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Toxicity of certain essential oils loaded on silica nanoparticles against *Tribolium castaneum* (Coleoptera: Tenebrionidae) adults

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Abstract:

Nano silica was chemically prepared from rice husk and characterized using X-Ray Diffractometer and Electron Dispersive Analysis instruments. The data indicated an amorphous structure with 96.9% of silica. In addition, the synthesized nanoparticles were coated with essential oils (EOs); either clove or peppermint (20%) to form solid lipid nanoparticles (SLNs/EO). Scanning Electron Microscopy (SEM) investigation indicated that, SLNs/EO was almost spherical.

Furthermore, the Fourier Transform Infrared Spectroscopy (FTIR) analysis of these formulations showed asymmetric vibration for Si-O, Si-OH as well as bands of alkyl, hydroxyl and carbonyl groups of EOs. Subsequently, toxicity of EOs and their SLNs/EO formulations were assessed against adults of red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). The mortality was recorded after 5, 7, 10 and 14 days of exposure. The data showed that, SLNs/clove was more toxic than clove oil alone (4.86-folds) after 7 days. On the other hand, no significant differences in the total protein, carbohydrate contents and percentages of germination were observed between treatments and control group. These findings suggest that, SLNs/EO could serve as alternative potent insecticides for controlling stored product insects. However, further researches are required to improve SLNs/EO efficacy and investigate their environmental impact.

Introduction

Post-harvest grain crops are exposed to many insects that decrease their quantity and quality. Despite the fact that, there is no accessible accurate estimation of the amount of grain loss during storage in Egypt, it is believable to range between (10 -20%) (FAO, 2015). Among the stored product insects, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a cosmopolitan and a serious pest of cereal grains and their products. Adult beetles and larvae feed on stored food stuff viz. dry fruits, pulses, bran, coat, germ, grain dust and prepared cereal foods (Khattak and Khatoon, 1999 and Dars *et al*., 2001).

Regarding the insecticide resistance, pesticide residues in food, and health/environment concerns it is obvious that, chemical control is not the appropriate strategy for controlling stored grain pest populations (Debnath *et al*., 2011). As an alternative approached Diatomaceous Earths (DEs) which composed mainly of amorphous silica and derived from fossilized phytoplankton are used against stored product insects (Subramanyam and Roesli, 2000 and Mewis and Ulrichs, 2001). DEs could be more effective against insects if they possessed high silica content, uniform size distribution and number of physical properties which could achieved by alter their size approach to nanoscale (Debnath *et al*., 2011).

Nanotechnology has been offered a powerful tool in modern agricultural practices throughout the past few years (Scott and Chen, 2013). Despite, nanoformulations are widely engaged in pharmaceutical and personal care industry, using nanomaterials in agriculture is still at a rudimentary stage (Anton and Vandamme, 2011 and Ptorchilin, 2006). Lately, nanoparticles have received great attention for controlling pathogens in agriculture (Guan *et al*., 2008; Kim *et al*., 2009 and Elek, 2010). Another alternative is the employing of essential oils (EOs) as potential toxic agents against stored product insects. They showed toxic, repellent and antifeedent effects against stored product insects (Regnault-Roger, 1997; Isman, 2006 and Regnault-Roger *et al*., 2012). Despite these promising properties, problems related to EO volatility, poor water solubility and aptitude for oxidation have to be resolved before application (Moretti *et al*., 2002).

Fortunately, nanoformulation of EOs could conquer these problems protecting them against degradation and evaporation achieving a controlled release of these products and facilitating their handling (Martín *et al*., 2014). Hence, nanoparticles (NPs) represent a new generation of promising technologies that could provide a cost-effective solution for the most challenges and could help to produce new pesticides, insecticides and pest repellents (Owolade *et al*., 2008 and Athanassiou, 2018). Nanopesticides are defined as any formulation that intentionally includes elements in nm range and/or claims novel properties associated with small size ranges (Kah *et al*., 2013). Solid Lipid Nanoparticles (SLNs) are typically spherical with an average diameter (10–100 nm). Since, they possess a solid lipid core matrix that can solubilize lipophilic molecules, while the lipid layers on solid particles is stabilized by surfactants. SLNs were demonstrated as modern approaches in drug delivery and pest control (Scheffel *et al*., 1972). In addition, nanomaterials hold promising properties for application in plant protection and nutrition due to their size-dependent qualities, high surface-to-volume ratio and unique optical properties (Puoci *et al*., 2008). For instance, Wan and Nain (2005) demonstrated that, mixtures of two NPs with insecticides were effective against mites, *Epitrimerus pyri* (Nalepa) (Acari: Eriophyidae). Also, Yng *et al.* (2009) stated that, nanoparticles loaded with garlic essential oil were effective against *T. castaneum*. As well, Stadler *et al.* (2010) showed that, nanoalumina could be successfully used to control stored grain pests and Khoobdel *et al.* (2017) suggested that formulated nano encapsulated essential oils from *Rosmarinus officinalis* were significantly more toxic against *T. castaneum* than the non-formulated oil.

However, few studies had been carried out to investigate the toxic effects of NPs against stored product insects.

The aim of this work is to prepare practical formulas of SLNs/EOs to control *T. castaneum* in comparison with either unaccompanied essential oils or silica salts.

Materials and methods

1. Essential oils:

Peppermint oil was obtained from Elgmhoria Company for Drug and Chemicals, while clove oil was supplied by Al-Kapten Company for Medical Products, Egypt.

2. Rearing insects:

A laboratory-susceptible strain of *T. castaneum* has been continuously reared in the laboratory for more than eight years at the Faculty of Agriculture, Alexandria University. The strain was maintained as described by Beeman *et al.* (2017) on whole wheat flour contains 5% (w/w) brewer's yeast at constant condition (28 ˚C±1, relative humidity 70±5 and photoperiod L/D 12:12 hr).

3. Nanosilica extraction:

Rice husk was burnt to white ash and the remaining ash was boiled in 2.5M NaOH solution for 3 hr. Then, a solution of 5M H_2SO_4 was added to produce highly purified silica. Subsequently, the continuous refluxing process with 3M HCl was proceeded for 6 hr to obtain nanosilica (SiO_2NPs) powder. Finally, the precipitated NPs were washed and dried at 50 ˚C for 24 hr (Awizar *et al*., 2013).

4. Solid lipid nanoparticles preparation:

SLNs/EO formulations were prepared using ultrasonic–solvent emulsification technique at 45-50 ˚C. Twenty percentage (w/w) of EOs were mixed with nanosilicate in diethyl ether (analytical grade) and sonicated for 1 hr. Then, 0.5 to 1% of emulsifier agent was dispersed into the mixture with continuous mixing. Finally, the sample was evaporated to dryness and stored in dissector at 4 ˚C until used.

5. Solid lipid nanoparticles characterization:

The samples of silica crystals and NPs were subjected to X-Ray Diffractometer (XRD) (APD 2000 PRO, GNR Co., Bonn, Germany), wavelength 1.54 A and scanning time 0.52 per sec, while, Electron Dispersive Analysis (EDA) was performed by X-ray Oxford (model 6647, England), at 5.9 Kev. On the other hand, the prepared formulations were scanned using Scanning Electron Microscopy (SEM) (JEOL, JSM-5300) at the Faculty of Science, Alexandria University. Regarding the active groups of the products, the formulated SLNs/EO was examined by Fourier Transform Infrared Spectroscopy (FTIR) (Tensor 27 Bruker) in comparison with silicate salt absorbance. The samples were ground and mixed with KBr to make pellets. FTIR spectra were obtained by the transmission mode (400 – 4000 cm^{-1}).

6. Susceptibly test:

In 500 ml glass Jars with tight glass cap, 50 g of sterilized wheat grains (Seeds 12) was well mixed with the appreciate weight of each silica salt, NPs and the SLNs/EO formulations by shaking and overturning for 1 min to give the desirable concentration as mg/kg. A series of 6-8 concentrations were tested for each material. The treated jars were left to set for 10 min, and adults of *T. castaneum* (25 beetles) were introduced to each jar and incubated at 28 ˚C±1, RH 70±5 and L/D 12:12 hr. Each concentration was replicated three times and mortality was recorded after 5, 7, 10 and 14 days of treatment. The LC_{50} values and the regression equations were calculated according to Finney (1971) using LdP Line ® software.

The same procedure mentioned above was followed except that, the appreciate weight of the tested EO was dissolved in 1ml acetone, then applied to 50 g of sterilized whole wheat grains to give the desirable concentration and mixed well as previously mentioned. Afterward, the cap was opened to allow acetone evaporate. After 15 min the procedure was completed as stated previously.

7. Grain quality:

7.1. Humidity determination:

A bulk of 100 g wheat grains treated with LC_{50} either NPs, SLNs/EO formulations or EOs as well as silica salt were stored for two weeks. Samples (2-3 g) from each treatment were weighed, grounded and heated at 130 ˚C for 1 hr. After that they were kept at room temperature to cool, and weighed again. The moisture content was calculated as percentages (ISTA, 1985).

7.2. Water absorption:

The effect of SLNs/EO formulations at LC_{50} level on the water absorption capacity of the treated grains was determined at the initial time of storage and after 2 weeks (Liu *et al*., 2006). Twenty five g of the treated grains (three replicates each) were immersed in 100 ml of water. The water swelling capacity was expressed as the difference in the weight of seeds before and after the chosen periods post submergence in water.

7.3 .Grain germination:

Sterilized whole wheat grains (50 g) were treated with LC_{50} concentration of each tested material as previously mentioned. The treated grains were stored for 14 days at the same rearing conditions. After the storage period, a number of 100 grain seeds were randomly collected treatment and divided by four. Afterward, each 25 seeds were placed on a moistened slight sheet of cotton in a Petri dish and incubated at 25±1 ˚C in a dark place. The seeds were sprayed if required with water to keep the moisture. The numbers of germinated seeds were counted after 4 and 7 days and the percentages of germination were calculated (Horwitz, 1980).

7.4. Total protein:

The treated grains and untreated (0.1 g) were extracted by 10 ml of borate buffer (28.63 boric acid+ 29.8 g KCl + 3.5 g NaOH in one litter of distilled water), kept overnight, then centrifuged at 3000 rpm for 10 min, filtered and completed to 10 ml. Protein concentration was determined according to Lowry *et al.* (1951) and expressed as mg/g dry weight.

7.5. Total carbohydrates:

The powder (0.1 g) of the treated and untreated grains was mixed with 4% of NaOH and boiled in water bath for 2 hr. After cooling, the samples were centrifuged at 3000 rpm for 10 min. An aliquot (0.5 ml) was mixed with the same volume of phenol reagent 5% and 2.5 ml of concentrated H_2SO_4 for 30 min at room temperature. The optical density was measured at wavelength 490 nm against blank on Spectronic 21D (Milton Roy Co. USA). Glucose was used as a standard to calculate the extension coefficient and expressed as % of total weight (Dubois *et al*., 1965).

7.6. Lipid peroxidation:

Lipid peroxidation was estimated as malondialdehyde (MDA) content following the method of De Vos *et al.* (1991). Half g of wheat grains from each treatment was homogenized with 5 ml of 5% (w/v) trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 10 min. Two ml of the supernatant were mixed with 3 ml of thiobarbituric acid (TBA) (0.65% w/v TBA in 100 mM HCl). After cooling, the sample was centrifuged as described before and the absorbance was recorded at 535 nm. MDA was quantified using an extinction coefficient of 156 $mM^{-1}$and its concentration was expressed as mmol/g dry mass.

8. Statistical analysis:

The LC_{50} values and the regression equations were calculated according to Finney (1971) using LdPLine®software. All data were presented as means ±SE and subjected to analysis of variance (ANOVA). The statistical analysis was performed using COSTAT , Costat User Manual, version 3. Cohort Tucson, Arizona, USA (1985).

Results and Discussion

1. Characterization of solid lipid nanoparticles:

The SEM images (Figure 1a) exhibit characteristic sharp edges for SiO_2NPs or agglomerates composed by NPs. Figure 1 (b and c) shows EOs layers loaded on NPs.

Additionally, XRD patterns of the formulated SiO_2NPs and SiO_2 crystals are plotted in Figure (2) showing board peaks in the range of 15-35 with Laser beam 2θ which indicate an amorphous structure. On the other hand, EDA pattern for elemental analysis is plotted in Figure (3) displaying the dominance of Si (96.9%) of the total contents. The FTIR spectra of SLNs/EOs are plotted in Figure (4) where, (a) and (b) show absorption bands at 1045 cm^{-1} and 964 cm^{-1} which may be attributed to the asymmetric vibration of Si-O and Si-OH, respectively. However, the band at 793 cm^{-1} can be referred to the symmetric vibration of Si-O (Beganskienė *et al*., 2004). As well as, the absorption bands of alkyl group are at 2396 and 2873 cm^{-1} (Figure 4, c and d) are attributed to bands of *cis* olefin group or carbonyl group from the EOs. The board absorption bands between 3200 and 3600 cm^{-1} can be attributed to the hydroxyl groups of the EOs and SiO_2. However, the presence of residual silanol (Si-OH) group is frequently observed in many derived materials reflecting the incomplete polycondensation (Lee *et al*., 2009).

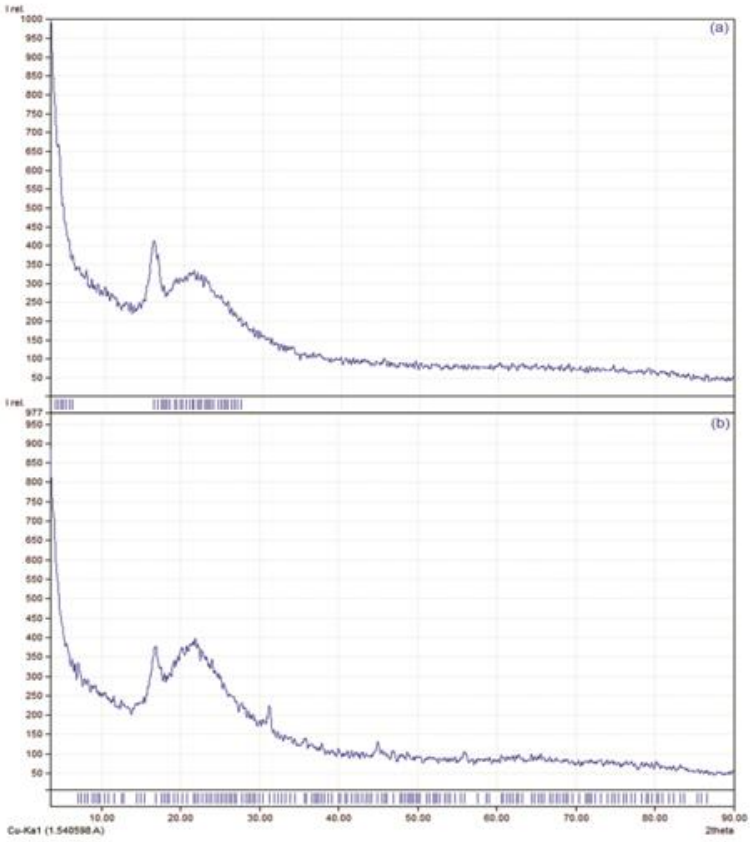
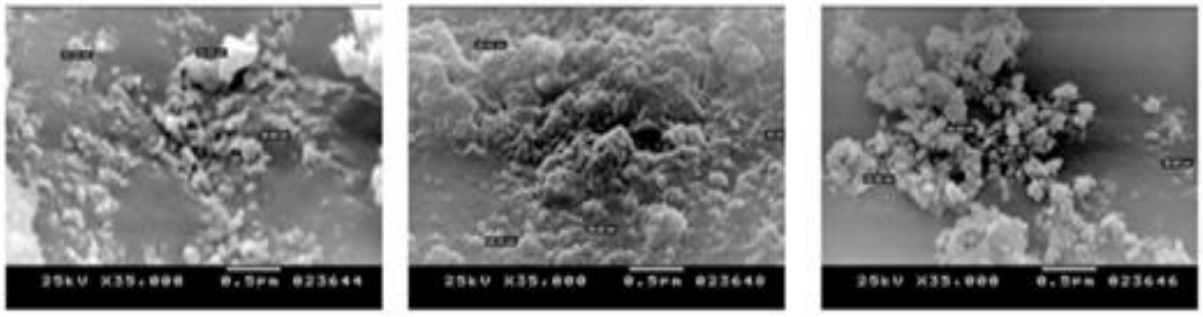


Figure (2): X-ray diffraction pattern graphics of SiO_2 crystals (a) and SiO_2nanaoparticles (b).



(a) (b) (c)

Figure (1): SEM photograph of Silica nanoparticles, NPs (a) and agglomerated nanosilica loaded essential oils peppermint, SNL/Peppermint(b) or clove, SNL/Colve(c).

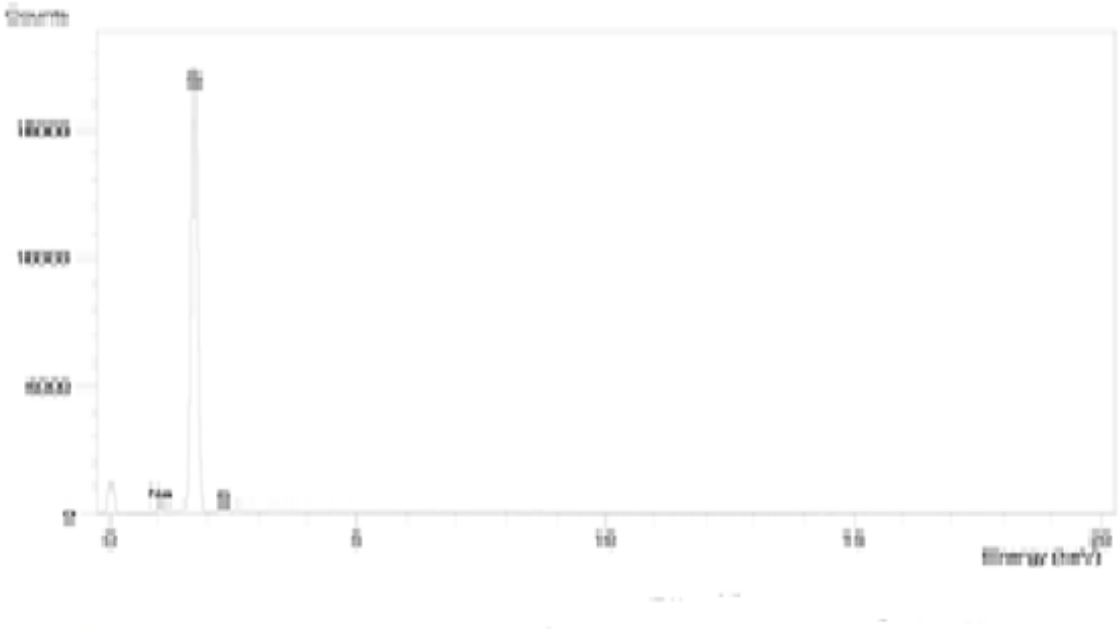


Figure (3): EDA of SiO_2 nanoparticles on X-ray elemental analysis instrument

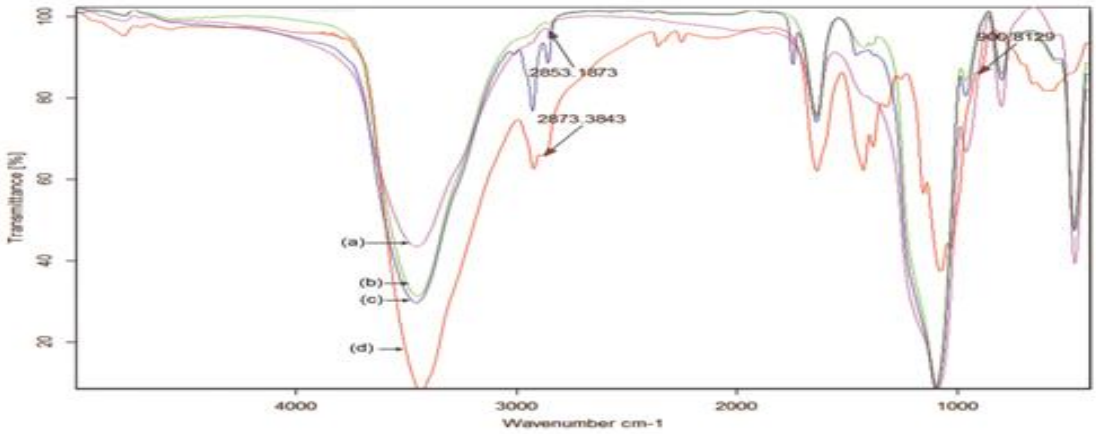


Figure (4): FTIR spectra of SiO_2 crystals (a), SiO_2 nanoparticles, NPs (b), SiO_2 nanaopaticles contained clove oil, SLN/clove of peppermint.

2. Insect susceptibility:

The toxicity values of SLNs formulations and EOs are presented in Table (1). The prepared SLNs/Clove formulation was more toxic to adults of *T. castaneum* compared with clove oil treatment, since LC_{50} values were 1156.26 and 5619.03 ppm after 14 days post treatment, respectively. Despite SLNs/Clove formulation was not effective up to five days after the treatment, its potential employed 4.86 folds after 7 days against clove oil treatment. Moreover, this difference was observed up to 14 days (the end of the experiment). On the other hand, no significant differences were observed between the SLNs/Peppermint formulation and peppermint oil alone. Since the LC_{50} values were 486.63 and 494.35 ppm, after 7 days of treatment, respectively and their confidence limits were overlapped. However, the silica salts and SiO_2NPs were obviously none effective against *T. castaneum* adults up to 50.000 ppm.

Table (1): Acute toxicity of some essential oils and their nanoformulations against *Tribolium castanuem* after different time intervals

Days after Treatment	LC_{50} (ppm)	Confidence limits		Slope	variance	Chi^2
		lower	upper			
5 day						
Peppermint oil	454.05	309.74	541.58	3.67	0.76	0.09
Clove oil	5619.03	5287.54	5938.83	4.31	0.34	8.63
SLNs/Peppermint	500.14	490.23	511.06	13.71	1.33	1.84
SLNs/Clove	None	None	None	None	None	None
7 day						
Peppermint oil	494.35	484.49	505.53	12.71	1.10	4.70
Clove oil	5619.03	5287.54	5938.83	4.31	0.34	8.63
SLNs/Peppermint	486.63	477.79	495.68	15.38	1.37	2.59
SLNs/Clove	1156.26	1014.74	1398.67	2.62	0.51	0.0024
10 day						
Peppermint oil	461.78	443.57	480.03	15.28	1.18	9.6
Clove oil	5619.03	5287.54	5938.83	4.31	0.34	8.63
SLNs/Peppermint	464.82	457.85	471.16	16.45	1.18	7.64
SLNs/Clove	1156.26	1014.74	1398.67	2.62	0.51	0.0024
14 day						
Peppermint oil	448.72	423.89	470.12	14.94	1.20	14.34
Clove oil	5619.03	5287.54	5938.83	4.31	0.34	8.63
SLNs/Peppermint	464.14	454.09	472.44	20.57	2.09	2.35
SLNs/Clove	1156.26	1014.74	1398.67	2.62	0.51	0.0024

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3. Biochemical quantifications:

The data of moisture contents, water absorption capacity and germination percentages of wheat grains are listed in Table (2) no significant differences of moisture contents were detected neither between all treatments nor the untreated wheat, where the percentages ranged between 10.88 and 11.58%. However, results of water absorption percentages indicated that, all treatments significantly enhanced the water absorption capacity after 5 and 24 hr compared with the control group. SLNs/clove treatment showed the maximum significant improvement of the water absorption capacity (253.53% and 392.40%) compared with the control (237.60% and 3 29.33%) after 5 and 24 hr, respectively. While no significant differences between the entire treatments and control were recorded after 1 hr. SiO_2 and SiO_2NPs showed high germination percentages after 4 days (80.67 and 83.50%, respectively), but no significant differences were observed between them and other treatments after 7 days. Meanwhile, the peppermint oil treatment showed the least germination percentage (67.33%) followed by SLNs/clove treatment (71.33%) and control (92.67%).

On the other hand, the data of total protein, total carbohydrates and lipid peroxidation of the treated grains are listed in Table (3). SLNs/clove formulation caused the highest significant enhancement of protein content (32.55%) but clove oil showed the least significant decreasing (10.16%). The other treatments were in the following order: peppermint oil (25.08%)> SiO_2NPs (23.67%) > SLNs/peppermint (22.28 %)> control (16.61)> and SiO_2 (15.89), respectively. Although the entire treatments showed increase in total carbohydrate contents compared, no significant differences were observed between them and the control group. However, significant increase in total carbohydrate contents was observed as

following SiO_2 (82.23%), clove oil (70.00%), peppermint oil (64.85%) and SiO_2NPs (62.10%), treatments, respectively. In case of peroxidation, malnodialdhyde (MAD) contents, there were no significant differences between SLNs/clove treatment and control (0.0007 mmol/g tissue). However, the highest MAD contents (0.0011 mmol/g tissue), was recorded for SLNs/peppermint treatment, while the least value was recorded for SiO_2 treatment (0.0002 mmol/g tissue).

Obviously, the challenges associated with applying synthetic insecticides such as insecticide

Table (2): Effect of nanoformulations of certain essential oils on moisture, water absorption and germination percentage of wheat grains after different storage periods.

Treatment	Moisture%	Water Absorption%			Germination%	
		After 1 h	After 5 h	After 24 h	after 4 day	after 7 day
Control	$11.32^{a} \pm 0.07$	$120.20^{a} \pm 1.74$	$237.60^{e} \pm 4.00$	$329.33^{f} \pm 10.21$	$78.56^{a} \pm 5.49$	$92.67^{c} \pm 1.66$
SiO_2	$10.88^{a} \pm 0.15$	$113.67^{c} \pm 2.13$	$247.43^{b} \pm 4.01$	$380.00^{d} \pm 9.64$	$80.67^{a} \pm 4.53$	$92.60^{c} \pm 1.48$
SiO_2NP_s	$11.13^{a} \pm 0.03$	$100.47^{e} \pm 3.00$	$240.13^{c} \pm 4.87$	$392.80^{c} \pm 36.34$	$83.50^{a} \pm 1.26$	$87.00^{bc} \pm 1.68$
Clove oil	$11.58^{a} \pm 0.32$	$116.10^{b} \pm 3.27$	$238.20^{d} \pm 1.23$	$394.63^{b} \pm 2.47$	$76.67^{a} \pm 0.95$	$90.50^{c} \pm 0.33$
Peppermint oil	$11.08^{a} \pm 0.04$	$112.43^{c} \pm 1.98$	$240.47^{c} \pm 0.73$	$346.23^{e} \pm 9.23$	$64.17^{a} \pm 0.95$	$67.33^{a} \pm 1.26$
SLNs/ Clove	$11.29^{a} \pm 0.03$	$115.00^{b} \pm 1.51$	$253.53^{a} \pm 3.20$	$392.40^{c} \pm 6.14$	$60.17^{a} \pm 3.50$	$71.33^{a} \pm 0.63$
SLNs/peppermint	$11.04^{a} \pm 0.21$	$105.10^{d} \pm 4.10$	$232.87^{f} \pm 4.03$	$403.30^{a} \pm 29.22$	$76.67^{a} \pm 3.82$	$83.75^{b} \pm 0.96$

Each value represents the mean of three replicates ± SE.
No significant difference obtained of the same letters at 0.05 levels.

Table (3): Biochemical alterations in treated wheat grains

Treatment	Total protein (%)	Total carbohydrates (%)	Lipid peroxidation (mmole/g tissue)
Peppermint oil	$25.05^{b} \pm 1.73$	$64.85^{b} \pm 0.31$	$0.0010^{c} \pm 0.00005$
Clove oil	$10.16^{d} \pm 0.83$	$70.00^{e} \pm 1.80$	$0.0007^{d} \pm 0.00001$
SLNs/peppermint	$22.28^{a} \pm 6.63$	$56.45^{c} \pm 1.31$	$0.0011^{b} \pm 0.00010$
SLNs/clove	$32.55^{a} \pm 3.66$	$66.75^{c} \pm 3.84$	$0.0010^{c} \pm 0.00019$
SiO_2NP_s	$23.67^{b} \pm 2.83$	$62.10^{e} \pm 0.31$	$0.0006^{d} \pm 0.00003$
SiO_2	$15.89^{b} \pm 2.37$	$82.23^{a} \pm 10.01$	$0.0002^{a} \pm 0.00004$
Control	$16.61^{c} \pm 0.27$	$53.60^{c} \pm 2.20$	$0.0007^{d} \pm 0.00002$

Each value is the mean of three replicates ± SE.
The same letters indicate no significant different at 0.05 levels.

resistance, residues in stored products and environment as well as health problems have been necessitated the seeking for more effective and environmentally friendly controlling agents such as Eos (Lorinia and Galleya, 1999 and Zettler and Arthur, 2000). EOs has been known as a natural source of insecticides (Gbolade, 2006). Their lipophilic nature gives them possibility to interfere with an assortment of vital functions of insects (Nishimura, 2001). Nevertheless, their high volatility and poor water solubility are difficulties, which confine their advancement as commercial pesticides. Besides, a principal disadvantage of using EOs as pesticides is their lack of persistence, which required two or more applications to exert a satisfactory management of the pests (Isman *et al*., 2011). Nanoformulation of pesticides aims toward measure releases of the necessary and sufficient amounts of the active ingredients for a period of time to obtain the fullest biological efficacy (Ghormade *et al*., 2011). As well, NPs have chemical activity higher than the bulk material (González and Alicia, 2014). Likewise, several investigators reported that, NPs could be applied to facilitate the management of stored product insects (Goswami *et al*., 2010; Zahir *et al*., 2012 and Rouhani *et al*., 2012). Moreover, it has been demonstrated that, application of SiO_2NPs could significantly increase the mortality of *Sitophilus oryzae* (L.) as a result of increasing the time of exposure (Debnath *et al*., 2011). Therefore, depending on the present results and previous reports the developed SLNs/EO could prove a possible solving for such problems. The prepared SLNs/EO

formulations in this study could protect the EOs through slowing down their rapid evaporation and degradation and successfully improve their stability. This is in agreement with what stated by Lai *et al.* (2006), where they reported that, a formulation of SLNs of *Artemisia arborescence* extract showed controlled release of the EO and decreased its rapid evaporation. Meanwhile, the presented formulations could enhance EOs toxicity *via* increasing their bioavailability as a consequence of the high mobility of the NPs.

The activity of EOs mainly relies on the synergistic effects of their major constituents, where 97.77% of peppermint oil contents are terpenes (Bazargania and Rohloff, 2016), but clove oil contains 85.2% eugenol (Rajkowska, 2016). Hence, it had been demonstrated that, terpenoids have a biological action against several post-harvest Coleopteran insects (Regnault-Roger *et al*., 2012; Sahaf *et al*., 2007 and Tripathi *et al*., 2009). The efficacy of the prepared SLNs/EO formulations against *T. castaneum* adults may be referred to terpenes, which represent the major components of the peppermint and clove oils loaded on NPs (Abdelgaleil *et al*., 2009; Ukeh and Umoetok, 2011 and Zhang *et al.*, 2011). These terpenes may act against insects throughout the interference with nervous system, including γ-aminobutyric acid (GABA)-gated chloride channels, acetylcholine esterase, sodium channels, octopamine receptors, tyramine receptors, nicotinic acetylcholine receptors (nAChR) and others (Tong , 2010). On the other hand, the diffusion and transport processes of the amorphous materials are considerably faster than the crystals (Hancock and Zografi, 1997). Therefore, the toxicity of the developed SLNs/EO formulations could be referred to the high mobility of NPs, which enable to penetrate into insect tissues. The penetration can be achieved either by means of faster penetration through the direct contact with the insect's cuticle, or by ingestion and diffusion through the digestive tract (Margulis and Magdassi, 2012). Despite the fact that, the unusual physicochemical and toxicological properties of NPs are attributed to their small size, chemical composition, and aggregation, yet the high surface area creates the opportunity for increasing the uptake and interaction with a biological target (Nel *et al*., 2006 and 2009).. For example, efficacy of SiO_2NPs against *T. castenum* adults could be attributed to impairment of the digestive tract (Smith, 1969) or to surface enlargement of the integument a consequence of dehydration or blockage of spiracles and tracheas. Also, their enormously increased exposed surfaces could allow more interaction with the insect cuticle resulting damage to insects' protective wax which coat on the cuticle, both by sorption and abrasion (Rouhani *et al*., 2011). NPs display large specific surface, resulting in higher adhesiveness of EO-NPs to insect's body, and increasing the exposure time. Furthermore, the detoxifying oxidation enzymes role had been reported in EOs detoxication process (Rossi *et al*., 2012).

Thus, we can assume that SLNs/EO formulations in particularly the SLNs/clove may decrease the detoxication rate compared with EOs alone, since NPs reserved the oil into the extracellular tissues and allowed it to reach its site of action (Sahaf *et al*., 2007 and Isman, 2000). Hence, the grain quality is an important concern in the stored product insect management. These findings revealed that the prepared formulations enhanced the efficacy of the EOs meanwhile they positively affected the grain quality with the exception of slight effect on the lipid peroxidation. So, further studies are required to determine the mode of action, enhance of the formulation efficacy and improve grain quality.

It is concluded that the benefits of SLNs/EO formulations evaluated in this work are: the efficacy enhancement due to the higher surface area, the lower expected detoxication rate and sustained controlled release. Also, the induction of systemic activity attributable to their smaller particle size, higher mobility and possible lower ecotoxicity are considerable advantages of SLNs/EO. These designed formulations may be useful to promote the massive use of the

EO in stored product insect controlling systems and develop sustainable environmentally friendly controlling agents.

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