Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(4):979-983

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Laboratory evaluation of superoxide dismutase (SOD) of methylamine avermactin control of the rice red weevil (*Sitophilus oryzae* L.) in stored wheat grains

Amal M. F. Al-Barty

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

---

ABSTRACT

Methylamine avermactine (Radical) is a novel semi-synthetic derivative of natural product abamactin in avermactin family. The efficiency of this bioinsecticide was evaluated on the adults of S. oryzae . Five concentrations were tested to control the weevil adults. Concentration 0.3 and 0.1 ppm produced weevil mortality as high as 100 % after 8 days, while only 60 and 48.7% mortality was recorded for the same concentration after 4 days. The present study was investigated the effects of Methylamine avermactine on the oxidative stress indicator, and antioxidant enzyme [superoxide dismutase (SOD)] activity in Sitophilus oryzae tissues. There were statistically significant increases in SOD activities in the LC50/48h concentration of Methylamine avermactine -treated Sitophilus oryzae compared to the control. These results indicated that Methylamine avermactine causes an increase in oxidative stress and we conclude that increasing oxidative stress induces antioxidant defense mechanisms.

Key words: Methylamine Avermactin, Bioinsecticide, *Sitophilus oryza*

INTRODUCTION

Chemical insecticides are the main method applied against many insect pests. Appearance of many problems such as insect resistance to chemical insecticides and environmental pollution led to search for effective and safe alternatives to be used in pest's control. The continuous use of chemical pesticides for control of pests has resulted in serious problems such as insecticide resistance *(Pacheco et al., 1990; Sartori et al., 1990)*. For these reasons the need of new chemical insecticides with new and different mode of action against pests is required. Methylamine avermactine (Radical) is a novel semi-synthetic derivative of natural product abamactin in avermactin family. Methylamine avermactine (Radical) is a novel semi-synthetic derivative of natural product abamactin in avermactin family. Abamactins (Avermactin B1) are a fermentation product from the soil microorganisms, *Streptomyces avermactitis (Burg et al., 1979)*. Avermactines have been shown to be effective against broad spectrum of arthropod pests *(Putter et al., 1981)*. This insecticide has been reported to have a good field activity against a number of pests as well as certain Homoptera and Coleoptera insects and exhibits reduced pesticide risk with low mammalian toxicity, *(Wing et al., 2000 and McKinley et al., 2002)*. Methylamine avermactine blocks post- synaptic potentials of neuromuscular junctions, leading to paralysis. Avermactin B1 has been shown to inhabit pheromones production *(Wright., 1984)* and inhabit feeding *(Pienkowski and Mehring., 1983)*. Abamectin also is more environmentally acceptable because it binds to soil, does not bioaccumulate, and degrades rapidly *(Lasota and Dybas., 1991)*. The relative toxicity of topically applied avermectin B1 (Abamectin) was studied by *(Corbitt et al., 1989)*.

Rice weevils (*Sitophilus oryzae*) are considered a primary stored-grain insect in warm climate areas. They cause significant losses to stored grains, especially cereals, at conditions favorable to their development (25–35 °C and low RH). The objectives of this research were: (i) assess the efficacy of Methylamine avermactine for the control of rice± weevils with different concentrations (ii)

EXPERIMENTAL SECTION

2.1. Insect rearing:

Adults of *S. oryzae* collected from infested stored wheat grains were reared on healthy wheat grains held in cloth mesh covered plastic pots (15 cm diameter by 20 cm high) at 28± 2 ° c, 70± 5% RH, and 16:8 L:D cycle. Newly emerged adults were used in the experiments.

2.2.Laboratory bioassays:

Radical 0.5 % EC was provided by a trade Mark or Agromen Chemicals Co. Itd.,-China. Radical 0.5 % EC, common name Methylamine Avermactin " 4-deoxy-4 (Methylamine)-(4 R) Avermactin Benzoate (salt) ", it was obtained from Plant Protection Research Institute (Egypt, Cairo). Different concentrations of Radical 0.5 % EC were prepared in the lab; five concentrations (0.3, 0.1, 0.07, 0.04 and 0.02 ppm) were prepared by using distilled water *(Yankanchi and Gadache., 2010)*. Each concentration was consisting of four replicates; replicates with 20 adults of *Sitophilus oryzae* were used in all experiments. Grains were dipped in the insecticide for 15 seconds. For control, grains were dipped in distilled water. All treatments allow drying under lab condition.

2.3. Preparation of homogenates and determination of enzymatic activities and the levels of Superoxide dismutase (SOD):

2.3.1. 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 Methylamine Avermactin. Thirty-insects were used to determine SOD levels. 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^{o}C. The cryotubes were kept at room temperature until the tissue began to thaw before using.

2.3.2. Sample Preparation

Extracts of *Sitophilus oryzae L* insects' homogenates were prepared at 4 ^{o}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 ^{o}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 ^{o}C (SOD assay), 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).

2.3.3. Measurement of SOD Activity

The total SOD (EC 1.15.1.1) activity was determined according to *Marklund and Marklund, (1974)* assaying the auto oxidation 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).

2.4. Statistical analyses

Means of percentage of adult mortality were statistically analyzed using ANOVA followed by Duncan-MSD test ***(Duncan., 1955),*** through software computer program. Statistical significant differences between individual means were determined by one way analysis of variance.

RESULTS AND DISCUSSION

3.1. Effectiveness of different concentrations on the adult of *Sitophilus oryzae* mortality:

Results showed in Table (1) summarized the efficacy of Methylamine Avermactin at different concentrations against the adults of *S. oryzae* . Obtained results refers to that the different applied concentrations of the present bioinsecticide clearly affected the percentage of adult mortality, increasing gradually with an increase with the tested concentration *(Loschiavo., 1976)*.

There were significant differences in the mean mortality of S. oryzae between concentrations (F 3.45, P < 0.001), the adult survival was significantly different between concentrations with times (2 days: F= 21, P < 0.001; 4 days: F= 17.2, P < 0.001; 6 days: F=7.13 P < 0.001; 8 days: F=4.6, P < 0.001). However, mortality was increased by an increase in radical concentration as shown in Table (1) Adult mortality varied and exposure periods indicating the mortality effect of the insecticides treatments were concentration and time dependent *(Fernando and Karunaratne., 2012)*, where concentration 0.3 and 0.1 ppm produced weevil mortality as high as 100 % after 8 days, while only 60 and 48.7% mortality was recorded for the same concentration after 4 days.

The Avermectins are both insecticides and acaricides which are effective by either contact or ingestion. The target for avermectins is the GABA receptor in the peripheral nervous system. Avermectins stimulate the release of GABA from nerve endings and enhance the binding of GABA on the post-junction membrane of muscle cells of insects and other arthropods. This eventually results in an increased flow of chloride ions into the cell, with consequent hyper polarization and elimination of signal transduction, resulting in an inhibition of neurotransmission *(Jansson and Dybas., 1996)*.

Table (1): The effectiveness of Methylamine Avermactin on the mortality of the adult of *Sitophilus oryzae*

Concentration (ppm)	After 2days of treatment		After 4days of treatment		After 6days of treatment		After 8days of treatment	
	Mean of dead adult ±se	Mortality (%)	Mean of dead adult ±se	Mortality (%)	Mean of dead adult ±se	Mortality (%)	Mean of dead adult ±se	Mortality (%)
0.3	5.25±0.37a	26.2	12±1.1a	60	18±1.25a	90	20±0a	100
0.1	4.25±0.37ab	21.2	9.7±0.28a	48.7	17±0.5a	86.2	20±0a	100
0.07	3±0.25bc	15	7.5±0.28a	39.5	13±0.62a	65	17±1.8a	88.75
0.04	2±0.25c	10	6.5±0.64b	32.5	11±0.62b	55	13.75±0.b87	68.22
0.02	2±0.25c	10	4.25±0.47bc	21.25	7.7±0.5b	38.7	10.7±0.75b	53.7
Control (distilled water)	0d	0	0d	0	0c	0	1.25±0.37c	6.25
LSD	1.79		2.6		4.2		4.6	

3.2. Antioxidant enzyme activities:

SOD activity was determined to be highly increased in *Sitophilus oryzae L* after exposure to Methylamine avermactine 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 and 0.23 and 0.1 ppm despite their increasing as compared to control group, respectively (Figures 1). There were statistically relevant and distinctive significant increases in the SOD activities in the concentrations: 1.87, 0.93 (ppm) concentrations respectively of Methylamine avermactine treated insects compared with the control (Table.2) and (Fig. 2).

Table (2): Antioxidant enzyme activities (mean ± SE) of (mean ± SE) of storage pest Rice weevil, *Sitophilus oryzae L.* (Coleoptera: Curculionidae) with Methylamine avermactine

Concentration (ppm)	SOD (U/mg Protein)
1.87	8.93 ± 2.77^{a}
0.93	6.62 ± 1.32^{b}
0.46	4.45 ± 1.13^{cd}
0.23	3.78 ± 1.78^{d}
0.11	3.15 ± 1.23^{e}
Control (distilled water)	1.51±0.57f

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.

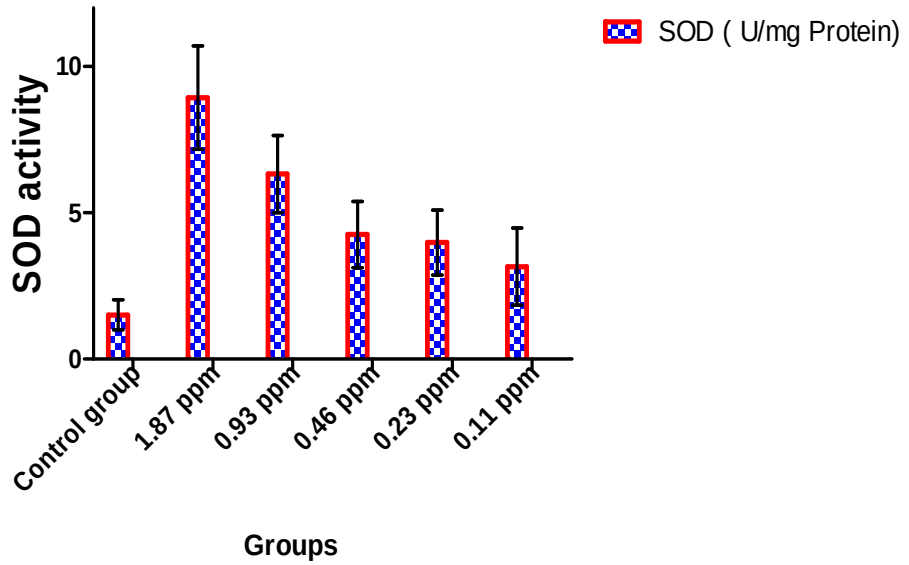

Fig.2: Antioxidant enzyme activities of Methylamine avermactine against storage pest Rice weevil, *Sitophilus oryzae L.* (Coleoptera: Curculionidae)

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 activity was found in insects' tissues homogenates after application of Methylamine avermactine. different concentrations. This suggested that Methylamine avermactine 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 et al., 2010; Wu et al., 2011)* and these results are greatly reinforced by the present findings explaining the high significant increase in SOD activities in different concentrations in *Sitophilus oryzae L.* 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 Methylamine avermactine induces the superoxide radical in the tissues of *Sitophilus oryzae L.*SOD activity significantly increased when the insects were exposed to Methylamine avermactine. Suggesting that SOD was stimulated by scavenging superoxide radical to protect the insect from Methylamine avermactine stress. It has been reported that an increase in SOD activity is probably a response towards increased ROS generation in rat erythrocytes *(John et al., 2001)*.

REFERENCES

[1]Pacheco IA; Sartori MR; Taylor RWD, **1990**. Levantamento de resistencia de insetospragas de graos armazenados a fos.na, no Estado de sao Paulo. *Coletanea do institudo de Technologia de Alimentos*, 20: 144-154.
[2] Sartori MR; Pacheco IA; Laderosa M; Taylor RWD,**1990**. Ocorrencia eespeci. ciadade de resistencia ao insecticide malathion em insetos-pragas de graos armazenados, no Estado de Sao Paulo. *Coletanea de Institudo de Technologia de Alimentos*. 20: 194-209.
[3]Putter I; MacConnell JG; Preiser FA; Haidri AA; Ristich SS; Dybas RA,**1981**. *Experientia* 37: 963-964.
[4]Burg RW; Miller BM; Baker EE; Birnbaum J; Currie SA; Hartman R; Kong YL; Monaghan RL; Olson G; Putter I; Tunac JB; Wallick H; Stapley EO; Oiwa R; Omura S,**1979**. *Antimicrob. Agents Chemothe*., 15:361-367.

[5]Wing KD; Sacher M; Kagaya Y; Tsurubuchi Y; Mulderig L; Connair M; Schnee M, **2000**. *Crop-Protection*. 19(8/10): 537-545.
[6] N McKinley; S Kijima; G Cook; D Sherrod, **2002**. Avaunt (Indoxacarb): A New Mode of Action Insecticide for Control of Several Key Orchard Pests. Proceedings of the 76th Annual Western Orchard Pest & Disease Management Conference 9-11 January (**2002**), Portland, Publ. By Washington State Univ., Pullman, Washington Chemical Control/New Products DuPont Crop Protection, Wilmington, DE.
[6]Pienkowski RL ;Mehring, PR, **1983**. *J. Econ. Entomol*. 76: 1167-1169.
[7]Wright JE,**1984**. *J. Econ. Entomol*.77: 1029-1032.
[8]Corbitt TS; AJ Stgreen; Wright DJ, **1989**. Relative potency of abamectin against larval stages of S. littoralis (Boisd.), Heliothis armigera (Hübn.) and Heliothis virescens (F.) (Lepidoptera: Noctuidae Crop Protection. 8(2):127-132.
[9]Lasota JA; Dybas RA,**1991**. *Annu. Rev. Entomol*. 36: 91-117.
[10]Dahi HF; El-Sayed, YA; El-Barkey NM ;Abd-El Aziz MF ,**2009**. *Egypt. Acad. J. biolog. Sci*., 2 (1): 103- 116
[11]Duncan B D,**1955**. *Biometric*, 11: 1-42.
[12]Jansson RK; Dybas,RA,**1996**. Avermectins: Biochemical mode of action biological activity and agricultural importance. In Insecticides with Novel Modes of Action: Mechanisms and Application; Ishaaya, I., Ed.; Springer-Verlag: New York, NY,
[13]Yankanchi SR; Gadache AH, **2010**. *J Biopesticides* 3: 511- 516.
[14]Loschiavo SR ,**1976**. *J Econo Entom* 69: 395-399.
[15]Marklund S; Marklund G, **1974**. *European Journal of Biochemistry*. 47:469-474.
[16] Bagchi D; Bagchi, M; Hassoun E A; Stohs S J,**1995**. *Toxicology*, 104: 129-140.
[17]Dubovskii IM; Olifrenko OA; Glupov VV, **2005**. *Journal of Evolutionary Biochemistry and Physiology*, 41(1): 20-25.
[18]Dubovskiy, IM; Martemyanow VV; Vorontsova YL; Rantala MJ; Gryzanova EV; Glupov VV,**2008**. Effect of bacterial infection on antioxidant activity and lipid peroxidation in the midgut of Galleria mellonella L. larvae (Lepidoptera, Pyralidae). *Comparative Biochemistry and Physiology*, Part C, 148: 1-5.
[19]Gultekin F; Ozturk M; Akdogan M, **2000**. *Archives of Toxicology*, 74: 533-538.
[20]Gupta SC; Mishra M; Sharma A; Deepak Balaji TGR; Kumar R; Mishra RK; Chowdhuri DK, **2010**. *Ecotoxicology and Environmental Safety*, 73 (6):1415-1423.
[21]Wu H; Liu J; Zhang R; Zhang J; Guo Y; Ma E, **2011**. *Pesticide Biochemistry and Physiology*, 100: 23-26.
[22]Bukowska B, **2004**. *Cell Biology International*, 28: 557-563.
[23]John S; Kale M; Rathore N; Bhatnagar D, **2001**. *Journal of Nutritional Biochemistry*, 12: 500-504.