Residual Efficacy of Certain Insecticides for Protecting Grain Stores from Infestation of Stored Product Insects

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ABSTRACT: Pesticides are relatively inexpensive and easy tool that can be used and applied for controlling stored-products insects attacking grain elevators, grain stores, flours mills and feed mills. Certain contact insecticides of low mammalian can be applied and sprayed in these stores of grains before or during storage to protect them from insect pests or to control established infestations. Four insecticides (lambda-cyhalothrin [Lambada- Magic®5% EC], primiphos- methyl [Actellic®50% EC], chlorpyrifos [Magic-phos®48% EC] and spinetoram [Radiant®12 %SC]) were tested against adults of *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) , using direct contact application. The response varied with chemical insecticide, insect species and exposure time. Filter paper diffusion method at different doses was used for assaying the different tested insecticides. Spinetoram was highly toxic against *T. castaneum* after 24 and 48 hrs of exposure. Lambda-cyhalothrin was highly toxic against *T. castaneum* followed by spinetoram; chlorpyrifos and primiphos-methyl after the period of exposure of 72 hrs. Nevertheless, it could be noticed that the toxic effect of spinetoram (LC_{50}=4.12ppm) was close to that of lambda-cyhalothrin (LC_{50} =3.28ppm) after exposure period of 72 hrs. Spinetoram was highly toxic against *S. oryzae* followed by chlorpyrifos, and primiphos-methyl after 24 and 48hrs. Spinetoram was also highly toxic against *S. oryzae* followed by chlorpyrifos, lambda-cyhalothrin and primiphos-methyl after 72hrs. The results indicated that spinetoram as a novel insecticide is highly toxic to both the red flour beetle *T. castaneum* and the rice weevil *S. oryzae*. Implications of these results for stored product insects' management programs would be beneficial.

Keywords: *Tribolium castaneum*, *Sitophilus oryzae*, lambda- cyhalothrin, primiphos- methyl, chlorpyrifos, spinetoram, Toxicity index, Relative potency

INTRODUCTION

Annual post-harvest losses resulting from insect damage, microbial deterioration and other factors such as humidity, temperature, aeration and cleanliness of the bulk storage, are estimated to be 10-25% of production worldwide **(Mohan and Fields, 2002)**. However, insects are the main problem in stored grain because they reduce the quantity and the quality of grains **(Madrid *et al.*, 1990)**.

The red flour beetle *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) is an important worldwide secondary insect-pest of stored products that is observed among several commodities. This pest may cause considerable economical losses if not adequately controlled because it has a very high rate of population increase **(Hill, 1990)**.The red flour beetle is a serious insect-pest species that attacks stored grain products such as flour, cereals, meal, beans and other dried food products; the larvae prefer cereal grain embryos . The female lays tiny white eggs (up to 450/female) that hatch after about 9 days (**Sokokoff, 1972**).

The rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) is a major main insect- pest of most stored cereal (rice, wheat, sorghum, barley and maize) worldwide before harvest and in store **(Ahmed, 2001; Sabbour, 2012)**. The adults of the rice weevil are around 2 mm long with a long snout and able to fly. The body color appears to be dark brown, but on close examination, four orange/red spots are arranged in a cross on the wing covers (**Halstead, 1964**).

Synthetic insecticides such as lambda-cyhalothrin, primiphos-methyl and chlorpyrifos are currently of the main chemical that can be used to protect stored grains from insects. Spinosad is a new introduced and currently registered compound that can be used in several countries as a grain protectant.

The spinosyns are a unique family of fermentation-derived insecticides having potent activity and lower environmental effect. Spinosad is a defined combination of the two principal fermentation factors, spinosyns A and D. Structure–activity relationships (SARs) have been extensively studied, leading to development of a semisynthetic second-generation derivative, spinetoram. The spinosyns have a unique mechanism of action involving disruption of nicotinic acetylcholine receptors **(Kirst, 2010).** Spinosad possesses a unique mode of action in insects and controls insect strains resistant to other grain protectants. When launched globally, spinosad will represent a valuable new addition to the limited arsenal of grain protectants and can positively impact global food security. Its combination of high efficacy, broad insect pest spectrum, low mammalian toxicity, and sound environmental profile is unique among existing products currently used for stored-grain protection (**Hertlein *et al.*, 2011).** Spinetoram is chemically similar to spinosad, a pesticide approved for use in organic agriculture with an established safety record. Spinetoram is a mixture of chemically modified spinosyns J and L. Formulations are sold under various trade names Delegate®, Exalt® and Radiant®. Spinetoram is a broad-spectrum insecticide used to control crop-damaging insects. It shows high-efficacy against target insects at a very low use rate, with a margin of safety toward beneficial insects. It acts by causing persistent activation of insect nicotinic acetylcholine receptors (**Anonymous, 2014).** Spinetoram can be an effective alternative to spinosad, and may be used as a grain protectant.

The objective of this investigation is to evaluate the insecticidal activity of lambda-cyhalothrin, primiphos-methyl, chlorpyrifos and the new insecticide "spinetoram" against both the rust red flour beetle, *Tribolium castaneum* and the rice weevil *Sitophilus oryzae*.

MATERIALS AND METHODS

Tested insects

Cultures of *S. oryzae* and *T. castaneum* were maintained in the laboratory without exposure to any insecticides on wheat grain and flour wheat, respectively, in glass jars containers kept under the conditions of 25ºC ± 3ºC and 65 ± 5 % R.H., and continuous daily darkness of 24 hrs, except when working inside the rearing cabinet.

Insecticides

Four formulated insecticides (lambda- cyhalothrin [Lambada- Magic®5% EC], primiphos- methyl [Actellic®50% EC obtained from Shora Chemicals, Egypt], chlorpyrifos [Magic-phos®48% EC] and spinetoram [Radiant®12 %SC]) were tested. Each insecticide was diluted with water to obtain serial concentrations to be tested against the suggested insects.

Bioassays

The insecticidal activity of evaluated lambda-cyhalothrin, primiphose methyl, chlorpyrifos and spinetoram was determined by direct contact application. One millimeter of each diluted and prepared concentrations (1 ml) was applied and regularly distributed on filter paper (9cm dia.). Each concentration was replicated 3 times. The filter papers were left over at room temperature to allow the water to evaporate and became dry. Each paper was handled carefully and fixed inside a Petri-dish. Ten adult insects were released into the filter paper and maintained in a Petri-dish that previously treated with the same concentration as that of the filter paper and left at constant room temperature along a period lasted for 72 hours. Mortality determination was done after 24, 48 and 72 hours. The insects were categorized to alive or dead (brittle and showing no movement over a 5 min observation period). This procedure would be easy and rapid method for evaluating the residual activity of a pesticide **(Saad *et al.*, 2011)**.

Statistical Analysis of bioassays data

Probit (mortality)/log con. (Dose) regression equations were calculated using the maximum likelihood algorithm described by **Finney (1971)** adopted as a computer program. Values of LC_{50} and LC_{95}'s and associated fiducial limits were also calculated by the method described by Finney. The correction of mortality percentages, if there were any control mortality was done using **Abbott's formula (1925)**. Also, the relative efficiency (Toxicity index and Relative potency) of the tested compounds was determined by the formula of Sun (1950) as follows:

$$\text{Toxicity index (\%)} = \frac{LC_{50} \text{ of the most effective compound}}{LC_{50} \text{ of the tested compound}} \times 100$$

$$\text{Relative potency} = \frac{LC_{50} \text{ of the least effective compound}}{LC_{50} \text{ of the tested compound}} = \ldots\ldots \text{ Fold}$$

RESULTS AND DISSCUSION

1. Efficacy of the tested insecticides against *T. castaneum*

Data in Table 1 show the toxicity of different tested insecticides (lambda-cyhalothrin [Lambada-Magic®5% EC], primiphos-methyl [Actellic®50% EC], chlorpyrifos [Magic-phos®48% EC] and spinetoram [Radiant®12 %SC]) against *T. castaneum* after 24, 48 and 72 hours of exposure. Table 1 show the LC_{50} (ppm), fiducial limits, slope value and regression equation of each of these tested insecticides against *Tribolium castaneum* adults. After 24 hour of exposure, spinetoram was proved to be the most toxic insecticide tested against *T. castaneum* followed by chlorpyrifos, primiphos- methyl and lambda-cyhalothrin. Also, the same trend as that after 24 hrs was achieved after 48 hrs of exposure. Although lambda-cyhalothrin was shown to be the least toxic tested compound within the first 24 hrs (LC_{50}= 86.29 ppm), it was shown that after 72 hrs of exposure that lambda-cyhalothrin was the most toxic compounds as compared with the other tested insecticides. The calculated LC_{50} of spinetoram was found to be 4.12 ppm (Table 1).

From the previous results, it could be also seen that spinetoram was highly toxic against *T. castaneum* after 24 and 48 hrs of exposure. Vice –versa lambda-cyhalothrin was highly toxic against *T. castaneum* followed by spinetoram; chlorpyrifos and primiphos-methyl after the exposure period of 72 hrs. Nevertheless, it could be noticed herein that the toxic effect of spinetoram (LC_{50}=4.12ppm) was merely equal to that of lambda-cyhalothrin (LC_{50} =3.28ppm) after that same period of 72 hrs exposure.

Table (1): Response of *T. castaneum* to primiphos- methyl, lambda- cyhalothrin, chlorpyrifos and spinetoram

Bioassay Time (hrs)	LC_{50} (ppm)	Fiducial limits (ppm) Lower-Upper	Slope	Regression Equation
Primiphose methyl				
24	35.91	30.33 – 42.55	2.12	Y=-3.29+2.12x*
48	22.93	19.47 – 26.99	2.09	Y=-2.85+2.09x
72	14.74	12.31 – 17.61	2.39	Y=-2.80+2.39x
Lambde cyhalothrin				
24	86.29	41.99 – 190.36	0.63	Y=-1.22+0.63x
48	16.78	8.99 – 31.33	0.52	Y=-0.64+0.52x
72	3.28	1.45 – 6.71	0.58	Y=-0.30+0.58x
Chlorpyrifos				
24	23.99	20.55 – 27.99	2.29	Y=-3.15+2.29x
48	14.63	11.94 – 17.89	2.08	Y=-2.42+2.08x
72	8.55	6.43 – 11.30	2.09	Y=-1.95+2.09x
Spinetoram				
24	18.64	14.56 – 23.80	1.47	Y=-1.87+1.47x
48	9.49	7.05 – 12.68	1.28	Y=-1.25+1.28
72	4.12	2.89 – 5.78	1.24	Y=-0.76+1.24x

* x=log concentration

These results supported the obtained results by **Huang and Subramanyam (2003)** who reported that spinosad at 0.5 or 1 mg/kg on white wheat was very effective against all the tested species except the red and confused flour beetle *T. confusum*). Also, **Arthur (1992)** mentioned that *Sitophilus zeamais* or *Tribolium castaneum* did not survive in the case of the application of deltamethrin + chlorpyrifos to corn. Meanwile, results of **Khashaveh *et al.* (2008)** revealed that the application of spinosad dust formulation at higher rates and for longer exposure intervals could control *T. castaneum* in different oilseed types.

Denloye *et al.* (2008) reported that both Sumithion® (fenitrothion) and Actellic® (primiphos-methyl) were effective for controlling *C. maculatus* and *S. zeamais* at concentrations higher than that of 5 mg/kg which have been recommended by manufacturers.

Table 2 shows the LC_{50} (ppm), Toxicity index (%) and Relative potency (fold) of the four tested insecticides against the rust red flour beetle adults (*T. castaneum)* after 24, 48 and 72 hours.

Table (2): LC_{50} values, Toxicity index and Relative potency of the tested insecticides against *T. castaneum* adults (after 24, 48 and 72 hrs bioassay)

Treatment	Calculated LC_{50} (ppm)	Toxicity index %	Relative potency (fold)
	After 24 hrs		
Primiphos-methyl	35.91	51.91	2.40
Lambade-cyhalothrin	86.29	21.60	1.00
Chlorpyrifos	23.99	77.70	3.60
Spinetoram	18.64	100.00	4.63
	After 48 hrs		
Primiphos-methyl	22.93	41.39	1.00
Lambade-cyhalothrin	16.78	56.55	1.37
Chlorpyrifos	14.63	64.87	1.57
Spinetoram	9.49	100.00	2.42
	After 72 hrs		
Primiphos-methyl	14.74	22.25	1.00
Lambade-cyhalothrin	3.28	100.00	4.49
Chlorpyrifos	8.55	38.36	1.72
Spinetoram	4.12	79.61	3.58

After 24 hrs bioassay, it was confirmed that lambde-cyhalothrin was the least efficient toxicant (LC_{50} = 86.29 with toxicity index equal to 21.6% and relative potency of 1.00 1fold, respectively). After 48 hrs, spinetoram still had a strong action on *T. castaneum* (LC_{50} = 9.49 ppm, toxicity index 100% and relative potency 2.42 fold), followed by chlorpyrifos (LC_{50} = 14.63 ppm, toxicity index 64.87 % and relative potency 1.57 fold). Primiphos-methyl was the lowest efficient toxicant (LC_{50} = 22.93 ppm with a toxicity index of 41.39% (of spinetoram) and relative potency of 1.00 fold). After 72 hrs, each of spinetoram and lambde-cyhalothrin gave strong action on *T. castaneum* represented by their high toxicity and reduced LC_{50} values (Toxicity index of 79.61 & 100% and relative potency of 3.58 & 4.49 folds, respectively.)

2. Efficacy of the tested insecticides against *S. oryzae*

Table 3 shows the extracted parameters of the toxicity of different tested concentrations of evaluated insecticides expressed as the LC_{50} value (ppm), fiducial limits, slope value and regression equation of each of these tested insecticides against *Sitophilus oryzae* adults after 24, 48 and 72 hours of exposure. After 24 hrs of exposure, chlorpyrifos and spinetoram were equally high toxic against *S. oryzae* adults showing merely the same LC_{50} values of 14.56 and 13.38ppm, respectively.

Again, the further exposure of the adults of the rice weevil *S. oryzae* to the tested insecticides up to 48 and 72 hrs revealed that chlorpyrifos was as toxic as spinetoram and comparatively were more toxic and superior to the other tested compounds (LC_{50}=5.73 and 5.29 ppm, respectively) after a 72 hrs bioassay versus lambda-cyhalothrin which was the least toxic compound (40.62ppm). Moreover, chlorpyrifos was as toxic as spinetoram.

From the previous results, it could be concluded that spinetoram was the utmost highly toxic insecticide against *S. oryzae* followed by chlorpyrifos, and primiphos-methyl after 24 and 48hrs. Also spinetoram was the superior and highly toxic one against *S. oryzae*, followed by chlorpyrifos, lambda-cyhalothrin and primiphos-methyl after 72hrs.

Samson and Parker (1988) found that deltamethrin was not effective against *Sitophilus* spp. Our results agree with those results reported by **Kljajic *et al.* (2007)** who found that the most toxic insecticides to *S. oryzae* adults were bifenthrin and dichlorvos, and the least toxic was pirimiphos-methyl. Also, **Getchell and Subramanyam (2008)** reported on the comparison of the time required for killing 50% (LT_{50}) and 95% (LT_{95}) and showed that *R. dominica* adults were consistently and significantly more susceptible and died quickly than *S. oryzae* adults when exposed to spinosad treated commodities.

Kavallieratos *et al.* (2010) stated that the lowest dose of spinosad was highly effective (>90%) against *R. dominica* and *S. oryzae*. In the case of *T. confusum* combination of longer exposures with higher doses was required for each formulation to be effective. Our results disagree with those arrived at by **Rumbos *et al.* (2013)** who found that *Sitophilus* species were highly susceptible to two pirimiphos-methyl formulations, since complete mortality (100%) was achieved while the present investigation showed that *S. oryzae* was more susceptible to spinetoram and chlorpyrifos.

Table (3): Response of *Sitophilus oryzae* to primiphos-methyl, lambde cyhalothrin, chlorpyrifos and spinetoram

Bioassay time (hrs)	LC_{50} (ppm)	Fiducial limits (ppm) Lower-Upper	Slope	Regression Equation
		Primiphos- methyl		
24	35.05	29.13 – 42.22	1.90	Y=-2.94+1.90x*
48	21.73	18.19 – 25.95	1.93	Y=-2.58+1.93x
72	14.13	11.64 – 17.12	2.25	Y=-2.59+2.25x
		Lambda- cyhalothrin		
24	82.04	47.59 – 146.01	0.85	Y=-1.63+0.85x
48	40.62	27.22 – 61.44	0.90	Y=-1.46+0.90x
72	9.86	6.34 – 15.05	0.82	Y=-0.81+0.82x
		Chlorpyrifos		
24	17.09	14.56 – 20.04	2.52	Y=-3.10+2.52x
48	9.96	7.67 – 12.87	2.06	Y=-2.06+2.06x
72	5.73	3.95 – 8.24	2.12	Y=-1.61+2.12x
		Spinetoram		
24	17.22	13.38 – 22.09	1.50	Y=-1.86+1.50x
48	9.33	7.14 – 12.09	1.53	Y=-1.48+1.53x
72	5.29	3.99 – 6.94	1.61	Y=-1.17+1.61x

*x=log concentration

The exhibited results in Table 4 show the calculated values of LC_{50} (ppm), Toxicity index (%) and Relative potency (fold) of the four tested insecticides against the rice weevil adults *S. oryzae* after 24, 48 and 72 hours. After 24 hrs, chlorpyrifos had the strongest action against *S. oryzae* ($LC_{50} =$ 17.09 ppm with a toxicity index of 100% and relative potency of 4.80 fold). Spinetoram was as effective as chlorpyrifos $LC_{50} =$ 17.22 ppm with a toxicity index of 99.24% and relative potency of 4.76 fold) followed by primiphos-methyl (LC_{50} = 35.05 ppm with toxicity index equal to 48.76% and relative potency of 4.27 fold). Lambde-cyhalothrin was the lowest efficient toxicant (LC_{50} = 82.04 with toxicity index equal to 20.83 and relative potency of 1.00 fold). After 48 hrs of exposure, spinetoram had a strong action on *S. oryzae* ($LC_{50} =$ 9.33 ppm with toxicity index of 100% and relative potency of 4.35 fold), while lambde-cyhalothrin was still the lowest efficient toxicant (LC_{50} = 40.62 ppm, with toxicity index of 22.97% and relative potency of 1.00 fold). Furthermore, after 72 hrs, spinetoram had a strong action against *S. oryzae* showing its high toxicity ($LC_{50} =$ 5.29 ppm with a toxicity index of 100% and relative potency of 2.67 fold). On the other hand, primiphos-methyl was the lowest efficient toxicant (LC_{50} = 14.13 ppm with toxicity index 37.44% and a standard relative potency considered for the least efficient compound that equal 1.00).

Table (4): LC_{50} values, Toxicity index and Relative potency of the tested insecticides against *S. oryzae* adults (after 24, 48 and 72 hrs bioassay).

Treatment	Calculated LC_{50} (ppm)	Toxicity index (%)	Relative potency (fold)
	After 24 hrs		
Primiphos- methyl	35.05	48.76	2.34
Lambde-cyhalothrin	82.04	20.83	1.00
Chlorpyrifos	17.09	100.00	4.80
Spinetoram	17.22	99.24	4.76
	After 48 hrs		
Primiphos- methyl	21.73	42.94	1.87
Lambde-cyhalothrin	40.62	22.97	1.00
Chlorpyrifos	9.96	93.67	4.08
Spinetoram	9.33	100.00	4.35
	After 72 hrs		
Primiphos- methyl	14.13	37.44	1.00
Lambde-cyhalothrin	9.86	53.65	1.43
Chlorpyrifos	5.73	92.32	2.47
Spinetoram	5.29	100.00	2.67

From the afore-mentioned results, it could be revealed that the spinosyns having potent activity, lower environmental effect and unique mode of action toward insects and can control insect strains resistant to other grain protectants (malathion, chlorpyrifos …etc). Therefore, the application of spinetoram would be realty valuable as a good protectant against stored-grain insect-pests. Though, its application could be recommended and involved within integrated stored- product pest management programs for protecting grain stores from the insect infestation.

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الملخص العربي

كفاءة بعض المبيدات في حماية المخازن من الإصابة بحشرات المواد المخزونة

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جمهورية مصر العربية

تعتبر مبيدات الآفات أداة رخيصة نسبياً وسهلة الإستخدام لمكافحة حشرات المواد المخزونة التي تهاجم مخازن الحبوب ومطاحن الدقيق والأعلاف. مما يمكن من إستخدام بعض المبيدات ذات التأثير المنخفض علي الثدييات لرشها في المخازن قبل أو أثناء عملية التخزين وذلك بغرض الحماية الوقائية من الآفات الحشرية أو مكافحة الحشرات الموجودة أصلاً في المخازن. وقد تم تقييم وإختبار أربعة مبيدات هي: لمبادا–سيهالوثرين (لمبادا –ماچيك ٥% مركز قابل للإستحلاب) ، بيريميفوس–ميثيل (أكتليك٥٠% مركز قابل للإستحلاب) ، كلوربيريفوس (ماچيك–فوس ٤٨% مركز قابل للإستحلاب) ومركب سبينتورام (رادينت ١٢% معلق مركز) ضد حشرة خنفساء الدقيق الحمراء وسوسة الأرز وذلك بإتباع تقنية تقييم فعالية طريق الأثر الباقي للمبيدات علي ورق الترشيح المعامل بالتركيزات المختلفة من هذه المبيدات المختبرة .

أظهرت النتائج أن تأثير وإستجابة الحشرات المعرضة للمبيدات تختلف بإختلاف المبيد المختبر ونوع الحشرة ووقت التعرض للمبيد حيث تبين أن مركب سبينتورام كان أكثر كفاءة وسمية ضد خنفساء الدقيق الحمراء بعد ٢٤، ٤٨ ساعة من التعرض. أما بعد ٧٢ ساعة أظهر مركب لمبادا–سيهالوثرين أعلي كفاءة وتبعه في ذلك كل من سبينتورام ، كلوربيريفوس ثم بيريميفوس– ميثيل . وفي هذا الصدد يمكن ملاحظة أن التاثير السام لمركب سبينتورام كان لحد كبير مقارباً ومساوياً لتأثير مركب لمبادا–سيهالوثرين. كما وتبين أيضاً أن مركب سبينتورام كان ذو تأثير سمي عالي ضد سوسة الأرز بعد فترة تعريض ٧٢ ساعة. يتضح من تلك النتائج المتحصل عليها أن مبيد سبينتورام الذي يُعد من المركبات الجديدة والحديثة كان أكثر سمية ضد الحشرات المختبرة (خنفساء الدقيق الحمراء وسوسة الأرز) مما يجعل في تطبيقه فائدة كبيرة في مكافحة أنواع حشرات المخازن المختلفة التي تُظهر المقاومة لبعض المبيدات المستعملة وكذلك في برامج المكافحة المتكاملة لحشرات المواد المخزونة .