

Effectiveness of Radiant SC 12% on superoxide dismutase (SOD) and catalase (CAT) activities in storage pest Rice weevil, *Sitophilus oryzae* L.

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ABSTRACT: 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. The highest effect of different concentrations in laboratory were recorded 55 and 31% adult mortality after 4 days for the two concentrations (1.87 and 0.93 ppm), while they caused 100% mortality of adult after 6 and 8 days respectively, 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 oxidative stress indicator, and antioxidant enzymes [superoxide dismutase (SOD) and catalase (CAT)] activities in *Sitophilus oryzae* tissues. There were statistically significant increases in SOD and CAT activities in the LC50/48h concentration of Radiant-treated *Sitophilus oryzae* compared to the control. These results indicated that Radiant causes an increase in oxidative stress and we inferred that increasing oxidative stress induces antioxidant defense mechanisms.

Keywords: *Sitophilus oryzae*, Rice weevil, Radiant SC 12%, bio-insecticides, SOD, CAT

INTRODUCTION

Wheat (*Triticum* spp) is the major source of protein in human foods, having higher protein content than maize or rice, the other major cereal grains. In terms of total production for food, it is currently second to rice as the main human food crop (Ileke and Bulus 2012).

Wheat is infested by various insect pests between harvest and storage. The most economically important insect pests of stored wheat are the granary weevils, *Sitophilus granaries*; maize weevils, *Sitophilus zeamais*; rice weevils, *Sitophilus oryzae*; lesser grain borer and *Rhizopertha dominica* (Ileke and Bulus 2012). The rice weevil feeds on rice, wheat, barley and on other raw or processed cereals such as pasta. The larva feeds within the kernel and consumes the endosperm. The adult leaves a large, ragged exit hole in the kernel and feeds on damaged kernels (Fang and Dolder 2002b).

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

Spinosad is considered a commercial insecticide used for management of many insect pests' species on a variety of crops (Thompson, 1997). Spinosad has been classified as a bioinsecticide (Copping and Menn, 2000). 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, 1997). Spinosad has low mammalian toxicity and little toxicity to non-target insects and it degrades quickly when exposed to sunlight (UV light) (Bret, 1997 and Sparks, 1998). The mode of action of spinosad is completely novel, making it a useful resistance management tool, it has unique mode of action on the insect nervous system at the nicotinic acetylcholine receptors and it has additional effects on gamma aminobutyric acid or GABA receptor sites, leading to continuous activation of motor neurons and causing cessation of feeding, tremors of most muscles in the body and later, paralysis and death (Salgado 1997, 1998 and Semiz, 2006).). It acts on various Lepidoptera pests of economic importance (Strong and Brown, 1987). Conventional toxicity tests indicate that spinosad has virtually no toxicity to birds and mammals.

It caused highly effective against stored product insects like *Sitophilus oryzae*, *Tribolium castaneum* and larvae of Indian meal moth *Plodia interpunctella* in stored wheat (Liang Fang & Frank 2002). It effect also on the house flies (Jeffery 1998). It has broad spectrum nematocidal, acaricidal and insecticidal properties (Putter, 1981).

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, 1999). Besides, under environmental stress, ultraviolet irradiation, bacterial infections, antibiotics and pesticides exposure, the ROS level may increase remarkably and result in oxidative stress in insects (Lopez-martinez, 2008; Buyukguzel and Kalender, 2009; Durak, 2009).

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-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidases (GPx) and non-enzymatic antioxidants-phenol containing compounds such as, vitamin E, vitamin C and molecular thiols (Dubovskii *et al.*, 2005). SOD catalyses the conversion of the superoxide radicals to H_2O_2 and oxygen and appears to be the main response to dietary pro-oxidant exposure (Ahmad and Pardini, 1990). CAT catalyzes the degradation of H_2O_2 to water and oxygen (Ahmad, 1991).

MATERIALS AND METHODS

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 uninfested wheat grains (*Triticum 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±3°C 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*. The five concentrations were (1.8, 0.93, 0.46, 0.23 and 0.11 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.

The dead adults were counted every 2 days after treatment. Dead adults were counted and used to calculate the percentage of adult mortality.

Preparation of homogenates and determination of enzymatic activities and the levels of Superoxide dismutase (SOD) and Catalase (CAT)

Tissue collection

For measurement of antioxidant enzyme activities in insect tissue homogenate, a separate test was arranged by application of the LC50/48h value of Radiant SC 12%. After 48 h.

Thirty-insects were used to determine SOD and CAT levels and antioxidant enzyme activities. Insects were collected into a chilled Eppendorf tube charged with a cold homogenization buffer [w/v 1.15% KCl, 25 mM K₂HPO₄, 5 mM ethylen-diaminetetraacetic acid (EDTA), 2 mM phenylmethylsulphonyl fluoride (PMSF), 2 mM dithiotreitol (DTT), pH 7.4] and stored at -20 °C. 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 °C 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 °C. The resulting cell-free extracts were collected for biochemical analysis of antioxidant enzymes activities. Supernatants were centrifuged at 1000g for 10 min at 4 °C (SOD and CAT assays), contents and antioxidant enzymes activities were determined by measuring the absorbance of the samples in a dual beam spectrophotometer (Shimadzu-1700, UV/vis, Kyoto, Japan). Essays were replicated six times with four insects each. All chemicals used were analytical grade and were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Measurement of SOD Activity

The total SOD (EC 1.15.1.1) activity was determined according to (Marklund and Marklund, 1974) assaying the autooxidation and illumination of pyrogallol at 440 nm for 3 min. One unit total SOD activity was calculated as the amount of protein causing 50% inhibition of pyrogallol autooxidation. The total SOD activity was expressed as units per milligram of protein (U mg⁻¹). A blank without homogenate was used as a control for non-enzymatic oxidation of pyrogallol in Tris-EDTA buffer (50 mM Tris, 10 mM EDTA, pH 8.2).

Measurement of CAT Activity

Before the determination of CAT (EC 1.11.1.6) activity, samples were diluted with 1:9 with 1% v/v Triton X-100. The enzyme activity was measured according to (Aebi 1984) assaying the hydrolysis of H₂O₂ and decreasing absorbance at 240 nm over a 3 min period at 25 °C. The CAT activity was expressed as millimoles of H₂O₂ reduced per minute per milligram of protein, using an extinction coefficient of 0.0394 mM⁻¹ cm⁻¹. A blank without homogenate was used as a control for non-enzymatic hydrolysis of peroxide in phosphate buffer (50 mM, pH 7.0).

Electron microscopy

The third portion of the pancreas was immediately cut into small cubes and transferred to ice-cold fixation buffer (1.25% v/v glutaraldehyde in 0.1 mM cacodylate- HC1 buffer, 0.1 M sucrose, and 2 mM calcium chloride pH 7.2) and prepared for transmission electron microscopy (Harris, 1991).

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 $\alpha = 0.05$ level (SAS Institute, 1988).

RESULTS AND DISCUSSION

Susceptibility of adult *Sitophilus oryzae* to 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, 2008) reported that mortality increased with the increase of concentration (Dahi 2009). 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: F= 7.2, P < 0.001; 4 days: F= 36, P < 0.001; 6 days: F=4.5 P < 0.001; 8 days: F=4.86, P < 0.001). As shown in Table (1), mortality increased by an increase in spinosad concentration (Huang and Subramanyam 2007).

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

Concentration (ppm)	After 2days of treatment Mean of dead adult \pm se	After 4days of treatment Mean of dead adult \pm se	After 6days of treatment Mean of dead adult \pm se	After 8days of treatment Mean of dead adult \pm se
1.87	4.5 \pm 2.7 ^a	7.7 \pm 0.4 ^a	15 \pm 0.25 ^a	15 \pm 00.10 ^a
0.93	1.5 \pm 1.2 ^b	5 \pm 0.2 ^a	14.7 \pm 0.4 ^a	15 \pm 0.01 ^a
0.46	1.25 \pm 0.7 ^b	1.25 \pm 0.72 ^b	6.75 \pm 1.5 ^{ab}	10.2 \pm 0.75 ^b
0.23	4.5 \pm 2.7 ^a	4.5 \pm 2.7 ^a	5.5 \pm 0.5 ^b	7.5 \pm 0.75 ^b
0.11	1.75 \pm 1.2 ^b	2 \pm 0.25 ^b	3.5 \pm 0.6 ^b	4.7 \pm 0.18 ^b
Control (distilled water)	0 ^b	0.5 \pm 0.2 ^b	1.25 \pm 0.3 ^c	1.5 \pm 0.42 ^c
LSD	1.87	4.5	4.5	2.42

*Means within a column for insect pest followed by different letters are significantly different ($P < 0.05$; by Fisher's Protected.LSD test).

Effect of the different concentrations of radiant introduced different significant effect on *S. oryzae* adults. Data in table showed that two concentrations (1.87 and 0.93 ppm) caused mortality reached to 100% after 6 and 8 days respectively (Amos, 1986) but for three concentrations (0.46, 0.23 and 0.11ppm), there were no significant differences recorded after 8 days,(Fernando and Karunaratne 2012).

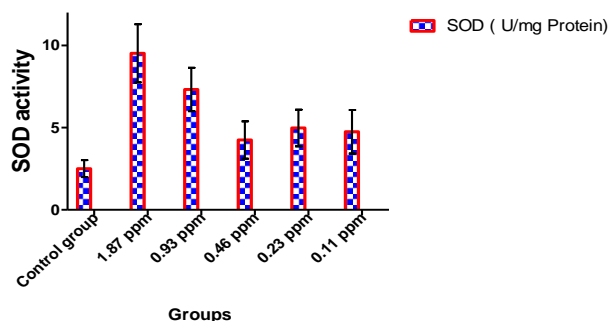
Antioxidant enzyme activities

SOD and CAT activities 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, However, there was non significant differences between concentrations 0.46, 0.23 and 0.11 ppm despite their increasing as compared to control group, respectively (Figures 1, 2). There were statistically relevant and distinctive significant increases in the SOD and CAT activities in the concentrations: 1.87, 0.93 (ppm) concentrations respectively of Radiant SC (12%) treated insects compared with the control (Table.2) and (Figs.1, 2).

 Table 2. Antioxidant enzyme activities (mean \pm SE) of (mean \pm SE) of storage pest Rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) with Radiant SC 12%

Concentration (ppm)	SOD (U/mg Protein)	CAT (mmol/mg Protein)
1.87	9.53 \pm 2.77 ^a	520.36 \pm 26.40 ^a
0.93	7.32 \pm 1.32 ^b	435.18 \pm 21.89 ^{1b}
0.46	4.25 \pm 1.13 ^d	385.25 \pm 15.72 ^{cd}
0.23	4.98 \pm 1.78 ^{cd}	375.62 \pm 14.97 ^d
0.11	4.75 \pm 1.23 ^d	320.85 \pm 11.37 ^a
Control (distilled water)	2.51 \pm 0.57 ^e	284.36 \pm 10.25 ^f

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


 Figure 1. Effect of Radiant SC 12% on SOD activity in *Sitophilus oryzae* L

Data represents the means \pm SD of seven samples. Values are mean \pm SD of seven rats in each group.

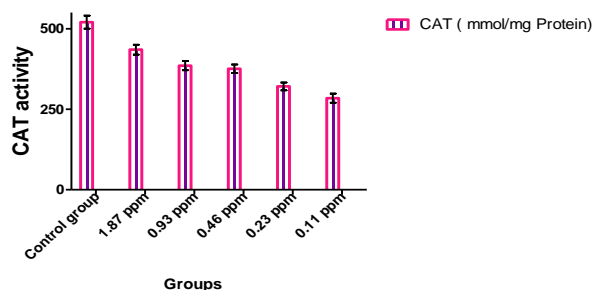


Figure 2. Effect of Radiant SC 12% on CAT activity in *Sitophilus oryzae L*

Data represents the means \pm SD of seven samples. Values are mean \pm SD of seven rats in each group.

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, 1995; Gultekin, 2000). It was reported that pesticides effected on antioxidant enzyme activities in insects (Dubovskii, 2005; Dubovskiy, 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 peroxidation and the alterations in the antioxidant defense enzymes of insect (Gupta, 2010; Wu, 2011).

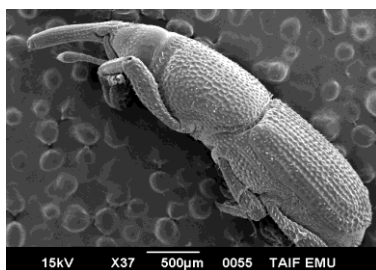
Under physiological conditions, intracellular antioxidant enzymes, such as SOD and CAT eliminate ROS, thereby playing an integral role in the oxidative stress defenses of the cell (Bukowska, 2004).

SOD plays an important role as an antioxidant enzyme by reducing high level of intracellular SOD activity suggested that Radiant SC 12 % induces the superoxide radical in the tissues of *Sitophilus oryzae L*. SOD activity significantly increased when the insects were exposed to Radiant SC 12%, suggesting that SOD was stimulated by scavenging superoxide radical to protect the insect from Radiant SC stress. It has been reported that an increase in SOD activity is probably a response towards increased ROS generation in rat erythrocytes (John, 2001).

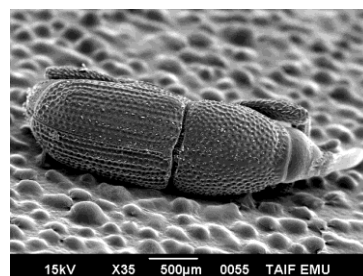
In the present study, CAT activity significantly increased in response to Radiant SC 12% induced oxidative stress in tissues of *Sitophilus oryzae L*. CAT is perfectly suited for reducing the high amount of H_2O_2 and regulated by the concentration of H_2O_2 (Fridovich, 1978). We conclude that increased SOD activity would result in an increased H_2O_2 concentration and consequently a further increase in CAT activity. Previous studies have shown that CAT can protect against oxidative stress and extend the lifespan of insects (Orr and Sohal 1994).

So, the present study was an attempt to clarify the effect of Radiant SC 12% on antioxidant defense system by measuring the levels of SOD and CAT activities in insect tissues homogenates after exposure to 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 SOD and CAT activities 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.

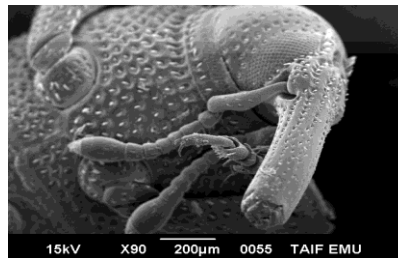
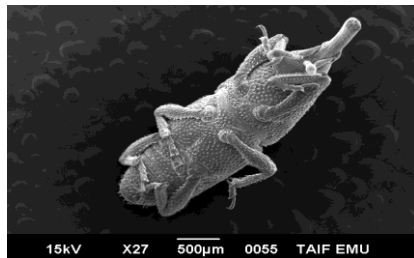
SEM (Scanning Electron microscope)



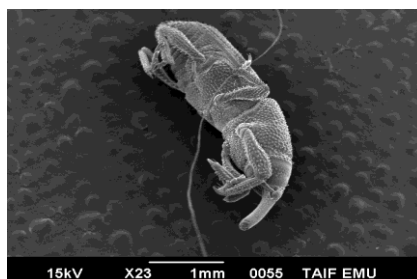
Group 1. Control group



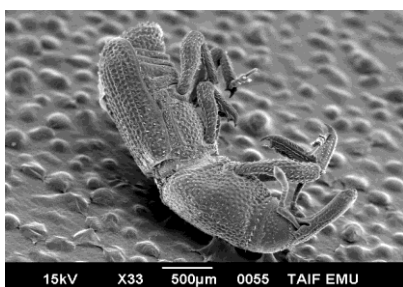
Group 2. Conc. 0.11 ppm



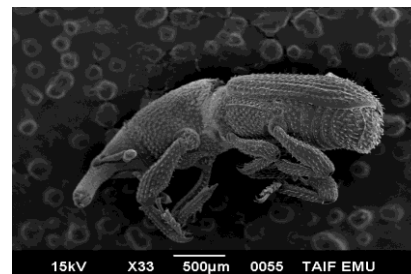
Group 3. Conc. 0.23 ppm



Group 4. Conc. 0.46 ppm



Group 4. Conc. 0.93 ppm



Group 5. Conc. 1.87 ppm

Group (1) Control group

Normal structure of *Sitophilus oryzae* L with normal size and appearance with pronotal dorsum and Pronotal punctures are separated by a flat, median, longitudinal puncture-free zone.

Group (2) Conc. (0.11 ppm)

Normal appearance of *Sitophilus oryzae* L but with less oxidative markers appearing by less volume than the normal and with small Curvature.

Group (3) Conc. (0.23 ppm)

Beginning of appearance of oxidative stress with more curvature in *Sitophilus oryzae* L than concentration 0.11 ppm with notice of more drooping in the rostrum that will affect on insect feeding and other biochemical functions.

Group (4) Conc.(0.46 ppm)

in this concentration treated group , the *Sitophilus oryzae* L appear as more smaller in size than that noticed in conc.(0.11 and 0.23 ppm) with curvature also of the body.

Group (5) Conc. (0.93ppm)

The more oxidative stress which appears greatly in *Sitophilus oryzae* L of this group than the higher concentration also with more drooping of the whole body and reduced in size of *Sitophilus oryzae* L.

Group (6) Conc. (1.87ppm)

oxidative stress also was greatly appeared in this group but with reduction in the fore and hind limbs of the insect and curvature of the rostrum of the insect with some degeneration in the mid region of the insect.

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