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Evaluation of Different Control Methods on the Rust Flour Beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract

The presented study aimed to evaluate the different control methods of the rusty flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). The experiments were conducted to evaluate the efficiency of a chemical pesticide (Malathion), biopesticide (Tracer 24% SC), plant oil (Lemongrass oil), plant extraction (Acetone extracts from Rosemary), powder (Diatomaceous earth) and modified Atmosphere (argon gas). Mortality percentage increased with increase of exposure times at all tested concentrations. The toxicity effect of different treatments against adults of *T. castaneum* at the LC_{50} after 7 days post-treatment at 30±1°C could be arranged in descending order as follows: Malathion, Tracer 24% and lemongrass oil, while, acetone extracts from Rosemary and diatomaceous earth (DE) were the least effective. The LT_{50} values against the adult stage of *T. castaneum* were 19.99, 2.33 and 4.86 hr. for Argon 100, 80 and 60%, respectively. In order to avoid the disadvantages of using chemical insecticides such as malathion, it is recommended to use other more safe control methods for the environment as Tracer 24% or lemongrass oil or Argon gas.

Keywords: *Tribolium castaneum*, Chemical pesticide, Biopesticide, Plant oil, Plant extraction, Powder, Modified atmosphere

1 Introduction

Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae), is considered an important stored grain insect pest (Saeed et al 2016, Sami et al 2018). It is popularly known as the beetle of the flour and it is considered a cosmopolitan insect, mainly prevalent in the tropics (Faroni and Sousa, 2006).

This beetle is categorized as a secondary insect pest because the adult and immature forms feed on pre-cracked or broken grains which were damaged by primary pests. However, reports in the literature describe the ability of this insect to survive even in the undamaged grains (White, 1982). It was reported as causing 15–20% damage to a wide range of commodities. It is capable of causing high losses of millions of pounds per year in many developing countries (Khattak and Shafique 1986).

This insect causes substantial loss during storage because of its high reproductive potential. In addition to direct feeding damage, product quality reduction also occurs due to the residues of insect bodies and skin, and the release of quinones with their unpleasant smell (Johnson, 2013). This study aims to evaluate the impact different control methods against the rust flour beetle. So used Malathion, Tracer 24% SC, Lemongrass oil, Acetone extracts from Rosemary, Diatomaceous earth and Aragon gas to evaluate the efficiency against the rust flour beetle.

2 Materials and Methods

Laboratory strain of the rust flour beetle *Tribolium castaneum (*Herbst.) used in these studies was obtained from the Plant Protection Dept. Fac. of Agriculture, Benha University and reared on wheat flour in a climate chamber at 28±1°C and 65±5% R.H. for about one year. Experiments were conducted in the laboratory.

2.1 Preparation of the test-insects for various treatments

Batches of 20 *T. castaneum* adults were used in all experiments. Adults 7-14 day-old were used in these tests. Three replicates were conducted in the various treatments.

2.2 Laboratory conditions

The experiments were conducted in the laboratory at 30 ± 1 ° C. and 65 ± 5 % R.H. to evaluate the efficiency of each of the following 5 treatments:

2.2.1 Malathion

Common name: Malathion 57% EC., Chemical name: diethyl [(dimethoxyphosphinothioyl)thio]butanedioate. Formula: $C_{10}H_{19}O_6PS_2$. The applied formulation: 57% EC, Source: purchased from Nasr Company for Intermediate Chemicals Abu Rawash – Egypt.

2.2.2 Tracer 24% SC

Biopesticide: Tracer 24% SC a formulation of spinosad that contains about 85% spinosyn A and 15% spinosyn D. It was obtained from Dow AgroSciences Company.

The concentrations of Malathion and Tracer 24% SC (v/w) used in this investigation were, 0.1, 0.05, 0.025, 0.0125 and 0.006%.

2.2.3 Lemongrass oil

Lemongrass oil (*Cymbopogaon Citratus*, Fam.: Poaceae) was bought from National Research Center.

2.2.4 Acetone extracts from Rosemary

Acetone extracts from Rosemary (*Rosmarinus officinalis*, Fam.: Lamiaceae) leaves were rinsed with distilled water, dried in room temperature (25˚c), then pulverized into plant powder using a small grinder. The leaves powder was weighed and soaked in aceton. The extract was filtered by Whatman No.4 filter paper. The solvent was removed by vacuum in a rotary evaporator separately at 40°C and the residue was dissolved in the least amount of solvent and used as a starting stock solution. Further dilutions in the distilled water were prepared in suitable concentrations used in treatments (Warthen et al 1984**).**

The concentrations of oil and acetone extracts from Rosemary (v/w) used in this investigation were 4, 3, 2, 1 and 0.5%.

2.2.5 Diatomaceous earth

Diatomaceous earth (DE): Obtained from Algae Diatomes in the form of rigid, Rocky Mountains and it was ground into fine powder. Dust powder formulation contains 97% silicon dioxide produced by Hedley Pacific Ventures Ltd., Fabrique par, Vancouver, B. C., Canada mixed with wheat grains and tested at 4, 3, 2, 1 and 0.5% (w/w) concentrations.

2.2.6 Gases Used

Argon was provided as pure gas in pressurized steel cylinders. The cylinder was connected to a pressure regulator. The dilution method was used to achieve the required argon concentration. For the atmosphere of nearly pure Ar (100%), the valve of each cylinder was opened for three minutes in order to fill the gastight Dreshel exposure flask with the gas. Modified Atmosphere (MA) of Argon concentrations 60 and 80 % in air were prepared using Gas Distribution device. Determination of the concentrations of Ar was monitored using a gas Analyser model 2(10-600 Gow-Mac-Instruments Company U.S.A.).

2.3 Preparing *T. castaneum* for bioassay tests of modified atmosphere

A number of 20 *T. castaneum* adults were placed into small cloth bags (4×8 cm) filled with about 25 g uninfected wheat flour and closed with rubber bands. Cloth bags were taken and introduced into the gastight Dreshel-flasks of 0.55L volume. *T. castaneum* in the gastight flasks were treated with the MA at mentioned concentrations for different exposure periods ranged from 3 to 144 hrs. at 27 ± 2°C. After the exposure periods, the flasks were aerated for 24 hrs. and the adults were transferred into Petri dishes and kept at 27 ± 2°C and 65 ± 5% R.H. Adults were examined daily to record mortality.

2.4 Statistical analysis

Mortality percentages were corrected by Abbott's formula (1925). The obtained mortality data were subjected to Probit analysis (Finney, 1971), using a computer program of (Noack and Reichmuth, 1978).

3 Results and Discussion

1. Concentration mortality data of all treatments are represented in **Tables 1-5** as mortality percentages among treated *Tribolium castaneum* at 30±1°C and 65±5% RH. The obtained results indicated that mortality percentage increased by increasing the used concentration and the prolongation of exposure period. The efficacies of different assayed materials against *T. castaneum* were as followed:

3.1 Malathion

As shown in **Table 1,** 1 day after *T. castaneum* adult treatments by Malathion at 0.1, 0.05, 0.025, 0.0125 and 0.006% concentrations, mortalities ameny adults reached 76.7, 48.9, 25.6, 17.8 and 10.8%. These percentages increased, successively, reached 100, 78.8, 46.8, 41.1 and 37.8%, respectively, after 3 days. As the applied concentration was decreased to 0.05, 100% mortality was recorded after 7 days exposure. As the exposure period was prolonged to 14 days, accumulative adults mortality % for treatments by 0.025, 0.0125 and 0.006 reached 80.8, 72.3 and 64.5%, respectively **(Table 1).**

Table 1. Effect of Malathion on adult mortality of *T. castaneum*

Concentrations % (v/w)	` Accumulative mortality (%) after indicated days post treatment						
	1	**2**	**3**	**5**	**7**	**10**	**14**
0.1	76.7	93.5	100	100	100	100	100
0.05	48.9	59.1	78.8	88.6	100	100	100
0.025	25.6	35.6	46.8	56.6	64.5	72.7	80.8
0.0125	17.8	37.5	41.1	48.6	55.6	68.5	72.3
0.006	10.8	22.2	37.8	43.3	51.9	60.2	64.5
Control	0	0	0	0	0	0	0

Table 2. Effect of Tracer 24% on adult mortality of *T. castaneum*

Concentrations % (v/w)	Accumulative mortality (%) after indicated days post treatment						
	1	**2**	**3**	**5**	**7**	**10**	**14**
0.1	10.8	25.5	36.6	46.6	76.6	93.3	100
0.05	7.3	17.4	20.5	25.5	45.5	54.5	74.8
0.025	5.4	10.8	18.4	18.4	24.4	33.3	51.6
0.0125	0.0	3.4	8.8	11.1	16.6	20.2	36.5
0.006	0.0	0.0	2.7	8.8	11.1	16.6	22.2
Control	0	0	0	0	0	0	0

Table 3. Effect of lemongrass oil on adult mortality of *T. castaneum*

Concentrations % (v/w)	Accumulative mortality (%) after indicated days post treatment							
	1	**2**	**3**	**5**	**7**	**10**	**14**	**21**
4	43.3	54.4	77.7	83.3	96.6	98.78	100	100
3	11.1	27.8	33.5	41.1	55.5	73.3	94.4	100
2	10	17.6	22.8	35.3	42.2	52.3	58.8	67.8
1	8.9	15.6	20.5	28.8	37.7	45.1	48.5	50.3
0.5	0.0	4.4	10.5	16.7	23.3	30.4	34.5	38.3
Control	0	0	0	0	0	0	0	0

Table 4. Effect of Rosemary acetone extract on *T. castaneum* adults mortality

Concentrations % (v/w)	Accumulative mortality (%) after indicated days post treatment							
	1	**2**	**3**	**5**	**7**	**10**	**14**	**21**
4	10	22.2	28.5	45.5	63.3	77.7	87.7	100
3	8.8	16.6	22.4	35.5	48.8	62.2	78.8	95.4
2	4.5	11.1	18.8	22.2	34.4	53.3	62.2	86.5
1	0.0	2.2	7.6	11.1	18.8	28.8	46.5	65.5
0.5	0.0	1.1	4.5	7.7	12.2	23.3	27.8	35.5
Control	0	0	0	0	0	0	0	0

Table 5. Effect of diatomaceous earth (DE) treatments on mortality percentages among *T. castaneum* adults

Concentrations % (w/w)	Accumulative mortality (%) after indicated days post treatment							
	1	**2**	**3**	**5**	**7**	**10**	**14**	**21**
4	6.9	8.3	11.6	21.2	51.6	71.6	83.3	100
3	3.3	6.9	8.3	14.2	25.2	39.2	48.6	77.3
2	0.0	3.3	6.9	11.6	17.8	25.8	32.2	68.9
1	0.0	1.6	5	8.3	11.6	18.3	22.2	43.4
0.5	0.0	0.0	1.6	4.2	6.9	14.2	24.3	33.3
Control	0	0	0	0	0	0	0	0

3.2 Tracer 24%

Application of Tracer 24% took place by the same concentrations as those of Malathion. Data showing mortality percentages in Table (1 and 2) showed that the bioinsecticide Tracer was, clearly, less efficient against *T. castaneum* adults than the chemical one Malathion.

At 0.1, 0.05, 0.025, 0.0125 and 0.006% concentrations, the adults mortality percentages were 10.8, 7.3, 5.4, 0.0 and 0.0% after 1 day exposure. These percentages increased, successively, during the subsequent days after treatment until reached the maxima of 100, 74.8, 51.6, 36.5 and 22.2 %, respectively, by the same concentrations, respectively. The two lower concentrations (0.0125 and 0.006) were completely ineffective (0.0 mortality) during the first day after treatment. This inefficacy extended for the second day after treatment by the lowest concentration.

3.3 Lemongrass oil

At 4, 3, 2, 1 and 0.5% concentrations adult mortality percentages were 43.3, 11.1, 10, 8.9 and 0.0%, respectively, after one day exposure. Mortality of all treated *T. castaneum* adults died after 14 days of lemongrass oil treatment at the highest concentration of 4% while this high mortality rate (100%) occurred 21 days post treatment at 3%. By treatment at lower concentrations (2.0, 1.0 and 0.5%), mortality percentages reached 67.8, 50.3 and 38.3%, respectively. All the exposed *T. castaneum* adults showed on effect of lemongrass oil at the lowest concentration (1%) for one day after treatment (**Table 3**).

3.4 Rosemary acetone extract

After one day of *T. castaneum* treatment by the Rosemary acetone extract at 4, 3, 2, 1 and 0.5% concentrations adult mortality percentages were 10, 8.8, 4.5, 0.0 and 0.0%, respectively, (**Table 4**). These mortality percentages reached their maxima of 100, 95.4, 86.5, 65.5 and 35.5%, respectively, after 21 days of treatments.

3.5 Diatomaceous earth (DE)

One day after treatment by diatomaceous earth 4, 3, 2, 1 and 0.5% concentrations adults mortality percentages were 6.9, 3.3, 0.0, 0.0 and 0.0%. These percentages increased, successively, throughout 21 days post treatment until reached their maxima values of 100, 77.3, 68.9, 43.4 and 33.3%, respectively, as shown in **Table 5**.

3.2 The lethal concentrations of the tested materials against *T. castaneum* adults

Data in **Table 6** show the values of the lethal concentrations (LC_{50}, LC_{90} and LC_{95}) of malathion, Tracer 24%, lemongrass oil, acetone extracts from Rosemary and diatomaceous earth (DE) to adults of *T. castaneum* after 7, 10 and 14 days post treatments.

3.2.1 Toxicity indices of different treatments against *T. castaneum* adults, 7 days post treatment

The toxicity effect of different treatments against adults of *T. castaneum* at the LC_{50} after 7 days post-treatment at 30±1° C could be arranged in descending order as follows: malathion, Tracer 24%, lemongrass oil, acetone extracts from Rosemary and diatomaceous earth (DE) which was the least effective **Table 7.**

According to the toxicity values (LC_{50}) of the 5 tested materials against *T. castaneum* adults after 7 days of treatment at 30±1° C (**Table 6**) and the toxicity indices presented in **Table 7,** the tested materials may be arranged descendingly according to their toxicities as: the chemical insecticide, Malathion, the bioinsecticide, Tracer 24%, Lemongrass oil, Rosemary acetone extract and the algae diatoms (Diatomaceous earth), respectively. It is clean that the chemical insecticide has the highest efficacy, while the diatomaceous earth had the lowest efficiency. The 3 remaining materials, Tracer 24%, Lemongrass oil and Rosemary acetone extract had intermediate efficacies on the rust flour beetle adults.

3.3 Effect of exposure of adults to MA containing Argon gas application on tested insect

Results concerning the evaluation efficacy of MA concentration 100, 80 and 60% of Argon on adult stages *of T. castaneum* are shown in **Table 8**. The results revealed that reduction in emerged adults increased gradually by increasing the exposure period. Complete reduction (100%) was observed in *T. castaneum* after 72 hrs. exposure to pure Argon, whereas, in the case of 80% Ar. complete reduction (100%) was estimated after 6 days exposure time. Whereas, by exposure of adults to atmosphere of 60% Argon, the highest reduction% reached 66.67% **(Table 8).**

3.4 The lethal times (LT) and parameters of probit regression line estimated for the tested adults of *T. castaneum* exposed to Argon MA at 60, 80 and 100%

As shown in **Table 9** the LT_{50}, LT_{90} and LT_{95} for adult stage of *T. castaneum* were 19.99, 65.10 and 90.67 hr. for Argon 100%, respectively. In the same trend, the LT_{50}, LT_{90} and LT_{95} values were 2.33, 6.15 and 8.09 days, respectively, for Argon 80%. While for exposure to MA 60% Ar. The LT values were 4.86, 12.16 and 15.77 days for Argon 60%, respectively.

Concerning the hazards of using Malathion, Badr (2020), Mamun et al (2015) and Varsha et al (2009) reported that treated with malathion has been associated with different toxicities that nearly affect every single organ in mammal bodies, with CNS toxicity being the most well documented. Malathion toxicity on liver, kidney, testis, ovaries, lung, pancreas, and blood were also reported. Moreover, malathion was considered as a carcinogenic and genotoxic chemical compound.

Table 6. Lethal concentrations of different treatments against adults of *T. castaneum* at 30±1° C, 65±5% R.H. and various exposure periods

Exposure period (Days).	Lethal concentrations (%v/w) and their 95% confidence limits.			Slope ± SD	R
	LC_{50}	LC_{90}	LC_{95}		
malathion					
7 days	0.008 (0.002-0.037)	0.071 (0.015-0.344)	0.133 (0.027-0.647)	1.33±1.92	0.872
10 days	0.005 (0.001-0.022)	0.051 (0.011-0.238)	0.099 (0.021-0.467)	1.24±1.79	0.894
14 days	0.004 (0.001-0.013)	0.034 (0.011-0.108)	0.063 (0.02-0.2)	1.37±1.25	0.935
Tracer 24%					
7 days	0.049 (0.028-0.086)	0.336 (0.190-0.596)	0.582 (0.328-1.030)	1.528±0.643	0.927
10 days	0.029 (0.018-0.047)	0.152 (0.095-0.242)	0.242 (0.152-0.386)	1.795±0.519	0.849
14 days	0.018 (0.010-0.032)	0.083 (0.047-0.147)	0.128 (0.073-0.226)	1.943±0.654	0.951
lemongrass oil					
7 days	1.519 (1.014-2.276)	7.395 (4.935-11.081)	11.582 (7.730-17.355)	1.864±0.461	0.649
10 days	1.150 (0.612-2.160)	5.618 (2.990-10.554)	8.809 (4.689-16.548)	1.860±0.760	0.836
14 days	0.928 (0.613-1.405)	2.981 (1.969-4.513)	4.150 (2.741-6.283)	2.528±0.451	0.820
acetone extracts from Rosemary					
7 days	3.003 (1.773-5.084)	18.388 (10.859-31.138)	30.737 (18.152-52.049)	1.628±0.610	0.963
10 days	1.704 (1.018-2.852)	10.568 (6.315-17.685)	17.728 (10.594-29.667)	1.617±0.612	0.944
14 days	1.112 (0.698-1.770)	5.422 (3.405-8.633)	8.496 (5.335-13.529)	1.862±0.535	0.976
diatomaceous earth (DE)					
7 days	6.125 (3.241-11.576)	45.955 (24.315-86.854)	81.367 (43.052-153.782)	1.464±0.684	0.853
10 days	3.292 (1.895-5.720)	22.830 (13.139-39.668)	39.530 (22.751-68.685)	1.524±0.639	0.776
14 days	2.250 (1.319-3.837)	16.843 (9.874-28.732)	29.806 (17.473-50.842)	1.466±0.634	0.655

R = Correlation coefficient of regression line
SD= Standard deviation of the mortality regression line

Table 7. Toxicity indices of different treatments on *T. castaneum* adults, 7 days post treatment at 30 ±1° C and 65±5% R.H.

Plant oils	LC_{50} after 7 days	Toxicity index at LC_{50}	Slope ± SD	R
Malathion	0.008	100	1.33±1.92	0.872
Tracer 24%	0.049	16.33	1.528±0.643	0.927
lemongrass oil	1.519	0.53	1.864±0.461	0.649
acetone extracts from Rosemary	3.003	0.27	1.628±0.610	0.963
diatomaceous earth (DE)	6.125	0.13	1.464±0.684	0.853

Table 8. Effect of MA enriched with Argon 100, 80 and 60 % on adult emergence of tested insects

Concentra-tions %	Reduction%						
	Exposure time (hr)						
	3	**9**	**12**	**18**	**24**	**48**	**72**
100	4.12	15.64	20.63	50.12	74.39	88.89	100
Exposure time (days)							
	0.5	**1**	**2**	**3**	**4**	**5**	**6**
80	3.39	14.78	24.48	58.49	65.51	89.03	100
60	0.0	2.12	12.26	22.22	35.59	48.15	66.67

Table 9. lethal time values (LT) and parameters of probit regression line estimated for the tested adult stage of *T. castaneum* exposed to Argon at 60, 80 and 100%

Argon concentration	Lethal times and their 95% confidence limits			Slope ± SD	R
	LT_{50} (hr)	**LT_{90} (hr)**	**LT_{95} (hr)**		
100%	19.99 (14.22-28.12)	65.10 (46.29-91.55)	90.97 (64.68-127.93)	2.50±0.43	0.91
Argon concentration	**LT_{50} (days)**	**LT_{90} (days)**	**LT_{95} (days)**	**Slope ± SD**	**R**
80%	2.33 (1.756-3.088)	6.15 (4.634-8.151)	8.09 (6.102-10.732)	3.04±0.36	0.93
60%	4.86 (3.68-6.41)	12.16 (9.21-16.05)	15.77 (11.94-20.82)	3.22±0.33	0.99

Plant oils showed relative grain protecting activity and could be included in integrated pest management (IPM) programs was the most striking. This result is in harmony with the results of Bilal et al (2015), Abdelgaleil et al (2016), Darwish (2016), Ibrahim (2016), Heidari et al (2017), Darwish et al (2018).

4 Conclusions

As a result of the severe toxicity caused by malathion and other chemical pesticides, it is fairly recommended to use other control methods that are more safe for living organisms in the environment as Tracer 24% or lemongrass oil or Argon gas which were assayed in the present investigation.

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