Efficacy of some natural products mixed with wheat flour on the survival and development of the red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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Abstract: This study aimed to evaluate the four natural products; Super Nano, Screen, Bentonite and Diatomaceous earth as protectants flour from *Tribolium castaneum* infestation. The direct toxicity of these products, the reduction (%) in populations and weight losses (%) of wheat flour diets were determined in laboratory bioassays. Moreover, different biological parameters such as the durations of the developmental stages and the reproductive performance were monitored. Results showed that Bentonite gave the highest direct toxicity against *T. castaneum* adults followed by diatomaceous earth, Super Nano and Screen at 24 h post treatment. However, the percentage of reduction in population numbers with 0.25 g/g diatomaceous earth was the most effective treatment, followed by Bentonite, Screen and Super Nano. All tested compounds reduced significantly the weight losses in flour (8.00-18.90%) comparing with that in control (27.84%). All tested compounds exhibited significant effects on egg-incubation period, larval duration, pupal duration and generation period. In all treatments, egg number per female, hatchability (%), and adult emergence (%) were significantly lower than those of control group. In conclusion, the two products bentonite and diatomaceous earth were the most effective against rust flour beetle.

Keywords: Flour beetle, natural products, bentonite, diatomaceous earth and kaolin.

1.INTRODUCTION

In many cases stored grain provides a perfect place for insects to live and grow because food, air and water are available in sufficient quantities. The two major insect pests in stored grains and pulses are beetles and moths. Minerals like fine sand, lime, bentonite, certain types of kaolin clay and diatomaceous earth can be used to protect stored grain against insects. The minerals are mixed with the threshed grain, they will fill the spaces between the grain kernels and thus prevent movement and dispersal of insects inside the stored grain. Damage will not be totally prevented but newly hatched weevils are hindered in their activities. Filling the space in between the grains with fine material is a traditional method to remove oxygen from the stored product. In this way there is less air mixed with the stored product making it hard for insects to get enough oxygen to live. The mode of action of these substances has not been completely explained. It is known shells of minerals damage the cuticle layer of the arthropod integument, as a result of physical properties and of their peculiar pointed, geometric shapes. This occurs because the shells absorb the lipids which make up the epicuticular waxy layer and abrade the exoskeleton, especially in its thinnest zones (flexible membranes and joints) **(Korunic, 1997)**. It has not yet been proved whether diatomaceous earth affects the respiratory system and whether it has a damaging action on the digestive system when ingested with food **(Alexander *et al*., 1944)**.

Kaolinite and bentonite based particle film repellents have been used to control insect pests in fruit trees **(Gleen *et al*., 1999)**. The kaolinite-based particle films may also have potential for use as a dry dust material in stored-product environments, especially in specialty organic markets. The potential of kaolinite-based particle films are important to control the red flour beetle, *Tribolium castaneum* (Herbst) and the confused flour beetle, *Tribolium confusum* Jacquelin du Val. These two species are major pests inside milling and processing facilities, food warehouses, and food plants. Most of the researches have been with diatomaceous earth on stored grain insects, including internal feeders. The survival of stored-grain insects treated with diatomaceous earth could vary by species **(Arthur, 2000a)** and in general increasing relative humidity has an adverse effect on the efficacy of diatomaceous earth products **(Fields and Korunic, 2000)**. The presence of food material can also have an adverse effect on the efficacy of inert dusts, primarily by enhancing insect survival either during or after exposure **(Loschiavo, 1988; Arthur, 2000b)**. **Fields *et al*. (1997)** demonstrated that the use of heat sterilization in combination with diatomaceous earth was more effective in controlling confused flour beetles (*T. confusum* Jacquelin du Val) than the use of heat sterilization alone. Diatomaceous earth has been used with varying effectiveness as a protectant on raw commodities **(Golob, 1997)**. Several diatomaceous earth formulations are also labelled for use as structural treatments and may be effective when used in combination with high temperatures. The efficacy depends on the source of diatomaceous earth used in the formulation; thus, some commercially available products are more efficacious than others **(Dowdy, 1999)**.

The objective of this work was to study the effect of different natural components such as kaolin, bentonite and diatomaceous earth on adult survival, the population density, duration of life cycle and weight loss of infested flour due to infestation by *T. castaneum*.

2.MATERIALS AND METHODS

Insect culture

This study was carried out in the laboratory of stored grain insects at the Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University. *Tribolium castaneum* larvae, pupae and adults were obtained from laboratory cultures maintained in large glass jars (30 cm high, 8 cm diameter) with flour at 26 ± 1 °C, 65 ± 5% r.h. and a photoperiod of 12 h light, 12 h dark.

Natural compounds

The tested natural compounds were Super Nano (kaolin (aluminosilicate mineral [$AI_4Si40lO(OH)8$]), Screen (kaolin ((aluminosilicate mineral [AI4Si40lO (OH)8]), Bentonite ($Al_2O_3SiO_2H_2O$), and diatomaceous earth (fossilized skeletal remains of diatoms and a silicon dioxide assay of about 90%).

Laboratory experiments

Adult insects used for all bioassays were of mixed sex (50% of each sex) and 10-20 days old. All experiments were carried out in the rearing room at 25 ±1 ºC and 65 ± 5% r.h. In order to evaluate the effect of the four natural components on adult survival and some biological aspect of *T. castaneum,* flour was mixed separately with this components at 0.05, 0.1,0.15,0.20 and 0.25 g product/ g wheat flour. Four replicates were performed for each mixture as well as additional four replicates containing flour free from any treatment was used as the control. Adult beetles (20 beetles per petri dish/ 5 g wheat flour) were placed on the treated flour in 10 cm petri dishes.

The effect of tested compounds on the adult survival

Petri dishes were examined daily to record mortality of adult beetles over a period of 25 days and corrected according to Abbott's formula **(Abbott, 1925)**.

Estimating the reduction percentages of population

All treatments were put into a petri dishes and stored at room temperature for two months. All replicates of the treatments and control group were weekly examined for the presence of insect population. The numbers of larvae in the treatments and control groups were counted and recorded to calculate the reduction percentages which cause by natural components. Reduction in the percentage of populations was calculated according to the formula of **Henderson and Tilton (1955)**.

Estimating the weight loss of flour

Corresponding to monthly examination of all treatments, flour mixed with natural components and control were weighted to calculate the losses caused by insect feeding. The percentage of weight loss was calculated by the following equation:

% weight loss = (weight flour of treatment- weight flour of control/ weight flour of treatment) × 100

The effect of tested compounds on the biology of *T. castaneum*

Ten pairs (10 males and 10 females) of the newly -emerged beetles/replicate/mixture were individually transferred into glass vials (4 × 10 cm) then tightly covered with muslin fitted in place with rubber band. Every day, vials were inverted over dish and number of oviposited eggs per female was recorded until all females were dead. Eggs deposited were kept into Petri dishes incubated under the optimum conditions 25 ° C and 60 ± 5% R.H) and the mean number of eggs laid/ female were calculated.

To determine the incubation period and hatchability (%), twenty newly laid healthy eggs were carefully transferred to a petri dish with the aid of a tiny camel hair brush. This procedure was repeated five times for each tested products. All replicates were incubation under the above mentioned conditions and checked daily until egg hatching.

To determine the duration of larval instars, the survived larvae reproduced from the previous experiment were introduced separately into the glass vials (1 × 3 cm) containing the tested foods using a camel brush. The tested foods were 0.05, 0.1, 0.15, 0.20 and 0.25 g component/ g wheat flour with fifty replicates for use. All petri dishes were incubated at 25 ºC and 60 ± 5 % RH. These replicates were daily checked until all larvae completely developed to pupae then, the durations of larval stage were recorded.

Pupae developed from previous larvae were removed carefully and transferred into glass vials (4 × 10 cm) covered with muslin and fitted in place with a rubber band. Vials were incubated under the previously mentioned optimum conditions and checked daily until adult emergence to record pupal duration.

Statistical analysis

The obtained data were subjected to two way (ANOVA), with the means separate during Duncan's Multiple Range test ($P<0.05$) to compare the response of insects to the different treatments. One way Anova was used in case of the comparisons of biological parameters in mixtures and control for each treatment **(Snedecor and Cochran, 1967)**.

3.RESULTS

3.1.Toxicity on *T. castaneum* adults

Table 1 shows that all sources; natural products, days post treatment, mixtures and interactions between a source and another exhibited significant effects on the corrected mortality. This means that the increase of days and mixtures led to significant increase in the corrected mortality. In the tested products, this means that there is a significant difference between the corrected mortalities recorded for each.

Table(1) Three way ANOVA parameters for main effects and interactions for corrected mortality of *T. castaneum* adults exposed to flour mixed with different natural products

Source	df	*F*	*P*
Natural products	3	71.430	<0.001
Days	4	74.965	<0.001
Mixtures	4	28.543	<0.001
Natural products * Days	12	8.831	<0.001
Natural products * mixtures	12	5.104	<0.001
Days * mixtures	16	3.337	<0.001
Natural products * Days * mixtures	48	0.937	0.593

Tables (2-5) show corrected mortality percentages of *T. castaneum* adults exposed to flour mixed with the four natural products; Super Nano, Screen, Bentonite and diatomaceous earth at 1, 3, 11, 20, and 24 days post treatment. On the 1st and 3rd days post treatment, no mortalities were observed in the most concentrations of the three products; Super Nano, Screen, and diatomaceous earth. Rare mixtures **(**0.05 and 0.1 g product /g wheat flour) of Super Nano and diatomaceous earth induced low levels of mortality (3.33 – 6.67%) after 1 and 3 days. However, Bentonite gave higher mortality than the other products at the 1st and 3rd days (3.33 – 20.0%). At 11 and 20 days post treatment, Bentonite was the most effective product recording 10 – 66.67% mortality. While the two products diatomaceous earth and Super nano revealed moderate affects recording 3.33 -26.67% mortality. The Screen was the weaker product recording 3.33 with 0.25 g product /g wheat flour only at 11 days and all mixtures at 20 days. At 24 days post treatment, it is clearly that Bentonite gave the highest toxicity against *T. castaneum* adults followed by diatomaceous earth, Super nano and Screen (Fig. 1).

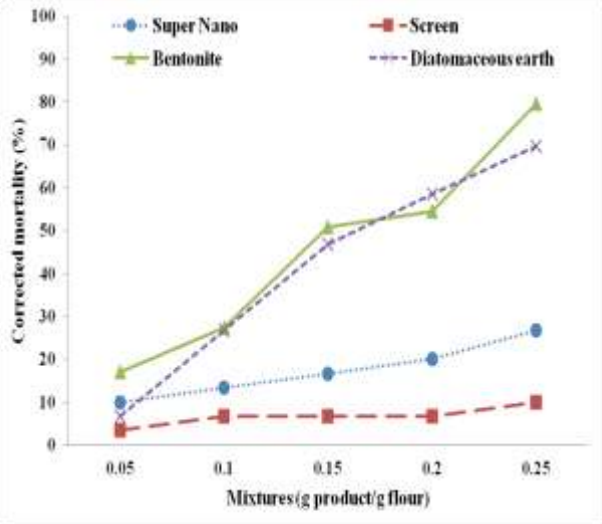

Fig. 1 The corrected mortality percentages of *T. castaneum* exposed to flour mixed with different mixtures of Super Nano, Screen, Bentonite and diatomaceous earth at 24 days after treatment.

3.2.The reduction in *T. castaneum* populations

Table (6) shows that the statistical analysis (three way ANOVA) for natural products, days post treatment, mixtures and their interactions. It was found that all sources and some interactions had a significant role in reduction of population of *T. castaneum*. By other meaning, the increase of days and mixtures caused significant increase in the reduction in populations. Moreover, the tested products recorded significant reductions in population of *T. castaneum* in comparing to each other.

Figure 2 shows the reduction percentages in *T. castaneum* population that weekly recorded for two months starting from the 12nd day until the 60th day post treatment. All the tested products recorded high reductions in populations with all mixtures through all days post treatment (29.28 – 100%). The ranges of reduction percentages were 59.73 – 72.65%, 59.30 – 76.60%, 65.98 – 79.33%, 68.78 – 80.70% and 69.70 - 88.08% with 0.05, 0.10, 0.15, 0.20, 0.25 g product /g wheat flour of Super nano, respectively (Fig 2a). The

Table(2) The corrected mortality percentages (Mean±SE) of *T. castaneum* exposed to flour mixed with Super Nano.

Mixtures (g product/ g flour)	Days after treatment 1	3	11	20	24
0.05	0.00±0.00^b	0.00±0.00^b	6.67±3.33	10.00±5.77	10.00±5.77
0.1	0.00±0.00^b	0.00±0.00^b	10.00±5.77	10.00±5.77	13.33±6.67
0.15	0.00±0.00^b	0.00±0.00^b	10.00±0.00	16.67±3.33	16.67±3.33
0.20	0.00±0.00^b	0.00±0.00^b	13.33±3.33	16.67±3.33	20.00±0.00
0.25	6.67±3.33^a	6.67±3.33^a	16.67±3.33	20.00±5.77	26.67 ±6.67
F	4.000	4.00	1.083	0.818	1.542
P	0.034	0.034	0.415	0.542	0.263

Within each column, different lowercase letters indicate significant differences among mixtures.

Table (3) The corrected mortality percentages (Mean±SE) of *T. castaneum* exposed to flour mixed with Screen.

Mixtures	Days after treatment				
(g product/ g flour)	1	3	11	20	24
0.05	0.00±0.00	0.00±0.00	0.00±0.00	3.33±3.33	3.33±3.33
0.1	0.00±0.00	0.00±0.00	0.00±0.00	3.33±3.33	6.67±3.33
0.15	0.00±0.00	0.00±0.00	0.00±0.00	3.33±3.33	6.67±3.33
0.20	0.00±0.00	0.00±0.00	0.00±0.00	3.33±3.33	6.67±3.33
0.25	0.00±0.00	0.00±0.00	3.33±3.33	3.33±3.33	10.00±5.77
F	-	-	1.000	0.000	0.357
P	-	-	0.542	1.000	0.833

Table (4) The corrected mortality percentages (Mean±SE) of *T. castaneum* exposed to flour mixed with Bentonite.

Mixtures	Days after treatment				
(g product/ g flour)	1	3	11	20	24
0.05	3.33±3.33	3.33±3.33	10.00±5.77	13.33±6.67b	17.03±2.97c
0.1	3.33±3.33	3.33±3.33	10.00±5.77	26.67±6.67b	27.40±11.84bc
0.15	10.00±10.00	10.00±10.00	16.67±16.67	33.33±8.82b	50.73±14.56ab
0.20	13.33±3.33	16.67±6.67	23.33±8.82	46.67±12.02ab	54.43±10.95ab
0.25	13.33±3.33	20.00±10.00	30.00±11.55	66.67±3.33a	79.63±5.46a
F	0.885	1.083	0.680	4.227	5.900
P	0.507	0.514	0.621	0.029	0.011

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Table(5) The corrected mortality percentages (Mean±SE) of *T. castaneum* exposed to flour mixed with diatomaceous earth.

Mixtures	Days after treatment				
(g product/ g flour)	1	3	11	20	24
0.05	0.00±0.00	0.00±0.00	3.33±3.33ba	6.67±3.33	6.67±3.33d
0.1	0.00±0.00	0.00±0.00	3.33±3.33b	20.7±5.77	26.67±6.67cd
0.15	0.00±0.00	0.00±0.00	6.67± 3.33b	23.33±12.02	46.67±6.67bc
0.20	0.00±0.00	6.67± 3.33	20.00±5.77a	23.33±3.33	58.53±1.47ab
0.25	3.33±3.33	6.67±6.67	23.33±3.33a	26.67±3.33	69.63±11.23
F	1.000	1.200	5.929	1.447	13.917
P	0.452	0.369	0.010	0.289	<0.001

respective ranges of reduction percentages with Screen were 55.25 – 75.48%, 58.58 – 81.75%%, 58.90 – 86.03%, 60.08 – 87.25%%, 69.88 – 87.50% (Fig 2b). The respective ranges of reduction percentages with Bentonite were 47.93 – 72.40%, 55.43 – 78.78%%, 64.00 – 90.25%, 81.40 – 92.60%%, 84.10 – 95.98% (Fig 2c). The respective ranges of reduction percentages with diatomaceous earth were 29.28 – 79.55%, 67.98 – 87.83%, 82.85 – 100%, 92.70 – 100%, 94.00 – 100% (Fig 2d). The highest mixture (0.25 g product /g wheat flour) induced the highest reduction % in population with all tested products ranging between 69.70 – 100%. According the resulted reduction% in populations with 0.25 g prod - g wheat flour, diatomaceous earth had the highest effect, followed by Bentonite, Screen and Super nano (Fig. 3).

3.3.Weight losses of flour

Figure 4 shows weight losses (%) of flour infested by *T. castaneoum* that serve as a control and compared with weight losses (%) of infested flour that treated with Super nano, Screen, Bentonite and Diatomaceous earth. The mixtures of all products reduced significantly the weight losses (8.00-18.90%) comparing with that in control (27.84%). The most protectant products were Bentonite and Diatomaceous earth those recorded the lowest weight losses (8.00-9.25%) with the mixture 0.25 g product / g wheat flour.

3.4. The durations of the developmental stages of *T. castaneum*

Table (7) shows the efficacy of the four tested compounds (Super Nano, Screen, Bentonite, and diatomaceous earth) on the durations of the developmental stages of *T. castaneum*. The durations were egg-incubation period, larval duration, pupal duration and generation period. All tested compounds exhibited significant effects on various durations comparing with those in control group. The incubation period of eggs laid from treated *T. castaneum* females extended 2.20 to 17.37 days comparing with eggs in control group. The mixture 0.05 g product /g wheat flour in all tested compounds recoded insignificant increase (2.20 – 4.23 days). The concentration 0.1 g product /g wheat flour in Bentonite only recorded significant increase (9.87 days). The

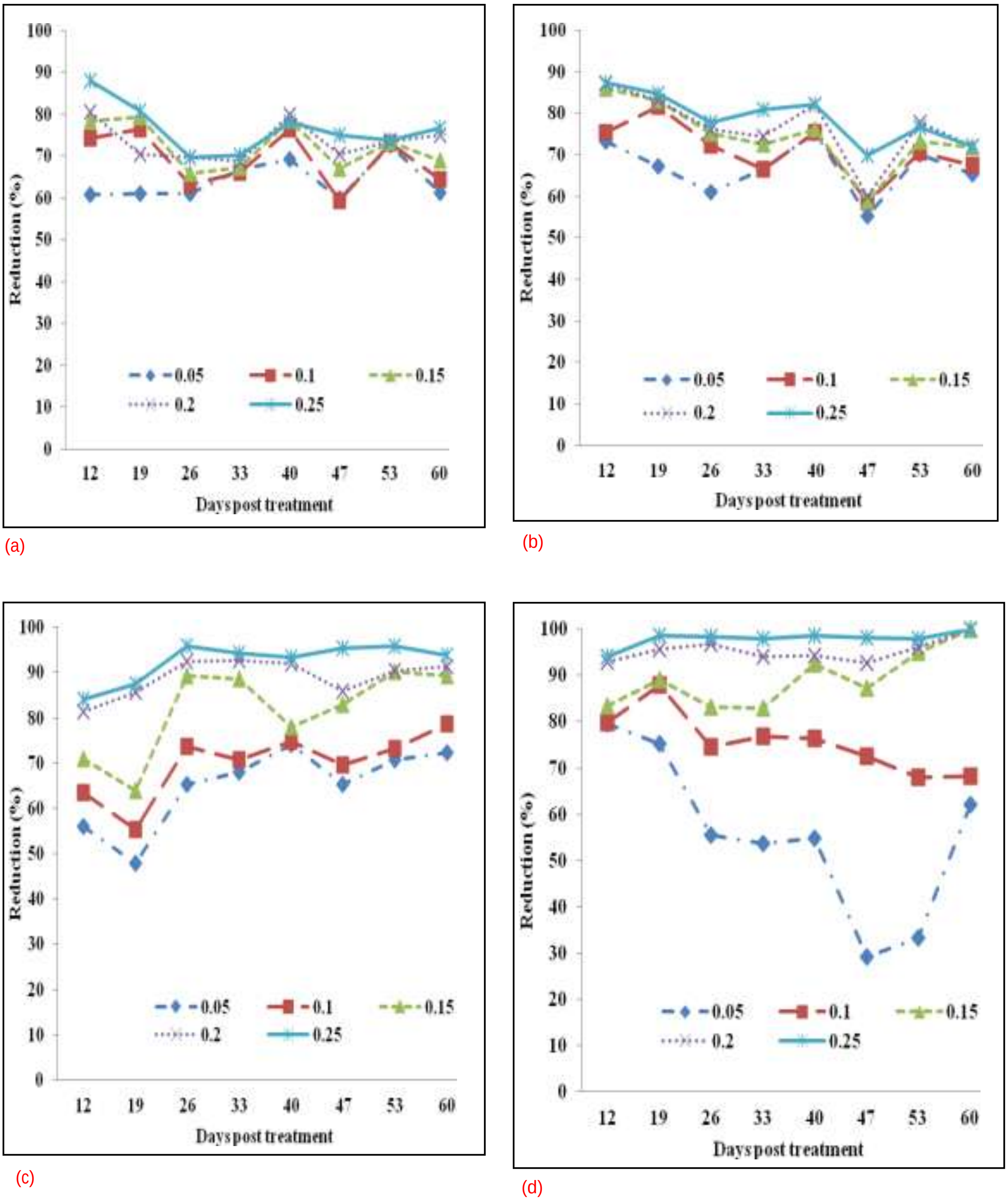

Fig 2 The reduction percentages in *T. castaneum* populations exposed to flour mixed with 0,05, 0.10, 0.15, 0.20 and 0.25 g product/g wheat flour: (a) Super Nano, (b) Screen, (c) Bentonite and (d) Diatomaceous earth through 60 days following treatment

Table 6 Three way ANOVA parameters for main effects and interactions for reduction percentages in population of *T. castaneum* exposed to flour mixed with different natural products

Source	df	*F*	*P*
Natural products	3	29.598	<0.001
Days	7	4. 236	<0.001
Mixtures	4	78.130	<0.001
Natural products * Days	21	4.054	<0.001
Natural products * Mixtures	12	8.555	<0.001
Days * Mixtures	28	0.406	0.997
Natural products * Days * Mixtures	84	0.885	0.751

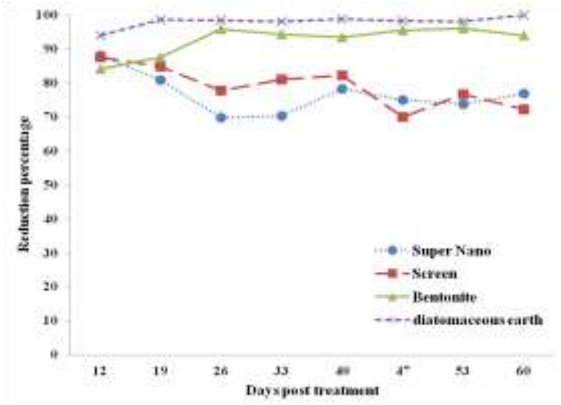

Fig. 3 The reduction percentages in population of *T. castaneum* exposed to flour mixed with 0.25 g product/g wheat flour of Super Nano, Screen, Bentonite and diatomaceous earth through 60 days following treatment

Table(7) Durations of different stages and generation of *T. castaneum* treated with different natural products.

Natural Product	Mixtures (g product/g flour)	Durations (Days) (Means + SE) Incubation period	Larval duration	Pupal duration	Generation period
Super Nano (kaolin).	0.05	15.93 ±0.89^{ab}	49.43±4.53^{a}	12.03±0.98^{b}	77.60±6.09^{a}
	0.1	17.83±1.56^{ab}	99.63±7.84^{b}	14.30±0.88^{bc}	117.43±9.23^{b}
	0.15	17.46±1.5^{ab}	109.26±8.87^{bc}	14.76±0.63^{c}	128.16±3.41^{b}
	0.20	19.73±1.65^{bc}	125.2±7.38^{c}	15.73±0.68^{c}	147.33±4.32^{c}
	0.25	24.23±3.05^{c}	130.06±5.38^{c}	19.93±0.56^{d}	160.90±5.00^{c}
	Control	13.13±1.80^{a}	96.56±7.24^{b}	7.36±0.98^{a}	87.26±6.60^{a}
	F	4.040	16.882	26.497	29.074
	P	0.022	<0.001	<0.001	<0.001
Screen (kaolin)	0.05	15.33±2.36^{ab}	78.03±4.71^{a}	7.56±0.51^{a}	101.80±10.05^{ab}
	0.1	18.66± 1.68^{abc}	87.0 6±3.44^{ab}	8.36±0.49^{a}	102.66±6.20^{ab}
	0.15	20.20±1.70^{bcd}	100.30±6.83^{bc}	10.36±0.86^{ab}	120.46±0.93^{b}
	0.20	22.20±1.90^{cd}	111.76±5.29c	13.26±1.85^{b}	145.16±4.71^{c}
	0.25	26.03±2.18^{d}	110.76±8.63^{c}	17.90±1.73^{c}	156.83±4.50^{c}
	Control	13.13±1.80a	96.56±7.24^{abc}	7.36±0.98^{a}	87.26±6.60^{a}
	F	5.672	4.455	11.709	19.519
	P	0.007	0.016	<0.001	<0.001
Bentonite	0.05	17.36±1.07^{ab}	83.63±2.93^{a}	18.83±1.90^{b}	115.16±2.66^{b}
	0.1	23.00±0.64^{bc}	95.80±5.41^{a}	20.93±0.81^{bc}	136.43±8.78c
	0.15	24.00±0.73^{c}	116.13±5.08^{b}	24.20±1.70^{cd}	158.16±3.27d
	0.20	26.90±1.93^{cd}	128.40±2.34^{b}	26.73±1.60^{d}	176.13±4.73^{e}
	0.25	30.10±3.37^{d}	143.53±1.73^{c}	31.20±1.15^{e}	184.23±4.05^{e}
	Control	13.13±1.80^{a}	96.56±7.24^{a}	7.36±0.98^{a}	87.26±6.60^{a}
	F	11.320	24.762	33.461	46.999
	P	<0.001	<0.001	<0.001	<0.001
Diatomaceous earth	0.05	16.30±1.09^{a}	87.13±4.41^{a}	10.96±1.07^{b}	118.93±2.4^{b}
	0.1	17.50±0.87^{a}	110.53±8.23^{bc}	16.70±1.40^{c}	139.66±5.80^{c}
	0.15	24.16±2.51^{b}	113.20± 5.67^{bc}	18.26±1.39^{c}	151.60±6.65c
	0.20	24.03±2.12^{b}	123.83±5.18^{c}	22.4±0.88^{d}	174.9±4.64^{d}
	0.25	30.50±1.73^{c}	128.96±4.97^{c}	26.7±1.14^{e}	186.46±6.63^{d}
	Control	13.13±1.80^{a}	96.56±7.24^{ab}	7.36±0.98^{a}	87.26±6.60^{a}
	F	12.936	6.787	37.786	41.520
	P	<0.001	0.003	<0.001	<0.001

Within each natural product, different lowercase letters indicate significant differences among mixtures.

Table (8) Number of eggs per female, hatchability percentages and adult emergence of *T. castaneum* treated with different natural products.

Natural Product	Mixtures (g product/g flour)	Numbers (Mean±SE) Number of eggs per female	Percentages (Mean±SE) Hatchability	Adult emergence
Super Nano (kaolin).	0.05	44.00±2.31^{b}	63.97±3.38^{b}	74.50±4.37^{b}
	0.1	43.33±2.03^{b}	54.90±2.45^{bc}	62.20±0.70^{c}
	0.15	36.00±0.58^{c}	51.27±3.61^{bcd}	55.30±1.50^{cd}
	0.20	32.00±1.15^{cd}	50.13±1.79^{cd}	47.47±1.27^{de}
	0.25	30.33±2.03^{d}	39.40±8.08^{d}	40.17±3.60^{e}
	Control	90.67±1.45^{a}	91.57±1.48^{a}	88.53±1.67^{a}
	F	176.376	19.260	48.690
	P	<0.001	<0.001	<0.001
Screen (kaolin)	0.05	35.33±0.88^{b}	65.63±1.16^{b}	72.10±2.19^{b}
	0.1	32.67±0.67^{bc}	56.60±2.80^{c}	67.10±2.19^{bc}
	0.15	31.67±0.88^{bc}	48.97±1.03^{d}	62.17±0.33^{cd}
	0.20	29.67±2.03^{c}	44.87±1.28^{d}	58.57±1.15^{d}
	0.25	25.33±1.76^{d}	36.77±0.73^{e}	47.83±4.37^{e}
	Control	90.67±1.45^{a}	91.57±1.48^{a}	88.53±1.67^{a}
	F	320.745	155.084	38.607
	P	<0.001	<0.001	<0.001
Bentonite	0.05	35.67±0.67^{b}	47.63±2.37^{b}	54.07±2.17^{b}
	0.1	31.00±0.58^{c}	46.63±1.93^{b}	42.87±2.05^{c}
	0.15	14.67±1.76^{d}	43.70±1.97^{b}	33.97±1.59^{d}
	0.20	12.00±1.15^{de}	41.53±0.83^{b}	27.23±1.19^{e}
	0.25	10.67±0.67^{e}	34.60±2.30^{c}	21.03±0.92^{f}
	Control	90.67±1.45^{a}	91.57±1.48^{a}	88.53±1.67^{a}
	F	711.349	117.055	217.089
	P	<0.001	<0.001	<0.001
Diatomaceous earth	0.05	32.67±1.45^{b}	50.93±5.20^{b}	45.50±1.78^{b}
	0.1	23.67±0.88^{c}	47.83±2.91^{bc}	35.40±1.66^{c}
	0.15	18.00±1.15^{d}	42.53±1.47^{c}	16.67±4.82^{d}
	0.20	10.00±1.15^{e}	40.23±0.50^{c}	00.00±0.00^{e}
	0.25	5.67±1.20^{f}	31.13±0.57^{d}	00.00±0.00^{e}
	Control	90.67±1.45^{a}	91.57±1.48^{a}	88.53±1.67^{a}
	F	640.585	66.355	212.858
	P	<0.001	<0.001	<0.001

Within each natural product, different lowercase letters indicate significant differences among mixtures.

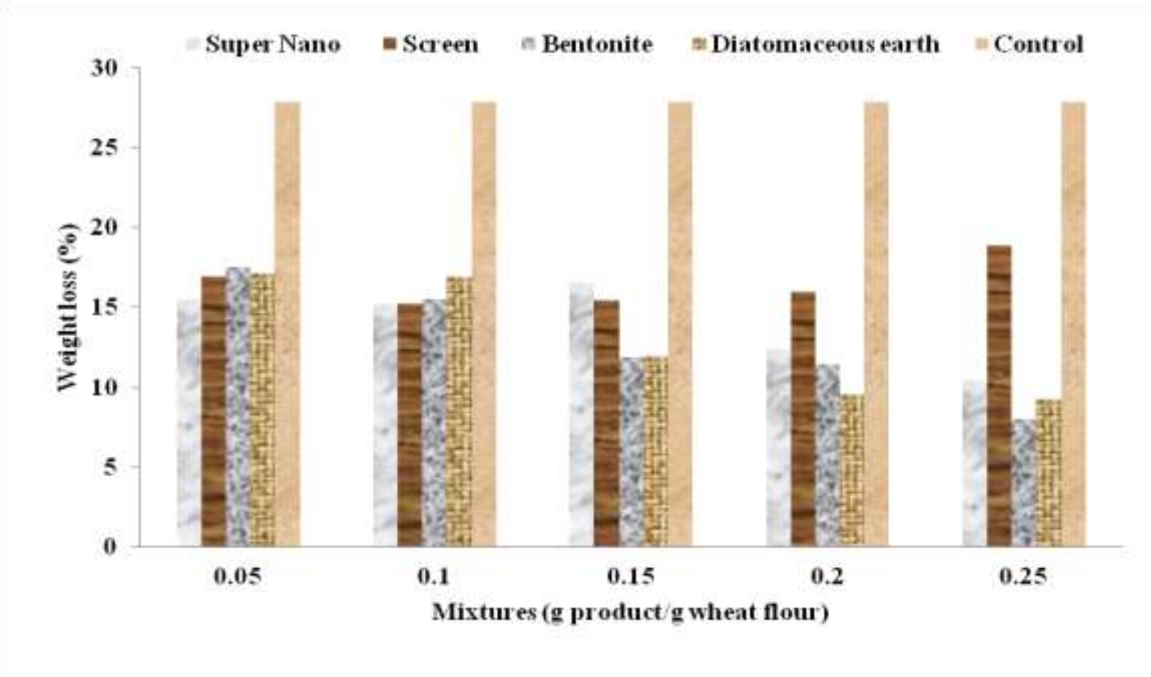

Fig. 4 The weight losses (%) of flour mixed with different natural products due to *T. castaneum* infestation.

The mixtures 0.20 and 0.25 g product /g wheat flour revealed significant increase in all tested compounds (6.6 -17.37 days). According to these results, the effect of the four tested compounds is ordered as Bentonite, followed by diatomaceous earth, Screen and Super Nano.In comparing with control group, all tested compounds induced significant increase or decrease excepting Screen that recorded insignificant increase or decrease in larval duration. The mixture 0.05 g product /g wheat flour revealed significant decrease (47.13 days) in Super Nano, while it recorded insignificant decreases in other compounds (9.43 – 18.53 days). The mixture 0.1 g product /g wheat flour recorded insignificant decreases (0.76 and 9.50 days in Bentonite and Screen, respectively) and insignificant increases (3.07 and 13.97 days in Super Nano and diatomaceous earth, respectively). The mixture 0.15 induced significant extending in larval duration (19.57

days) in Bentonite only while other compound recorded insignificant increases (3.74 – 16.64 days). The mixtures 0.20 and 0.25 g product /g wheat flour revealed insignificant increases 14.20 and 15.20 days, respectively, in

Within each natural product, different lowercase letters indicate significant differences among mixtures.

screen while they recorded significant increases in other compounds (27.27 – 46.97 days). These results indicate to the effect of the tested compounds on the larval duration is ordered as Bentonite followed by Super Nano, diatomaceous earth and Screen.

In comparison with control group, all products caused significant extending in pupal duration (3.7 – 23.8 days) excepting the mixtures, 0,05, 0.1 and 0.15 g product /g wheat flour of Screen recorded insignificant increase (0.2 – 3.0 days). The two highest mixtures (0.20, 0.25 g product /g wheat flour) in all products achieved more significant increase (5.9 – 23.8 days) in pupal duration than the others (0.05, 0.1 and 0.15 g product /g wheat flour). The results of pupal duration confirmed that the effect of the tested products is ordered as follows: Bentonite > Diatomaceous earth > Super nano > Screen.

All products gave longer generation in comparison with control excepting the 0.05 g/g of Super Nano and 0.05 and 0.1 g/g of Screen. The lowest concentration of Super Nano was the unique concentration that gave an insignificant reduction in generation time (9.7 days). However, the two lowest concentrations of Screen induced a significant increase in generation times reached to 15.4 days. The mixtures 0.20 and 0.25 g product /g wheat flour of the two products Bentonite and diatomaceous earth were the most effective on generation time. They extended the generation time more than a fold (87.6 – 99.2 days) in that of control group. Meanwhile, they recorded lower increasing in generation time in case of the Super Nano and Screen (56.9 – 73.6 days).

3.5.The reproductive performance of *T. castaneum*

Table (8) shows the efficacy of the four tested compounds (Super Nano, Screen, Bentonite, and diatomaceous earth) on the reproductive performance of *T. castaneum*. The reproductive performance included egg number per female, hatchability (%), and adult emergence (%). In all treatments, the elements of reproductive performance were significantly lower than those of control group. The values of these elements reduced gradually with increasing the mixture in all products. The range of egg number per female was 32 .67 – 44.00, 23.67 – 43.33, 14.67 – 36.00, 10.00 – 32.00 and 5.67 – 30.33 eggs, with 0.05, 0.10, 0.15, 0.20 and 0.25 g product /g wheat flour, respectively, comparing with 90.67 eggs in control group. The range of hatchability was 47.63 – 63.97%, 46.63 – 56.60%, 42.53 – 51.27%, 40.23 – 50.13% and 31.13 – 39.40% with 0.05, 0.10, 0.15, 0.20 and 0.25 g/g, respectively, comparing with 91.57% in control group. The range of adult emergence was 45.50 – 74.50%, 35.40 – 67.10%, 16.67 – 62.17%, 0.00 – 47.47% and 0.00 – 47.83% with 0.05, 0.10, 0.15, 0.20 and 0.25 g product /g wheat flour, respectively, comparing with 88.53% in control group. The results revealed that diatomaceous earth was more effective in reduction of egg number per female and hatchability (%) followed by Bentonite, Screen and then Super Nano. In adult emergence (%) the product Screen archived the lowest reduction. *Tribolium castaneum* failed to reach the adult stage in the media that treated with 0.20 and 0.25 g product /g wheat flour of diatomaceous earth.

4.DISCUSSION

Chemical insecticides have been used extensively in grain storage facilities to control stored-product insect pests. The red flour beetle is one of the serious insect pests of stored products in Egypt. The management of this insect pest mainly depends on the use of fumigants and systemic insecticides since long time **(Rajasri *et al.*, 2014)**. Before the invention of these synthetic insecticides, there were several of non-chemical methods used in grain stores and food processing facilities. Today, there is an increased interest in these methods, because of the development of resistance to synthetic insecticides, the concerns about worker safety and the demands by consumers for finished products free of insecticide residues **(Fields, 2000)**. Inert dusts are used to control insect pests of field crops, stored products and animals. They have low mammalian toxicity. They are stable on the grain and protection as long as the dusts remain dry. Unlike organophosphate grain protectants they don't leave toxic residues. Grains treated with inert adults can be cleaned prior to milling to remove most of the dust particles **(Mahdi and Khaleq 2006)**.

In recent years, consumer awareness of health hazards from residual toxicity and the growing problem of insect resistance to these conventional insecticides have led the researchers to look for alternative strategies for stored grains protection. With the ever increasing emphasis on reduction of environmental contaminations inert dusts will play a significant role in replacing synthetic conventional chemicals as grain protectants. Kaolin and bentonite are considered to be used as an alternative to the traditional insecticides in a long term. Diatomaceous earth (DE), kaolin and bentonite are very low mammalian toxicity. **Athanassiou *et al.* (2008)** observed that DEs general acted as desiccants when applied on the cuticle of insects and ultimately insect dies due to the excessive loss of water from the body. Inert dusts such as DE disrupt the absorption of lipids through the epicuticle, and insects become vulnerable to desiccation **(Gleen *et al*., 1999)**.

The results that were summarised in estimating the infestation which showed that the effect of the different tested natural components (kaolin, bentonite and DE) of five mixturs. Reduction percentage means at two products of kaolin (Super Nano and Screen) were the lowest (64.1- 76.6 %) in Super Nano and (66.8 - 81.3 %) in Screen product but the bentonite and DE product were the highest effective. Exposure to DE and bentonite formulation cause greater beetle reduction percentage than exposure to kaolin. The data presented here agree with those reviewed by **Athanassiou *et al*., 2007** who found that, the DE was effective against *T. castaneum* adult emergence in maize treated with the rate of 1 g / kg recoding reduction (98-100%). The results of the present study were agree with **Mahmoud *et al.* (2010)** who found that the adults of *Callosobruchus maculatus* (F.) and *C. chinenesis* (L.) were exposed to broad bean seeds treated with kaolin-based particle film dusts (powder) at different mixtures . The effect of kaolin powder film was clearly effective on the first month of storage period of the treated seeds resulted a 100% protection of treated seeds at high mixtures from 1.0 to 0.2 w/w for both tested insects. After three months of storage of the treated seeds only the highest two mixtures 1.0 and 0.8 w/w gave a 100% protection for both tested insects. After six months of storage of the treated seeds, kaolin powder still could protect the broad bean seeds against *C. maculatus* and *C. chinensis* attacks although the efficacy of kaolin powder decreased with aging. **Baldassari *et al*., 2004** showed that the product (DE) can prevent infestations of *T. castaneum*. In this study, DE could prevent insect infestation. It was lethal for the early larval instars insects living outside grains. This is due not only to the capacity of the exoskeleton structure to retain the produce on its surface by causing desiccation , water is lost because the dusts remove the waxy layer of the cuticle of the exoskeleton by adsorption but also the chemical composition of different physiology of each development stage **(Mewis and Ulrichs 2001)**. Stored product insects show a wide range of susceptibility to DE **(Carlson and Ball 1962)**. **Fields *et al*. (2001)** stated that the effectiveness of inert dusts may depend on the speed and amount of waxy cuticle that the dust can absorb. As some insects move on the grain storage area or on the stored grain, behavior also is a factor and **(Korunic, 1997)** showed that DE can be abrasive causing additional damage to the cuticle and while the particle film may be less abrasive, its effects on the cuticle are similar, when plant surfaces are treated with the particle film kaolin, the material acts primary as a protective barrier but once the insects become coated with the film, different physiological effects may be produced. The film could inhibit breathing through plugging of the spiracles and cause water loss through damage to the cuticle. These effects would be mitigated or r.h. increases. Different physiological effects may be produced when the insects become coated with film of dusts, thus film inhibits breathing through plugging of the spiracles **(Gleen *et al*., 1999)**. Commercial formulations of DE vary in toxicity **(Fields and Korunic 2000)** and therefore it is difficult to compare kaolinite-based particle films to all commercial DE products. However, many DE contain either amorphous or crystalline silica which can preclude use in some organic markets. The mortality percentage of adults increased gradually by increase of mixture in all products. This data agreed with **Frank and Puterka (2002)** who exposed *T. castaneum* to kaolinite – based particle film at the rate of 0.1-0.5 mg/cm^2. They found all the red flour beetles were dead after 3 days. In the present study, results show that, all components at all mixtures decreased the weight losses. The protective cuticle wax varies among insect species. Some insects have a hard cuticle while others have a softer cuticle, Therefore most natural inert dust are more effective against some species and less effective against others **(Al-Iraqi and Al -Naqib 2006)**. In Pakistan, **Chattha *et al*. (2015)** applied Straw-clay bins retain low moisture conditions for extended wheat storage duration better than conventional bulk covered store. Seed stored in bulk covered store suffered severe deterioration caused by fungi, while straw-clay bins protected seed from these losses. Wheat seed stored for 12 months in straw-clay bins maintained better seed germination percentage, test weight, protein content and ash/mineral content than seed stored in bulk covered store. There was a minimum loss of quality of wheat in straw-clay bin as compare to bulk covered store.

REFERENCES

ABBOTT, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, **18**: 265-267.

ARTHUR, F.H. (2000a). Toxicity of diatomaceous earth to red flour beetles and confused flour beetles: effects of temperature and relative humidity. *Journal of Economic Entomology* **93**: 526–532.

ARTHUR, F.H. (2000b). Impact of food source on survival of red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae) exposed to diatomaceous earth. *Journal of Economic Entomology **93***: 1347–1356.

ALEXANDER, P., KITCHENER, J. A. and BRISCOE, H.V.A. (1944). Inert dust insecticides. Part I. Mechanism of action. *Annals of Applied Biology* **31**: 143-149.

AL-IRAQI, R.A. and AL-NAQIB S.Q. (2006). Inert dusts to control adults of some stored product insects in stored Wheat. *Raf. Jour. Sci.***17**(10 26-33.

ATHANASSIOU, C.G., KAVALLIERATOS, N.G. and MELETSIS, C.M. (2007). Insecticidal effect of three diatomaceous earth formulations, applied alone or in combination against three stored product beetle species on wheat and maize. *Journal of Stored Products Research* **43**: 303–334.

ATHANASSIOU, C.G., KAVALLIERATOS, N.G., VAYIAS, B.J. and PANOUSSAKIS, E.C. (2008). Influence of grain type on the susceptibility of different *Sitophilus oryzae* (L.) populations, obtained from different rearing media, to three diatomaceous earth formulations. *Journal of Stored Products Research* **44**: 279–284.

BALDASSARI, N., BERLUTI, A., MARTIN, A. and BARONIO, P. (2004). Analysis of the sensitivity of different stages of *Rhyzopertha dominica* and *Tribolium castaneum* to diatomaceous earth. *Bulletin of Insectology* **57**: 95-102.

CARLSON, S.D. and BALL, H.J. (1962). Mode of action and insecticidal value of a diatomaceous earth as a grain protectant. *Journal of Economic Entomology* **55**: 964-970.

CHATTHA, S.H., CHE MAN HASFALINA, TEANG SHUI LEE, BENISH NAWAZ MIRANI and MUHAMMAD RAZIF MAHADI (2015). Effective and economic storage of wheat seed in straw-clay bin. *International Journal of Biosciences* **6**: 83-93.

DOWDY, A.K. (1999). Mortality of red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae) exposed to high temperature and diatomaceous earth combinations. *Journal of Stored Products Research* **35**: 175-182.

FIELDS, P., DOWDY, A. and MARCOTTE, M. (1997). Structural pest control: the use of an enhanced diatomaceous earth product combined with heat treatment for the control of insect pests in food processing facilities. Canada United States Working Group on Methyl Bromide Alternatives, http://res.agr.ca/winn/Heat-DE.htm.

FIELDS, P., KORUNIC, Z. and FLEURAT-LESSARD, F. (2001). Control of insects in postharvest: Inert dusts and mechanical means. In: Vincent C., Panneton B., Fleurat F. eds. 2001. Physical control methods in plant protection. Springer-Verlag, Berlin. http:// home. cc. umanitoba.ca /N fieldspg /field-DE-review-pdf .

FIELDS, P. (2000). Alternatives to chemical control of stored-product insects in temperate regions. *9th International Working Conference on Stored Product Protection*. 653-662.

FIELDS, P. and KORUNIC, Z. (2000). The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored- product beetles. *Journal of Stored Products Research* **36**: 1-13.

FRANK, H. A. & PUTERKA, G. J. (2002). Evaluation of kaolinite-based particle films to control *Tribolium* species (Coleopteran) Tenebrionidae. *Journal of stored products Research* **38**: 341-348.

GLEEN, D.M., PUTERKA, G.J., VANDERZWET, T., BEYERS, R.E. and FELDHAKE, C. (1999). Hydrophobic particle film: a new paradigm for suppression of arthropod pest and plant diseases. *Journal of Economic Entomology* **92**: 759-771.

GOLOB, P. (1997). Current status and future perspectives for inert dusts for control of stored product insects. *Journal of Stored Products Research* **33**, 69–79.

HENDERSON, C.F. and TILTON, E.W. (1955). Test with acaricides against the brown wheat mites. *Journal of Economic Entomology* **48**: 157 –161.

KORUNIC, Z. (1997). Rapid assessment of the insecticidal value of diatomaceous earth without conducting bioassays. *Journal of Stored Products Research* **33**: 291-229.

LOSCHIAVO, S.R. (1988). Availability of food as a factor in the effectiveness of a silica aerogel against the merchant grain beetle (Coleoptera: Cucujidae). *Journal of Economic Entomology* **81**: 1237–1240.

MAHDI, S.H.A.M. and KHALEQ U. M. (2006). Toxicity studies of some inert dusts with the cow pea beetle *Callosobruchus maculatus* (Fabricius) (Coleopteran : Brucinidae). *Journal of Biological Sciences* 6: 402-407.

MAHMOUD, A.E.M., EL-SEBAI, O.A., SHAHEN, A.A. & MARZOUK, A.A. (2010). Impact of kaolin-based particle film dusts on *Callosobruchus maculatus* (F.) and *C. chinenesis* (L.) after different storage periods of treated broad bean seeds. 10th *International Working Conference on Stored Product Protection* 638-646.

MEWIS, I. and ULRICHS, C.H. (2001). Treatment of rice with diatomaceous earth and effects on the mortality of the Red flour beetle *Tribolium castaneum* (Herbst).- *Anzeiger fürSchädlingskunde* **74**: 13-16.

RAJASRI, M., RAO, P.S., MEENA KUMARI, K.V.S. (2014). Inert dusts - better alternatives for the management of angoumois grain moth, *Sitotroga cerealella* in stored rice. *International Journal of Science and Research* **3**: 278-283.

SNEDECOR, G.W. and COCHRAN, W.G. (1967). Statistical Methods, sixth ed. Iowa State Univ. Press, Ames, Iowa, USA.

تأثير بعض المنتجات الطبيعية المختلطة بالدقيق علي حيوية وتطور ضد خنفساء الدقيق الصدئية *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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الملخص العربى

لقد هدفت هذه الدراسة الى تقييم فعالية أربعة منتجات طبيعية هى سوبر نانو وسكرين و البنتونيت و دياتومات الارضية كمواد واقية للدقيق من الاصابة بحشرة خنفساء الدقيق الصدئية *Tribolium castaneum*. تم التقييم الحيوى المعملي لتلك المنتجات بدراسة تأثير السمية المباشرة و النسبة المئوية للخفض في تعداد الحشرة والنسبة المئوية للفقد في وزن دقيق القمح. وكذلك تم تقدير بعض النواحى البيولوجية المختلفة وهى فترة حضانة البيض و مدة العمر اليرقى وفترة تطور العذراء وكذلك مدة الجيل . ولقد اوضحت النتائج ان خام البنتونيت يسبب اعلي نسبة موت للحشرات الكاملة بعد 24 ساعة من المعاملة يليه الدياتومات الارضية ثم السوبر نانو والسكرين على التوالى. تغذية الحشرات الكاملة على الدقيق المعامل بالدياتومات الارضية بمخلوط 0.25 جرام مادة واقية / جرام دقيق قمح حققت أعلى نسبة خفض فى تعداد الحشرات الناتجة فى الجيل الثانى يليها البنتونيت ثم السكرين والسوبر نانو. أدت كل المعاملات الي خفض الفقد في وزن الدقيق بصورة معنوية (7 - 18.9 %) وذلك عند مقارنتها بالكنترول (27.84%) .ادت جميع المواد المختبرة الى إطالة فترة الحضانة و مدة الطور اليرقي ومدة طور العذراء ومدة الجيل بصورة معنوية. انخفض عدد البيض الموضوع/ انثي وكذلك النسبة المئوية لخروج الحشرات الكاملة بصورة معنوية عند المعاملة بجميع التركيزات المختبرة لكل المواد المختبرة عند مقارنتها بمجموعة الكنترول. ومن النتائج المتحصل عليها يمكن استنتاج انه يعتبر كلا من البنتونيت والدياتومات الارضية أكثر المواد فاعلية ضد حشرة خنفساء الدقيق الصدئية.

الكلمات الدالة: خنفساء الدقيق ، المنتجات الطبيعية ، البنتونيت ، الدياتومات الارضية، الكاولين