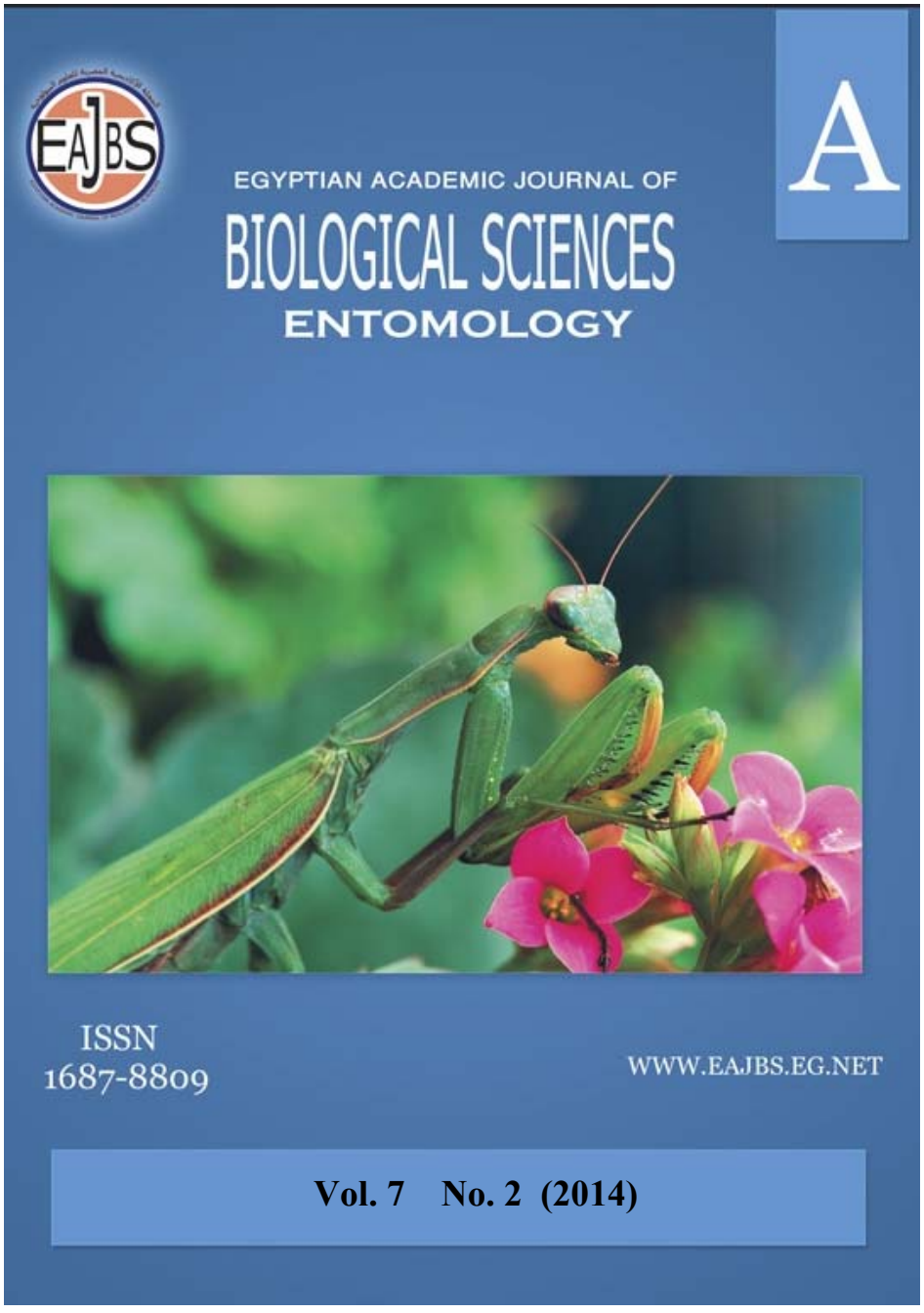

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Insecticidal potential of the bioagent radiant sc 12% against stored rice weevil, *Sitophilus oryzae* L. (Curculionidae: coleoptera)

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ABSTRACT

The bioagent, Radiant SC12% (Spinetoram) is a commercial reduced-risk pesticide that is naturally derived. Efficacy of five concentrations (1.8, 0.93, 0.46, 0.23 and 0.11ppm) of spinosad against adults of the rice weevil, *Sitophilus oryzae* was studied under laboratory conditions. Data obtained demonstrated that the highest concentration of the bio-agent Radiant SC 12% (1.87 and 0.93 ppm) achieved the highest mortality percent of adults (55 and 31 %, respectively) after 4 days of treatment and 100 mortality percent for both two concentrations after 6 and 8 days of treatment. Antioxidant defense components protect insects by scavenging reactive oxygen species, leading to oxidative stress. The present study also 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. The use of the bio- agent lead to statistically significant increases in SOD and CAT activities after 48h of application as compared to untreated *Sitophilus oryzae*. These results indicated that Radiant causes a significant increase in oxidative stress, that increasing oxidative stress induces antioxidant defense mechanisms, which lead to mortality action.

Keywords: Rice weevil, *Sitophilus oryzae*, 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 the other major cereal grains. (Ileke and Bulus 2012).

Wheat is infested by various insect pests between late stage of maturation in field, 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* and lesser grain borer *Rhizopertha dominica* (Ileke and Bulus 2012).

The rice weevil adult gathers and reproduces in stored grains and cause a great damage and loss according storage period (Asawalam*et al*., 2012).

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Spinosad has been classified as a bio-insecticide (Copping and Menn, 2000), it considered a commercial insecticide used for management of many insect pests' species on wide varities of crops (Thompson *et al*., 1997). 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), it 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). Spinosad has unique mode of action on the insect nervous system at the nicotinic acetylcholine receptors and it has additional effects on gamma aminobyturic 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 (Semiz *et al*., 2006)

Pesticides produce reactive oxygen species (ROS), leading to oxidative stress and alterations in radical scavenging enzymes in insects (Buyukguzel, 2009). 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. They play an important role in cell signaling and the induction of host defense genes (Kamata and Hirata 1999; Dalton *et al.,* 1999)

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 *et al*., 1991).

This work was conducted to investigate the insecticidal potential of the bioagent Radiant SC 12% against stored rice weevil, *Sitophilus oryzae* under laboratory conditions.

MATERIAL 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 (*Triticium aestivum*). Adults of rice weevils were introduced into plastic jars containing wheat grains, and 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 the bio-insecticide:

A liquid formulation of Radiant 12% was obtained from Plant Protection Research Institute (Egypt, Cairo). The bio- insecticide was diluted in distilled water to make solutions of different concentrations for grain treatment. Different concentrations of the bio-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 0.11ppm), wheat grains were dipped in each concentration for 15 seconds (Fang *et al*.); 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 which dipped in distilled water; adults were allowed to feed on treated grains. The dead adults were counted every2 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, 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-diaminetetra acetic acid (EDTA), 2 mM phenylmethylsulphonyl fluoride (PMSF), 2 mM dithiotreitol (DTT), pH 7.4] and stored at -20^{o}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 ^{o}C by a homogenizer (HEIDOLPH Silent Crusher M) at 10 seconds in the homogenization buffer and subsequent centrifugation (MinispinPlus Eppendorf) at 10,000g for 15 min at 4 ºC. The resulting cell-free extracts were collected for biochemical analysis of antioxidant enzymes activities. Supernatants were centrifuged at 1000g for 10 min at 4^{o}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 H_2O_2 and decreasing absorbance at 240 nm over a 3 min period at 25^{o}C. The CAT activity was expressed as millimoles of H_2O_2 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).

Scanning Electron microscopy:

Sitophilus oryzae was 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 scanning electron microscopy described by (Harrison 2012)

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). Mortality was subjected to the Abbott formula (Abbott, 1925) for correction wherever required.

RESULTS AND DISCUSSION

Efficiency of different concentrations of radiant SC 12% against adult of *Sitophilus oryzae*:

Data in Table (1) summarizes the obtained results about the effect of different concentrations of the bio-agent, Radiant SC 12% on the rice weevil, *Sitophilusoryzae,* expressed as mortality percent in adult stage. Data revealed that mortality in the treated adults increased with the increase of concentration and also with elapse of time (Dahi 2009)., where the highest mortality percent (29.2 & 29.2, 51.4 & 33.26, 99.9 & 98.2 and 99.9 and 99.9 %) were achieved at the rates 1.8 and 0.93 ppm of Radiant SC 12% ,respectively after 2, 4, 6 and 8days of treatment.

Statistical analysis of the data confirmed the insignificant differences between the two high concentrations (0.93 and 1.8ppm). Meanwhile, the other three low concentrations (0.46, 0.23 and 0.11ppm) seem to be less potent against rice weevil with highly significant differences as compared to the previously mentioned two high concentrations.

Table 1: Adult mortality (mean ± SE) of the Rice weevil, *Sitophilus oryzae L.* induced with Radiant SC 12% treatment.

Consentration (ppm)	After 2days of treatment		After 4days of treatment		After 6days of treatment		After 8days of treatment	
	Mean of dead adult ±se	Corrected mortality (%)	Mean of dead adult ±se	Corrected Mortality (%)	Mean of dead adult ±se	Corrected mortality (%)	Mean of dead adult ±se	Corrected mortality (%)
1.8	4.5±2.7^{a}	29.2	7.7±0.4^{a}	51.4	15±0a	99.9	15±0^{a}	99.9
0.93	4.5±2.7^{a}	29.2	5±0.2^{a}	33.26	14.7±0.4a	98.2	15±0^{a}	99.9
0.46	1.75±1.2^{b}	10.8	4.5±2.7^{a}	29.9	6.75±1.5ab	44.9	10.2±0.75^{b}	67.9
0.23	1.5±1.26^{b}	9.8	2±0.25^{b}	13.2	5.5±0.5b	36.5	7.5±0.75^{b}	49.9
0.11	1.25±0.7^{b}	8.2	1.25±0.72^{b}	8.2	3.5±0.6b	23.2	4.7±0.18^{b}	31.5
Control (distilled water)	0^{b}	0	0.5±0.2^{b}	3.3	1.25±0.3c	8.3	1.5±0.42^{c}	9.1
LSD	1.87		4.5		4.5		2.42	

*Means within a column for insect pest followed by different letters are significantly different ($P < 0.05$; using Duncan's multiple range clarifying by LSD test).

Antioxidant enzyme activities

Table (2) shows SOD and CAT activities to be highly increased in *Sitophilus oryzae* after exposure to Radiant SC 12%, 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 decreasing as compared to control group, respectively. These results are appeared at Figures 1 and 2.

Table 2: Antioxidant enzyme activities (mean ± SE) induced in Rice weevil, *Sitophilus oryzae* as a result of treatment by Radiant SC 12%.

Consentration (ppm)	SOD (U/mg Protein)	CAT (mmol/mg Protein)
1.87	9.53±2.77^{a}	520.36±26.40^{a}
0.93	7.32±1.32^{b}	435.18±21.891^{b}
0.46	4.25±1.13^{d}	385.25±15.72^{cd}
0.23	4.98±1.78^{cd}	375.62±14.97^{d}
0.11	4.75±1.23^{d}	320.85±11.37^{e}
Control(distilled water)	2.51±0.57e	284.36±10.25^{f}

SOD: superoxide dismutase, CAT: catalase

*Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range tests

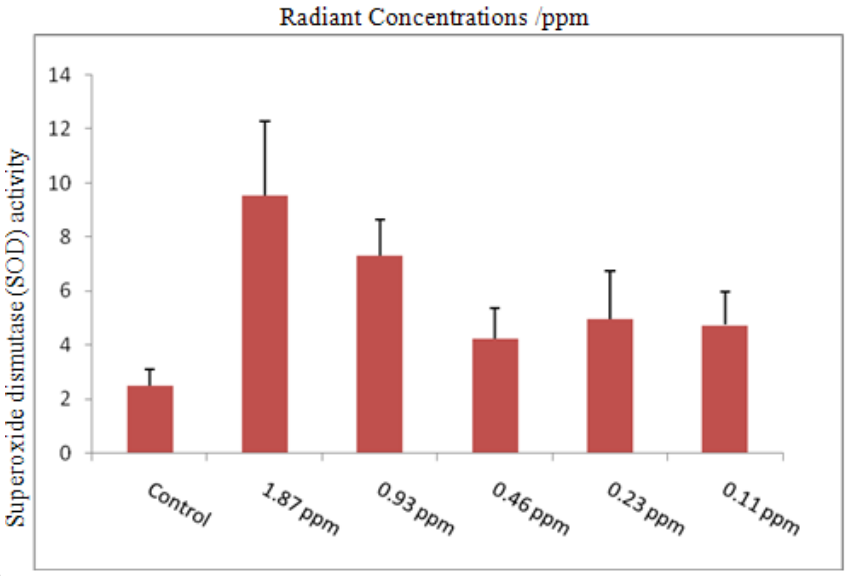


Fig. 1: Effect of different concentrations of Radiant SC 12% on SOD activity in the adult of *Sitophilus oryzae*

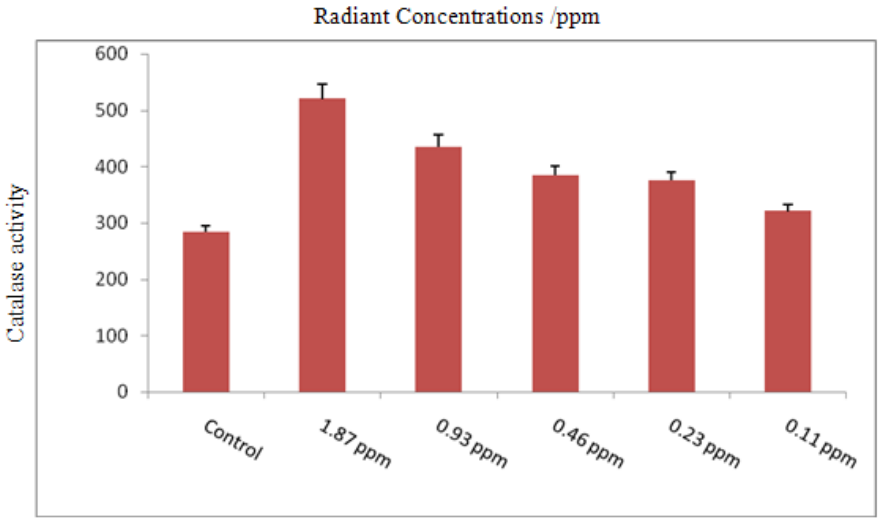


Fig. 2: Effect of different concentrations of Radiant SC 12% on CAT activity in the adult of *Sitophilus oryzae*

Some studies have also showed 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 (Huang and Subramanyama 2007). It was reported that pesticides effected on antioxidant enzyme activities in insects (Dubovskii*et al.,* 2005; Kamata and Hirata 1999). 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* by producing ROS in insect tissues. Other studies reported that pesticides caused lipid peroxidation and the alterations in the antioxidant defense enzymes of insect (Yankanchi and Gadache 2010 and Harris 1991).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 (Buyukguzel 2009).

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,* 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 (Copping and Menn 2000).

In the present study, CAT activity significantly increased in response to Radiant SC 12% induced oxidative stress in tissues of *Sitophilus oryzae* (Amos *et al.*). We conclude that increased CAT 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 (Das 2013).

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% , 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* and increasing oxidative stress in insect's tissues.

As shown in Fig. (3), scanning electron microscope revealed the effect of different concentrations of radiant on the adult of *Sitophilus oryzae*, Where:

Group (1) Control group: Normal structure of *Sitophilus oryzae* with normal size and appearance of the insect body.

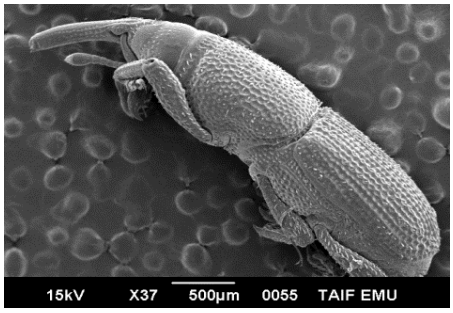
Group (2) Conc. (0.11 ppm): Normal appearance of *Sitophilus oryzae L* but with less oxidative markers appearing by less volume than the normal and with small Curvature.

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.

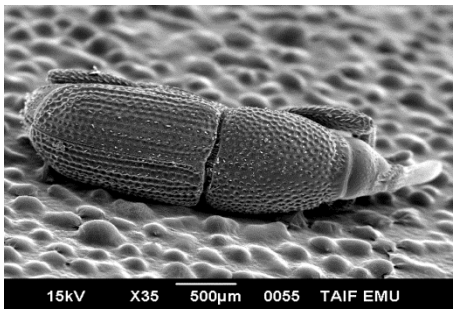
Group (4) Conc.(0.46 ppm):In this concentration treated group , the *Sitophilus oryzae* appears as more smaller in size than that noticed in conc.(0.11 and 0.23 ppm) with curvature also of the body.

Group (5) Conc. (0.93ppm):The more oxidative stress which appears greatly in *Sitophilus oryzae L* of this group, more drooping of the whole body and reduced in size of *Sitophilus oryzae* Prothorax of insect started to separate from the rest of the body.

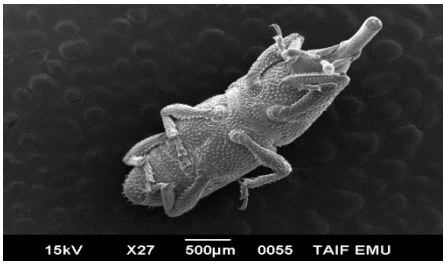
Group (6) Conc. (1.87ppm): oxidative stress also was greatly appeared in this group with generally shrinkage of the whole body of the insect and curvature of the rostrum of the insect with increase degeneration in the prothorax of the insect.

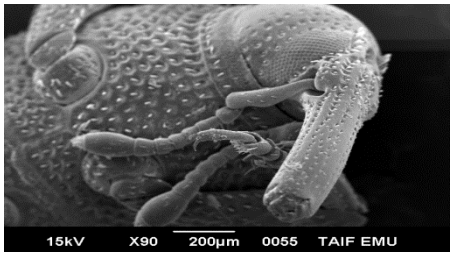


Group (1): Control

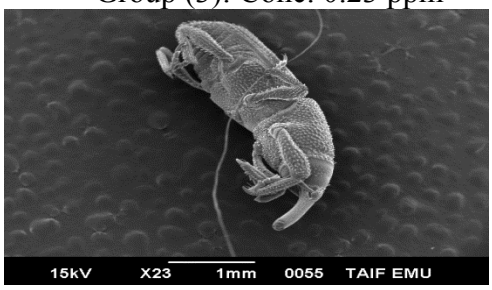


Group (2): Conc. 0.11 ppm

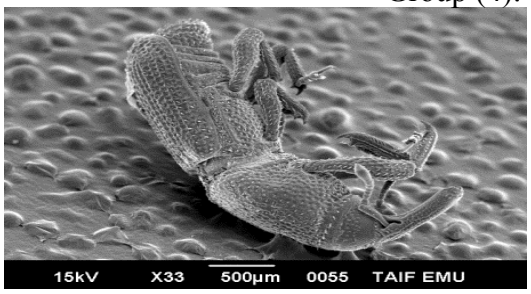


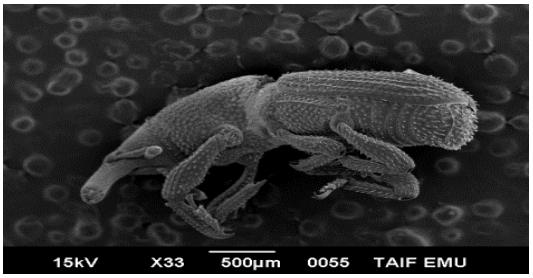


Group (3): Conc. 0.23 ppm



Group (4): Conc. 0.46





Group (5): Conc. 0.93 pp

Group (6): Conc. 1.87 ppm

Fig. 3: Scanning electron microscope showing the effect of different concentrations of Radiant SC 12% on the adult of *Sitophilus oryzae*

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ARABIC SUMMARY

التأثير الإبادي للمركب الحيوي راديانت اس سي ١٢% على سوسة الأرز

رانيا صلاح رشوان [١] و آمال فاضل البرطي [٢] و ريهام زكريا حمزة [١&٣]

١- قسم وقاية النبات- كلية الزراعة- جامعة عين شمس- مصر

٢- قسم الأحياء- كلية العلوم- جامعة الطائف – المملكة العربية السعودية

٣- قسم الحيوان- كلية العلوم- جامعة الزقايق- مصر

المركب الحيوي راديانت اس سي ١٢% يعتبر مبيد تجاري طبيعي وله تأثير منخفض المخاطر. تمت دراسة تأثير خمسة تركيزات من المركب وهي (١.٨و ٠.٩٣و ٠.٤٦و ٠.٢٣و ٠.١١ جزء في المليون) على الحشرات الكاملة لسوسة الأرز وذلك تحت ظروف المعمل. وقد أوضحت النتائج ان التركيزين (١.٨٧ و ٠.٩٣ جزء في المليون) قد حققا أعلى نسب موت في الحشرات الكاملة بعد ٤ أيام من المعاملة حيث وصلت نسب الموت الى (٥٥ و ٣١ % على التوالي)، وبعد ٨ أيام وصلت نسبة الموت الى ١٠٠% لكلا التركيزين. اما المركبات المضادة للأكسدة التي تحمي الحشرة وتعمل كخط دفاعي تؤدي في النهاية الى ضغط التأكسدي. فقد اجريت الدراسة لمعرفة تأثير الراديانت ١٢% على مؤشرات الضغط التأكسدي و تأثيره على نشاط الانزيمات المضادة للأكسدة سوبر اوكسيد ديسميوتيز والكتاليز داخل انسجة حشرة سوسة الأرز.

أدى استخدام المركب الحيوي الى زياده معنويه في نشاط كلاً من الإنزيمين سوبر اوكسيد ديسميوتيز والكتاليز بعد ٤٨ ساعة من التطبيق وذلك مقارنة بالحشرات الغير معاملة. كما تشير النتائج الى ان الراديانت يسبب زيادة معنوية في الضغط التأكسدي والتي تعمل على زيادة ميكانيكية مضادات الأكسدة والتي تؤدي في النهاية الى موت الحشره.