

TOXICITY AND REPELLENT ACTIVITY OF SPINOSAD AND ORANGE OIL AGAINST *Rhizopertha dominica* F. AND *Tribolium castaneum* (HERBST)

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ABSTRACT

The lesser grain borer, *Rhizopertha dominica* Fabricius and the red flour beetle, *Tribolium castaneum* Herbst, are of the most insect species which cause the highest grain damage. Many chemical insecticides related to different groups used as protectants to stored products presented serious problems to human health and environment. To avoid these disadvantages, laboratory experiments were carried out to evaluate two materials namely, plant oil (Orange oil) and bioinsecticide, spinosad as stored wheat grain protectants against *T. castaneum* and *R. dominica* using two bioassays technique, mixing with medium and repellency at different exposure periods (72 h for mixing with feeding medium and 24 h for repellency). Results obtained revealed that the two tested materials, orange oil and spinosad had moderately action on the two tested insects either by mixing with medium or by repellent bioassay methods. Data cleared that the effect of the tested materials increased with the increasing of concentration and period of exposure especially with mixing bioassay. For repellent effect, the highest level of concentration had the most action on both *T. castaneum* and *R. dominica*. Except orange oil against *T. castaneum* the effect of materials tested decreased through 24 h of exposure. Spinosad showed nearly similar effect on the two tested insects while *R. dominica* was found to be more tolerant than *T. castaneum* with orange oil. Finally, our findings suggest that spinosad and orange oil may be potential protectants against *R. dominica* and *T. castaneum* in stored wheat grain principally with mixing bioassay technique.

INTRODUCTION

Major insect pests of stored wheat include *Rhizopertha dominica* Fabricius, *Sitophilus oryzae* L., *Cryptolestes ferrugineus* Stephens, *Tribolium castaneum* Herbst and *Oryzaephilus surinamensis* L. The first two species cause the most grain damage because the immature stages develop inside the grain kernel (Hagstrum and Subramanyam, 2006). Stored grain insect pests have been damaging our economy by infesting agricultural stored products. These are responsible for worldwide loss of 10-40% in the stored grain annually (Matthews, 1993). In such a situation, protection of stored grain and agricultural products against insect infestation is an urgent need, various synthetic insecticides have been used, but insects have acquired resistance against most of these synthetic pesticides. Also, these insecticides causes great hazards for environment and consumers due to residual property. Thus, it

is an urgent need to develop new alternatives that must be ecologically sound with no residual activity and adverse effect on other non-target animals to control stored product insect pests. In this regard, many plant products have been evaluated for their toxic properties against different stored grain pests (Su, 1990; Mukherjee and Joseph, 2000) especially in form of essential oils (Shaaya *et al.*, 1991;; Ngamo *et al.*, 2007). Spinosad is a naturally occurring mixture of two active compounds spinosyn A and spinosyn D (salgado, 1998). As a part of future strategies for stored product insect control essential oils with repellent and/or insecticidal properties should be studied. therefore, the aim of the present work was to study the toxicity as well as repellent activity of orange oil and spinosad against the red flour beetle, *T. castaneum* and the lesser grainborer, *Rhizopertha dominica*.

MATERIALS AND METHODS

Insects:

Two important coleopteran stored grain pests were assessed in the current investigation, Lesser grain borer, *R. dominica* (F.) (Bostrychidae: Coleoptera) and red flour beetle, *T. castaneum* (Herbst) (Tenebrionidae: Coleoptera) . The original stock culture of the two insects were obtained from stored product pest laboratory, Plant Protection Research Institute, Sakha Agricultural Research Station. The insects were reared on wheat grain and wheat flour for *R. dominica* and *T. castaneum*, respectively in laboratory at 30±2°C and 75±5% R.H.

Chemicals

Bioinsecticide

Tracer:

Common name: Spinosad

Chemical name: Mixture of spinosyn A and spinosyn D

Spinosyn A:

(2R, 3as, 5bs, 9s, 13s, 14R, 16bR) -2-(6-deoxy-2,3,4-Tri-o-methyl- α -L-Mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetra-deoxy- β -D-erythro-pyranosyloxy)-9-ethyl 2, 3, 3a, 5a, 5b, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16a, 16b-hexadecahydro 14-methyl-1H-8-oxacyclododeca[b]as-indacene-7, 15-dione

Spinosyn D:

(2s, 3aR, 5as, 5bs, 9s, 13s, 14R, 16as, 16bR)-2-(6-deoxy-2, 3, 4-tri-o-methyl- α -L-Mannopyranosyloxy)-13-(4-dimethylamino-2, 3, 4, 6 tetra-deoxy- β -D-erythro-pyranosyloxy)-9-ethyl-2, 3, 3a, 5a, 5b, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16a , 16b-hexadecahydro-4, 14-dimethyl-1H-8-oxacyclododeca[b]as-indacene-7, 15-dione

The applied formulation: Tracer 24% SC .

Source: Nile valley for Agricultural Development, Giza, Egypt.

Plant oil

PREV- AM

Common name: Orange oil

Source: Nile valley for Agricultural Development, Giza, Egypt.

Methods

Grain treatments (mixing with feeding medium):

Batches of whole grain were weighed (10 gm) and placed in glass jars (250 ml) for *R. dominica* or 20 g of cracked wheat grains for *T. castaneum*. The tested insecticide (Spinosad) was diluted in water and added to grains at rates which give the required concentration 10, 50, 100 and 150 ppm), while orange oil was diluted with acetone and added to the grains at rate which gave the required concentration (40000, 20000, 10000, 5000 and 2500 ppm) for *T. castaneum*, (1250, 625, 312.5 and 156.25 ppm) for *R. dominica*, jars were shaken by hand and grains were allowed to dry at room temperature. Ten unsexed adults of both insects (1-2 weeks old) were introduced to jars containing treated grain, 3 replicates were set up for both treatment and control. Mortality counts were recorded daily and corrected by

Abbott's formula (1925).

Repellent activity :

A choice bioassay system was used to evaluate repellency of orange oil and spinosad. Half filter paper disks of 8 cm diameter were treated with 0.5 ml acetic solutions of the orange oil (40000, 20000, 10000 and 5000 ppm) and water solutions of spinosad (100, 50, 25 and 12.5 ppm). Solution of the spinosad was left to dry. Half of the bottom of a Petri dish was covered with the treated filter paper, while the other half was covered with a filter paper disk impregnated with 0.5 ml acetone in orange case but with spinosad impregnated with 0.5 ml water in spinosad. Ten unsexed adults were put into each Petri dish and the lid was sealed within place with parafilm. Three replicates were run for each tested concentration. So that 30 adults were assayed per concentration. The test was carried out in an incubator. The numbers of insects on the two half paper disks were recorded after 2, 3, 8, 16 and 24 h from the beginning of the test. Percentage of repellency (PR) was calculated as follows: $PR = \frac{[C-T]}{[C+T]} \times 100$ where C = number of insects on the untreated area T = treated

RESULTS AND DISCUSSION

Two bioassay methods, contact repellent activity were investigated in the present study on the two tested insects. *T. castaneum* and *R. dominica* to obtain the most suitable method and to compare between the two tested materials.

Toxicity of spinosad and orange oil :

To avoid the risk results in attacks of *T. castaneum* and *R. dominica* on wheat grains either quantity and quality effects, a laboratory experiments were carried out to study the potential activity of two materials one is a bioinsecticide, spinosad and the other is a plant oil, orange oil. The aim of this experiment was seeking safe alternatives replaced with hazardous chemical insecticides. Adults of *T. castaneum* and *R. dominica* were exposed to wheat and cracked wheat grain, respectively treated by different rates of the above toxicants wt/wt. Mortality counts were recorded after 24, 48 and 72 hr.

Data obtained in Table (1) summarized results in mixing with medium bioassay method of spinosad. Based on the LC₅₀ values, mortality percentage increased with the increasing of concentrations and exposure periods for the two tested compounds with both the investigated insects.

Results in Table (2) showed that the effect of orange oil increased with the increasing of concentration and exposure period. Data cleared that, *R. dominica* was more susceptible than *T. castaneum*. Also, results obtained exhibited that spinosad was more toxic than orange oil against both *T. castaneum* and *R. dominica* (Table 1 and 2) .

Repellent effect of spinosad and orange oil :

According to results obtained in Tables (3,4,5 and 6), both the two tested materials had moderately deteriorate effect against the two tested insects, the highest rate of the tested materials had the most repellent effect on both *T. castaneum* or *R. dominica* where the percentage repellency at 100 ppm ranged from 74 to 34%, 60 to 40%, 94 to 100% and 86 to 66% with spinosad and orange oil against *T. castaneum* and *R. dominica* through the time of exposure (24 h), respectively. Except orange oil against *T. castaneum* the effect of materials tested decreased through the 24 h time of exposure. Also, results showed that the effect decreased with the decreasing of concentration and increasing time of exposure either with orange oil or spinosad against both *T. castaneum* or *R. dominica*. Spinosad showed nearly similar effect on the two tested insects while *R. dominica* was found to be more tolerant than *T. castaneum* with orange oil.

Studies in the United States of America on several species of insects show that among stored grain insecticides, spinosad is particularly effective against *R. dominica* (F.) (Fang *et al.*, 2002 a,b; Toews and Subramanyam, 2003).

Although spinosad breaks down quickly in sunlight, limited published data suggest that spinosad in stored grain will be stable and loss of efficacy will be negligible (Fang *et al.*, 2002 a).

Subsequently, spinosad may be exploited for insect control of stored product insects and is likely to be a safe alternative of chemical insecticides. The search of anti-insect chemicals naturally occurring in plants has received special attention in recent years. The biorational insecticides, those based on natural products and synthesized analogues of naturally occurring biochemicals, are more acceptable than other conventional chemical pesticides; because of assumed reputation for being environmentally innocuous, available and less hazardous to humans and non-target organisms (Me Closky *et al.*, 1993 and Prakash and Rao, 1997).

There is now overwhelming evidence that many plant species exert diverse biological effects on insects, i.e., killing, attracting, repelling, feeding deterring, growth inhibiting and sterilizing effects (Lichtenstein and Cosida 1963; Abbassy, 1969, 1974, 1981 and 1982; Ogendo *et al.*, 2008; Derbalah and Ahmed, 2011 and Abdelgaleil *et al.*, 2012).

Our findings are consistent with those reported in literature for stored product insect pests tested with different insecticides (Mishra *et al.*, 2011).

Table (3): Repellency of spinosad to *T. castaneum* through 24 hrs of exposure time.

% PR (h)	Concentration (ppm)			
	100	50	25	12.5
2	74%	46%	34%	20%
4	66%	.0	26%	6%
8	66%	26%	20%	14%
16	54%	34%	26%	6%
24	34%	26%	26%	0%

Table (4): Repellency of spinosad to *R. dominica* through 24 hrs of exposure time.

% PR (h)	Concentration (ppm)			
	100	50	25	12.5
2	60%	46%	34%	26%
4	34%	34%	26%	20%
8	74%	34%	6%	6%
16	74%	46%	26%	0%
24	40%	40%	34%	0%

Table (5): Repellency of orange oil to *T. castaneum* through 24 hrs of exposure time.

% PR (h)	Concentration (ppm)			
	100	50	25	12.5
2	94%	74%	43%	40%
4	86%	46%	34%	40%
8	80%	74%	46%	40%
16	80%	60%	40%	34%
24	100%	77%	34%	34%

Table (6): Repellency of orange oil to *R. dominica* through 24 hrs of exposure time.

% PR (h)	Concentration (ppm)			
	100	50	25	
2	86%	80%	40%	46%
4	66%	66%	40%	66%
8	46%	66%	40%	66%
16	60%	60%	40%	60%
24	66%	26%	26%	34%

Toxicity of the tested materials varied dependence on insect species and bioassay method. This variation may be connected the alimentary habit of insect species, morphology and genetic agents. Also, the type of the tested material plays an important role for affecting on the tested insects, where the

chemical elements of each differ from compound to another. In addition to another factors such as the vapor pressure, molecular weight of each compound, which influence the level of toxicity.

The results clearly indicated that higher concentrations of the investigated materials for short periods were more effective than lower concentrations for longer periods. Insecticidal activity in the tested materials was related to their chemical composition, activity decreasing with the time because of component volatility. The differences between chemical composition of tested materials could be explain the variations observed in the insecticidal activities of these materials.

Beeman and Speirs (1986) found that avermectin_{B1} (abamectin) was extremely effective against 6 beetles and 3 moth pests of stored products. At dose 320 ppb in wheat, all adults of 3 species of Coleoptera were killed in 3 weeks. For most of the coleoptera and Lepidoptera 96-100% suppression of progeny was achieved at doses of 10-160 ppb.

Abo-Arab and El-Hamady (1998) carried out studies to evaluate the efficiency of ivermectin as a protectant against three important stored-grain insects, namely, the rust red flour beetle, *T. castaneum* (Herbst), the rice weevil, *S. oryzae* L. and the cowpea weevil, *Callosobruchus maculatus* F. using the technique of exposure to feeding medium, ivermectin exhibited considerable toxicity nearly equal to that of malathion. *C. maculatus* showed the highest susceptibility to ivermectin followed by *S. oryzae* and *T. castaneum*, respectively. The compound also showed potential toxicity to the immature stage inducing reduction in the progeny. Thus, number of offspring and number of eggs (laid by *C. maculatus*) or their hatchability were greatly reduced.

The repellent effect of *Ocimum gratissimum* L. was evaluated against adults of *S. oryzae* (L.), *T. castaneum* (Herbst), *Oryzaephilus surinamensis* (L.), *R. dominica* (F.) and *Collasobruchus chinensis* (L.). The repellence of the oil in acetone was evaluated in choice bioassay at five rates (0, 1, 2, 3 and 4 μ l/ 2 g grain). Results showed that repellence of the oil was significantly influenced by concentration and time after treatment. *T. castaneum* was more tolerant than the other tested insects. All tested insects had percentage repellence (PR) values which ranged from 37.5% to 100%. *O. gratissimum* oil is potential alternative to synthetic fumigants in the treatment of durable agricultural products (Ogendo *et al.*, 2008).

Essential oils from plants are valuable secondary metabolites which have already been used as raw materials in many fields, including perfumes, cosmetics, phytotherapy and nutrition. These oils also offer potential as sources of insecticides with environmental compatibility (Katz *et al.*, 2008). Recently, many studies have focused on the possibility of using plant essential oils for application to stored grain to control insect pests (Collins, 2006; De Garvalho and De Fonseca, 2006).

The extracts and secondary metabolites of plants are among the most promising alternatives. These botanical pesticides have the advantage of providing novel modes of action against insects that can reduce the risk of

cross-resistance as well as offering new leads for the design of target-specific molecules (Isman, 2006). Essential oils and their major constituents, mainly monoterpenoids, attracted research attention in recent years as potential alternatives to synthetic insecticides (Aslan *et al.*, 2004).

Our findings suggest spinosad may to be a potential protectant against *R. dominica* and *T. castaneum* in stored grain. This potential use would be in combination with another protectant capable of controlling other members of the pest complex.

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تأثير السمية وقوة الطرد لمبيد سبينوساد وزيت البرتقال ضد حشرة ثاقبة الحبوب الصغرى وخنفساء الدقيق الصندنية
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تعتبر ثاقبة الحبوب الصغرى *R. dominica* وخنفساء الدقيق الصندنية الحمراء *T. castaneum* من أشد الأنواع الحشرية التي تسبب ضررا عظيما للحبوب. لذلك استخدمت مبيدات كيميائية عديدة تنتمي لمجاميع مختلفة لمكافحة هذه الآفات مما نتج عنه مشاكل خطيرة على صحة الإنسان والبيئة المحيطة به. لتلافي هذه العيوب التي ظهرت في المواد الكيماوية أجريت تجارب معملية لتقييم اثنين من المواد كمواد واقية للحبوب وهما زيت نباتي (زيت البرتقال)، مبيد حيوي وهو سبينوساد بطريقتين هما الخلط مع البيئة الغذائية. وأيضا دراسة التأثير الطارد لهذه المواد ضد حشرتي خنفساء الدقيق الصندنية، ثاقبة الحبوب الصغرى وذلك عند فترات تعرض مختلفة (٧٢ ساعة للخلط مع البيئة، ٢٤ ساعة مع التأثير الطارد لكلا المادتين المختبرتين وكذلك باستخدام تراكيز مختلفة (١٠-١٥٠ جزء في المليون) لطريقة الخلط مع البيئة ضد كلا الحشرتين وذلك باستخدام مبيد سبينوساد. أما مع زيت البرتقال فقد استخدمت تراكيز تتراوح بين ١٢٦.٢٥-١٢٥٠ جزء في المليون وكذا ٢٥٠٠-٤٠٠٠ جزء في المليون مع حشرتي ثاقبة الحبوب الصغرى وخنفساء الدقيق الصندنية على الترتيب. أظهرت النتائج أن كلا المادتين المختبرتين كان لها تأثيرات معقولة على كلا الحشرتين وذلك سواء بطريق الخلط مع البيئة الغذائية أو طريقة التأثير الطارد. وايضا النتائج أظهرت أن تأثير المواد المختبرة زاد مع زيادة التركيز وكذا فترة التعرض خصوصا بطريقة الخلط مع البيئة. أخيرا هذه النتائج تقترح أن كلا من المادتين المختبرتين يمكن إستخدامهما كواقيات محتملة لحبوب القمح ضد الحشرتين موضوع الدراسة خصوصا بطريقة الخلط مع البيئة الغذائية.

قام بتحكيم البحث

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Table (1): Toxicity of spinosad using mixing with feeding medium against *T. castaneum* and *R. dominica* at different periods.

Treatment	Conc. ppm	%mortality			LC ₅₀			Slope value			Confidence limits					
		24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h		48 h		72 h	
											Lower	Upper	Lower	Upper	Lower	Upper
<i>T. castaneum</i>	10	7	10	33	604.594	173.717	33.131	0.924	1.217	1.010	296.214	324.405	108.1	278.3	22.758	44.536
	50	10	17	50												
	100	23	33	67												
	150	33	57	80												
	0	0	0	0												
<i>R. dominica</i>	10	17	30	60	100.205	35.798	7.225	1.023	1.089	0.958	74.461	149.521	25.655	47.092	3.94	13.20
	50	33	50	67												
	100	50	67	90												
	150	60	80	93												
	0	0	0	0												

Table (2): Toxicity of orange oil using mixing with feeding medium against *T. castaneum* and *R. dominica* at different induction periods.

Treatment	Conc. ppm	%mortality			LC ₅₀			Slope value			Confidence limits					
		24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h		48 h		72 h	
											Lower	Upper	Lower	Upper	Lower	Upper
<i>T. castaneum</i>	40000	13.3	33.3	47.0	290680	80241.8	42423.4	1.292	1.225	1.416	110770	6687100	50151	128623	31845.4	64645.2
	20000	6.7	23.3	33.3												
	10000	3.3	20.0	20.0												
	5000	0.0	3.3	10.0												
	2500	0.0	0.0	3.3												
	0	0.0	0.0	0.0												
<i>R. dominica</i>	1250	29	44.4	70.3	3052.5	1746.8	559.7	1.398	1.758	1.257	1861.01	8384.2	1298.2	2850.9	113.2	737.7
	625	18	15.0	48.1												
	312.5	7.1	11.1	37.0												
	156.25	3.5	3.7	26.0												
	0	6.7	10.0	10.0												

