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TOXICITY OF *ROSMARINUS OFFICINALIS* ESSENTIAL OIL AGAINST IRRADIATED *TRIBOLIUM CASTANEUM*

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ABSTRACT

Management of stored-product insect using gamma radiation could be enhanced by other feasible control methods such as essential oils as potent alternatives to chemical insecticides. In this study, the efficiency of sub-lethal doses of essential oils from *Rosmarinus officinalis* L. combined with gamma radiation was verified to assess their enhanced toxicity against the adults of *Tribolium castaneum* (Herbst). Irradiated adult insects by 720, 890 and 1200 Gy doses (LD_1, LD_5 and LD_{25}) were subjected to three levels of essential oil (LC_1, LC_5 and LC_{25}). It was shown that using of essential oil from *R. officinalis* could be statistically increased 2-4 times the intensity of gamma radiation and high mortality levels has been observed in this combination. Also the results showed a synergistic effect between rosemary essential oil and gamma radiation combinations. The results indicated that insecticidal activity of essential oils would be altered to varying degree if the insects already were exposed to radiation. The potential use of integrating essential oils and gamma radiation treatment are discussed for management of the stored-product pests.

Keywords: Sub-lethal dose, essential oil, gamma radiation, *Rosmarinus officinalis*, synergistic effect, *Tribolium castaneum*

INTRODUCTION

In order to control of *Tribolium castaneum* (Herbst), fumigants such as methyl bromide and phosphine were widely used. Wide use of these fumigants has raised residue levels and has lead to the development of resistance in certain species (Rossi et al., 2010). Due to environmentally unfavorable effects of the fumigants, their use has recently been banned in many countries (Carpenter et al., 2000). Irradiation and essential oils have become approved and feasible alternatives to conventional method for the direct control of stored product pests; because of residue free advantages over chemical fumigation. *Tribolium castaneum* was observed to be sensitive to irradiation (Misra and Paravathy, 1998; Tuncbilek et al., 2003). In addition, there are several reports on insecticidal effects of rosemary essential oil on stored-product pests. Clemente et al. (2003) observed that extracts of *Rosmarinus officinalis* L. have an insecticidal effect on *T. castaneum*. Ahmadi et al. (2008b) also reported that *R. officinalis* essential oil could be utilized to control *T. castaneum* due to its fumigant toxicity.

On the other hand the use of gamma radiation and essential oils alone at high doses is very expensive and time-consuming; therefore to reduce costs, it is important to look for a strategy to use low doses without reducing the efficacy. One of the alternative possibilities is using a combination of the two methods looking for synergistic effect. Combination of gamma radiation with other treatments like, microwave, infra-red radiation and insecticides has also been previously reported (Cogburn and Spiers, 1972; Tilton et al., 1972; Ramesh et al., 2002; Mehta et al., 2004). Ahmadi et al. (2008a) discovered that combination of gamma radiation with essential oils of *Perovskia atriplicifolia* (Benth) may result in synergistic interactions that would enhance the potential for control of *T. castaneum*.

In this study the toxicity of doses of gamma radiation combined with *R. officinalis* essential oil against *T. castaneum* adults was tested.

MATERIALS AND METHODS

Extraction of essential oils

Arial parts of *R. officinalis* were collected at full flowering stage from Tehran in April 2011. Essential oils were extracted from dried plant samples using a Clevenger-type apparatus. Conditions of extraction consisted of 40 g of air-dried sample (1:10 plant material: water volume ratio) and 4 h distillation.

Irradiation

Irradiation of the tested insects was administered by using ^{60} cobalt gamma sources at the Nuclear Science and Technology Research Institute at a dose rate of 0.4 Gy sec^{-1}.

Fumigant toxicity

In this experiment, filter paper was impregnated with oil and then the filter paper was attached under the surface of the screw cap of a glass vial volume 280 ml (Negahban et al., 2007). The cap was screwed tightly on the vial containing 50 adults (1-3 d old). Five replicates were setup for treated and controlled adults. A series of dilutions was prepared to evaluate mortality of the insects. Insects were exposed to the oil for 24 h and then the number of dead and live insects in each bottle was counted 48 h after fumigation. When no leg or antennal movements were observed, insects were considered dead. Probit analysis (Finney, 1971) was used to estimate LC_1, LC_5 and LC_{25} values.

Interaction of gamma radiation and essential oil

In the first part of the experiment, where irradiation was used 24 and 48 h after fumigation by essential oil, adults were exposed to *R. officinalis* (close to LC_{25}) then treated with different doses of gamma radiation within 24 and 48 h after fumigation. In the second part of the experiment, insects were treated with gamma radiation (close to LD_{25}) and then survivors after 24 and 48 h were subsequently exposed to essential oil. In the third part in which insects were subjected to irradiation, and immediately exposed to the *R. officinalis* oil. Each experiment was conducted with five replications consisting of 50 insects for each replication. Percentage insect mortality was calculated 72 h after the initial treatment, using the Abbott (1925) correction formula for natural mortality in the untreated control.

Calculation of synergistic effects

Synergistic effect in combination of 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: additivity; S<1: synergism)

Where d_a and d_b are the concentration of each treatments (gamma radiation, essential oil) used in combination mode. D_a and D_b are their single concentrations which yielding the same effect level, when administered alone as the mixture.

RESULTS

Effects of different levels of gamma radiation on *T. castaneum* showed that 1-3 d old adults were susceptible to irradiation. Doses of 720, 890 and 1200 Gy caused 1.66%, 6.2% and 24.8% mortality 3 d post-irradiation, respectively. It was shown that mortality was increased with the increasing doses.

Results of fumigation by *R. officinalis* oil showed that LC_1, LC_5 and LC_{25} values were 4.2, 4.84 and 5.93 µl l^{-1} air respectively. In this experiment, mortality was noticeably increased as doses of essential oil increased. Comparative analyses for these 3 experiments testing the combination of gamma radiation with *R. officinalis* oil are shown in Fig 1. The results showed a significant synergistic effect between oil concentration and gamma treatment (Tukey, $P<0.05$) especially when fumigation was used 48 h after irradiation. Doses of 4.2, 4.84 and 5.93 µl l^{-1} air of *R. officinalis* alone resulted in average adult mortality of 1.33, 5.25 and 27.6% respectively. whereas average mortality rate increased to 18.3, 38.15 and 43.15% for treatment of 720 Gy of irradiation 24 h after fumigation and to 27.33, 34.13 and 45.2% for treatment of 720 Gy irradiation 48 h after fumigation. When the insects were exposed to 720 Gy of gamma radiation before fumigation of adults at the dose of 4.2, 4.84 and 5.93 µl l^{-1} air of *R. officinalis*, the mortality rate reached to 42.5, 51.33 and 62.5% (irradiation applied 24 h before fumigation) and 65.33, 75.5 and 75.3% respectively (irradiation applied 48 h before fumigation). During the third part of the experiment when irradiation by 720 Gy and essential oil (LC_1, LC_5 and LC_{25}) were exposed to the adults at the same time, mortality percentages increased to 27.33, 34.13 and 45.2% respectively. The results obtained from the application of gamma radiation in 890 and 1200 Gy doses with *R. officinalis* essential oil are shown in Fig 1.

Although it was shown that the combination of gamma radiation and rosemary essential oils could be provided significant increase in mortality rate of *T. castaneum*, but according to the synergism formula, synergistic effect was observed only when fumigation by 4.2 and 4.84 µl l^{-1} air applied 48 h after irradiation by 720 Gy dose and also irradiated adults by 890 Gy dose exposed to 4.2 µl l^{-1} air of *R. officinalis* after 48 h.

DISCUSSION

Currently, the combination of several independent techniques for the control of pest as integrated pest management (IPM) is one of the main strategies. Irradiation and fumigation by essential oil are two main methods that could be used as combined treatment in IPM. There are several reports on interaction of irradiation with other methods like fumigants (Mehta et al., 2004); thiodicarb (Ramesh et al., 2002); azadirachtin (Sharma and Seth, 2005) and *Perovskia atriplicifolia* Benth essential oil (Ahmadi et al., 2008a) to achieve adequate control of pests. Data on the toxicity of *R. officinalis* oil to adults after or before exposure to gamma

radiation indicated that a delay of 1-2 d between irradiation and fumigation could affect their susceptibility to the essential oil. Synergistic effect between gamma radiation and *R. officinalis* oil was observed when irradiated adults exposed to fumigation after 48 h.

Results obtained in our study are in agreement with the findings of El-Sayeed et al. (1988). They observed that *Callosobruchus maculatus* (Fabricius) adults are more susceptible to fenvalerate after irradiation. Similarly, Moustafa and Abdel Salam (1991) reported the synergistic effects of gamma radiation and chlorpyrifos on larvae of *Spodoptera littoralis* (Fabricius). Our findings further suggest that the fumigation of *T. castaneum* adults after irradiation is much more effective than the reverse method. The reason for this phenomenon is not clearly understood, but it seems that irradiation might have altered the cell resistance against non-favorable conditions and so had increased their susceptibility towards fumigation.

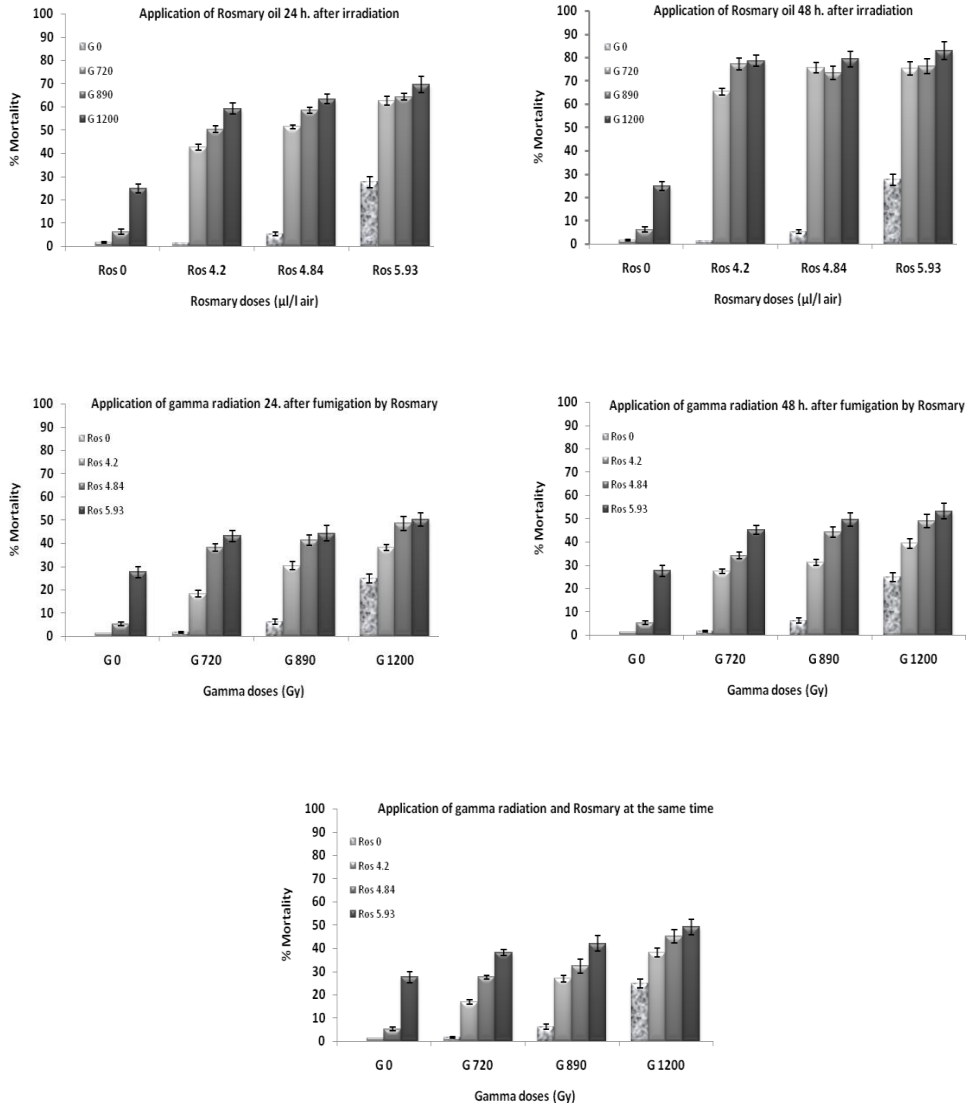

Fig. 1- Combined effect of gamma radiation and *Rosmarinus officinalis* essential oil against mortality of *Tribolium castaneum* adults.

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