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Effect of spinosad and malathion on esterase enzyme activities of *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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Abstract
Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) is a major pest belongs to the most widespread and destructive pests of stored products. This pest may cause considerable economic losses therefore numerous insecticides have been used for its management. This pest resistance to chemical insecticides and the management programs failure have been reported in several documents. Bioassay and detoxifying enzymes levels in *T. castaneum* were evaluated using different spinosad and malathion insecticides concentrations in this study. Insects were exposed to contact toxicity of the insecticides for 24 h in laboratory condition (25±5 °C in darkness). Bioassay results showed that the spinosad had had more effects than malathion. Also LC_{50} ratio range showed the insecticides LC_{50} was significantly different against *T. castaneum.* Detoxifying enzymes assay was performed in four insecticides concentrations (LC_{10}, LC_{25}, LC_{50} and LC_{75}) and showed that level of esterase enzymes exposed to malathion was higher than spinosad in using alpha and beta-naphtyl acetate as substrate. It is concluded that susceptibility of the *T. castaneum* against both insecticides were different and these differences were obvious in their enzymes activity as well.

Keywords: bioassay, detoxifying enzymes, malathion, spinosad, *Tribolium castaneum*

1. Introduction
Stored grain loss in weight and quality due to pests is a serious problem all over the world. It is estimated that 10% of stored grain loss is occurred due to insects throughout the world, each year [1]. *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a major pest of several stored grains all over the world. This pest may cause considerable economic losses if not controlled [2]. The species has gained economic importance because infested products may contain insect fragment and cast skin in addition to live insect pest. Numerous insecticides have been used to control of this pest [3]. However, using chemical pesticides against *T. castaneum* has become ineffective due to the development of resistance in the pest populations [4]. Resistance of stored product pests to chemical insecticides and failure of management program have been reported in some studies [5, 6, 7].
Development of resistance to chemical insecticides is a slow process, however numerous studies confirmed the occurrence of resistance to them in insect pests [8, 9]. The metabolic resistance is one of the most common strategies of resistance due to enhanced levels of detoxifying enzymes activity [10]. Levels of esterase enzymes, glutathione S-transferases (GSTs) and multifunction oxygenases (MFOs) are identified as main biomarkers in enzymatic resistance to insecticides. Studying the level of detoxifying enzymes could approve resistance in insect populations [11, 12].
In this study we have compared effect of spinosad and malathion against detoxifying enzyme activities of *T. castaneum*. spinosad is a bacterial insecticide with low mammalian toxicity and is very effective against a wide range of pest species [13, 14, 15]. Recently, spinosad was registered for use in stored products at USA as an alternative to traditional grain protectants [16]. Malathion (S-1,2-di (ethoxycarbonyl) ethyl, dimethyl phosphorothioate) is a nonsystemic, wide spectrum organophosphate insecticide. It was one of the earliest organophosphate insecticide introduced in 1950. It is used for agricultural and non-agricultural purposes [17, 18].

2. Material and methods
2.1 Insects
All insects were obtained from the laboratory culture of stored product pests, Plant Protection Department, University of Tehran, Iran. *T. castaneum* was reared on flour mixed with yeast

(5% w/w) that were kept in oven (60 °C) for half an hour to remove all probable pest pollutants. Then, culturing media and insects were transferred to incubator with 65±10% R.H. and 27±5 °C in darkness for mass reproduction. All experiments are done in 2014 (September-December).

2.2 Chemicals
Spinosad was purchased from Dow Agro Science (http://www.dowagro.com/). Malathion was purchased from Gyah Corp. Ingredients for electrophoresis techniques were obtained from Sigma-Aldrich.

2.3 Bioassay
Insecticide concentrations (50, 75, 100, 150, 200 ppm of malation) (10, 25, 50, 75, 100 ppm of spinosad) were applied in contact toxicity study on a filter paper (Whatman No. 1, with 7cm diameter) that totally covered the experimental arena (7cm Petri dishes) [19]. Thirty adult beetles (1 day old) were placed in air ventilated Petri dishes (2.5 cm hole was on top of each one that covered by 40 mesh net). In the control dishes only distilled water was poured on the filter paper. All experiments were performed in laboratory condition (25±5 °C and Darkness) [20, 21]. Mortality was determined after 24h exposure time. Each experiment was replicated five times. Insects were considered as dead when no leg or antennal movements were observed.

2.4 Enzyme sample preparation
Fifty adult beetles were used for enzymes assay exposed to four concentrations (LC_{10}, LC_{25}, LC_{50} and LC_{75}) of two insecticides. Survived insects (10 beetles per concentration) were homogenized in 100 µl of ice-cold sodium phosphate buffer (10 mM, pH 7, containing 0.1% Triton X-100) before (control) and after (treatment) the use of concentrations of the insecticides, in phosphate buffer 0.1 M (180 µL) at 4 °C, 24 h after treatment [22]. The homogenated mixture was spun (15,000 g for 10 min at 4 °C) in a microcentrifuge (eppendorf 5417 R). The resulting supernatants were used as the enzyme source in all enzyme assays [23].

2.5 Detoxification Enzymes assay
Evaluation of esterase activities were performed based on van-Asperen method (1962) [24] with little modifications. Alpha-naphthyl acetate (30 mM) and beta-naphthyl acetate (30 mM) were used as substrate (diluted in phosphate buffer 0.02 M (ratio 1:99)). Enzyme samples (15 µL for alpha-naphthyl and 10 µL for beta-naphthyl), plus alpha-NA or beta-NA substrate (200 µL) and 50 µL of fast blue RR (solved in distilled water ratio of 10:1) were poured in microplate wells. Finally, absorbance was taken at 450 nm for alpha-naphthyl and 540 nm for beta-naphthyl every 2 min for 10 minutes, continuously.

2.6 Data analysis
Mortality corrections were done by the Abbott correction formula for natural mortality in the untreated control [25]. Data of bioassays were analyzed for calculating lethal and sublethal concentrations by PoloPlus 2.0 (LeOra Software) and mean comparisons were performed using SPSS 22.0. Tukey test ($P \leq 0.05$) was used to compare means in enzymes activity [26].

3. Results
3.1 Bioassay
Bioassay results showed that the spinosad had had more effects than malathion. Also LC_{50} ratio range showed the insecticides LC_{50} was significantly different against *T. castaneum* (table 1). Also, Bioassay results showed that the spinosad was more effective than malathion against *T. castaneum* adults (Fig. 1).

Table 1: Lethal and sublethal concentrations of malathion and spinosad against *T. castaneum*.

Insecticide	LC_{10}*	LC_{25}*	LC_{50}	LC_{75}	b±S.E.	χ^2 (df)	*P*	LC_{50} ratio†
malathion	37.169	54.309	82.767	126.137	3.686±0.35	3.772 (13)	0.791	3.235
spinosad	5.087	10.933	25.585	59.871	1.827±0.198	7.444 (13)	0.873	(2.663-3.929)

*ppm
† Fiducial limit of 95% using PoloPlus 2.0

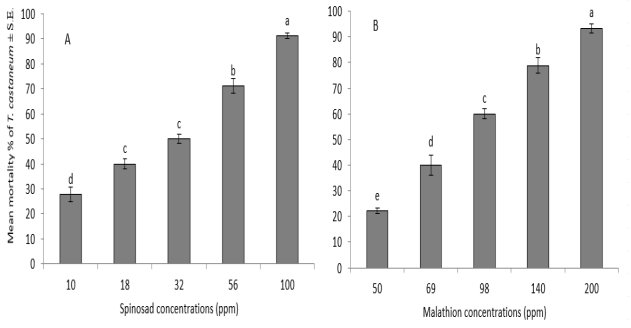

Fig 1: Mean mortality percent (±S.E.) of *T. castaneum* exposed to different concentrations of malathion (A) and spinosad (B) (Different letters over columns indicate significant differences according to Tukey test at α= 0.01; Columns with the same letter are not significantly different; Vertical bars indicate standard error (±)).

3.2 Enzymes assay
Esterases activity assay in *T. castaneum* exposed to malathion and spinosad have been shown in figure 2 and 3. During the experiment, it was found that the red flour beetle, *T. castaneum* was more resistant to the malathion and esterase enzymes activity were more than spinosad treatment.

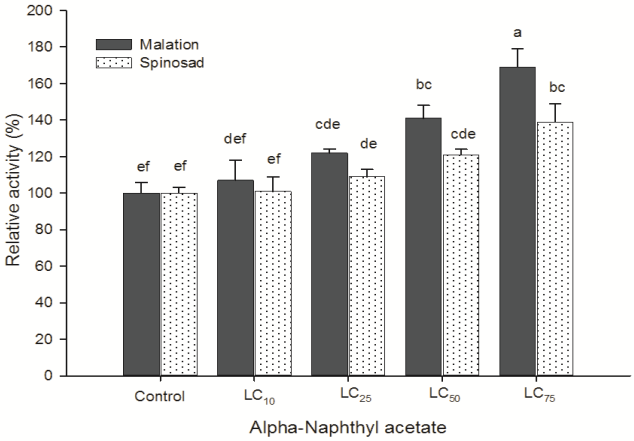

Fig 2: Esterases activity (using alpha-naphtyl acetate), exposed to different concentrations of malathion and spinosad in *T. castaneum* (Different letters over columns indicate significant differences according to Tukey test at α= 0.01; Columns with the same letter are not significantly different; Vertical bars indicate standard error (±)).

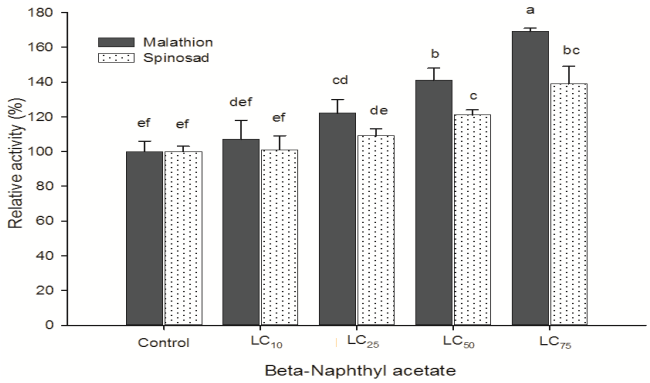

Fig 3: Esterases activity (using beta-naphtyl acetate), exposed to different concentrations of malathion and spinosad in *T. castaneum* (Different letters over columns indicate significant differences according to Tukey test at α= 0.01; Columns with the same letter are not significantly different; Vertical bars indicate standard error (±)).

4. Discussion

The most common resistance mechanisms in insects are increased levels or activities of esterase detoxification enzymes that metabolize a wide range of insecticides. Detoxification enzyme based resistance occurs when enhanced levels or modified activity of esterases and glutathione s-transferase prevents the insecticide from reaching its site of action. Our results showed esterases have an important role in resistance of *T. castaneum* to malathion in comparison with spinosad. These enzymes probably sequester or degrade insecticide esters before they reach their target sites in the nervous system. This mechanism seems to be important in the insecticide resistance of *Culex* mosquitoes [27, 28, 29] and *Aphis gossypii* [30]. The relationship between the enzymes which catalyze hydrolysis of β-NA of malathion was studied in resistant and susceptible strains of *Tetranychus kanzawai* [31]. Their results showed that resistance to malathion was associated with increased esterase activity at E3 and E4 bonds on which the main peak of malathion degradation was detected.

Obtained results showed the activity of esterases were dose-dependent with both control and treatments. Ahammad-Sahib *et al.* [32] showed that glutathione S- transferases and monooxygenase activities were increased against xenobiotics in the resistant Colorado potato beetle, while no increase in carboxyesterase activity was seen.

The activities of specific detoxification enzymes could be dependent on the species [33]. The results of our study showed that esterases had an important role in metabolism or in detoxification of malathion and spinosad in *T. castaneum*. According to slight increase in esterases activity at high concentrations of both insecticides, these enzymes might be effective in their detoxification. Our results suggest that esterases involved in detoxification of malathion and spinosad in *T. castaneum* and this involvement should be considered in management of the mentioned pest.

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