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Toxicity comparison of Three IGR Agents on *Oryzaephilus surinamensis* L. (Col., Silvanidae) Under Laboratory Conditions

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Abstract

The saw-toothed grain beetles, *Oryzaephilus surinamensis* L. (col., Silvanidae) is one of the most important pests that damage to stored products in Iran. The efficacies of three IGR insecticides, Tebufenozide, Chromafenozide and Lufox® have been evaluated on eggs and larval stages of *O. surinamensis* during this study. The Efficiencies of these insecticides have been considered by different concentrations on one day old eggs, 5 day old (young) and 19 day-old larvae. The results of bioassay tests showed that Lufox® has the most and Tebufenozide has the least efficacy on eggs and larval stages. Also, the mortality effects directly increased by raising the IGRs concentrations. In the other aspect, the eggs of *O. surinamensis* were the most susceptible stage in response to IGR insecticides. The LT_{50} value of Lufox® was shorter than that of Tebufenozide and Chromafenozide. Finally the high toxicity and strongest efficiency can be resulted by using of Lufox® in comparison with Tebufenozide and Chromafenozide.

Key word: *Orizaephilus surinamensis,* IGRs*,* Tebufenozide, Chromafenozide, Lufox®

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Introduction

Approximately, 10-20 percent of the agricultural products which have been stored in storages and thesauruses are lost by different kinds of pests such as insects and plant pathogens in Iran (Moharramipour *et al*., 2003). The species, *Oryzaephilus surinamensis* (Col., Silvaninae) who is called the saw-toothed grain beetle is the most important insect pest in the world can damage to dried fruits, stored grains and flour productions (White & Leesch, 1995; Jovanović *et al*., 2007).

Most economic storage insect pests can be controlled by using phosphine, methyl bromide fumigation ingredients and other synthetic insecticides. Recently, usages of high risk insecticides are limited because they have high toxicity for vertebrates and invertebrates animals specially humans and natural enemies. The other side effects of chemical pesticides consist of the pest resistance, the pest resurgence, the environmental pollutions, causing unacceptable pesticide residues in food, living animal bodies and trophic circles and finally, the lethal effects on non-target organisms (Bell & Wilson, 1995; White & Leesch, 1995; Daglish & Collins, 1999; Jovanović et al., 2007).

Recently, almost studying and research processes are focused on finding new compounds by less damages to non-target living organisms which are living in deferent parts of environment. The Insect growth regulators (IGR), e.g. Teflubenzuron, Chromafenozide and ®Lufox®, are commonly used as the practical compounds with less poisonous effects on human, domestic vertebrates and environmental aspects. These cheap compounds can be easily produced on an industrial scale. Additionally, these materials will be disintegrated quickly and completely under normal environmental condition and caused weakly effects on insect's resistance. (Mc Gregor & Kramer, 1977). The IGRs are suitable for controlling store pests (Loschiavo, 1976). The IGRs play the important role in destruction of different developmental stages in insects; The IGRs are mainly known as the larvicides and ovicides compounds (Lee *et al*., 1996). But, these agents can be affected latently on longevity, fecundity and fertility capacity of the insect's adult stages (Marco et al., 1998; kandil *et al*., 2012). The IGRs are more selective insecticides for target pests and less toxicity for non-target organisms and natural enemies rather than other synthetic conventional insecticides in contrast to living organisms (Perry *et al*., 1998).

Otherwise, some researchers have reported the effects of IGRs on insects' chitin synthesis. They found that the chitin has been synthesized from glucose, glucosamine or N-acetyl glucosamine, protein and carbohydrate witch are the immediate precursor of chitin (Candy & Kilby, 1962; Marks & Sowa, 1976). Tebufenozide is a novel synthetic non-steroidal ecdysteroid agonist, can be blocked the site of the molting hormone action. It is an agonist of ecdysone that causes premature molting in larval stage pests. It can be affected with high selectivity and low toxicity on the target pests. The company that discovered Tebufenozide (which is known as Rohm and Haas), was given a Presidential Green Chemistry Award for its development (Carlson, 2000). Chromafenozide is a novel dibenzoylhydrazine andis which is categorized as an insect hormone ecdysone (moulting hormone agonists) that was developed in the collaborative research project between Nippon Kayaku Co., Ltd., and Sankyo Co., Ltd., and is categorized to be an insect hormone ecdysone agonist. Chromafenozide is found to be significantly the potent compound against various insects. This material is completely non-dangerous for pollinators, predators and parasitoids insects. Also, Chromafenozide has a low toxicity for mammals and causes the minimum impact on the environmental aspects. It would be an ideal agent for integrated pest management (Smagghe *et al*., 2012)

Lufox® is the potent IGRs with proven activity on many pests of horticultural and urban environments (Charmillot *et al*., 2006). Lufox® is mixture of juvenile hormone mimic (Fenoxycarb 7.5% EC) and chitin synthesis inhibitor (Lufenuron 3% EC) (Reda *et al*., 2010 a,b). Lufox® is known as the ovicidal and larvicidal component with serious effects on various insect pests . Adult of the orders, Diptera and Orthoptera can be controlled by using this material (Cantus *et al*., 2008; Reda *et al*., 2010 a,b). Lufox® is relatively safe agent for natural enemies (Hosseinzadeh *et al*., 2011).

Our objective was to evaluate toxicity of three IGR insecticides, Tebufenozide, Chromafenozide and Lufox® on different growth stages of *O. surinamensis*. Therefore, the present study was designed to describe the acute toxicity of this IGRs compounds. This study report preliminary results of which IGRs can be more effective on The saw-toothed grain beetles. Also the obtained results support more environmentally friendly inseceticides that can be used in stored productions.

Materials and Methods

The effects of Tebufenozide, Chromafenozide and Lufox® compounds have been tested on one day old eggs, young and old larvae (4 days old and 20 days old) of *Oryzaephilus surinamensis.* This study has been done in agricultural college laboratory of Islamic Azad University–Arak branch during 2013-2014.

Rearing of *Oryzaephilus surinamensis*

The specimens of *Oryzaephilus surinamensis* were reared in standard containers which had been filled by 10grs of wheat flour. The containers were chosen by the following dimensions: 18 centimeters height and 8 centimeters diameters, which used as the foodstuffs. The rearing specimens are maintained for three generations at the fixed temperature and humidity under dark condition (28 ± 2 °C; 65 ± 5% relative humidity). Different developmental stages are randomly chosen from healthy individuals as the biotests with the same cohort.

Insecticides

Three Insect growth regulators were experimentally used in this project those are written here-in-after:

Mimic is a IGR insecticide and has a common name (Tebufenozide) with chemical name(N'-(4-Ethylbenzoyl)-3,5-dimethyl-N-(2-methyl-2-propanyl)benzohydrazide), of molecular formula ($C_{22}H_{28}N_2O_2$).

Matric is a IGR insecticide and has a common name (Chromafenozide) with chemical name(3, 4 dihidro-5-methyl-2H-1-benzopyran-6-Carboxylic acid 2- (3, 5-dimethylbenzoyl)-2-(1, 1-dimethyl) hydrozide), of molecular formula ($C_{24}H_{30}N_2O_3$).

Lufox is a IGR insecticide (mixture of juvenile hormone mimic (Fenoxycarb 7.5% EC) and chitin synthesis inhibitor (Lufenuron 3% EC) with chemical name (ethyl [2-(4- phenoxyphenoxy) ethyl] carbamate (Fenoxycarb) + N-[[[2,5- dicholoro-4-(1,1,2,3,3,3- hexafluoropropxy) phenyl] amino] carbonyl] -2,6-difluorobenzamide (Lufenuron)).

Toxicity bioassays

The toxicity of Tebufenozide, Chromafenozide and Lufox® has been considered on eggs of *O. surinamensis* with one day old and two different larval stages. The eggs are obtained from adults who were fed by wheat flours. The eggs separated by a 60-mesh sieve. The toxicity consideration will be done after preliminary tests. The separated eggs weresubmerged by solution concentrations with 500, 250, 125, 62, 31, 15, 7 and 3 ppm. Also, the efficacies of different doses, comprising of 1000, 500, 250, 125, 62, 31, 15 and 7 ppm are tested on young larvae (4-5 day) and 3000, 1500, 750, 375, 187, 93, 46 and 23 ppm checked on old larvae (20 days). Each concentration will be replicated at three times on three different biotests. The 10 numbers of eggs and larvae were placed in the Petri dishes which were covered by a damp filter paper as lower layer and 10 grs of wheat flours as upper layer. Each petri dish will be dried during 3 hour under laboratory conditions before inserting specimens.

The Petri dishes which carry specimens are placed into incubators by fixed temperature (28°C), constant relative humidity (65±5%) and dark conditions. The mortality effects on eggs was recorded during 6 days after treating and larval mortality recorded during 10 days after exposing biotests by each compound. The eggs hatching and larval movement are the signs of death, e.g. dead Larvae did not move when were prodded by a soft paint brush and dead eggs did not hatch.

Statistical analysis

The data are transformed [arcsin sqrt (x+0.001)] before analysis for stabilizing variance. The rate of mortality is analyzed by using ANOVA software. The means can be separated based on the results of analyzing by ANOVA software. The doses with mortality response of different concentrations can be estimated by using duncan analysis in SAS 9.13. The LC50 and LT50 factors and their related statistics estimated separately for each insecticide by using the Polo-pc software.

Results

Toxicity of the IGR compounds on *O. surinamensis* eggs:

Lufox® compound have the highest mortality effects on *O.surinamensis* eggs. Furthermore, the lowest mortality percentage is caught by Tebufenozide compound usage. The variance analysis results of the mortality percentage show that there is a significant different between the three difference compounds (df= 2; F=120.75, P<0.0001). Tebufenozide has caused 6.66% mortality at 3.9 ppm which is the lowest rate and 250 and 500 ppm of Lufox® have the strangest mortality effects with the rate 100%. There is another significant difference between the dose efficacies on eggs mortality based on the resulted data (df= 7; F=321.76, P<0.0001). The hatching rates decreased in contrast to increasing of the compounds concentration (Fig. 1). The LC_{50} values are 37.9, 21.15 and 9.15 ppm for Tebufenozide, Chromafenozide and Lufox®. The details of LC_{50} values those are resulted from the tests on the one day old eggs of *O.surinamensis* are shown at table (1).

Toxicity of the IGR compounds on young and old larvae of *O.surinamensis*

The IGR, Lufox® has the highest mortality effects on the young and old stages of the species *O.surinamensis* in comparison with Tebufenozide and Chromofenozide compounds. Tebufenozide compound has the lowest mortality effects. The results of the analysis showed that there are significant differences between the compounds on young larvae (df= 2; F=45.32, P<0.0001) and also on old larvae (df=2; F=126.41, P<0.0001). Also, the results of the analysis showed that there are significant differences between the effects of different doses on young larvae (df= 7; F=148.1, P<0.0001) and on old larvae (df= 7; F=126.44, P<0.0001).

The effects of Tebufenozide usage show that 10% mortality will be occurred by using of 7.81 ppm on young larvae and 13.75% mortality at 23.43 ppm on old larvae, which are the lowest rates during this study. Lufox® compound has the highest toxicity on young larvae by concentrations of 500 and 250 ppm with 100% mortality based on the final results, while 85 and 92.5% mortality is occurred at the high concentrations of Tebufenozide and Chromofenozide compounds for young larvae. The resulted data based on bioassay tests show that Lufox® by the concentrations 3000, 1500 and 750 ppm has the strongest effects on the old larvae, with the rate 100%, while Tebufenozide and Chromofenozide compounds can be killed just 81.25 and 92.5% of the biotests. Larval mortality decreased in contrast to increase of the compounds concentration (Fig. 1; 2).

The bioassay data with respect to the LC_{50} values are presented in tables 1. According to the obtained results, Lufox® has the better and efficiently functional response for controlling the young and old larval than that of Tebufenozide and Chromafenozide. The results indicate that the LC_{50} value of Lufox® for the young and old larvae are 1.00 and 29.2 ppm, respectively with slop (± SE) value of 1.15±0.14, 1.37±0.15 and chi-square value of 5.75 and 5.16 (table 1).

LT50 values for the young and old larval stages are presented in Table 2 and 3 which calculated after exposing by the three compounds. The LT50 values of Lufox® at highest concentration (1000 and 3000 ppm) are shorter (2.6 and 2.75 days) than that of Tebufenozide by same concentrations (5.32 and 5.17 days). The LT50s values of Lufox® are 10.02 and 7.84 days for low concentrations (7 and 23 ppm), while Tebufenzide LD50s are 17.51 and 15.6 days for low concentrations (7 and 23 ppm). The mortality rate of larval stages of *O. surinamensis* is calculated about 10 days after exposing by the different doses of IGR compounds those are shown in figures 2 and 3.

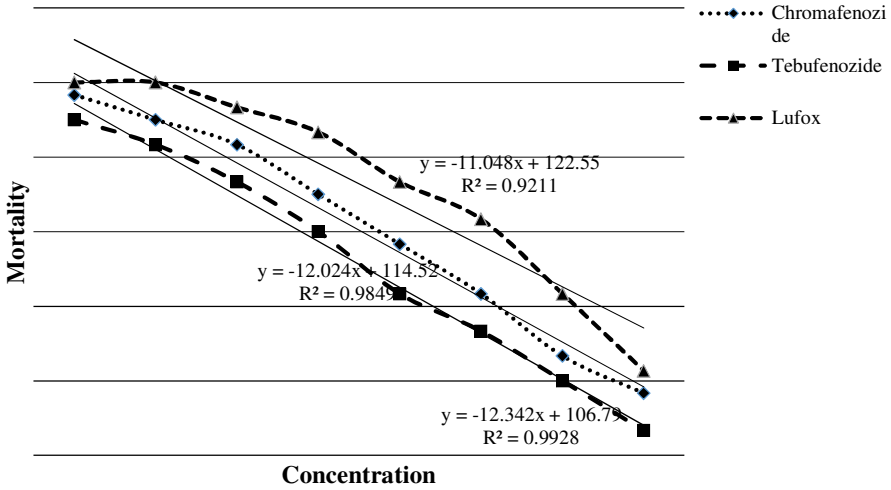

Fig. 1- Mortality of *O. surinamensis* eggs in different concentrations of Tebufenozide, Chromafenozide and Lufox®

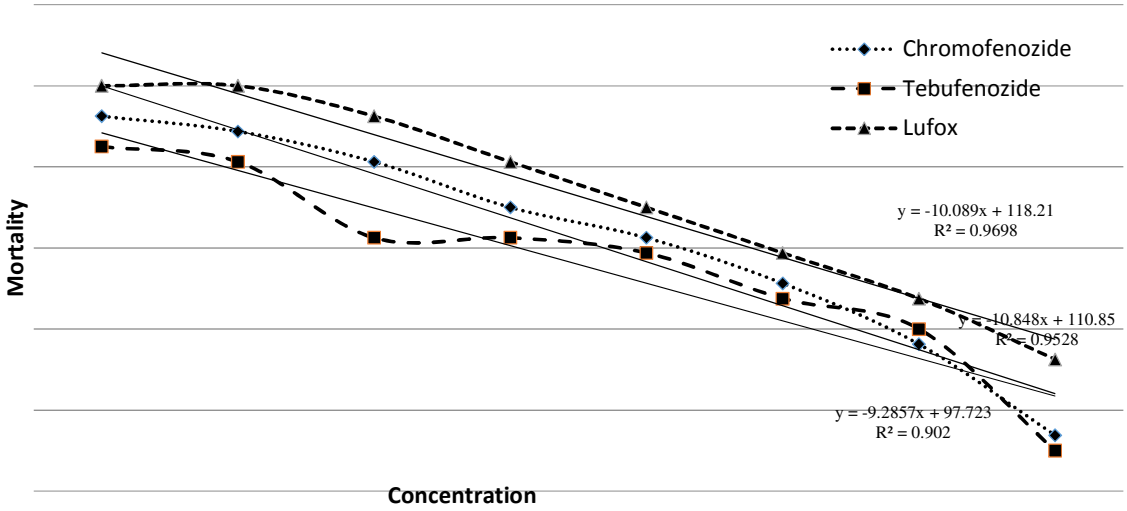

Fig. 2- Mortality of *O. surinamensis* young larvae in respect to the different concentrations of Tebufenozide, Chromafenozide and Lufox®

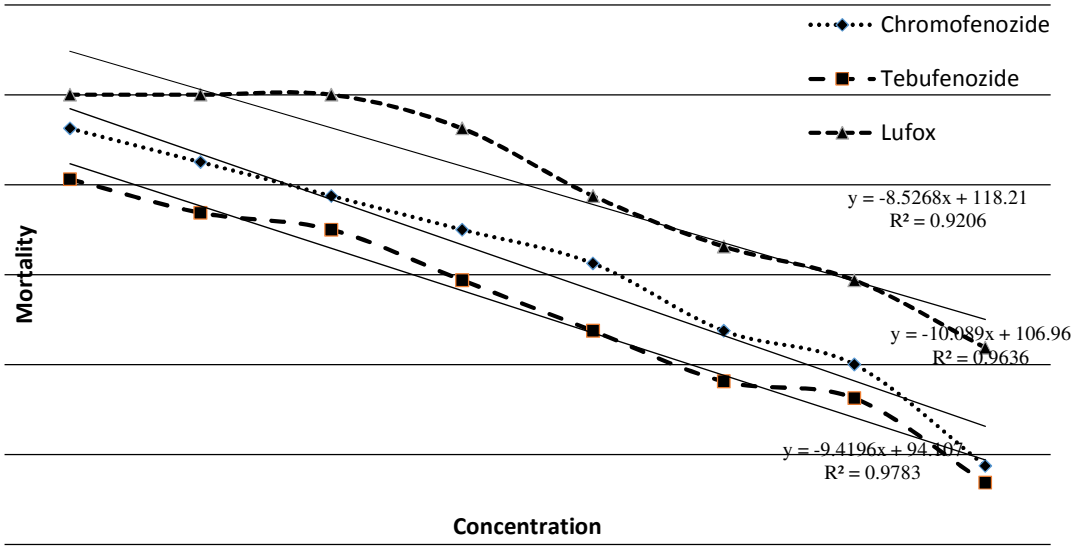

Fig. 3- Mortality of *O. surinamensis* old larvae in respect to the different concentrations of Tebufenozide, Chromafenozide and Lufox®.

Table 1- Estimated LC_{50} of Tebufenozide, Chromafenozide and Lufox® on *Oryzaephilus surinamensis*

Compound	Stage	Number	Lc_{50}(Ml/l)	Df	Chi-squar	%95 Upper	%95 Lower	Slop±SE
Tebufenozide	Egg	270	37.90	2	2.33	54.35	26.18	1.16±0.141
	Young Larvae		33.9082		6.30	56.78	16.76	0.767±0.12
	Old Larvae		143.03	2	3.94	237.14	76.31	0.764±0.12
Chromafenozide	Egg	270	21.15	2	3.66	29.86	14.32	1.22±0.14
	Young Larvae		19.33		3.92	32.14	9.10	0.868±0.13
	Old Larvae		76.82	2	5.29	122.12	40.38	0.913±0.13
®Lufox	Egg	270	9.159	2	4.642	12.868	5.94	1.42±0.18
	Young Larvae		1.002		5.75	18.85	8.09	1.154±0.14
	Old Larvae		29.203	2	5.167	39.89	20.404	1.375±0.15

Table 2- Estimated LT_{50} of Tebufenozide, Chromafenozide and Lufox® on young larvae

Compound	Concentration ppm	LT_{50}(day)	df	Chi-squar	%95 Upper	%95 Lower	Slop±SE
Tebufenozide	1000	5.32	2	7.37	5.8	4.84	4.66±0.5
	125	7.22		4.45	8.09	6.55	4.09±0.53
	7	17.51	8	6.74	49.62	12.5	3.06±0.83
Chromafenozide	1000	4.17	2	7.67	4.57	3.74	4.67±0.48
	125	5.84		8.43	6.37	5.35	3.74±0.45
	7	15.49	8	7.87	32.52	11.57	2.83±0.67
®Lufox	1000	2.6	2	16.01	2.84	2.36	5.69±0.65
	125	3.5		11.11	4.00	2.97	2.92±0.32
	7	10.02	8	11.92	13.43	8.43	2.74±0.47

Table 3- Estimated LT_{50} of Tebufenozide, Chromafenozide and Lufox® on old larva

Compound	Concentration ppm	LT_{50}(day)	df	Chi-squar	%95 Upper	%95 Lower	Slop±SE
Tebufenozide	3000	5.17	28	6.08	5.72	4.64	3.86±0.43
	375	7.05		6.32	8.07	6.3	3.39±0.12
	23	15.6		5.39	33.34	11.67	2.98±0.72
Chromafenozide	3000	4.77	28	58.49	5.5	4.04	4.25±0.44
	375	6.14		7.28	6.81	5.56	3.93±0.43
	23	15.91		8.26	33.88	11.76	2.73±0.64
®Lufox	3000	2.75	28	26.13	3.05	2.45	5.34±0.55
	375	3.71		13.49	4.1	3.3	4.64±0.47
	23	7.84		8.25	9.48	6.83	2.8±0.42

Discussion

Oryzaephilus surinamensis is recognized as a most cosmopolition pest who attacks to stored products and causes serious damages in quantity and quality scales through feeding and contaminating the products with cast skin and depositing frass (Ja Hyun & Ryoo, 2000), Here, we investigated the mortality of eggs, young and old larval stages of *O.surinamensis* with reference to the toxically effects of different IGRs insecticides. The Comparison of the different insecticides LC_{50} which is explained in this research process indicates that Lufox® have the lowest LC_{50} in comparison with the other both insecticides and observes that the ovicide and larvicide efficacy of Lufox® on *O. surinamensis* is the highest one. According to the obtained results, Lufox® has better and stronger effects on controlling of the saw-toothed grain beetle than the other both components. Lufox® is the mixture of juvenile hormone mimic (Fenoxycarb) and Chitin Synthesis inhibitor hormone (Lufenuron). During the previous investigations, it was cleared that the Lufenuron has a destructive effects on the egg stage, larval stages and adults while, Fenoxycarb is a Juvenile hormone mimic, its impact is devastating on eggs and last larval age (Minguez *et al*., 2004).

The final resulted data of this project is agreed by the reported results by Hosseinzadeh *et al*. (2011) who reported that Lufox® gave the best effects in controlling process of the population of *Lobesia botrana* Den & Schiff. Previously, it has been submitted by Minguez *et al*., (2004) that Lufox® have the best performance in controlling of grape pests. Gelbic *et al*. (2011) who has been represented the comparative studies on the effects of tebufenozide and lufenuron on *Spodoptera littoralis*, reported that Lufenuron has more activity than tebufenozide.

the mortality is directly increased by raising the compounds concentrations. With due attention to the LC_{50} values of Tebufenozide, Chromafenozide and Lufox®, it would be suggested that Lufox® has more toxicity on storage pests than the other insecticides which have been mentioned above. This result is confirmed with Yasir *et al*. (2012) who found that Lufenuron caused significant effects on larval mortality, by using the species *Tribolium castaneum*, as the biotest. Also, Saenz de Cabezon *et al*. (2006) found that lufenuron has the high chitin synthesis inhibitory effects on *Lobesia botrana* eggs. The highest effect can be observed on eggs. In contrast, El-Shennawy (2009) recorded that LC_{50} for lufenuron is 2.276 ppm when 4-day old eggs of *Pectinophora gossypilla* were treated (kandil *et al*., 2012). Yasir *et al*., (2012) recorded that the adult's fecundity and eggs hatchability rates of *T. castaneum* larvae were reduced by deferent doses of Lufenuron.

The LT_{50} values of 1000 and 3000 ppm of the poison materials are shorter for controlling young and old larval stages than that of the concentrations 7 and 23 ppm of same materials. These resulted times are matched by the results of Rashid *et al.* (2012) who reported 1000 ppm of hexaflumuron can kill *A. vilis* larvae during about 7 days. Finally, the LT_{50} value of Lufox® is shorter than that of Chromofenozide and Tebufenozide.

Additionally, the presence differences in LC_{50} values between these three IGR insecticides indicate that the required concentration for killing 50% of the different stages of *O.surinamensis* by using Lufox® is lower than that of the other compounds. Therefore, the toxicity effects of Lufox® are higher than that of Chromofenozide and Tebufenozide. These results revealed that Lufox® is the most effectively compound that can be used safely for controlling insect pests.

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مقایسه سمیت سه عامل IGR در Oryzaephilus surinamensis L. (Col., Silvanidae) تحت شرایط آزمایشگاهی

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چکیده

سوسک‌های Oryzaephilus surinamensis L. (col., Silvanidae) یکی از مهم‌ترین آفاتی است که به محصولات انباری در ایران آسیب می‌رساند. اثر سه حشره کش IGR، تبوفنوزید، کرومافنوزید و لوفوکس® در طی این مطالعه بر روی تخم و مراحل لاروی ارزیابی شده است. کارایی این حشره کش‌ها با غلظت‌های مختلف در تخم‌های یک روزه، ٥ روزه (جوان) و ١٩ روزه O. surinamensis مورد ارزیابی قرار گرفت. نتایج آزمایشات زیست سنجی نشان داد که Lufox® بیشترین و تبوفنوزاید کمترین اثر را بر روی تخم‌ها و مراحل لاروی دارند. همچنین اثرات مرگ و میر مستقیما با افزایش غلظت IGR افزایش یافت. از جنبه دیگر، تخم‌های O. surinamensis حساس ترین مرحله در پاسخ به حشره کش‌های IGR بودند.مقدار LT50 در Lufox کوتاه‌تر از Tebufenozide و Chromafenozide بود. در نهایت سمیت بالا و قوی ترین کارایی را می توان با استفاده از Lufox® در مقایسه با تبوفنوزید و کرومافنوزید به دست آورد.

واژه‌های کلیدی: Orizaephilus surinamensis، IGRs، تبوفنوزید، کرومافنوزید، Lufox®

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