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Comparative Toxicity of Four Formulations of Commercially Used Control Agents to The Red Flour Beetle, *Tribolium Castaneum* (Herbst) Under Laboratory Conditions

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ABSTRACT

Four formulations of commercially used control agents were tested under laboratory conditions on adults of the red flour beetle, *Tribolium castaneum*. The bioassays were carried out on wheat grains, at 25 ± 1 °C and 70 ± 5% RH. Compounds were applied at the dose rates of 250, 500, 750 and 1000 mg/L for Fenvalerate, 2500, 5000, 10000 and 20000 mg/L for Biofly, 25, 50, 100 and 200 mg/L for Neem and 25, 50, 75 and 100 mg/L for Diazinon. Adult mortality was assessed every day for 10 days and LC_{50}, LC_{95}, LT_{50} and LT_{95} were calculated. Mortality was 100% on wheat treated with 1000 mg/L of Fenvalerate, and 50, 75 and 100 mg/L of diazinon 10 days after exposure. Diazinon was the most effective against *T. castaneum* followed by Neem, Fenvalerate and then Biofly, where the recorded LC_{50} values were 46.25, 78.30, 271.12 and $3.05x10^4$ mg/L after 4, 8, 7 and 6 days of exposure, respectively. Generally , for all tested compounds, the LT_{50} values decreased as concentrations increased and Diazinon provided the shorter exposure time required for mortality. In conclusion, the biological method would not be as efficient as chemicals. However, the use of an effective plant extracts such as Neem on the stored grains would decrease the frequency with which insecticide was required. Thus, potentially remaining suitable pest control with a reduction in chemical residue. The present results encourage the use of Neem in control of stored product pests such as *T. castaneum* as alternative for synthetic insecticides or as a part in the integrated pest management programms.

INTRODUCTION

The red flour beetle, *Tribolium castaneum* (Herbst) is a serious pest of stored grain and grain products in many parts of the world. The beetles feed both as larvae and adults, and cause damage resulting in high economic losses. *Tribolium castaneum* secretes specific compounds called *p*-benzoquinones such as methyl-1,4-benzoquinone (MBQ) and ethyl-1,4-benzoquinone (EBQ) that are major components of defensive secretions used as repellents and irritants (Blum, 1981; Howard, 1987; Eisner *et al.*, 1998), these benzoquinones are hazardous to human health.

The chemical control has focused on very few compounds as control agents for stored product insects. Currently there is renewed interest in developing reduced-risk low toxicity chemicals, including new formulations of insect growth regulators (IGRs), to replace older conventional products used in many agricultural systems, including post-harvest protection in areas where food is stored. Oberlander *et al.* (1997) reviewed many studies involving IGRs and stored-product insects. Most of them involved exposure of eggs, 1st instars, or adults on treated grains, in diet, or in glass vials, with subsequent measurements of progeny production. The efficacy of hydroprene (an IGR) on *T. castaneum*, was studied by Arthur (2003) and Arthur and Hoernemann (2004). One of the frequently used IGR is lufenuron ((RS)-1-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxyl) phenyl]3-(2,6-difluorobenzoyl) urea (Kedar *et al.*, 2008). This class of chemicals acts as chitin synthesis inhibitor (CSIs). Recently, CSIs have gained significant popularity due to their low mammalian toxicity and absence of mutagenic and teratogenic effects on warm-blooded animals. The residual analysis of lufenuron in wheat grains as well as the biological efficacy opens up a new vista for its possible use in protection of the stored food commodities (Kedar *et al.*, 2008).

Other chemical agents like synergized pyrethrins and the organophosphate dichlorvos are two insecticides that have historically been used for aerosol applications inside processing facilities and food warehouses. More recently, new pyrethroid and insect growth regulators have been registered as aerosols for use against stored-product insects, but most documented reports of efficacy describe tests done under laboratory conditions (Arthur and Gillenwater, 1990; Arthur, 1993). Recently methyl

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bromide as an efficient fumigant to stored product insects was banned because it causes depletion of the ozone layer in the stratosphere.

Therefore, the control of red flour beetle has taken several approaches as mentioned by many scientists in that field. Su (1983) found that acetone extracts of *Piper nigrum* L. and the hexane extract of dry fruit of *P. guineese* was toxic to four species of stored product insects. Sighamony *et al.* (1984) also reported that acetone extracts of black pepper seeds were repellent to *T. castaneum* (Herbst). The essential oils of several spices like anise (*Pimpinella anisum* L.) and peppermint (*Mentha piperita* L.) were found to have fumigant toxicity to four major stored product pests, *Rhyzopertha dominica* (Fab.), *T. castaneum, Sitophilus oryza* (L.) and *Orzyaephilus surinamensis* (L.) (Shaaya *et al.*, 1991).

The non-polar extracts of the flower buds of clove, *Syzygium aromaticum* (L.) and star anise (*Illicium verum* Hook f.) have insecticidal activity to *T. castaneum* and *S. zeamais* Motsch as well as suppress progeny production (Ho *et al.*, 1994 and 1995). Moreover, the essential oil of garlic was found to be potent against *T. castaneum* and *S. zeumais* (Ho *et al.*, 1996), while chopped garlic and garlic extracts were repellent to the two beetles species (Ho and Ma, 1995). Huang and Ho (1998), found that the essential oil of *Cinnamomum aromaticum*, was toxic to both *T. castaneum* and *S. zeamais* with contact fumigant and antifeedant activities.

The metabolite of the actinomycete *Saccharopolyspora spinosa* Mertz and Yao, Spinosad, is currently used on several stored products (Thompson *et al.*, 1997). It has low mammalian toxicity and it is very effective against a wide range of pest species (Cloyd and Sadof, 2000; Peck and McQuate, 2000). Recently, spinosad was registered for use in stored products in the USA (Subramanyam *et al.*, 2003), as an alternative to traditional grain protectants.

Due to the urgent need to develop safe, convenient, environmental and low-cost alternatives to control stored product pests, the present study was designed to assess the efficacy of four control agents which belong to different classes, namely Fenvalerate (pyrethroid), Diazinon (organophosphate), Neem (Natural product) and Biofly (Entomopathogenic fungus).

MATERIALS AND METHODS

Chemicals:

Fenvalerate, 20% EC (cyano(3-phenoxyphenyl) methyl 4-chloro-α-(1-methylethyl) benzene acetate; Neem (azadirachtin), 0.09% EC (dimethyl [2a*R* [2aα,3β,4β(1a*R**,2*S**,3a*S**,6a*S**,7*S**,7a*S**),4aβ,5α,7a*S**,8β(*E*),10β,10aα,10bβ]]-10-(acetyloxy)octahydro-3,5-dihydroxy-4-methyl-8-[(2-methyl-1-oxo-2-butenyl) oxy]-4-(3a,6a,7,7a-tetrahydro-6a-hydroxy-7a-methyl-2,7-methanofuro[2,3-*b*] oxireno [*e*] oxepin-1a(2*H*)-yl)-1*H*,7*H*-naphtho[1,8-*bc*:4,4*a*-*c*']difuran-5,10a(8*H*)-dicarboxylate; Diazinon, 60% EC (*O,O*-diethyl *O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate) and Biofly, 100% (W/W) (Entomopathogenic fungus, *Beauveria bassiana*) were purchased from the local market.

Insect rearing:

The red flour beetle *Tribolium castaneum*, used throughout the present work was maintained under laboratory conditions at 25 ±1°C and 70 ± 5% relative humidity on wheat grains for several generations.

Toxicity of control agents against *T. castaneum*

Tested concentrations were 250, 500, 750 and 1000 mg/L for Fenvalerate, 2500,5000,10000 and 20000 mg/L for Biofly, 25, 50, 100 and 200 mg/L for Neem and 25, 50, 75 and 100 mg/L for Diazinon. Concentrations were thoroughly incorporated in wheat grains using water as carrier solvent. The treated grains were kept for 24 hr. at 25°C till complete evaporation of the solvent before starting the experiments. Ten adults of *T. castaneum* were put on 25 gm of the treated wheat grains for 10 days or until 100% mortality was obtained. The data was subjected to probit analysis in order to determine the LC_{50}, LC_{95}, LT_{50} and LT_{95} values. All experiments were carried out in triplicates at 25 ± 1°C and average 70 ± 5% relative humidity.

Statistical analysis

Data were calculated as mean ± standard deviation (SD) analyzed using analysis of variance (ANOVA). Probability of 0.05 or less was considered significant. The statistical package of Costat Program (1986) was used for all chemometric calculations.

RESULTS AND DISCUSSION

Four commercially used control agents, Fenverelate, Neem, Biofly and Diazinon, were tested against the adult stage of *T. castaneum* (Tables1-4) under the laboratory conditions. The results indicated that the mean values of the percent mortality were significantly different. The average mortalities were 41.66, 47.99, 53 and 62% at 250, 500, 750 and 1000 mg/L of Fenvalerate, respectively (Table1). Less average mortality values were recorded in the case of Biofly with values being 10, 10, 21.70 and 23.33% at 2500, 5000, 10000 and 20000 mg/L, respectively (Table 2). In the case of Neem, the recorded average mortality values were 26.95, 34.33, 35.19 and 37.84 at 25, 50, 100 and 200 mg/L, respectively (Table 3). The highest average mortality values were recorded with Diazinon of 45.64,

Table 1. Activity of Fenvalerate on the survival of *T. castaneum* adults, during 10 days

Dose	% Mortality[1] after										Average mortality[2]
(ppm)	1 day	2 day	3 day	4 day	5 day	6 day	7 day	8 day	9 day	10 day	
0	0	0	0	0	2	3	3	3	4	4	1.9±1.70a
250	16.7	20	20	33.3	43.3	50	53.3	53.3	56.7	70	41.66±18.30b
500	13.3	20	20	33.3	50	53.3	56.7	66.7	80	86.6	47.99±25.73c
750	20	26.7	30	33.3	53.3	60	66.7	70	80	90	53±24.32d
1000	30	33.3	33.3	36.7	56.7	60	86.7	90	93.3	100	62±28.10d

[1]Each data point is mean ± S.D. of three independent experiments. The mortality in the control was less than 5%.
[2]Means followed by the same letters are not significantly different ($P \leq 0.05$, Duncan's multiple-range test).

Table 2. Activity of Biofly on the survival of *T. castaneum* adults

Dose	% Mortality[1] after						Average mortality[2]
(ppm)	1 day	2 day	3 day	4 day	5 day	6 day	
0	0	0	0	2	2	3	1.2±1.33a
2500	10	10	10	10	10	10	10±0.0b
5000	10	10	10	10	10	10	10±0.0b
10000	20	20	20	20	20	30	21.70±4.10c
20000	20	20	20	20	20	40	23.33±8.20c

[1]Each data point is mean ± S.D. of three independent experiments. The mortality in the control was less than 5%.
[2]Means followed by the same letters are not significantly different ($P \leq 0.05$, Duncan's multiple-range test).

Table 3. Activity of Neem on the survival of *T. castaneum* adults

Dose (ppm)	% Mortality[1] after										Average mortality[2]
	1 day	2 day	3 day	4 day	5 day	6 day	7 day	8 day	9 day	10 day	
0	0	0	0	0	3	3	4	4	4	4	2.2±1.93a
25	3	13.3	13.3	13.3	20	30	33.3	40	50	53.3	26.95±17.10b
50	10	23.3	23.3	26.7	33.3	36.7	40	46.7	50	53.3	34.33±13.71c
100	10	20	20	20	30	30	33.3	56.7	63.3	66.7	35±19.10c
200	10	17	17	26.7	36.7	40	47.7	53.3	60	70	37.84±20.10c

[1]Each data point is mean ± S.D. of three independent experiments. The mortality in the control was less than 5%.
[2]Means followed by the same letters are not significantly different (P≤ 0.05, Duncan's multiple-range test).

Table 4. Activity of Diazinon on the survival of *T. castaneum* adults

Dose (ppm)	% Mortality[1] after							Average mortality[2]
	1 day	2 day	3 day	4 day	5 day	6 day	7 day	
0	0	1	1	2	3	4	4	2.14±1.57a
25	13.3	13.3	30	33.3	60	83.3	93.3	45.64±32.60b
50	30	30	33.3	43.3	70	96.7	100	57.61±31.10c
75	30	30	46.33	70	90	100		66.62±31.30c
100	30	30	56.7	76.7	90	100	-	69.10±30.60c

[1]Each data point is mean ± S.D. of three independent experiments. The mortality in the control was less than 5%.
[2]Means followed by the same letters are not significantly different (P≤ 0.05, Duncan's multiple-range test).

Table 5. Statistical parameters for the toxicity of Fenvalerate against *T. castaneum* adults

Dose (ppm)	LT_{50} (Day)	LT_{95} (Day)	Slope	Regression Equation	X^2	P	Fiducial Limits	
							Upper	Lower
250	6.50	75.10	1.55	-1.26+1.55X	13.66	9.11	7.43	5.71
500	4.96	26.65	2.26	-1.57+2.26X	24.74	3.82×10^{-4}	10.92	2.26
750	4.04	23.12	2.17	-1.32+2.17X	48.38	0.24	30.61	0.50
1000	3.15	16.68	2.47	-1.13+2.47X	99.54	0	484.29	1.32×10^{-2}

Table 6. Statistical parameters for the toxicity of Biofly against *T. castaneum* adults

Dose (ppm)	LT_{50} (Day)	LT_{95} (Day)	Slope	Regression Equation	X^2	P	Fiducial Limits	
							Upper	Lower
10000	4.08×10^{3}	1.50×10^{25}	0.25	-0.904+0.25X	3.38	0.50	7.38×10^{25}	4.59×10^{-7}
20000	98.00	2.44×10^{5}	0.49	-0.96+0.484X	12.28	0.06	5.88×10^{-39}	1.70×10^{38}

Table 7. Statistical parameters for the toxicity of Neem against *T. castaneum* adults

Dose (ppm)	LT_{50} (Day)	LT_{95} (Day)	Slope	Regression Equation	X^2	P	Fiducial Limits	
							Upper	Lower
25	11.50	90.44	1.83	-1.94+1.83X	7.86	0.211	18.86	7.15
50	10.42	24.24	1.24	-1.26+1.24X	3.10	0.870	13.79	8.00
100	9.21	106.35	1.55	-1.49+1.55X	27.04	1.42×10^{-4}	66.50	1.54
200	7.75	70.66	1.71	-1.52+1.71X	7.69	0.356	9.07	6.66

Table 8. Statistical parameters for the toxicity of Diazinon against *T. castaneum* adults

Dose (ppm)	LT_{50} (Day)	LT_{95} (Day)	Slope	Regression Equation	X^2	P	Fiducial Limits	
							Upper	Lower
25	3.78	12.91	3.08	-1.78+3.08X	36.44	$2.60x10^{-2}$	$4.53x10^{2}$	$3.06x10^{-2}$
50	2.74	12.42	2.51	-1.10+2.51X	100.83	0	$5.56x10^{5}$	$2.50x10^{-2}$
75	2.23	7.60	3.09	-1.08+3.09X	63.52	0	$9.42x10^{2}$	$2.06x10^{-3}$
100	2.10	6.91	3.18	-1.03+3.18X	50.40	0.198	2.31	1.91

Table 9. Statistical parameters for the toxicity of the selected four pesticides against *T. castaneum* adults

Compound	LC_{50} (ppm)	LC_{95} (ppm)	Slope	Regression Equation	X^2	P	Fiducial Limits	
							Upper	Lower
Fenvalerate	271.12	4202	1.38	-3.36+1.4X	9.73	$4.5x10^{-2}$	$1.13x10^{6}$	$3.9x10^{-1}$
Biofly	$3.05x10^{4}$	$1.71x10^{10}$	1.30	-5.84+1.3X	3.50	0.174	$5.03x10^{4}$	$1.86x10^{4}$
Neem	78.30	$2.25x10^{5}$	0.476	-0.9+0.48X	0.131	0.936	144.12	42.68
Diazinon	46.25	304.64	2.02	-3.4+2.02X	4.99	$8.3x10^{-2}$	53.96	39.63

57.61, 66.62 and 69.10% at 25, 50, 75 and 100 mg/L, respectively (Table 4). The 100% mortality was observed at 75 and 100 mg/L of Diazinon and 1000 mg/L of Fenvalerate after 6 and 10 days, respectively, compared with the control which showed less than 5% mortality in all cases. These results clearly indicate the high potential of Diazinon as a recommended treatment of wheat grains for controlling *T. castaneum* infestation.

As for the LT_{50} values for the adult stage of *T. castaneum*, they were found to be 6.50, 4.96, 4.04 and 3.15 days at 250, 500, 750 and 1000 mg/L of Fenvalerate (Table 5), while the LT_{50} values for Biofly were 4.08×10^3 and 98.00 days at 10000 and 20000 mg/L, respectively (Table 6). The LT_{50} values for Neem were 11.50, 10.42, 9.21 and 7.75 days at 25, 50, 100 and 200 mg/L, respectively (Table 7). For Diazinon, it was found to have lower values of LT_{50} being 3.78, 2.74, 2.23 and 2.10 days at 25, 50, 75 and 100 mg/L, respectively (Table 8).

The LC_{50} values for the *T. castaneum* adult were found to be 46.25, 78.30, 271.12 and 3.05×10^4 mg/L, while LC_{95} values were 304.64, 2.25×10^5, 4.20x103 and 1.71×10^{10} mg/L after 4, 8, 7 and 6 days of exposure for Diazinon, Neem, Fenvalerate and Biofly, respectively (Table 9). These results indicated that Diazinon was the most effective insecticide on *T. castaneum*, while the Biofly was the least.

CONCLUSION

Storage of grains is part of the post-harvest practice through which food material passes on its way from fields to consumer. There is a continuous need to protect stored products against deterioration, especially loss of quality and quantity during storage. The use of pesticides is one means of preventing some losses during storage. However, the use of pesticides for storage pest control is very limited because of the strict requirement imposed on the use of synthetic insecticides on or near food (Padín *et al.*, 2002). The continuous use of chemical pesticides for control of stored-grain pests has resulted in serious problems such as resistance (Pacheco *et al.*, 1990). The present results demonstrated that Diazinon was most effective against *T. castaneum* followed by Neem, Fenvalerate and then Biofly. Furthermore, the efficacy of the insecticides against storage pest varies greatly after treatment (Suchita *et al.*, 1989 and Pinto *et al.*, 1997). Also, chemicals used for stored product pests, or as protectants, need also to be compared with the suitability and effectiveness of alternative control method. Non-chemical methods are attractive since they neither have chemical residues in the commodity nor do they cause resistance in insects. The public awareness and concern for environmental quality, has led to more focused attention on research and development of biological agents, (Hidalgo *et al.*, 1998) and plant extract (Jbilou *et al.*, 2008) either as alternative or in integrated programs. A promising strategy with good potential to minimize the adverse effects of synthetic insecticides is the use of entomophagous fungi and other microbial control agents. The possibility of using fungal pathogens to control insects has been studied for many years but little attention has been paid to use of fungi as control agents against storage pests (Khan and Selman, 1988; Rodrigues and Partissoli, 1990; Adane, 1994; Adane *et al.*, 1996; Padín *et al.*, 1997; Hidalgo *et al.*, 1998; Moino *et al.*, 1998 and Padín *et al.*, 2002). The present results are in parallel with the results of Padín *et al.* (2002) who indicated that *B. bassiana* was not effective against *T. castaneum*, while *S. oryza* was very susceptible to *B. bassiana* isolate.

In general, the biological methods would not be as efficient as chemicals. However, the use of effective plant extracts such as Neem on the stored grain would reduce the need for synthetic insecticides. Thus, potentially remaining suitable pest control with a reduction in chemical residue. The present results encourage the use of Neem in control of stored product pests such as *T. castaneum* as alternative for synthetic insecticides or in the integrated programs.

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الملخص العربي

مقارنة التأثيرات السامة لأربعة من المركبات التجارية ضد خنفساء الدقيق الصدئية تحت الظروف المعملية

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تم اختبار فاعلية أربعة تجهيزات مختلفة من المبيدات المتوفرة تجارياً تحت الظروف المعملية ضد الحشرات الكاملة لخنفساء الدقيق الصدئية حيث تم إجراء التقييم الحيوي على درجة حرارة ٢٥±١°م و رطوبة نسبية قدرها ٧٠ ±٥ %، بواسطة خلط مبيد الفنفاليريت بمعدل ٢٥٠ و٥٠٠ و٧٥٠ و١٠٠٠ مجم/لتر ، ٢٥ و٥٠ و ٧٥ و ١٠٠ مجم/ لتر لمبيد الدايازينون ، ٢٥٠٠ و ٥٠٠٠ و ١٠٠٠٠ و ٢٠٠٠٠مجم/ لترللمبيد الميكروبي البيوفلاي ، ٢٥ و ٥٠ و ١٠٠ و ٢٠٠ مجم/ لترلمبيد النيم ذو الأصل الطبيعي مع حبوب القمح . وقد تم تقدير النسبة المئوية للموت يومياً لمدة ١٠ أيام ثم حساب التركيز اللازم لقتل٥٠%و٩٥%وكذلك حساب الزمن اللازم لقتل٥٠%و ٩٥% من يرقات خنفساء الدقيق الصدئية.

أوضحت النتائج أن النسبة المئوية للموت قد بلغت١٠٠% بعد ١٠ أيام من المعاملة بتركيز قدره ١٠٠٠ مجم/لتر من الفنفاليريت و بتركيز قدرها ٥٠ و٧٥ و١٠٠مجم/لتر من الدايازينون. كما أوضحت النتائج أن مبيد الدايازينون كان الأكثر فعالية ضد يرقات خنفساء الدقيق الصدئية يليه النيم ثم الفنفاليريت ثم البيوفلاي حيث بلغت قيم التركيز اللازم لقتل٥٠% بما يعادل ٤٦,٢٥ و ٧٨,٣٠ و ٢٧١,١٢ و ٣,٠٥×$١٠^{٤}$مجم/ لتر بعد ٤ و ٨ و ٧ و ٦ أيام من التعرض، على التوالي.

وبصفة عامة ولكل المركبات المختبرة فإن مقدار الزمن اللازم لقتل٥٠% من يرقات خنفساء الدقيق الصدئية تتناقص بزيادة التركيز و أن مركب الدايازينون قد احتاج لوقت أقل من التعرض لإحداث الموت.

ويمكن القول بأن استخدام المبيدات الميكروبية غير فعال مقارنة بالمبيدات الكيميائية، إلا أن استخدام النيم كمستخلص نباتي على الحبوب المخزونة قد يقلل من الاعتماد على استخدام المبيدات. وتوصي الدراسة الحالية باستخدام تجهيزه النيم لمكافحة خنفساء الدقيق الصدئية كبديل لاستخدام المبيدات الحشرية أو استخدامها ضمن برامج الإدارة المتكاملة للآفات.