECI significantly (P<0.05) decreased by increasing irradiation doses (7.14, 5.79, 18.73, 15.34 and 17.92% reduction rates by using of 100, 200, 300, 400 and 500 Gy doses, respectively). The data shows that gamma radiation has significant effect on the FDI of treated larvae and adults. The FDI of the adults and larvae increased from 0 to 2.39 and 60.9% respectively after exposure to 100 Gy of gamma ray. Generally the FDI amount increased by exposure to gamma ray, and the effect value among the larvae was much greater than that for the adults (Fig. 1).

Essential oil

The RGR significantly (P < 0.05) declined at all *P. atriplicifolia* essential oil concentrations used in this study. The treatment with the essential oil at doses of 5.27, 7.65 and 13.11 µl/l caused 41, 45.7 and 54.2% in larvae and 33.9, 34.4 and 67.7% reduction in adults respectively. This result indicates that the reduction rate gradually increased with increasing oil concentration. The results also indicate that the RCR in treated larvae and adults decreased significantly (P < 0.05) with increasing oil concentration. The reduction rate of RCR was higher in larvae than adults. In contrast, the same doses, significantly reduced the ECI in adults (30.7, 32.5 and 53.9%) more than in larvae (16.3, 16.9 and 22.49%) respectively. Similar to the RGR trend, the reduction rate of the ECI increased with increasing the oil concentrations (Fig. 2). The FDI values of the treated larvae and adults with essential oil increased with increasing the concentration. At doses of 5.27, 7.65 and 13.11 µl/l the FDI increased from 0 to 35.05, 45.12 and 49.55 in larvae and to 21.25, 28.22 and 35.45 in adults respectively. The data indicate that the increasing rate of the FDI is higher in treated larvae than adults (Fig. 2).

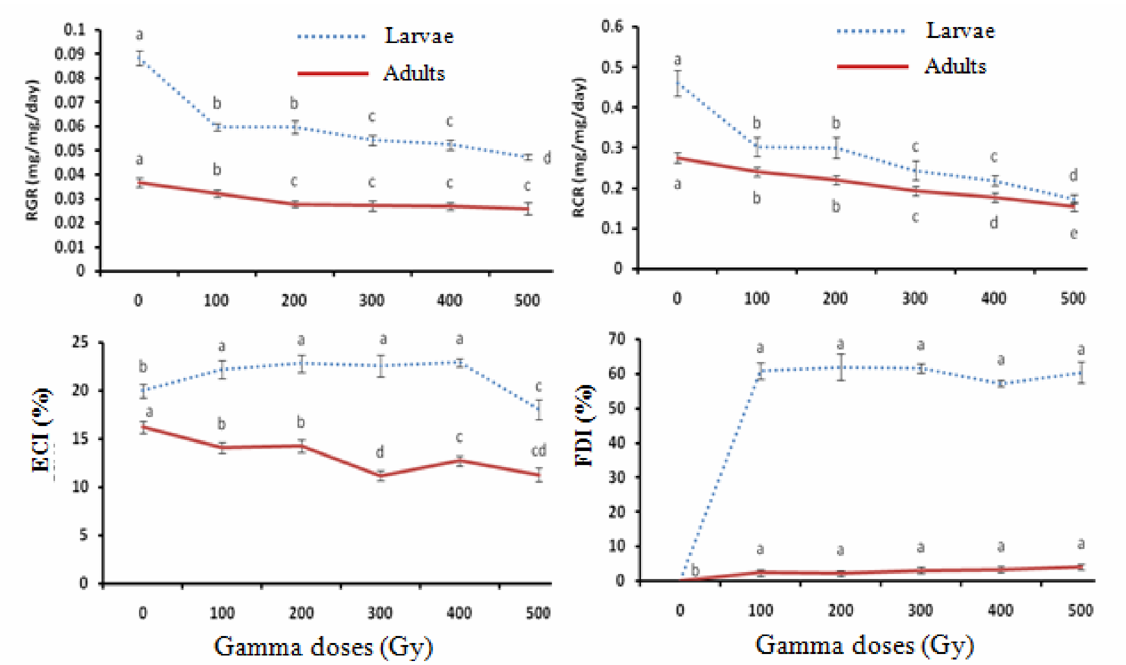

Figure 1 Effects of gamma radiation on relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion ingested food (ECI) and feeding deterrence index (FDI) of *Tribolium castaneum* adults and larvae. Means followed by the same letters in each line are not significantly different (Tukey's HSD test at 5% level). Vertical bars indicate standard error of mean.

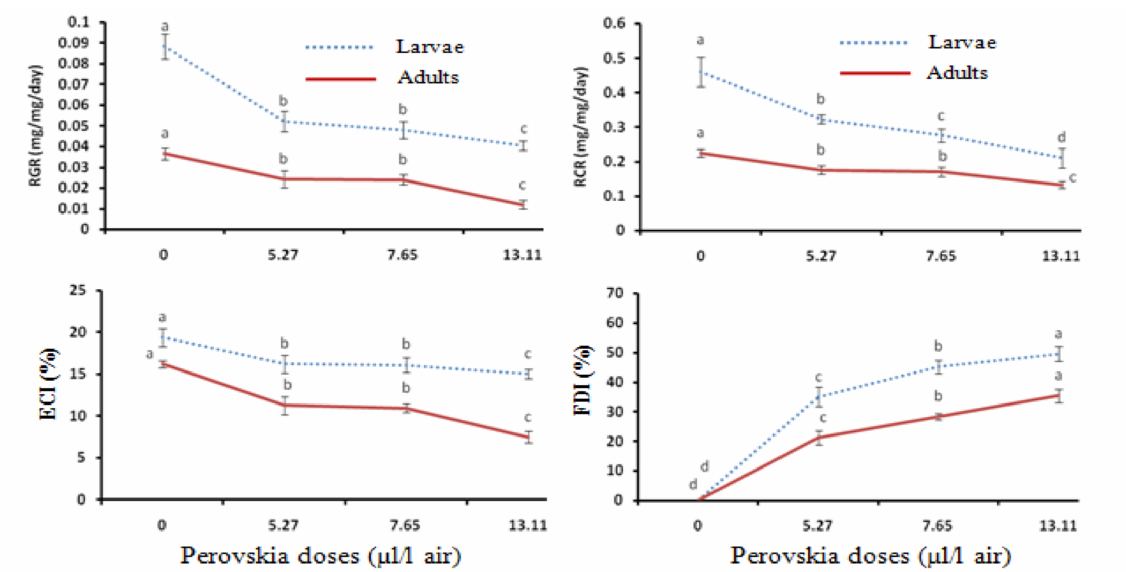

Figure 2 Effects of *Perovskia atriplicifolia* essential oil on relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion ingested food (ECI) and feeding deterrence index (FDI) of *Tribolium castaneum* adults and larvae. Means followed by the same letters in each line are not significantly different (Tukey's HSD test at 5% level). Vertical bars indicate standard error of mean.

Combination of radiation and essential oil
The antifeedant effect of gamma radiation in combination with *P. atriplicifolia* on *T. castaneum* increased markedly with an increase of the doses. As the exposure to 100 Gy reduced RGR of the larvae and adults from 0.0884 and 0.0366 to 0.0596 and 0.0332 respectively, while when combined with doses of *P. atriplicifolia* essential oil the reduction rate reached 0.051-0.388 and 0.224-0.01 respectively. These combinations especially at high doses were found to be appropriate for the purpose of the experiments (Tables 1 and 2). In all irradiation doses (100, 200, 300, 400 and 500 Gy), significant reduction in the RGR of the larvae and adults occurred when they combined with 13.11 µl/l of oil (34.2, 45.6, 47.9, 57.1 and 48.1% in the larvae and 69.8, 66.9, 67.4, 71.57 and 74.1% in the adults respectively). The results pointed out those adults had more reduction rate in their RGR compared with larvae. In the RCR of the treated larvae, low and high reductions were observed in combination of 200 Gy-5.27 µl/l and 200 Gy-13.11 µl/l respectively. Also in the treated adults, low and high reductions were observed in combination of 100 Gy-5.27 µl/l and 500 Gy-13.11 µl/l respectively. Combined doses of the gamma radiation-*P. atriplicifolia* essential oil which induced low and high reduction of the ECI were 100 Gy-5.27 µl/l and 400 Gy-13.11 µl/l for the larvae and 100 Gy-5.27 µl/l and 200 Gy-13.11 µl/l for the adults, respectively (Tables 1 and 2). The results showed that the combined treatments had greater effect on the ECI of the adults than the larvae. On the other hand, combined dose of 400 Gy-13.11 µl/l of gamma radiation and essential oil increased the FDI of larvae from 57.3 to 93.22. Also combined doses of 500 Gy-13.11 µl/l increased the FDI of the adults from 4.01 to 69.5. These results demonstrate the high increasing of the FDI among all of the combination treatments (Tables 1 and 2). In all experiments, the antifeedant effects of gamma radiation or essential oil separately were lower than the antifeedant rate of combination of these two treatments.

Discussion

Irradiation
Based on the results presented in this study, it was determined that gamma radiation has significant effects on the nutritional indices of *T. castaneum* at different doses and it was more effective on the larvae compared with the adults. The RGR and RCR indices were significantly reduced with irradiation; furthermore, the reduction rate was greater among larvae than adults. These differences could be due to high feeding rate of the treated larvae and their high sensitivity to gamma radiation compared with the treated adults. No significant differences were observed between the effect of 100 and 200 Gy doses on the RGR in larvae or 200 and 300 Gy in adults which indicates that the growth response of larvae and adults against different doses of radiation are different. In addition, the effect of radiation on the adult RCR was not significant which could be explained by the low feeding of irradiated and non-irradiated adults in comparison with the larvae. The effect of gamma irradiation on the ECI in larvae and adults was different to some extent. Except 500 Gy, in other doses the ECI percentage in the larvae was increased, whereas in the adults the use of gamma radiation caused significant decrease in amount of the ECI. It can be inferred that irradiation causes high reduction in the larval feeding, then the larvae which have a small part of food attempt to increase the ECI.

Table 1 Combined antifeedant effect of gamma radiation and *Perovskia atriplicifolia* essential oil on *Triboilum castaneum* larvae.

Essential oil (µl / Disk)	Gamma (Gy)	Mean ± SE[1]			
		RGR (mg/mg/day)	RCR (mg/mg/day)	ECI (%)	FDI (%)
0	0	0.0884 ± 0.0029 a	0.4603 ± 0.0321 a	19.38 ± 1.15 bc	0 j
5.27	0	0.0521 ± 0.005 cd	0.3224 ± 0.0122 b	16.21 ± 1.05 d	35.05 ± 3.25 i
7.65	0	0.0480 ± 0.0041 def	0.2755 ± 0.0196 bcd	16.1 ± 1.08 d	45.12 ± 2.2 h
13.11	0	0.0405 ± 0.0022 hi	0.2105 ± 0.0286 e	15.05 ± 1.55 def	49.55 ± 2.45 h
0	100	0.0596 ± 0.002 b	0.2825 ± 0.0232 bc	21.22 ± 1.15 ab	60.90 ± 2.42 g
5.27	100	0.0510 ± 0.0025 cd	0.2322 ± 0.011 bcde	16.05 ± 0.95 de	78.30 ± 2.85 f
7.65	100	0.0420 ± 0.0013 ghi	0.1825 ± 0.0122 cde	15.58 ± 1.28 de	80.66 ± 3.22 ef
13.11	100	0.0388 ± 0.0028 i	0.1524 ± 0.0145 cde	14.25 ± 1.05 defg	88.23 ± 3.25 bcd
0	200	0.0598 ± 0.0036 b	0.2809 ± 0.0266 bc	21.83 ± 1.92 a	62.01 ± 3.71 g
5.27	200	0.0485 ± 0.0025 def	0.231 ± 0.0215 bcde	15.55 ± 0.75 de	79.45 ± 4.22 ef
7.65	200	0.0455 ± 0.0025 efg	0.1752 ± 0.0125 cde	14.65 ± 0.85 def	82.10 ± 3.56 def
13.11	200	0.0325 ± 0.0015 j	0.1422 ± 0.0225 e	14.2 ± 0.95 defg	85.15 ± 2.85 cde
0	300	0.0553 ± 0.0031 bc	0.2444 ± 0.0236 bcde	21.56 ± 1.68 a	61.65 ± 2.24 g
5.27	300	0.0440 ± 0.0028 fgh	0.1850 ± 0.0189 cde	14.55 ± 0.85 def	85.12 ± 3.21 cde
7.65	300	0.0325 ± 0.0026 j	0.1820 ± 0.0188 cde	14.25 ± 1.57 defg	89.36 ± 4.21 abc
13.11	300	0.0288 ± 0.0024 jk	0.1385 ± 0.0222 e	13.85 ± 0.89 efg	92.25 ± 3.05 ab
0	400	0.0524 ± 0.0023 cd	0.2392 ± 0.0123 bcde	21.93 ± 0.43 a	57.30 ± 0.89 g
5.27	400	0.0445 ± 0.0025 efgh	0.1888 ± 0.0135 cde	14.21 ± 0.65 defg	89.63 ± 3.55 abc
7.65	400	0.0332 ± 0.0011 j	0.1756 ± 0.0111 cde	14.05 ± 0.85 defg	89.95 ± 4.55 abc
13.11	400	0.0225 ± 0.0018 l	0.1355 ± 0.0215 e	13.1 ± 0.58 fg	93.22 ± 4.33 ab
0	500	0.0492 ± 0.0011 de	0.2132 ± 0.0114 bcde	19.02 ± 1.05 c	60.42 ± 3.09 g
5.27	500	0.0325 ± 0.0022 j	0.1765 ± 0.0205 cde	13.25 ± 1.55 fg	88.50 ± 4.33 bc
7.65	500	0.0333 ± 0.0036 j	0.1655 ± 0.0233 de	12.25 ± 0.78 g	91.22 ± 5.24 abc
13.11	500	0.0255 ± 0.0036 kl	0.1345 ± 0.0195 e	12.10 ± 1.25 g	95.25 ± 5.05 a

[1] Means followed by the same letters within a column are not significantly different (Tukey's HSD test at 5% level).

Table 2 Combined antifeedant effect of gamma radiation and *Perovskia atriplicifolia* essential oil on *Triboilum castaneum* adults.

Essential oil (µl / Disk)	Gamma (Gy)	Mean ± SE[1]			
		RGR (mg/mg/day)	RCR (mg/mg/day)	ECI (%)	FDI (%)
0	0	0.0366 ± 0.0019 a	0.2253 ± 0. 01 a	16.23 ± 1.43 a	0 m
5.27	0	0.0242 ± 0.004 d	0.1755 ± 0.0117 b	11.25 ± 1.05 cd	21.25 ± 2.33 k
7.65	0	0.0240 ± 0.0025 d	0.1699 ± 0.0129 bc	10.95 ± 1.35 d	28.22 ± 1.05 j
13.11	0	0.0118 ± 0.0011 g	0.1322 ± 0.01 def	7.48 ± 0.88 efg	35.45 ± 2.25 i
0	100	0.0332 ± 0.0016 b	0.2199 ± 0.027 a	15.07 ± 1.27 ab	2.39 ± 0.53 lm
5.27	100	0.0224 ± 0.0015 de	0.1725 ± 0.01 bc	9.25 ± 1.55 de	39.45 ± 1.25 h
7.65	100	0.0219 ± 0.0016 de	0.1555 ± 0.019 bcd	8.25 ± 1.58 ef	41.25 ± 1.66 h
13.11	100	0.010 ± 0.0014 gh	0.1185 ± 0.012 efgh	5.55 ± 0.63 ghi	46.35 ± 2.33 g
0	200	0.0318 ± 0.0025 bc	0.2202 ± 0.019 a	15.29 ± 2.46 ab	2.11 ± 0.13 lm
5.27	200	0.0205 ± 0.0018 e	0.1666 ± 0.018 bc	8.65 ± 0.58 e	41.25 ± 1.59 h
7.65	200	0.0195 ± 0.001 e	0.1325 ± 0.018 def	5.33 ± 0.65 ghi	45.85 ± 2.25 g
13.11	200	0.0105 ± 0.0012 gh	0.1300 ± 0.011 def	4.26 ± 0.75 hi	51.25 ± 2.33 f
0	300	0.0322 ± 0.0021 bc	0.2231 ± 0.015 a	13.19 ± 1.25 bc	3.03 ± 0.89 lm
5.27	300	0.0215 ± 0.0019 de	0.1450 ± 0.018 cde	6.33 ± 0.87 fgh	48.88 ± 1.25 fg
7.65	300	0.0156 ± 0.0018 f	0.1255 ± 0.016 efg	5.23 ± 0.85 hi	55.35 ± 3.56 e
13.11	300	0.0105 ± 0.0015 gh	0.1125 ± 0.015 fghi	5.20 ± 0.65 hi	59.25 ± 2.45 d
0	400	0.0299 ± 0.0014 bc	0.2175 ± 0.014 a	13.74 ± 1.21 b	3.32 ± 0.61 lm
5.27	400	0.0115 ± 0.0015 g	0.1133 ± 0.015 fghi	6.25 ± 0.94 fgh	52.23 ± 1.22 ef
7.65	400	0.0090 ± 0.0008 gh	0.11 ± 0.014 fghi	4.29 ± 0.85 hi	61.25 ± 2.22 cd
13.11	400	0.0085 ± 0.0021 gh	0.0955 ± 0.018 hi	4.12 ± 0.83 hi	63.44 ± 3.85 bc
0	500	0.0289 ± 0.0025 c	0.2140 ± 0.011 a	13.32 ± 2.51 b	4.01 ± 0.56 l
5.27	500	0.0119 ± 0.0013 g	0.1055 ± 0.016 fghi	4.25 ± 0.45 hi	61.25 ± 2.33 cd
7.65	500	0.0095 ± 0.0008 gh	0.0985 ± 0.017 ghi	3.82 ± 0.55 i	66.33 ± 4.25 ab
13.11	500	0.0075 ± 0.0017 h	0.08555 ± 0.018 i	3.25 ± 0.25 i	69.5 ± 3.05 a

[1] Means followed by the same letters within a column are not significantly different (Tukey's HSD test at 5% level).

The results also showed that gamma radiation had feeding deterrence effects on either adults or larvae of *T. castaneum* and the effect on FDI was higher in larvae than adults; this fact illustrates that with the lowest dose of gamma radiation, sharp reduction on feeding of the larvae, (the most active stage) will be produced. Reduction in the feeding by stored-product beetles has been observed after radiation treatments, with a corresponding decrease in feeding damage (Cornwell, 1964; Watters and MacQueen, 1967; Brower and Tilton, 1973a, b). Johnson and Vail (1988, 1989) reported that feeding and damage caused by navel orange worm larvae could be reduced by as much as 78% after 300 Gy treatments; this treatment in our study reduced the feeding of larval stages of *T. castaneum* about 61.65%.

Antifeedant effects of gamma radiation can likely be due to insect's physiology and sensitivity of cells to irradiation. Sensitivity of any tissue to radiation is dependent on the degree of cellular development activity and differentiation (Bergonie and Tribondeau, 1959). Cells of the midgut epithelium of feeding insects are frequently replaced by undifferentiated regenerative cells. Radiations usually destroy these regenerative cells resulting in histolysis of the midgut epithelium (Ashraf *et al.,* 1971), which can affect the feeding amount of irradiated insects.

Essential oil

This study demonstrates that the essential oil of *P. atriplicifolia* had toxic and significant effect on the nutritional indices of the larvae and adults of *T. castaneum* at different doses. *P. atriplicifolia* essential oil can significantly reduce the RGR and RCR of the adults and larvae, also the treated larvae showed more sensitivity to the oil effect compared with the treated adults. There are several reports on antifeedent activity of essential oils. Huang and Ho (1998) reported the antifeedant action of the *Cinnamomun aromaticum* Nees, on the red flour beetle adults and larvae. Also Abbasipour *et al.* (2011) stated that *Datura stramonium* L. (Solanaceae) extract had significant effects on the nutritional indices and mortality of *T. castaneum* adults at different concentrations. They showed that this oil significantly reduced the growth rate and food consumption and utilization of *T. castaneum* adults at higher concentrations. Negahban and Moharramipour (2007) also verified that *Artemisia scoparia* Waldst. & Kit and *Artemisia sieberi* Besser extracts had antifeedant property on *T. castaneum* adults. These studies confirm the validity of our experiments however, *P. atriplicifolia* essential oil had a more antiffedant effect on *T. castaneum* than did *C. aromaticum* and *D. stramonium* but, it's inhibition of feeding was less than that of *A. sieberi* and *A. scoparia*. Although contact toxicity of *P. atriplicifolia* was verified (Ahmadi *et al.,* 2013) and its EO was shown to affect the feeding of the treated larvae and treated adult of *T. castaneum*, however the applied doses were high. Therefore it may not be feasible to apply high just doses of this essential oil for insect control purposes.

Combination of radiation and essential oil

Similar to other food processing methods, irradiation can make certain changes in the commodity that can modify the composition and nutritive values of food (Wiendl, 1984). The use of low irradiation doses combined with other treatments is a way to minimize the negative effects of irradiation on food quality (Dionisio *et al.,* 2009). The study of Ahmadi *et al.* (2013) indicated that *P. atriplicifolia* essential oil could be combined with gamma radiation to enhance the mortality effect of irradiation. They showed that the effect of the essential oil and gamma radiation can be increased and the doses can be reduced. The results demonstrated when a combination of *P. atriplicifolia* and gamma radiation was employed, insect mortality increased not only by combined toxicity of both treatment but also by their adverse effects on *T. castaneum* feeding behavior.

The antifeedant effects of some combined treatments to control the insects have been previously reported. Huang *et al.* (2002) specified that the toxicity of the deltamethrin could be enhanced to kill insects effectively. When a mixture of deltamethrin and eugenol or one of its analogues is applied, insects die due to the combined toxic and antifeedant effects. Moreover, the study of Ahmadi and Moharramipour (2011) indicated that the combination of gamma radiation and *R. officinalis* essential oil could reduce the feeding of *T. castaneum* larvae. They showed that relative growth rate had significantly decreased ($P < 0.05$) by combining the gamma radiation and *R. officinalis* and that the severity of the reduction rate increased by increasing doses. Also relative food consumption rate decreased when gamma radiation and *R. officinalis* were combined and its reduction severity had convert relative with increasing of doses. This study demonstrates the validity of our results. Our data suggest that a combination of treatments with essential oils and irradiation can play a major role in management of stored-product insects. However, the vapor pressure of essential oils is too low to enable them to penetrate into commodities to kill pests, and the treatment can be employed only on a small scale. In order to make practical use of essential oils, these compounds should be formulated. The present study shows that *P. atriplicifolia* essential oil when applied with gamma radiation can control *T. castaneum* by killing its two active and harmful life stages by means of fumigant and antifeedant actions.

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تأثیر ضد تغذیه‌ای تلفیق پرتو گاما با اسانس برازمبل *Perovskia atriplicifolia* روی شپشه آرد *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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چکیده: کنترل آفات انباری با استفاده از پرتو گاما می‌تواند با کمک سایر روش‌ها مانند اسانس‌های گیاهی که خود جایگزینی برای آفت‌کش‌های شیمیایی می‌باشند، بهبود یابد. در این تحقیق، کارایی تلفیق پرتو گاما با اسانس برازمبل *Perovskia atriplicifolia* به‌منظور افزایش تأثیر ضدتغذیه‌ای آنها روی شپشه آرد *Tribolium castaneum* مورد ارزیابی قرار گرفت. جهت ارزیابی شاخص‌های تغذیه‌ای مانند: نرخ رشد نسبی (RGR)، نرخ مصرف نسبی (RCR)، کارایی غذای خورده شده (ECI) و شاخص بازدارندگی تغذیه‌ای (FDI)، از آزمایش زیست‌سنجی با کمک دیسک‌های آردی استفاده گردید. نتایج نشان داد که پرتو گاما، اسانس برازمبل و نیز تلفیق این دو روش مقدار RGR، RCR و ECI را در لارو و حشرات کامل به‌طور معنی‌داری کاهش می‌دهند. به‌طوری‌که دز ۱۰۰ گری از پرتو گاما مقدار RGR لاروها و حشرات کامل را از ۰/۰۸۸۴ و ۰/۰۳۶۶ میلی‌گرم بر میلی‌گرم وزن حشره به ۰/۰۵۹۶ و ۰/۰۳۳۲ میلی‌گرم بر میلی‌گرم وزن حشره کاهش می‌دهد، این درحالی است که تلفیق این دز پرتو با اسانس‌های گیاهی میزان کاهش را تا ۰/۳۸۸-۰/۰۵۱ و ۰/۲۲۴-۰/۰۱ میلی‌گرم بر میلی‌گرم وزن حشره می‌رساند. هم‌چنین مشخص گردید که شدت و میزان کاهش در حالت تلفیق در لاروها نسبت به حشرات کامل شپشه آرد شدیدتر است. نتایج نشان داد که مقدار FDI لاروها و حشرات کامل در اثر پرتو گاما، اسانس برازمبل و نیز تلفیق هر دو روش، افزایش معنی‌داری داشته است. هم‌چنین شدت افزایش در FDI در حالت تلفیق بیشتر از زمانی است که هر دو روش به تنهایی مورد استفاده قرار گرفته بودند. بنابر این از یافته‌های به‌دست آمده می‌توان دریافت که پرتودهی می‌تواند اثرات ضدتغذیه‌ای اسانس‌های گیاهی را افزایش دهد.

واژگان کلیدی: برازمبل، نرخ رشد نسبی، نرخ مصرف نسبی، کارایی غذای خورده شده، شاخص بازدارندگی تغذیه