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Effectiveness of Radiant SC 12% on Glutathione Peroxidase Activities in Storage Pest Rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae)

Amal M. F. Al-Barty*

Biological science Department, Faculty of Science, Taif University, Taif, El-Hawyeia 888, Saudi Arabia.

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Radiant SC12% (Spinetoram) is a new generation of spinosyn group. Evaluated the efficacy of different concentrations of spinosad against adults of the rice weevil, *Sitophilus oryzae* were studied in the laboratory. Antioxidant defense components protect insects by scavenging reactive oxygen species, leading to oxidative stress. The present study was investigated the effects of Radiant SC 12%, on the GPX activity in *Sitophilus oryzae* tissues. There were statistically significant increases in GPX activity in the highest concentration of Radiant-treated *Sitophilus oryzae* compared to the control. These results indicated that Radiant causes an increase in oxidative stress thus increasing GPX activities.

Key words: *Sitophilus oryzae*, Rice weevil, Radiant SC 12%, GPX.

The rice weevil (Figure 1) is small, 1/10 inch (2 to 3 mm) and stout in appearance. It is very similar in appearance to the granary weevil. However, the rice weevil is reddish-brown to black in color with four light yellow or reddish spots on the corners of the elytra (the hard protective forewings). The snout is long (1 mm), almost 1/3 of the total length. The head with snout is as long as the prothorax or the elytra. The prothorax (the body region behind the head) is strongly pitted and the elytra have rows of pits within longitudinal grooves. The larva is legless and stays inside the hollowed grain kernel. It is fat with a cream colored body and dark head capsule (Koehler., 1994).

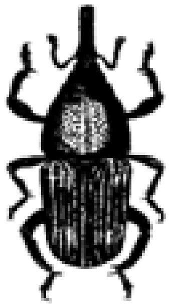

Fig. 1. Rice weevil

The rice weevil adult gathers and reproduces in stored grains. The objectives of this research work were to evaluate the efficacy of Radiant SC 12% as bioinsecticides against the adult mortality of *Sitophilus oryzae* and its toxicological studied that affect on different components in this insect pest.

* To whom all correspondence should be addressed.
E-mail: aalbarty@yahoo.com

Different biopesticides have been used to control the rice weevil infestations (Asawalam *et al.,* 2012) and (Yankanchi and Gadacge 2010).

The activity of spinosad is attributed to the metabolites spinosyns A and D, which are fermentation products of the soil actinomycete bacterium, Saccharopolyspora spinosa (Mertz and Yao, 1990). The active ingredient is composed of Spinosyn A and Spinocyn D, have strong insecticidal activity (Thompson *et al*., 1997). Spinosad has low mammalian toxicity and little toxicity to non-target insects and it degrades quickly when exposed to sunlight (UV light) (Bret *et al*., 1997 and Sparks *et al*., 1998).

Pesticides produce reactive oxygen species (ROS), leading to oxidative stress and alterations in radical scavenging enzymes in insects (Felton and Summers, 1995; Buyukguzel, 2006). ROS include oxygen ions, free radicals and peroxides, both inorganic and organic. These molecules are generally very small and highly reactive, because of the presence of unpaired electrons. ROS are formed as a natural byproduct of the normal metabolism of oxygen. They play an important role in cell signaling and the induction of host defense genes (Kamata and Hirata, 1999; Dalton *et al*., 1999).

To neutralize the toxicity of ROS, insects have developed a suite of antioxidant enzymes like other eukaryotes to overcome oxidative stress. Several antioxidant enzymes may decrease the level of lipid peroxidation in insects (Felton and Summers, 1995). In animals, including insects, various important components of the antioxidant system are identified. They are divided into enzymatic antioxidants glutathione peroxidases (GPx) (Dubovskii *et al.,* 2005).

MATERIALS AND METHOD

Culture of *Sitophilus oryzae*

Stock of *S. oryzae* was obtained from the infested wheat bought from the local market. Laboratory cultures of *S. oryzae* were maintained on uninfected wheat grains (*Triticium aestivum*). Adult of rice weevils were introduced into plastic jars containing wheat grains. These plastic jars were then covered with a muslin cloth to prevent insects escaping and to allow ventilation. After two weeks the adults were removed and the wheat grains were kept in ambient laboratory conditions for the emergence of S. *oryzae* adults (Huang and Subramanyam 2007). For all the experiments 1-7 days old, adult weevils were selected from cultures. All the experiments were kept aside at ambient temperature 26+3oC and 65+5% relative humidity.

Preparation of Insecticide

A liquid formulation of Radiant 12% was obtained from Plant Protection Research Institute (Egypt, Cairo). Insecticide was diluted in distilled water to make solutions of different concentrations for grain treatment. Different concentrations of the insecticide were prepared to test its effect on the adults of *S.oryzae*. Three concentrations were (1.8, 0.93, 0.46 ppm), wheat grains were dipped in the insecticide for 15 seconds; the treated grains were then left to dry under laboratory conditions. Each concentration consists of four replicates with 15 adults /replicate. Control adult were fed on grains were dipped in distilled water; adults were allowed to feed on treated grains.

Preparation of homogenates and determination of the levels of GPX

Tissue collection

For measurement of antioxidant enzyme activities in insect tissue homogenate, a separate test was arranged by application of the value of Radiant SC 12%. Insects were used to determine GPX levels. Insects were collected into a chilled Eppendorf tube charged with a cold homogenization buffer [w/v 1.15% KCl, 25 mM K_2HPO_4, 5 mM ethylen-diaminetetraacetic acid (EDTA), 2 mM phenylmethylsulphonyl fluoride (PMSF), 2 mM dithiotreitol (DTT), pH 7.4] and stored at -20 ^oC. The cryotubes were kept at room temperature until the tissue began to thaw before using.

Sample Preparation

Extracts of *Sitophilus oryzae L* insects' homogenates were prepared at 4 ^oC by a homogenizer (HEIDOLPH SilentCrusher M) at 10 seconds in the homogenization buffer and subsequent centrifugation (Minispin plus Eppendorf) at 10,000g for 15 min at 4^oC. Supernatants were centrifuged at 16000g for 20 min at 4 ^oC (GPX assays).

Measurement of GPX Activity:

The GPx (EC 1.11.1.9) activity was measured with H_2O_2 as substrate according to Paglia and Valentine (1987). This reaction was monitored indirectly as the oxidation rate of

NADPH at 340 nm for 3 min. Enzyme activity was expressed as nmol of NADPH consumed per minute per milligram of protein, using an extinction coefficient of 6.220 M-1cm-1. A blank without homogenate was used as a control for the non-enzymatic oxidation of NADPH upon addition of hydrogen peroxide in Tris buffer (0.1 M, pH 8.0).

Statistical analysis

Data were subjected to analysis of variance where significant differences existed, treatment means were separated using the Fisher's Protected LSD test at the ± = 0.05 level (SAS Institute, 1988). For beetle species, untransformed means and standard errors are presented in the tables.

RESULTS AND DISCUSSION

Susceptibility of adult *Sitophilus oryzae* to three different concentrations of radiant SC 12%

Data in table (1) illustrated the effect of radiant SC 12% on the mortality of adult *S. oryzae* treated with different concentrations. Data investigated the mean number of dead adults and its percentage mortality, (Das 2013 and Elbarky *et al*. 2008) reported that mortality increased with the increase of concentration. There were highly significant differences in the mean mortality of *S. oryzae* between concentrations (F 3.10, P < 0.001). The adult was significantly different between concentrations in both the exposure times (2 days:

Table 1. Adult mortality (mean ± SE) of storage pest Rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) with Radiant SC 12%

Consentration (ppm)	After 2days of treatment Mean of dead adult ±se	After 4days of treatment Mean of dead adult ±se	After 6days of treatment Mean of dead adult ±se	After 8days of treatment Mean of dead adult ±se
1.87	5.42±1.17[a]	8.75±2.44[a]	16.10±2.45[a]	14.20±3.70[a]
0.93	2.15±1.22[b]	6.28±1.72[b]	14.27±3.14[b]	13.25±3.81[b]
0.46	1.25±0.37[c]	2.45±0.93[c]	8.75±2.15[c]	12.20±2.75[c]
Control(distilled water)	0[d]	0.5±0.2[d]	1.25±0.63[d]	1.5±0.42[d]

*Means within a column for insect pest followed by different letters are significantly different (P < 0.05)

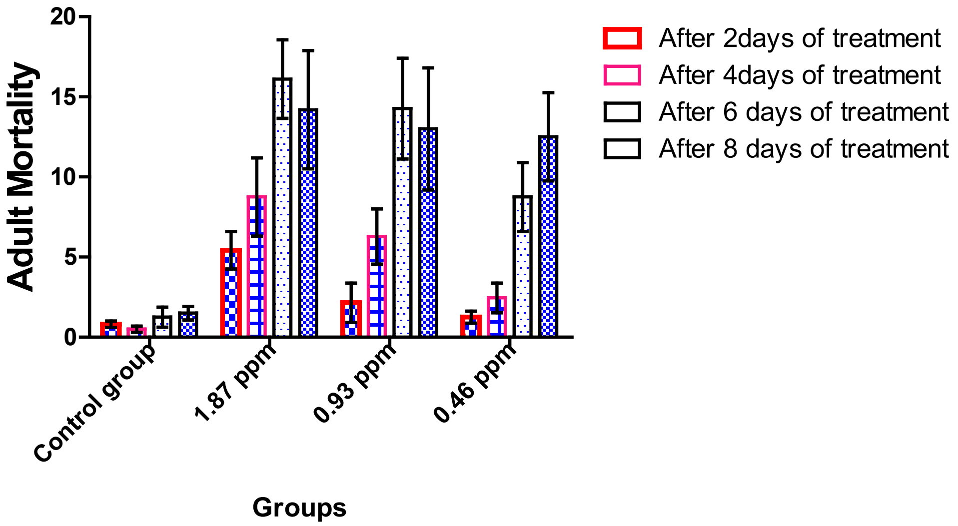

Fig. 1. Adult mortality of storage pest Rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) with Radiant SC 12%

F= 7.2, P < 0.001; 4 days: F= 36, P < 0.001; 6 days). As shown in Table (1) and Fig (1), mortality increased by an increase in spinosad concentration (Huang and Subramanyam 2007).

GPX activities

GPX activity were determined to be highly increased in *Sitophilus oryzae* L after exposure to Radiant SC 12% and the highly significant increase was observed in the concentration 1.87 ppm followed by concentration 0.93 ppm, (Figures 2). There was statistically relevant and distinctive significant increase in the GPX activity in the concentrations: 1.87, 0.93 (ppm) concentrations respectively followed by concentration 0.46 (PPm) of Radiant SC (12%) treated insects compared with the control (Table.2) and (Fig. 2).

Table 2. GPX activity (mean ± SE) of (mean ± SE) of storage pest Rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) with Radiant SC 12%

Concentration (ppm)	GPX (mmol/mg protein)
1.87	9.53±2.77[a]
0.93	7.32±1.32[b]
0.46	4.25±1.13[c]
Control (distilled water)	2.51±0.57[d]

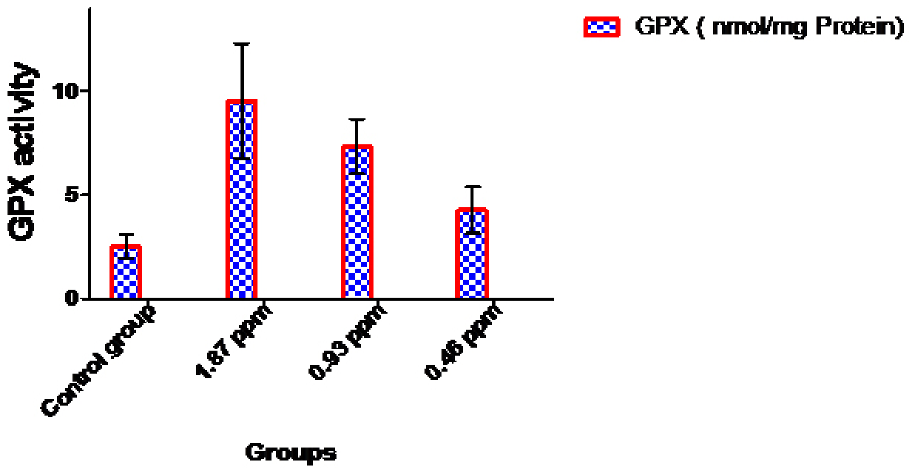

Fig. 2. Effect of Radiant SC 12% on GPX activity in *Sitophilus oryzae* L.
Data represents the means ± SD of seven samples. Values are mean ± SD of seven rats in each group

Means within the same column in each category carrying different litters are significant at (P ≤ 0.05) using Duncan's multiple range tests, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically

Some studies have also shown that oxidative stress could be an important component of the mechanism of toxicity of insecticides. Insecticides may induce oxidative stress leading to a generation of free radicals and alterations in antioxidants or reactive oxygen species (ROS)-scavenging enzymes in vivo and in vitro (Bagchi *et al*., 1995; Gultekin *et al*., 2000). It was reported that pesticides effected on antioxidant enzyme activities in insects (Dubovskii *et al*., 2005; Dubovskiy *et al*., 2008). In this study a change in SOD and CAT activities was found in insects' tissues homogenates after application of the Radiant SC % different concentrations. This suggested that Radiant SC12 % caused oxidative damage in *Sitophilus oryzae* L. possibly by producing ROS in insect tissues. Other studies reported that pesticides caused lipid per oxidation and the alterations in the antioxidant defense enzymes of insect (Gupta *et al*., 2010; Wu *et al*., 2011).

GPx catalyzes the glutathione-dependent reduction of lipid hydroperoxides and hydrogen peroxide for detoxification. In this study, GPx activity significantly increased in insect tissues homogenates of *Sitophilus oryzae* L.

So, the present study was an attempt to clarify the effect of Radiant SC 12% on antioxidant defense system by measuring the level of GPX activity in insect tissues homogenates after exposure to three different concentrations of Radiant SC 12% and our results revealed that Radiant SC 12% had significantly increased the oxidative stress in insect tissues which reflected by increasing the level of GPX activity to scavenge the free radicals produced which proved the efficient effect of Radiant SC 12% against *Sitophilus oryzae* L and increasing oxidative stress in insect's tissues.

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