Joint Action of Peppermint Oil on Diatom against *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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Abstract: A diatom-based formula (POF) of Peppermint oil (PO), 12.5% (w/w), was prepared and its toxicity was assessed against adults of *Tribolium castaneum* and *Sitophilus oryzae*. The toxicity was also compared with the toxicity of either commercial diatom (Celatom®) or PO alone using mixing with food bioassay technique. The POF formula significantly enhanced the toxicity of PO against *T. castaneum* and *S. oryzae* adults (1.33 and 8.58) and (4.31and 13.42) times after 5, 10 days of exposure, respectively. Whereas, POF was significantly more toxic to both *T. castaneum* (LC_{50} = 372.79 ppm) and *S. oryzae* (LC_{50} = 28.8ppm) compared with PO alone (LC_{50} = 495.66 and 246.85 ppm), respectively after five days of exposure. Furthermore, POF remained effective up to the end of the experiment (15 days), while the PO efficacy vanished within 5 days. The enhanced toxicity and the relative persistence of POF might be attributed to the physical and chemical properties of Celatom® and PO as well their integration. The analysis of PO by GC_MS indicated the predominance of the menthol [monoterpene] (43.43%). Also, the Scan Electron Microscopy (SEM) photographs of the *T. castaneum* and *S. oryzae* adults treated with Celatom® or POF showed adhesion of their particles on all body parts, corrosive and cracked cuticle unlike the untreated insects. These results suggested that the POF formula could provide a cost-effective, easy utilized and environmentally friendly for grain protection.

Keywards: *Mentha piperita*, diatom, rice weevil, red flour beetle, environmentally friendly insecticide

1.Introduction

There is an undeniable concern globally about the state of world's food security and its future demands. On one hand, one third of the global food production is loss during postharvest process cost the world a trillion US dollars annually **(FAO, 2011)**. On the other hand, world's population is expected to escalate up to 9.1 billion by the year of 2050. This creates great demand on the food supply that needs to be increased up to 70% **(Godfray et al., 2010)**. Consequently, both increasing agricultural productivity and reducing pre- and post-harvest crop loss are crucial measures to achieve the food security, which is one of the most current global concerns **(Obeng-Ofori, 2015)**. Among postharvest operations, storage is the most critical step particularly in the developing countries. For example, in Egypt, wheat loss during storage ranged between 20-30% **(Wally, 2015)**. A sort of pests infest grains and their products during storage amongst them the Rice Weevil, *Sitophilus oryzae* (L.), and the Red Flour Beetle, *Tribolium castaneum* are the most dominant and threat **(Champ and Dyte 1977)**. They cause deterioration of the cereal grain affecting quantity and quality. Their control principally depends on the synthetic insecticides (e.g. organophosphorus and pyrethroids) and the fumigants (e.g. methyl bromide and phosphine). Several problems are attributed to the continuous application of chemical insecticides and fumigants such as; resistance development **(Benhalima *et al.*, 2004, Kljajiæ and Periæ, 2009 and Attia *et al.*, 2017),** insecticide residues in food and negative environmental effects **(Bomzan *et al.*, 2018)**. Therefore, looking for eco-friendly alternatives to protect grains is in highly demanded. Essential Oils (EO) is a proficient alternative that showed contact and fumigant toxicity **(Abdelgaleil *et al.*, 2016)** as well repellant effects **(Abo-El-Saad *et al.*, 2011 and Guo *et al.*, 2016)** against stored product insects. Peppermint oil, *Mentha piperita* L. (PO) is one of the most popular among EOs, which is utilized in foods, pharmaceutical and cosmetic products **(McKay and Blumberg, 2006)**. Furthermore, PO showed toxicity to different insects includes stored-grain insect pests **(Mohamed and Abdelgaleil, 2008 and Mishra *et al.*, 2014)**. Also, **Lougraimzi *et al.*, (2018)** reported that, the EO and leaf powder of *Mentha pulegium* exhibited insecticidal activity against *S. oryzae* and *T. castaneum* adults. The PO properties like high volatility and low toxicity for mammals **(McKay and Blumberg, 2006)** elected PO as a promising alternative for stored grains protection. Nevertheless, its ability for oxidation and persistent odor that remains in food at high dosages causing an unpleasant taste limits its use for stored product insects control **(Moretti *et al.*, 2002)**. Another traditional alternative is the Diatomaceous Earth (DE) which is probably one of the most risk-free and efficient natural insecticides studied since the 20th centenary **(Koruniæ, 2016)**. DEs are fossils residues that consist of around 90% SiO_2 and aluminum in addition to magnesium, iron oxide and sodium lime. They have low mammalian toxicity without any chemical residues in cereal **(Baldassari and Martini, 2014)** as well they are inflammable, cheap and long persistence **(Korunic *et al.*, 1998)**. Additionally, Less or few resistance problems have been reported to DEs possibly only as behavioral resistance rather than physiological resistance

(Vayias *et al.*, 2008). These advantages besides safety for workers and cost effective made them useful for grain protecting IPM programs for controlling stored product insects **(Obeng-Ofori, 2011)** and **(Sabbour and Abd El- Aziz, 2015)**. Nevertheless, the use of DEs in stored grain protection still limited due to the mandatory high rate **(Islam *et al.*, 2010)**. Moreover, this high rate reduces the grain bulk density, which creates a greater friction between cereals affecting their test, weight and flow properties **(Korunic, 2016)** .It has been reported that, when DEs is combined with other materials their toxicity could be enhanced for instance insecticides **(Athanassiou, 2006)**, plant extracts **(Athanassiou *et al.*, 2007)** ,monoterpenoids **(Islam *et al.*, 2010)** ,essential oils **(Campolo *et al.*, 2014)**, natural insecticide spinosad **(Kavallieratos *et al.*, 2010)** and IGRs **(Arthur, 2004)**. A possible approach to overcome peppermint associated problems is delivering PO in a dust formulation using DE as a carrier which could utilize application of the EO and increase the benefits of both materials **(Ulrichs and Mewis, 2000, Islam *et al.*, 2010 and Yang *et al.*, 2010)**. This work is an approach to explore an environmentally friendly formulation for stored grains protection via enhancing the efficacy of the peppermint oil and overcome the associated problems that limits its employing in stored grain protection. The particular objective of this work is to upload PO on a commercial diatom forming a dust formulation and compare its toxicity with the toxicity of either EO or DE alone against adults of *T. castaneuim* and *S oryzae*.

2.Materials and methods

2.1.Tested Insects

The red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and the rice weevil *Sitophilus oryzae* (Coleoptera: Curculionidae) were cultured in the laboratory at 28 °C ±1, r.h. 70 ±10 and photoperiod L/D 12:12 h over than nine years at the Faculty of Agriculture, Alexandria University. *T. castaneum* was reared on a mixture of 90% whole wheat and 10% brewer's yeast (wt/wt) as described by **(Beeman *et al.*, 2009)**, while *S. oyrzae* was reared on whole wheat according to the method explained by **(Strong *et al.*, 1967)**.

2.2.Peppermint oil (PO)

A commercial product was obtained from El Gomhouria Company for Trading Chemicals and Medical Appliances, Alexandria, Egypt.

2.3.Diatom (DE)

A commercial formula of diatom, "Celatom® LCS_3", a product of Ep Mineral Europe, Verwaltungs, Luneburg, Germany. The chemical composition and physical properties of Celatom® are listed in table (1).

2.4.The peppermint-diatom formula, (POF) preparation

A formulation of peppermint (12.5%) carried on diatom was prepared by adding 0.25g peppermint oil, dissolved in 10 ml n-hexane, to 2.0 g of Celatom®. Then the mixture was homogenized by a magnetic stirrer for 2 min and kept overnight at the room temperature before application.

2.5.Oil analysis:

Peppermint oil was analyzed by Gas Chromatography- Mass Spectroscopy (Trance GC ULRA, Thermo) coupled with ISQ Single Quadruple mass spectroscopy with direct capillary column TG-5MS (30 m, 0.23 mm, 0.25 film thickness). The GC conditions were as following: initial temperature 45ºC for 2 min, then programmed to 165ºC at 3 ºC min^{-1} and held for 10 min. Then increased to 280 at 15ºC min^{-1} and held for 10 min then increased to 350 ºC up to the end. The carrier gas was Helium at a constant flow rate of 1 ml min^{-1}. The sector mass analysis was set at scan mod (30-500 aum /0.2 Sec). Spectra were obtained in EI mod with70 ev ionisation energy. The components were identified by comparison of mass spectra of each peak with those of the library.

2.6.Wheat grains:

A local wheat Variety (Gemmiza 11) was obtained from experimental extension farm, faculty of Agriculture, Alexandria University. The grains were washed, purified and sterilized. The grain humidity was determined and adjusted to 12% during the experiments.

2.7.Bioassay procedures:

A series of concentrations were tested for Celatom®, PO or POF. The desirable weight of PO corresponding to the required concentration was dissolved in acetone (1 ml) and applied to the 20 g of wheat in 0.5 litter glass jar with tight glass cap. The jars were stirred continuously for 5 min to ensure even spread of oil over the grains surface, and then they were set aside without caps for 15 min to allow the solvent evaporation completely. Twenty adults of each insect were separately placed in each jar and were covered with nylon mesh. Control treated with acetone alone, each concentration was replicated three times. The jars were incubated at the same condition of rearing. Mortality percentages were recorded after 5, 10 and 15 days of exposure. The appreciate weight of the Celatom® and POF formula were added to 20 g of sterilized wheat (in glass containers 500 ml with tight glass cap), then shaked up and overturning manually for 5 min to distribute the powder on the whole wheat mass. Then the containers were set aside with their caps for 15 min to allow the powder fine particles to sit-down. Then the bioassay procedure was completed as described previously. The co-toxicity coefficient (CTC) for POF formula were calculated according **(Sune and Johnson, 1960)** CTC = LC_{50} of PO alone / LC_{50} of PO in the POF formula.

2.8.Scanning electron microscopy

Adult insects (after 5 days exposure to Celatom® and POF as well unexposed insects) were washed by ethanol. Then, insects were coated with gold and subjected to Electron Microscopy (SEM) (JEOL, JSM-5300) at the Faculty of Science; Alexandria University

Table (1): Physical and chemical properties of Celatom®

Physical properties		
Color		off-white
G.E. Brightness		83.0
Sieve analysis (Tyler)	150 +Mesh (> 105 µm)	0.0
	325Mesh (> 44 µm)	0.7
Median particle diameter		11.0µm
pH (10% Slurry)		7.5
Free Moisture		%5.0 <
Wet bulk density		350g\cm^3
Dry bulk density		152g\cm^3
Oil Absorption (Gardner-Coleman)		170.0g/100g
Hegman		2.0
Specific gravity		2.0
Refractive index.		1.46
Chemical analysis		
Composition		percent
Silicon dioxide (SiO_2)		89.2
Aluminum oxide (Al_2O_3)		4.0
Iron(III) oxide (Fe_2O_3)		1.5
Calcium oxide (CaO)		0.5
Magnesium oxide (MgO)		0.3
Other Oxides		5.0
Loss on Ignition		4.0

2.9.Bulk density determination

The free and compact bulk densities were determined as described by **(Subramanian and Viswanathanin, 2007)**. To calculate the free bulk density, a grading cylinder (100 ml) was filled up to its top with wheat sample (untreated, treated with the LC_{50} of Celatom®, 6266 ppm or POF, 372.79 ppm) and was weighed. For measuring the compact bulk density, the cylinders were filled up to their top with the wheat samples, then moderately tapped 100 times on a wooden surface. The volume and weight were measured for each sample; the bulk density was calculated as the ratio of mass to volume. The test was repeated five times and both the free and compact bulk density was calculated as average of the five replicates.

2.10.Germination test

A sample of wheat grains, that treated with LC_{50} concentration (495.66, 6266 and 372.786 ppm) of PO, Celatom® and POF, respectively were stored for 15 days at the rearing conditions. From each treated bulk 25 seeds were collected and soaked in water for 24hr (5 replicates of each treatment). Then the swelling seeds were filtrated and placed on a moistened thin sheet of cotton (laid in a 9 cm Petri dish) and incubated at 25±1°C in a dark place. The seeds were watered as required. The numbers of seedling were counted after 4 and 7 days and the percentages of germination were calculated **(Horwitz, 1980)**.

2.11.Statistical analysis:

Statistical analyses: were performed using the IBM SPSS statistics version 2.0 software package.Before analysis the mortality percentages were transformed into arcsine-square-root and submitted to analysis of variance UNIANOVA. Means were separated by the Tukey-Kramer honestly significant differences (HDS) at the 5% level **(Sokal and Rohlf, 1995)**. The bulk density and germination data were subjected to one way ANOVA. The LC_{50}, LC_{90} and their confidence limits as well as the slopes and their variances were estimated using Ldp Line® software for probit analysis according to **(Finney, 1971)**.

3.Results

3.1.Peppermint oil constitutes:

The peppermint oil (PO) chemical compositions are listed in table (2). The menthol was the predominant monoterpene oxygenated compound (43.43%) in addition, the presence of D-menthone (19.17%), 2-isopropyl-5-methylcyclohexanone (13.36%) and Eucalyptol (11.26%). As well, γ-terpineol, caryophyllene, sabinen, orthodene and pulegone were existed. Besides, traces of trans-carane, 3-methylcyclohexanone and 3-Menthene were detected.

3.2.Bioassay results: Toxicity of peppermint oil (PO), Celatom® and the formulated oil (POF) against *Tribolum castaneum* and *Sitophilus oryzae*

Table (2): The relative percentage of the components of the peppermint essential oil analyzed with GC-MS

Components	RT (min)	Peak area%
Orthodene	5.87	2.04
-3Methylcyclohexanone	6.49	0.52
Sabinen	7.28	3.25
-3Menthene	7.80	0.43
trans-Carane	7.95	0.73
Eucalyptolss	9.45	11.26
D-menthone	11.94	19.17
-2Isopropyl-5-methylcyclohexanone	15.36	13.36
Menthol	16.07	43.43
γ-Terpineol	16.21	8.07
Pulegone	18.61	1.53
Caryophyllene	26.40	4.29

Data is presented in table (3 and 4). The mortality percentages were ≤ 10% within the first four days of exposure; therefore, the mortality recording was started on the fifth day. In addition, observing mortality daily showed insignificant changes within (6 – 9) and (11 -14) days of exposure (unpublished data). Consequently, the displayed data here was limited to the mortalities that counted after 5, 10 and 15 days of exposure.

Regarding the three tested materials, the mortality towards both insect species increased in a concentration dependent manner. Furthermore, adults of *S. oryzae* were more susceptible to all tested materials than those

Table (3): Toxicity of Celatom®, Peppermint oil (PO) and POF formula against adults of *Tribolium castaneum* after 5, 10 and 15 days of exposure

Treatment	Days	LC_{50} ppm	confidence limits	LC_{90} ppm	confidence limits	Slope±S.E	χ^2	CTC*
	5	495.66	485.1- 504.7	632.62	603.8 - 674.2	12.09 ±1.1	3.97	--
PO	10	495.66	485.1-504.7	632.62	603.8 - 674.2	12.09 ±1.1	3.97	--
	15	495.66	485.1 - 504.7	632.62	603.8 - 674.2	12.09 ±1.1	3.97	--
	5	6266	4701.8 - 9706.9	74732.47	28067.9 - $2.3E^{+2}$	1.19 ± 0.3	1.13	--
Celatom®	10	1118.90	1004 - 1241.9	2900.96	2449.0 - 3656.3	3.09 ± 0.3	2.69	--
	15	758.72	663.3 - 830.4	1738.17	1480 - 2208.8	3.50 ± 0.4	2.23	--
	5	372.79	324.0- 417.3	1293.95	1014.5- 1934.7	2.37 ± 0.3	3.56	1.33
POF	10	115.05	104.8 -125.9	259.06	224.6 - 313.8	3.63 ± 0.3	2.16	4.31
	15	83.49	74.7 - 92.4	193.74	163.3 - 250.7	3.50 ± 0.4	1.78	5.94

*Co-Toxicity Coefficient (CTC) = LC_{50} of the peppermint oil alone / LC_{50} of the peppermint oil in the POF formula (Sun and Johnson 1960)

of *T. castaneum*. The mortality of *S.oryzae* adults significantly affected by the tested material ($F^{2, 16}$ = 402.14; *P* <0.005) and the concentration ($F^{5, 40}$ = 24.40; *P* <0.005). While the effect of the exposure period was insignificant ($F^{2, 16}$ = 1.88; *P*= 0.185). In addition, there were significant interaction between [material x concentration] ($F^{10, 80}$= 81.27; *P* <0.005), [time x concentration] ($F^{10, 80}$= 7.13; *P* <0.005) and [material x time x concentration] ($F^{20, 108}$ = 308.16; *P* <0.005).

After 5 days of exposure, POF was significantly the most toxic compound among the tested materials ($F^{2, 87}$ = 23.18; *P* <0.005) against *S. oryzae* adults. At the lowest concentration 10 ppm, 34.35% mortality was recorded for adults exposed to POF, although no mortality was observed with Celatom® or PO at the same concentration. The pair wise comparison showed a significant higher mortality with POF compared with PO or Celatom®) *P* <0.005). Furthermore, the mortality percentages increased in a time dependent manner for adults exposed to POF and Celatom® while no additional mortality recorded for adults treated with the PO in up to the end of the experiment (15 days).

Concerning *T. castaneum,* both main effects and their associated interaction were significant (tested material: $F^{2, 16}$ = 153, 18; *P* <0.005; Concentration: $F^{5, 40}$ = 25.54; *P* <0.005; time $F^{2, 16}$= 11.04; *P* <0.001; [material

Table (4): Toxicity of Celatom®, Peppermint oil (PO) and POF formula against adults of *Sitophilus oryzae* after 5, 10 and 15 days of exposure

Treatment	Days	LC_{50} ppm	confidence limits	LC_{90} ppm	confidence limits	Slope±S.E	χ^2	CTC*
				Tribolium castaneum				
	5	246.85	232.1-260.1	375.23	351.39 - 408.9	7.04±0.66	0.59	--
PO	10	246.85	232.1-260.1	375.23	351.39 - 408.9	7.04±0.66	0.59	--
	15	246.85	232.1-260.1	375.23	351.39 - 408.9	7.04±0.66	0.59	--
	5	455.68	295.6 - 571.7	1877.31	1437 -3212.4	2.08 ± 0.4	0.12	--
Celatom®	10	282.05	251.8 - 311.7	692.99	603.82 - 83053	3.28 ± 0.3	1.31	--
	15	282.05	251.8 -311.7	692.99	603.82 - 83053	3.28 ± 0.3	1.31	--
	5	28.78	11.9 - 43.0	210.63	150.93 - 419.3	1.48 ± 0.3	3.29	8.58
POF	10	18.39	4.5 - 30.3	71.81	54.69 - 90.8	2.16 ± 0.5	0.05	13.42
	15	18.39	4.5 - 30.3	71.81	54.69 - 90.8	2.16 ± 0.5	0.05	13.42

*Co-Toxicity Coefficient (CTC) = LC_{50} of the peppermint oil alone / LC_{50} of the peppermint oil in the POF formula (Sun and Johnson 1960) x concentration]: $F^{10, 80}$= 23.32; P <0.005; and[Material x time x concentration]: $F^{20, 108}$= 289.60; P <0.005. Conversely, the effect of interaction between [time x concentration] was insignificant ($F^{10, 80}$ = 0.75; P= 0.68). After 5 days of exposure, the efficacy of the three tested materials were significantly different ($F^{35, 72}$= 5.65; P <0.005). The lowest concentration of POF (150 ppm) showed 20.05% mortality whilst no mortality was obtained with the same concentration of Celatom® and PO. The pair wise comparison showed significant differences in mortality between all pairs (P <0.005). After 10 days of exposure, the mortality percentages increased as the exposure period increased and this pattern continued up to the end of the experiment (15 days) except with the PO treatment.

Taking into account the LC_{50} values, after five days of exposure POF was more toxic to both *T. castaneum* (372.79 ppm) and *S. oryzae* (28.78ppm) compared with PO alone (495.66 and 246.85 ppm), respectively, with no overlap in the 95% fiducial limits. The toxicity of PO against *T. castaneum* adults vanished after 5 days of exposure while the LC_{50} of POF significantly decreased to 83.49 ppm after 15 days of exposure. Likewise, the toxicity of POF against *S.oryzae* significantly decreased after two weeks of exposure to 18.39 ppm while no changes of PO toxicity was recorded after 5 days of treatment.

3.3.Scanning electron microscopy:

The Scan Electron Microscopy (SEM) photographs of the *S. oryzae* and *T. castaneum* adults treated with celatom® or POF compared with the untreated insects are illustrated in Figures (1) and (2). The SEM micrograph of a whole body ventral surface view of untreated *S. oryzae* adults shows clear waxy surface of the cuticle and opened genital opening (Fig1-a), while the same view of the insects treated with celatom® demonstrates diatom particles on all body surface, leg joints and the genital opening (Fig 1-b). Likewise, the view of the insects treated with POF shows particles of the formula completely adhesive on all body parts and totally closed genital opening. In addition, the SEM micrograph view of the details of the dorsal surface of the untreated *S. Oryzae* adults shows plain waxy cuticle of the elytrum tegument has pores with entire small and large sensilla (Fig1-d). Although, the same view of the treated insects with celatom® shows diatom particles stick to the sensillas or clogged the cuticle pores. Moreover, the sensilla within the external surface of the elytra are partly absent, injured, or hidden by the diatom particles (Fig1-e). Similarly, the alike view for *S. Oryzae* adults treated with POF shows particles of the formula totally covering the elytra pores, the majority sensella are absent and cracked cuticle parts (Fig1-f).
Parallel, The SEM micrographs of a dorsal pronotum view of *T. castaneum* adults and the same view focus of untreated insect show obvious waxy surface, and intact cuticle have sensilla in sockets (Fig1-a), whereas, the same views of the *T. castaneum* adults treated with celatom® show diatom particles clustering under and around the sensilla causing abrasion of the cuticle **(**Fig1 **-c)**. Furthermore, the exact views of the *T. castaneum* adults treated with POF display waxy layer erosion, missing sensilla, irregularly many gaps in socket and dissipation in epicuticle of the elytra.

3.4.Bulk density:

The free and compact bulk density data is presented in table (5). No significant variances were observed between both free and compact bulk density of the wheat treated with Celatom®, POF or the untreated wheat ($F^{2,4}$= 15.55; P= 0.013 and $F^{2,4}$= 0.57; P= 0.61, respectively). The untreated wheat free and compact bulk density values were 700 .33 and 753.25 Kg m -3 followed by POF formula 694.67 and 755.17Kg m -3, respectively.

3.5.Germination:

The germination percentages of wheat treated with PO, Celatom® or POF after 15 days storage period data are shown in table (6). In general, POF exhibited insignificant deference of seed germination percentages (88.33 and 91.66%) compared with that of the control (80.00 and 93.33%) after 4 and 7 days germination period, respectively. On the other hand, Celatom® and PO treatments significantly decreased germination percentages after 4 and 7 days of germination ($F^{3,6}$= 18.32; P= 0.002 and $F^{3,6}$= 28.63; P= 0.0006, respectively).

4.Discussion

Evidently, results demonstrated that, the prepared peppermint oil formula (POF) significantly enhanced the toxicity of peppermint oil (PO) against both *Sitophlus oryzae* and *Tribolium Castaneum* adults.

Table (5): Effect of celatom® and the diatom based formula of peppermint oil (POF) treatments on the bulk density of wheat grains

Treatment	Free bulk density kg m^{-3} ± SD	Compact bulk density kg m^{-3} ± SD
Control	33. 700 (±6.81)	753.25(± 6.49)
Formulated peppermint oil	694.67(± 14.05)	755.17(± 14.77)
Celatom®	675.22(± 54.80)	746.00(± 5.00)
$LSD_{(0.05)}$	30.16	19.48
$F^{2,6}$	2.28	
P	0.183	

Although several studied suggested that, *Mentha* species oil is toxic to *S. oryzae* and *T. castaneum* **(Mohamed and Abdelgaleil, 2008, Mishra *et al*., 2014 and Lougraimzi *et al*., 2018)**; up to this study there are no published studies investigated the combined effect of PO and diatom. However, previous studies showed similar results with other essential oils (EO); **Islam *et al*., (2010)** reported synergistic effects of admixing eugenol or cinnamaldehyde with some commercial diatom products against *S. oryzae*. Similarly, **Ulrichs and Mewis, (2000)** reported additive effects of mixing diatom and neem against *S. oryzae* and *T. castaneum*. Another study suggested that, the combination of garlic oil and diatom was more toxic to both S. *oryzae* and *T. castaneum* than either material alone and considerably de-

Table (6): Effects of peppermint, celatom® and the diatom based formula of peppermint oil treatment on grain germination

	Germination % ± SD	
Treatment	**After 4days**	**After 7days**
Control	80(± 0.0)	93.33 (± 2.87)
POF	88.33(±5.77)	91.66(± 2.89)
Celatom®	68.67(± 3.75)	72.5(± 4.33)
Peppermint oil	74.17(±0.95)	87.5(± 2.22)
LSD $_{(0.05)}$	7.16	6.85

creased the concentration of garlic oil mandatory for efficient treatment **(Yang *et al*., 2010)**. Conversely, **Islam *et al*., (2010)** reported that, mixing of two commercial DE formulations with eugenol or cinnamaldehyde showed antagonistic effects to *Callosobruchus maculates* adults as well as the combination of one of the tested commercial diatoms with cinnamaldehyde resulted antagonistic effects against *S. oryzae*. Also, **Campolo *et al*., (2014)** found that, the combination of sweet orange oil and DE showed an antagonistic effect on mortality of *R. Dominica.*

Moreover, The POF formula remained effective for extended periods, while the PO efficacy did not change after 5 days. This finding is in agreement with the prior suggestion of the mandatory multiple applications of oils for successfully grain protection **(Isman *et al*., 2011)**. In addition, **Ziaee *et al*., (2014)** reported that, the toxicity of *Carum copticum* EO against *S. granarius* and *T. confusum* lessened in the presence of wheat grains. Consequently, we can assume that POF formula could overcome the low persistence of the PO which limits its utilization in stored grain protection. Apparently, the relatively longer persistence of POF might be attributed to the absorption of PO on Celatom® particles and pores as a consequence the oil sustained and released gradually. Besides, the small size of Celatom® particles might be improve the persistence of PO as **Ziaee *et al*., (2014)** stated that, combining EO and DE with particle size less than 37 µm enhanced the toxicity to *T. Confusum*. On the other hand, the POF toxicity might be originated from the predominance of menthol in PO component **(Gershenzon *et al*., 2000)**. Practically, the same as **Lee *et al*., (2002)** reported that, menthone was the most toxic fumigant constituent against the adults of *T. castaneum*. In the current study, the PO analysis confirmed the existence of terpens (menthol and menthone) that had been reported to possess biological effective on stored grain insects **(Regnault-Roger *et al*., 2012 and Saad *et al*., 2019)** In addition the toxicity of POF formula may also attribute to the physical and chemical properties of DE, such as high SiO_2 content, oil sorption capacity, and medium particle diameter **(Ebeling, 1971 and Korunic 1997).** , Furthermore, The Scan Electron Microscopy (SEM) photographs showed strong adhesion of POF on insect cuticle and severe damage of all body parts. These findings are consistent with results obtained by **Ebeling, (1971) and Malia *et al*., (2016),** who said that, the particles of DEs injured the insect sensilla and close up the sensilla pores in the epicuticular layer of the insect cuticle, which may overlap with insect behaviour. Also, DE corrupts the sensory organs of the olfaction and gustation, water balance, and gas exchange. Besides, *T. castaneum* and *S.oryzae* morphology have an important role in the efficacy of DEs rough and hairy insect body surface collect more DE particles and the thin waxy layer is more sensitive than a thicker layer **(Baldassari *et al*., 2004 and Korunic, 2013)**. Adults of *S. oryzae* were more susceptible to the three tested materials than adults of *T. castaneum*. Earlier studies classified *Tribolium* species among other stored-grain insect species as the most tolerant to DE **(Arthur, 2004).**In addition, **Lougraimzi *et al*., (2018)**reported that, the *Mentha pulegium* oil was more toxic to adults of *S.oryzae* compared with *T. castaneum* adults. Consequently, the enhanced toxicity of the POF formula could be due to the faster penetration of PO thought the damaged parts of the cuticle. Moreover, the enhanced toxicity of POF could be attributed to the insects' behaviour since **zaiee *et al*., (2014)** suggested that, the enhanced toxicity of a combination of *C. copticum* essential oil and diatomaceous earth to S. *granarius and T. confusum* is attributable to the increase of the insects' locomotor activity. SEM photographs suggested strong adhesion of the POF as a result of PO absorbance on DE particles and pores compared to the adhesion of DE alone that may be accredited to the POF toxicity enhancement. Alike, the coherence of POF to the wheat grains increased which suggested to be directly correlated with DE toxicity **(Korunic, 1997 and Abdelaziz, and Sherief, 2010)**.

On the other hand, the relatively high toxicity and persistence of POF could be a consequence of the PO conservation provided by Celatom® particles that distributed the oil through the damaged body parts of the insects into the action site faster and effectively compared with PO alone **(Sahaf, *et al*., 2007)**. Moreover, the absorption of PO on Celatom® particles and pores might be decrease its oxidation rate, seeing that the oxidative enzymes are involved in the detoxification process of the EO **(Rossi *et al*., 2012)**.

Previous reports suggested that, the addition of DE to grains negatively affects their bulk density (test weight and flow properties) **(Korunic, 2016)** the most effective DE dusts caused the greatest bulk density reduction **(Subramanyam and Roesli, 2000)**. In view of the fact that, the grain bulk density and germination percentage are important grain quality parameters; fortunately, the POF had no significant effects on both parameters. However, further studies may be required to evaluate these parameters at different temperature degrees, humidity percentages and commodity type, since these conditions had been reported to affect bulk density and grain vitality **(Korunic *et al*., 1998)** and **(Subramanian and Viswanthan, 2007)**.

Accordingly, The POF formulation could be a promising alternative of chemical insecticides. Howev-

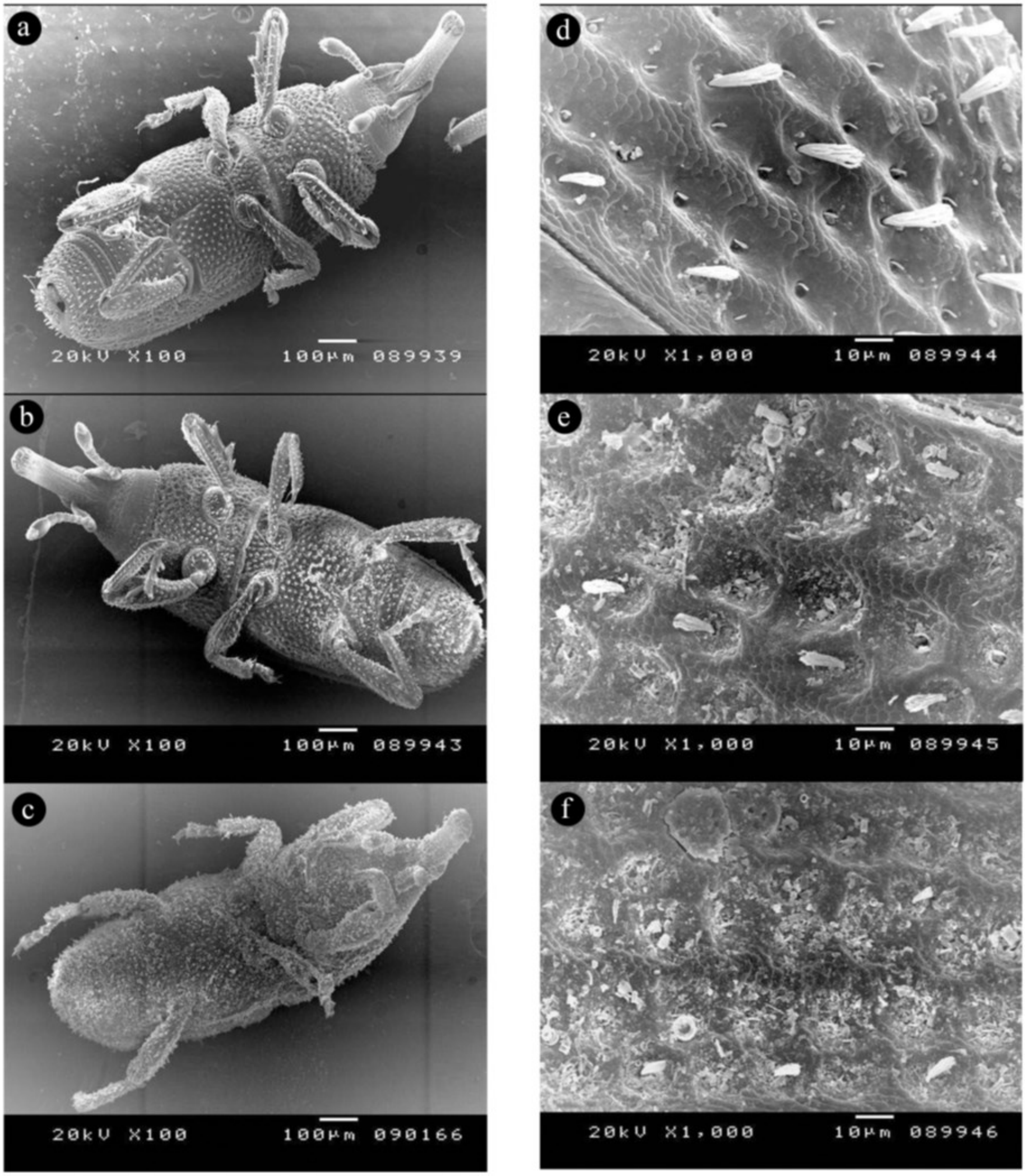

Fig. (1): Left: Scanning electron micrographs of the whole body ventral surface of *Sitophilus oryzae* adults (100x) (a): Untreated insect shows the clear waxy surface of the cuticle and opened genital opening, (b): Treated with celatom® shows diatom particles on all body surface, leg joints and the genital opening (c): Treated with POF shows particles of the formula completely adhesive on all body parts and totally closed genital opening.
Right: Scanning electron micrographs View of the details of the dorsal surface of *Sitophilus oryzae* adults (1000x) (d): untreated insect shows plain waxy cuticle of the elytrum tegument has pores with entire small and large sensilla, (e): Treated with celatom® shows diatom stick on the sensillas or clogged the cuticle pores. The sensilla within the external surface of the elytra are partly absent, injured, or hidden by the material and (f): Treated with POF shows particles of the formula totally covering the elytra pores, most sensella are absent and cracked cuticle parts.

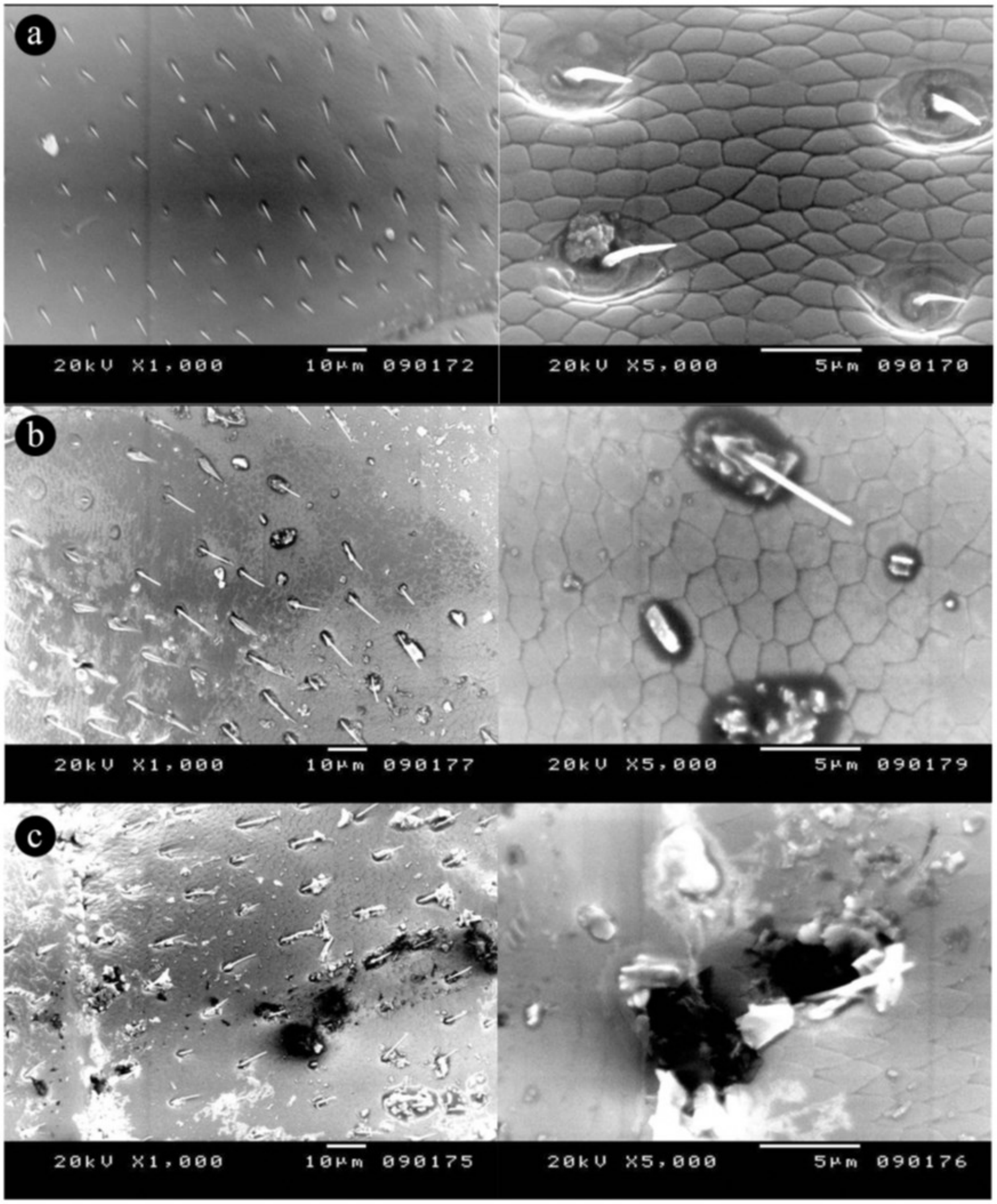

Fig. (2): Scanning electron micrographs of a dorsal pronotum view of *Tribolium castaneum* adults (1000x, Left) and the same view focus (5000x, Right) (a): Untreated insect shows, (b) treated with celatom® shows celatom® particles clustering under and around the sensilla causing abrasion of the cuticle and (c): Treated with POF shows Waxy layer erosion, missing sensilla, irregularly many gaps in socket and dissipation in epicuticle of the elytra.

er, further, studies are required to evaluate this formula at different conditions and the field-level as well to investigate its mode of action and persistence.

Conclusion

This study, throw the light on the benefits of the essential oils and diatoms combination since the developed peppermint oil formula (POF) significantly enhanced the toxicity and persistence of the oil compared with either the peppermint oil or Celatom® alone without adverse effects on the grain quality. The POF formula could serve as a cost effective, easy utilized and environmentally friendly insecticide.

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الفعل المشترك لزيت النعناع المحمل على الدياتوم ضد الحشرات الكاملة من خنفساء الدقيق الصدائية و سوسة الارز

تر انديل و هبة و منال عطية

قسم الإختبارات الحيوية ، المعمل المركزى للمبيدات ، مركز البحوث الزراعية ، الأسكندرية ، مصر

الملخص العربى

تم تحضيرتجهيزة من زيت النعناع (POF) بتحميله على مستحضر تجاري من الدياتوم (12.5٪ وزن / وزن) ، كما تم تقييم سميتها ضد الحشرات الكاملة من خنفساء الدقيق الصدائيه و سوسة الارز. أيضًا تمت مقارنة سمية تجهيزة POF مع سمية كل من الدياتوم اوزيت النعناع كل على حدى بإستخدام طريقة الخلط مع البيئة الغذائية. حيث رفعت تجهيزة POF سمية زيت النعناع للحشرات الكاملة من خنفساء الدقيق الصدائيه بمقدار (1.33 و 8.58) ضعف و سوسة الارز بمقدار (4.31 و 13.42) ضعف بعد التعرض لمدة 5 ، 10 أيام على التوالي. كما اظهر تعرض الحشرات الكاملة من سوسة الارز لتجهيزة POF او الدياتوم او زيت النعناع بتركيز 300 جزء في المليون لمدة 5 أيام نسب موت 100٪ و 36.80٪ و 71.23٪ على التوالي. بينما وصلت نسبة الموت الى100% من حشرات خنفساء الدقيق الصدائيه نتيجة التعرض لمدة 5 أيام لتركيزات 500 و 1000 جزء في المليون من تجهيزة POF او زيت النعناع على التوالي. وبمقارنة قيم LC_{50} كانت تجهيزة POF أكثر سمية لكل من خنفساء الدقيق الصدائيه (LC_{50} =372.79) و سوسة الارز (LC_{50}=28.8) مقارنةً بـزيت النعناع بمفرده (LC_{50}= 495.66) و (LC_{50} =246.85) جزء في المليون على التوالي بعد خمسة أيام من التعرض. وقد ظلت تجهيزة POF فعالة حتى نهاية التجربة (15 يومًا) ، بينما فقد زيت النعناع سميته خلال 5 أيام. وقد يعزى الارتفاع النسبى لكل من سمية وثبات تجهيزة POF إلى الخواص الفيزيائية والكيميائية للدياتوم و زيت النعناع وكذلك فعلهما المشترك. واظهر تحليل زيت النعناع بواسطة جهاز الكتلة الطيفيه GC_MS أن المنثول (مونوتربين) هو المكون الرئيسي بنسبة 43.43٪ من مكونات الزيت. كما أظهرت صور الميكروسكوب الالكترونى للحشرات المعاملة بكل من تجهيزة POF او الدياتوم التصاق جزيئات تلك المواد على جميع أجزاء الجسم حيث بينت تأكل وتشقق الكيوتيكل مقارنة بالحشرات غير المعاملة ، وتشير النتائج الى امكانية استخدام تجهيزة POF كبديل آمن و اقتصادي لحماية الحبوب المخزونة كما إنها سهلة الاستخدام وصديقة للبيئة.