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Evaluation of Toxic Effect of Methylamine Avermactin on Malondialdhyde Activity in the Rice Red weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae) in Stored Rice Grains

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The rice weevil, *Sitophilus oryzae* is one of the major pests of stored rice in the world. Methylamine avermactine (Radical) is a novel semi-synthetic derivative of natural product abamactin in avermactin family. This study investigates the effectiveness of the methylamine avermactine in controlling infestations of the rice weevil. Five concentrations were tested to control the weevil adults. Concentrations 0.3, 0.1, 0.07, 0.04 and 0.02 ppm produced weevil mortality as high as 100, 100, 88.75, 68.22 and 53.7 % respectively after 8 days. LC25, LC50 LC80, and LC90 values were calculated from the plotted toxicity regression lines from the mortality percentages as a result of treatment. Methylamine avermactine afforded significant increase in MDA level in *S.oryzae* after exposure to different concentrations especially conc.0.3 ppm which showed highly significant increase in MDA level which revealed the occurrence of lipid peroxidation in insect tissues and affect on insect antioxidant capacities and thus revealed its efficiency in controlling the infestations of the rice weevil.

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

The biopesticides have been extensively studied because the chemical pesticides used for the control of the insect pests of the stored food grain can be harmful due to the persistence of the toxic residues on the food grains and development of resistance in the insects (Wing *et al* 2000 and McKinley *et al* 2002). 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). Avermactins have been shown to be effective against broad spectrum of arthropod pests (Putter *et al*., 1981). This materials act by interfering with the action of gamma aminobutyric acid (GABA) (Fritz *et al*., 1979). It blocks post- synaptic potentials of neuromuscular juncations, leading to paralysis. Avermactin B1 has been shown to inhabit pheromones production (Wright 1984) and inhabit feeding (Pienkowski and Mehring 1983). Radical shows high potential effect against lepidopterous larvae (Mrozik 1994 and White *et al*., (1997). Abamectin also is more environmentally acceptable because it binds to soil, does not bioaccumulate, and degrades rapidly (Lasota and Dybas 1991).

Rice weevils (*Sitophilus oryzae*) are considered a primary stored-grain insect in warm

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climate areas. They cause significant losses to stored grains, especially cereals, at conditions favorable to their development (25–35 f C and low RH). Direct-feeding damage by *S. oryzae* reduces grain weight, nutritional value, and germination of stored grain. Infestations also cause contamination, odor, mold, and heat-damage problems that reduce the quality of the grain. Weevils feed mainly on the endosperm and germ of the grain, reducing the carbohydrate content, thus removing a large percentage of the protein and vitamins.

The objectives of this study were assessing the efficacy of Methylamine avermactine for the control of rice weevils with different concentrations.

MATERIALS AND METHODS

Insect culture and experimental conditions

Stock of *S. oryzae* was obtained from infested white rice (*Oryza sativa*) bought from the local market. Laboratory cultures of *S. oryzae* were maintained on uninfected white rice. Adult rice weevils were introduced into plastic jars containing 500g of rice. These plastic jars were then covered with a muslin cloth to prevent insects escaping and to allow ventilation. Experiments were maintained at laboratory conditions (27 ± 2 ˚ C and relative humidity of 65 ± 5 %) for the emergence of *S. oryzae* adults. For all the experiments 1-7 days old, adult weevils were selected from cultures.

Preparation of insecticides

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). Five concentrations were prepared in the lab., the concentrations were (0.3, 0.1, 0.07, 0.04 and 0.02 ppm) and prepared by using distilled water (Yankanchi and Gadache 2010). Four replicates with 20 adults of *Sitophilus oryzae* were used in all experiments. Grains of rice were dipped in the insecticide for 20 seconds to allow coating all grains with delicate film of the insecticide (Arthet 1996). Only distilled water was used to dip the grains for the control. Replicates containing grains were left to dry under lab conditions.

Preparation of *S.oryzae* tissues homogenates and determination of enzymatic activitiy and the level of Malondialdhyde (MDA)

Tissue collection

For measurement of antioxidant enzyme activities in insect tissue homogenate, a separate test was arranged by application of the LC25, LC50, LC80 and LC90 values of Methylamine Avermactin . Thirty-insects were used to determine MDA level and antioxidant enzyme activity. 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 tissues began to thaw before using.

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. MDA contents and antioxidant enzymes activities were determined by measuring the absorbance of the samples in a dual beam spectrophotometer (Shimadzu-1700, UV/vis, and Kyoto, Japan). Essays were replicated six times with six midguts each. All chemicals used were analytical grade and were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Measurement of MDA activity

MDA is secondary product of lipid peroxidation (LPO). *Sitophilus oryzae* tissues were incubated at 95 °C with thiobarbituric acid under aerobic conditions (pH 3.4). The pink colour produced by these reactions was measured spectrophotometrically at 532 nm to measure MDA levels (Ohkawa *et al*., 1979). MDA levels were defined as nanomole per gram tissue.

Data analysis

Recorded data were corrected by using the Abbott's formula (Abbott 1925) to correct the percent of mortality. Data obtained were subjected to analysis of variance (ANOVA) followed by Duncan-MSD test (Duncan, 1955) by using SAS program, values were represented as mean ± SEM.

RESULTS AND DISCUSSION

The percentage mortality of *S. oryzae* depended on the concentration of formulation and exposure time. All the treatments with the different concentrations showed significant level of toxicity to the *S. oryzae*. The analyzed results of the present study indicated significant differences in the mean mortality of *S. oryzae* between five concentrations (F 3.45, P < 0.001), the adult survival was significantly different between concentrations with exposure 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). For all concentrations, percentage mortality increased as concentration used increase, was significantly higher than the control at P<0.05 (Fig.1), where concentration 0.3, 0.1, 0.07, 0.04 and 0.02 ppm produced weevil mortality as high as 100, 100, 88.75, 68.22 and 53.7 % respectively after 8 days, while they recorded 90, 86.2, 65, 55 and 38.7% respectively after 6 days. As shown in figure (1), no mortality was observed in the control through the exposed time except after 8 days due to the natural mortality.

Data in Table 1 and Fig. 2 showed that the LC25 values of Methylamine Avermactin

Table 1. Toxicity values (ppm) of Methylamine Avermactin 1.9 % EC against *S. oryzae* adult after 2, 4, 6 and 8 days of treatment.

Days after Treatment	LC_{25}	LC_{50}	LC_{80}	LC_{90}	r	Slope
2 Days	0.25850	2.83481	56.27275	268.32883	0.95	0.62
4 Days	0.02525	0.14064	1.19883	3.67471	0.99	0.9
6 Days	0.01102	0.03168	0.11832	0.23562	0.95	1.4
8 Days	0.01161	0.02122	0.04506	0.06680	0.90	3.0

Table 2. Malondialdhyde level of Methylamine Avermactin 1.9 % EC against *S. oryzae* adult after 2, 4, 6 and 8 days of treatment

Days after Treatment	Control	0.02	0.04	0.07	0.1	0.3 conc.
2 Days	1.90 ± 0.12^{e}	4.53 ± 0.52^{d}	4.55 ± 1.52^{d}	6.53 ± 1.52^{c}	7.63 ± 1.52^{a}	7.52 ± 1.52^{b}
4 Days	1.98 ± 0.35^{f}	4.96 ± 0.42^{e}	5.63 ± 1.63^{d}	7.63 ± 1.63^{c}	10.35 ± 1.20^{b}	11.32 ± 1.63^{a}
6 Days	1.96 ± 0.14^{f}	6.52 ± 0.63^{e}	7.52 ± 1.87^{d}	9.86 ± 1.96^{c}	12.65 ± 1.36^{b}	13.25 ± 1.52^{a}
8 Days	1.96 ± 0.41^{f}	8.85 ± 0.52^{e}	9.21 ± 1.98^{d}	10.85 ± 1.75^{c}	13.63 ± 1.75^{b}	14.25 ± 1.96^{a}

Means within the same column carrying different superscripts are significant at P≤0.05 (M±SE) (n=10)

against S. *oryzae* adult after 2, 4, 6 and 8 days of treatment recorded 0.25, 0.025, 0.011 and 0.0116 ppm respectively, while LC_{50} after 2, 4, 6 and 8 days of treatment were 2.83, 0.14, 0.03 and 0.02 ppm. LC80 was determined to be 56.2, 1.198, 0.11 and 0.04 ppm. LC90 values revealed 268.3, 3.67, 0.23 and 0.066 ppm respectively.

Data in table (2) and Fig (3) revealed that of Methylamine Avermactin against S. *oryzae* adult after 2, 4, 6 and 8 days of treatment afforded significant increase in MDA level after exposure of *S.oryzae* to concentrations: 0.3, 0.1, 0.07, 0.4, 0.2 ppm respectively which approve presence of lipid peroxidation in *S.oryzae* tissues due to exposure of Methylamine Avermactin which prove it's successding in causing lipid peroxidation in insect tissues and then finally lead to insect death with the less side effects.

MDA, a major oxidation product of peroxidized polyunsaturated fatty acids, has been used to determine the degree of lipid peroxidation and as a biological marker of oxidative stress (Rael *et al*., 2004; Del rio *et al*., 2005). It has been shown previously that pesticides increase MDA level in human erythrocytes and insects (Durak *et al*., 2009; Buyukguzel, 2009). In our study, MDA level was increased in tissues of LC50/48h value of Methylamine Avermactin treated *S.oryzae*, which suggests that MDA levels could be used as a marker of Methylamine Avermactin injury.

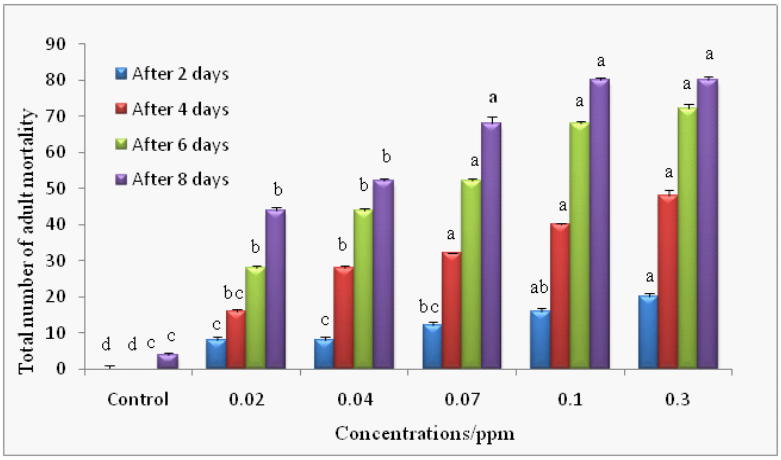

Fig. 1. Total mortality of *Sitophilus oryzae* exposed to different concentrations of the insecticide after 2, 4, 6 and 8 days. Different letters on top of columns indicate significant differences according to Duncan's test

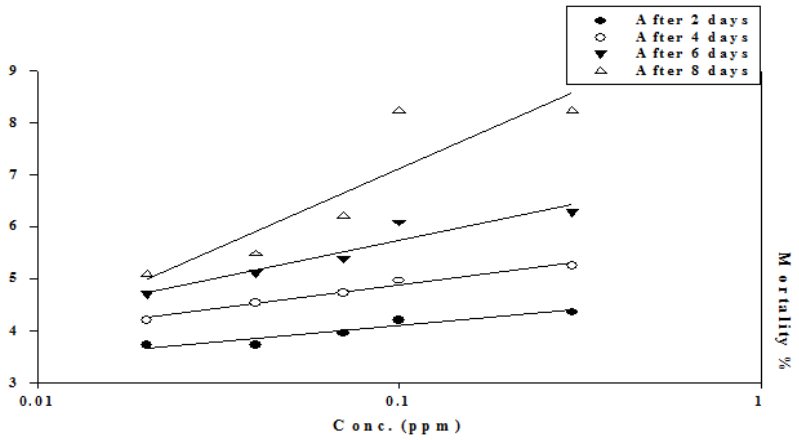

Fig. 2. Toxicity regression lines of Methylamine Avermactin 1.9 % EC against *Sitophilus oryzae* adult after 2, 4, 6 and 8 days of treatment

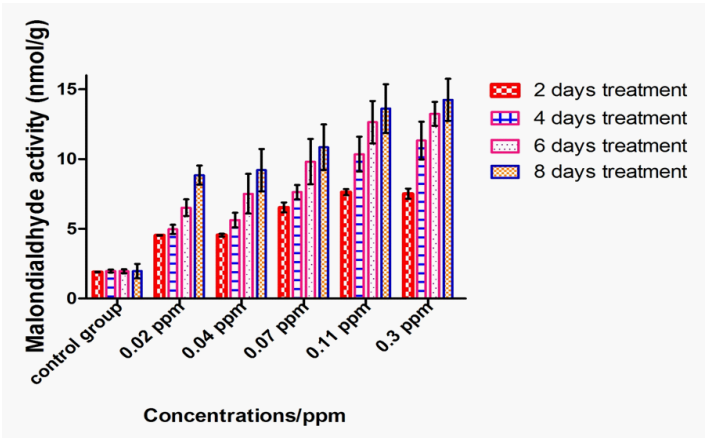

Fig. 3. Malondialdhyde level of Methylamine Avermactin 1.9 % EC against *S. oryzae* adult after 2, 4, 6 and 8 days of treatment

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