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Research Article

Interaction between essential oil of *Rosmarinus officinalis* and gamma radiation against *Callosobruchus maculatus*

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Abstract: In order to examine possible integration between irradiation and botanical pesticides, combined effects of essential oil from *Rosmarinus officinalis* L. and gamma radiation were determined on mortality of *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). Experiments were conducted by pretreatment with essential oil fumigation followed by irradiation and inversely, pretreatment with irradiation followed by essential oil fumigation. The mortality rate was assessed 72 h after last treatment. Integration of gamma radiation and *R. officinalis* oil enhanced the mortality of *C. maculatus* compared with control treatments of either irradiation or fumigation alone. Synergistic effects of mortality on 1-2 days old adults of *C. maculatus* were observed when exposed to combination of gamma radiation and essential oil. It was found that pretreatment with fumigation followed by irradiation was perfectly effective. The study showed that either of the pre-treatments could enhance the susceptibility of the pest to the other treatment. Our findings led to a conclusion that the combination of gamma radiation and *R. officinalis* oil fumigation has a potential for application in integrated management of *C. maculatus*.

Key words: Irradiation, fumigant toxicity, medicinal plants, *Callosobruchus maculatus*, synergism

Introduction

Callosobruchus maculatus F. (Coleoptera: Bruchidae) is the major insect pest of cowpea seed in Iran causing low yield and poor seed quality (Bagheri Zenouz, 1986). In order to control this pest, insecticides and fumigants is widely used for disinfestation of stored products. However, the wide use of these fumigants has led to the development of resistance in certain species (Armes *et al*., 1997). Since the application of these chemicals becomes increasingly restricted, integrated pest management (IPM) strategies where various techniques are combined to reduce the pest density have been developed as an environmentally safer alternative. Irradiation and essential oil utilization are two methods that could be used in IPM (Sharma and Seth, 2005). Both genera *Bruchus* and *Callosobruchus*, the main stored-product pests, are observed to be sensitive to irradiation (Diop *et al*., 1997). Roy and Prasad (1993) noticed that a dose of 1 kGy of gamma radiation killed all the adults of *Callosobruchus chinensis* L. within a week. There are several reports on insecticidal effects of Rosmary oil on stored-product pests. Clemente *et al*. (2003) observed that extracts of *R. officinalis* have an insecticidal effect on *Tribolium castaneum* (Herbst). In addition, Ahmadi *et al*. (2008a) have

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reported that *R. officinalis* essential oil could be utilized to control *T. castaneum* due to its fumigant toxicity.

The application of irradiation has been recommended not only as a possible alternative but also as a supplement for other control methods (Cornwell, 1966). The combination of gamma radiation with other treatments like microwave, infra-red radiation and insecticides 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*. (2008b) discovered that combination of gamma radiation with essential oils of *Perovskia atriplicifolia* may result in synergistic interactions that would enhance the potential for control of *T. castaneum*. Therefore, it seems likely that low doses of gamma radiation combined with an essential oil would enhance mortality in *C. maculatus* population. In this study, we examined interactions between these two treatments with the aim of reducing the cost of effective management of *C. maculatus* under different conditions by means of lower doses of gamma radiation and lower concentrations of essential oils.

Materials and Methods

Plant materials

Foliage of *R. officinalis* were collected at full flowering stage from Tehran in April 2010. The plant material was dried out naturally on laboratory benches at room temperature (23-24 ºC) for 5 days. The dried material was stored at -24 ºC until used.

Extraction of essential oils

Essential oils were extracted from the dried plant samples using a Clevenger-type apparatus where the plant material was subjected to hydrodistillation as described by Negahban *et al*. (2007). Portions of the material (40 g of an air-dried sample, 1: 10 plant/water ratio) were distilled for 4 h. Anhydrous sodium sulphate was used to remove water after extraction. The extracted oil was stored in a refrigerator at 4 ºC.

Insect rearing

C. maculatus was reared on mung bean. The adult insects (1-2 days old) were used for fumigant toxicity and gamma radiation. The cultures were maintained in growth chamber set at 28 ± 1 ºC, 60 ± 5% r. h and in darkness. All experiments were carried out under the same environmental conditions.

Irradiation

The insects were irradiated by means of a ^{60}Co-gamma source with a dose rate of 0.4 Gy/sec. The required dose rate was obtained by varying time of exposure of the insects. The present experiment required the use of gamma radiation at several doses sufficient to result in a reasonable range of mortality among the adults. To determine the LD_{10}, LD_{20} and LD_{50} values of irradiation, the healthy and active adult insects (1-2 days old) were irradiated with doses between 25 and 300 Gy (25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275 and 300 Gy). For each dose the experiment was replicated five times and each replicate consisted of 50 adults. Subsequently, the insects were kept in glass vials with perforated lids. The controls were similarly maintained but were not subjected to gamma radiation. The insects were then maintained in the dark in a growth chamber. The mortality rate was observed 72 h after treatment.

Fumigant toxicity

In this experiment, filter paper (2 cm in diameter) was impregnated with oil and then it was attached under the surface of the screw cap of a 280 ml glass vial (Negahban *et al*., 2007). The cap was screwed tightly on the vial containing 50 adults (1-2 days old). Five replicates were created for the treated and controlled adults. A series of dilutions was prepared to evaluate the mortality of the insects after an initial dose setting experiment. Concentrations of the oil tested were 0.3-7.5 µl/l air (0.3, 0.5, 0.8, 1, 1.2, 1.8, 2.2, 2.8, 3.2, 3.8, 4.2, 4.8, 5.2, 5.8, 6.2, 6.8, 7.2, 7.5 µl/l air). In this way, the mortality for each dose was investigated independently. The insects were exposed to the oil for 24 h and the number of dead and live insects in each vial was counted 48 h after fumigation. When no leg or antennal movements were observed, the insects were considered dead. Probit analysis (Finney, 1971) was carried out to estimate LD_{10}, LD_{20} and LD_{50} values.

Interaction of gamma radiation and essential oil

The interaction experiments of gamma radiation and essential oil on the adults of *C. maculatus* were set up as follows:

In the first experiment, where irradiation was used after fumigation by essential oil, 1-2 days old adults were exposed to *R. officinalis* (close to LC_{50}). The insects were then treated with different doses of gamma radiation within 24 and 48 h after fumigation. In the second experiment the insects were first treated with gamma radiation (close to LD_{50}) and then the survivors were subsequently exposed to essential oil doses after 24 and 48 h. In the third experiment, the irradiated insects were immediately exposed to the *R. officinalis* oil. Each experiment was conducted with five replications consisting of 50 insects each. The percentage of insect mortality was calculated 72 h after the first treatment, using the Abbott (1925) correction formula for natural mortality in the untreated control.

Calculation of synergistic effects

Synergistic effect in combination with gamma radiation and essential oil was calculated by the following formula (Berenbaum, 1989):

$$S = \frac{d_a}{D_a} + \frac{d_b}{D_b}$$

S = synergistic effect
S > 1: antagonism
S = 1: additive
S < 1: synergism

where d_a and d_b are the concentrations of each treatment (gamma radiation, essential oil utilization) used in combination mode. D_a and D_b are their single concentrations reaching the same effect level when administered alone.

Results

Effects of different levels of gamma radiation on *C. maculatus* show that 1-2 days old adults were susceptible to irradiation. Doses of 48, 78 and 194 Gy caused 10%, 20% and 50% mortality 3 days after irradiation, respectively (Table 1). In the control no mortality was observed during that period of experiment. There was a significant difference in the mortality of the irradiated adults at different levels of irradiation. Mortality was increased with the increasing doses. The results indicated that the doses higher than 300 Gy caused complete mortality (100%).

Table 1 Regression parameters of probit analysis for mortality of the adults *Callosobruchus maculatus* exposed to gamma radiation and essential oil of *Rosmarinus officinalis*.

Probit parameters	Gamma radiation	*Rosmarinus officinalis*
n	250	200
Slope ± SE	2.117 ± 0.377	3.544 ± 0.584
χ2 (df)	2.739 (3)	2.780 (3)
P-value	0.433	0.427
LD_{10} (95% fiducial limits)	48 (Gy) (25.81-66.53)	1.38 (µl/l air) (0.89-1.74)
LD_{20} (95% fiducial limits)	78 (Gy) (52.43-97.37)	1.83 (µl/l air) (1.34-2.19)
LD_{50} (95% fiducial limits)	194 (Gy) (161.36-254.42)	3.17 (µl/l air) (3.04-3.94)

The biological activity of *R. officinalis* oil showed that LC_{10}, LC_{20} and LC_{50} values were 1.38, 1.83 and 3.17 µl/l air respectively (Table 1). In this experiment, the mortality rate was noticeably increased as the doses of essential oil increased.

Statistical analyses for these 3 experiments as regards the combination of gamma radiation with *R. officinalis* oil are summarized in Tables 2 and 3. The results showed a significant synergistic effect between oil concentration and gamma treatment (Tukey, $P < 0.05$) especially when gamma radiation was used 48 h after fumigation (S = 0.59 and S = 0.63). In the presence of gamma radiation, there was increasing proportional mortality with increasing *R. officinalis* concentration (Fig. 1). The synergistic effect values (S) of the interaction between these two factors are listed

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for each experiment (Tables 2, 3). In most of S values of the interactions, S < 1 indicates confidence in the significance of the interactions. S values for using gamma radiation and fumigation simultaneously ranged between 0.66-0.83, 0.66-0.83 for using irradiation 24 h after fumigation and also 0.59-0.75 for administering irradiation 48 h after fumigation. Based on lower LC values, using gamma radiation 48 h after fumigation was more effective than other cases (Fig. 1).

Table 2 Synergistic effect of *Rosmarinus officinalis* on gamma irradiated adults of *Callosobruchus maculates.*

Gamma radiation (Gy)	Essential oil concentration (µl/l air)	S (Synergism)
48	1.38 (1)	1.05
	1.38 (2)	1.05
	1.38 (3)	0.92
	1.83 (1)	0.90
	1.83 (2)	1
	1.83 (3)	0.81
78	1.38 (1)	1.04
	1.38 (2)	1.04
	1.38 (3)	0.83
	1.83 (1)	1.08
	1.83 (2)	0.88
	1.83 (3)	0.88

1. Use of irradiation and fumigation simultaneously.
2. Irradiation 24 h before fumigation with essential oil.
3. Irradiation 48 h before fumigation with essential oil.
S = synergistic effect: S > 1: antagonism, S = 1: additive, S < 1: synergy.

Table 3 Synergistic effect of gamma radiation on Fumigated adults of *Callosobruchus maculatus* by *Rosmarinus officinalis.*

Essential oil concentration (µl/l air)	Gamma radiation (Gy)	S (Synergism)
1.38	48 (1)	0.83
	48 (2)	0.83
	48 (3)	0.75
	78 (1)	0.66
	78 (2)	0.66
	78 (3)	0.59
1.83	48 (1)	0.66
	48 (2)	0.66
	48 (3)	0.66
	78 (1)	0.78
	78 (2)	0.70
	78 (3)	0.63

1. Use of irradiation and fumigation simultaneously.
2. Irradiation 24 h after fumigation with essential oil.
3. Irradiation 48 h after fumigation with essential oil.
S= S = synergistic effect: S > 1: antagonism, S = 1: additive, S < 1: synergy.

Concentrations 1.38 and 1.83 µl/l air of *R. officinalis* given without the irradiation caused death of 10% and 20% of the adults. However, when they were combined with irradiation (48 and 78 Gy which individually caused only 10% and 20%) after 24 h, the mortality increased to 40%, 55% and 53%, 61.25% respectively. Also when irradiation was administered 48 h after fumigation, the mortality percentages increased to 47%, 55.25% and 59%, 65% respectively (Fig. 1).

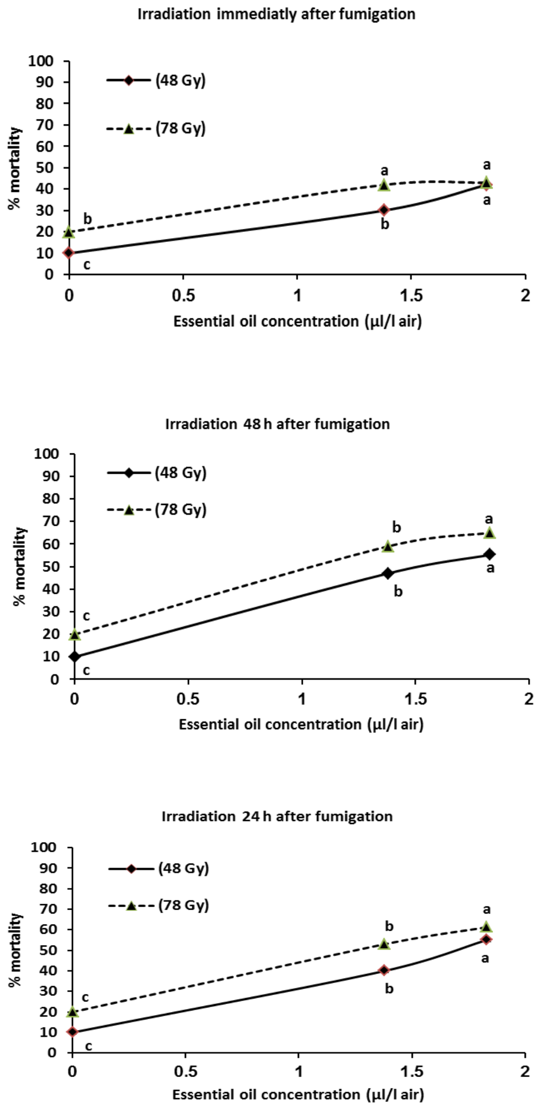

Figure 1 Effects of pretreatment with *Rosmarinus officinalis* essential oil followed by gamma radiation on mortality of *Callosobruchus maculatus* adults.

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During the second experiment where adults were exposed to the essential oil 24 h after irradiation, the mortality percentage increased from 10% and 20% to 30% and 42% by exposure to 1.38 µl/l air and to 41% and 54.25% by exposure to 1.83 µl/l air of *R. officinalis* (Fig. 2).

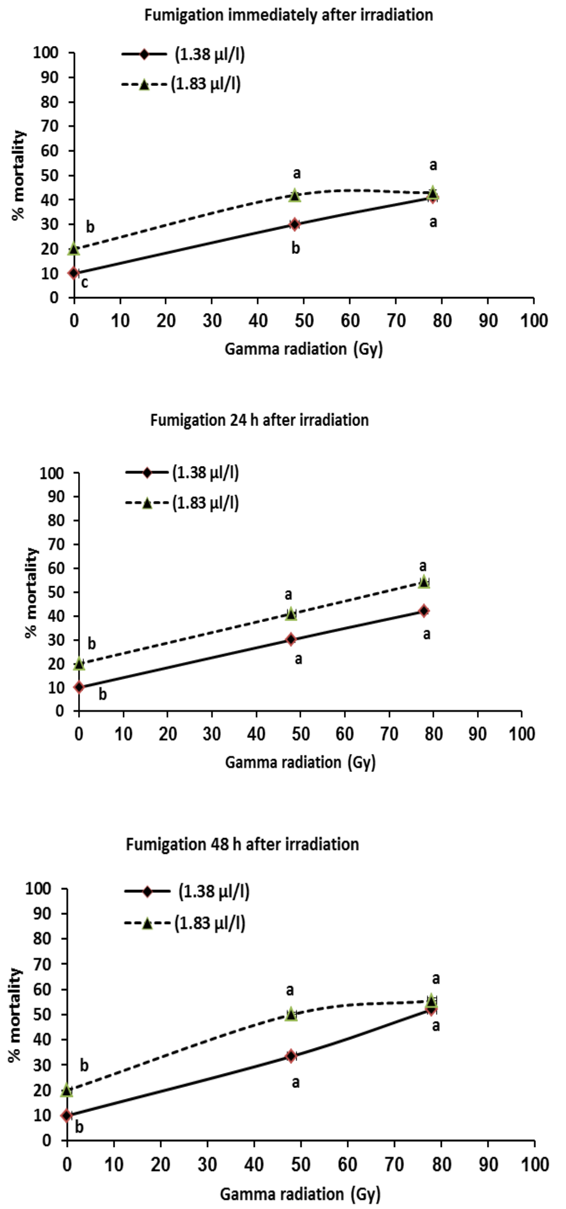

Figure 2 Effects of pretreatment with gamma radiation followed by *Rosmarinus officinalis* essential oil on mortality of *Callosobruchus maculatus* adults.

Also the results of fumigation after 48 h of irradiation showed acceptable interactions between *R. officinalis* and gamma radiation (Fig. 2). In another experiment where the irradiated adults were immediately exposed to *R. officinalis* oil, the mortality rates increased to 30% and 41% respectively. Similarly, when these irradiated adults were fumigated with oil at a dose of 1.83 µl/l air, mortality reached 42% and 43% respectively as compared with control (10% and 20%) and also in this case there were synergistic, antagonistic and additive effects between these two parameters (Table 2).

Discussion

Because of the harmful effects on the environment and on human health conventional fumigants were phased out by 2005 in the developed countries (Anon, 1997). Irradiation is a highly effective alternative to such fumigants whenever the use of these chemicals is banned (Marcotte, 1998). Our results indicate that *C. maculatus* is susceptible to irradiation and as expected, the mortality rate is correlated to the doses. At every dose higher than 48 Gy, 3 days after irradiation, the percentage of the adult mortality was found to be significantly different than control. These results are consistent with those by Diop *et al.* (1997), who demonstrated that the development of *C. maculatus* in cowpea seeds could be prevented by exposing the seeds to ionizing radiation at doses less than 100 Gy without leading to unfavorable nutritional consequences. Similar results were reported by Supawan *et al.* (2005) who revealed that a dose of 800 Gy (Gamma Cell 220) was efficient for controlling the natural infestation of mung beans by *C. chinensis* alone. Also Sutantawong (1991) showed that a dose of 1000 Gy caused 100% mortality in *C. maculatus* 7 days after irradiation. On the other hand, higher plants provide an excellent source for insecticides (Benner, 1993). Essential oils have been used as medicines in many countries; however only in recent years have these oils been commercialized as pest control products (Isman, 2000). Most of these oils are environmentally non-persistent, so they are safe to humans and other mammals (with some exceptions) (Hjorther *et al*., 1997), while being effective against several pest species (Choi *et al*., 2004). *R. officinalis* oil is an effective antioxidant used in food stuffs, such as oil antioxidant; and has the ability to maintain food in good condition and help to increase general health. Our results clearly indicate that Rosmary oil may be considered as an insecticide against *C. maculatus*. Similarly, Papachristos and Stampoulos (2004) showed the insecticidal activity of *R. officinalis* on *Acanthoscelides obtectus* at concentrations ranging from 0.8 to 47.1 mg/l air. Also Ahmadi *et al*. (2008a) reported a LC_{50} value of 6.84 µl/l air for *R. officinalis* to *T. castaneum* adults. In this study 95% mortality of insects was resulted with a concentration of 9.66 µl/l air.

Nowadays, the combination of several independent techniques for the control of a given pest as integrated pest management (IPM) is one of the basic strategies. Irradiation and fumigation by the essential oils are the two main methods that could be used as a co-treatment in IPM. There are several reports on the interaction of irradiation with other methods like fumigants (Mehta *et al*., 2004); infra-red and microwave (Kirkpatrich *et al*., 1973); microbial control (Jafari, 1967); thiodicarb (Ramesh *et al*., 2002); azadirachtin (Sharma and Seth 2005); *P. atriplicifolia* essential oil (Ahmadi *et al*., 2008b) and *R. officinalis* essential oil (Ahmadi *et al*., 2013) to gain adequate control of pests. The data obtained in our study have shown that the combination of radiation and essential oil can be used as an effective control method. The data on the toxicity of *R. officinalis* oil to the adults of *C. maculatus* post- or pre-exposure to gamma radiation indicated that a delay of 1-2 days between irradiation and fumigation could affect their susceptibility to the essential oil. In our experiment when the essential oils were used before irradiation, the mortality rate of *C. maculatus* was increased significantly. Susceptibility of the adults to irradiation 48 h after fumigation was higher than after 24 h. It seems that susceptibility to irradiation of pre-fumigated adult insects was highly enhanced. The results obtained in our study are in agreement with the findings of El-Sayeed *et al*. (1988) who observed that *C. maculatus* adults were more susceptible to fenvalerate after irradiation, whereas the opposite effect was found with cypermethrin, under similar condition. Similarly, Moustafa and Abdel Salam (1991) reported the synergistic effects of gamma radiation and chlorpyrifos on larvae of *Spodoptera littoralis*. Our findings further suggest that the irradiation of *C. maculatus* adults after fumigation is more effective than the reverse method. The reason for this phenomenon is not clearly understood, but it seems that fumigation might have altered the immune responses of insects and hence rendere them more susceptibility to irradiation. The synergistic effects of gamma radiation and essential oil of rosemary were demonstrated in this study; however, to determine the effectiveness of this combination at lower doses and pest mortality assessment over longer periods of time after the treatments need further studies.

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تأثیر هم‌افزایی اسانس *Rosmarinus officinalis* و پرتو گاما روی سوسک چهارنقطه‌ای حبوبات *Callosobruchus maculatus*

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چکیده: به‌منظور بررسی امکان تلفیق پرتو و سموم گیاهی، اثرات تلفیقی اسانس گیاهی رزماری *Rosmarinus officinalis* با پرتو گاما بر میزان مرگ‌ومیر سوسک چهارنقطه‌ای حبوبات *Callosobruchus maculatus* مورد بررسی قرار گرفت. آزمایشات به‌صورتی طراحی گردید که در مرحله اول ابتدا حشرات در معرض اسانس قرار گرفته و سپس پرتو استفاده می‌گردد، در مرحله بعد حشرات از قبل پرتو دیده در معرض اسانس قرار داده شدند. مرگ‌ومیر حشرات ۷۲ ساعت پس از پرتودهی مورد ارزیابی قرار گرفت. تلفیق اسانس رزماری با پرتو گاما میزان مرگ‌ومیر سوسک چهارنقطه‌ای حبوبات را در مقایسه با کاربرد مجزای هر کدام از آنها افزایش داد. در این آزمایشات زمانی‌که اسانس گیاهی با پرتو گاما تلفیق گردید، در میزان مرگ‌ومیر حشرات کامل ۱-۲ روزه سوسک چهارنقطه‌ای حبوبات اثر سینرژیستی ظاهر گردید. مشخص شد که روش پرتودهی حشرات اسانس داده شده از قبل، مؤثرتر از روش دیگر بود. نتایج نشان داد که هر روش کنترلی می‌تواند حساسیت آفت به تیمار دیگر را افزایش دهد. یافته‌های ما نشان می‌دهد که تلفیق پرتو گاما و اسانس گیاه رزماری دارای قابلیت استفاده در مدیریت تلفیقی آفات می‌باشد.

واژگان کلیدی: آفت‌کش گیاهی، سمیت تنفسی، گیاهان دارویی، حشرات آفت انباری، سینرژیسم

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