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Formulation, Characterization and Insecticidal Effect of Two Volatile Phytochemicals Solid-lipid nanoparticles against some Stored Product Insects

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Abstract

Stored product insect pests are responsible for considerable quantitative and qualitative losses of agricultural products, mainly cereals and legumes. Solid-lipid nanoparticles (SLNs) by neem (*Azadirachta indica*) and castor oil (*Ricinus communis*) were synthesized using the ultrasonic solvent emulsification technique. The particle size and morphology of produced nanocapsules were characterized using transmission electron microscopy (TEM) and evaluated in the laboratory. This study aimed to evaluate the contact and residual toxicities of the two natural oils, castor and neem oil, loaded into solid lipid nanoparticles pre and post loaded-SLNs against the adults of three stored product pests, *Sitophilus oryzae*, *Tribalium castaneum*, and *Oryzaeaephilus surinamensis*, and their progeny production. Results indicated that the neem and castor oils pre and post loaded had insecticidal activities, but the prepared neem and castor (SLNs) formulation exhibited more toxicity against *S. oryzae*, *T. castaneum*, and *O. surinamensis* than that of bulk oil with low concentrations under laboratory conditions. Also, castor (SLNs) has the highest insecticidal activity with LC_{50} of 405.5 ppm, followed by neem (SLNs) (785 ppm) against S. oryzae. While in the case of O. surinamensis, insect neem (SLNs) had the highest insecticidal activity with LC_{50} (157.5 ppm), followed by castor (SLNs) (356.5 ppm). The results were also conducted to show that the direct and residual effects of neem and castor (SLNs), when mixed with wheat grains, were more stable and gave a high percentage of mortality at the concentration of 4.5% used even after one and two weeks. It also caused a significant reduction in emerged adults after six weeks and three months of treatment compared with bulk oil and control. These findings may highlight the role of solid-lipid nanoparticles the second generation of nanoparticles could be used successfully as an alternative to synthetic chemical insecticides in stored wheat as a protectant and can be used in integrated pest management programs for controlling the different stored products insect pests.

Keywords: Stored-product insects**;** Neem and Castor oil**;** solid lipid nanoparticles**;** contact toxicity **;** residual effect**.**

1. Introduction

Stored foods are prone to post-harvest loss in quality and quantity due to infestation by different groups of stored product insects. Among the stored products, the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera, Curculionidae), is a serious pest of various food grains under storage. Both adults and larvae feed on whole cereal grains including wheat, rice, barley, corn, groundnuts, and sorghums [1, 2, 3]. The females can lay eggs and develop solid products made of cereals such as pasta [4]. The rust-red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is an important pest of stored products [5]. Both adults and larvae feed internally on maize grains, feed mainly on the germ of cereal. It is a major pest of the following crops: maize, groundnut, oats, rice, beans, peas, and wheat [6, 7]. The saw-toothed grain beetle, *Oryzaephilus surinamensis* Linnaeus (Coleoptera: Silvanidae), is one of the key stored grain pests that occur globally [8]. It is one of the most common pests of cereal grains and other grain products. It was once classified as a secondary pest because of its inability to damage whole cereal grains. Nowadays, however, it also feeds on whole wheat grains [9, 10]. This beetle is difficult to control because of its high fertility, the

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short time needed for complete development, and its significant emigration potential. Moreover, because of its small body size and great mobility, it can effectively hide in many places of the granaries [11]. Control of these insects is mainly based on the use of synthetic insecticides and fumigants, which has resulted in the development of resistance to commonly used grain protectants and fumigants [12].

There are two main chemical control methods against stored product insect pests, fumigation with very toxic gases and grain protection by residual contact insecticides. It is a fact that synthetic insecticides have been regarded as the most effective method to combat insect pests in stored grains. The random use of these chemicals induced many serious problems, involving insect resistance, toxic residues, and bad effects on humans and the environment [13, 14].

As a result, nowadays, the worldwide trends are to reduce and prevent the wide use of insecticides, which have high toxicity to humans and harm to the environment. Thus, there is an urgent need to apply an eco-friendly, safe, and low-cost alternative to chemical pesticides to protect the stored grains and seeds against these insect pests. Plant essential and natural oils may be used as biopesticides as an alternative or complementary method in crop production and integrated pest management [15].

Neem oil is extracted from the neem tree *Azadirachta indica* A. Juss (Family Meliaceae) that originates in the Indian subcontinent and is now valued worldwide as an important source of phytochemicals for use in human health and pest control [16, 17]. The popularity of neem products grew over time and this plant is now known as the village pharmacy or plant of the twenty-first century [18]. The main neem product is the oil extracted from its seeds by different techniques. Neem oil protects stored products up to five or six months when applied at the rate of 1% [19].

The chemical composition of neem seed oil is very complex and rich in terpenoids, limonoids, and volatile sulphur compounds [20]. Until now, more than 300 compounds have been isolated from various parts of *Azadirachta indica* [21]. However, neem seed oil alone contains more than 100 biologically active compounds [22]. The key chemical constituents reported from neem essential oil can be divided into Hydrocarbons, Fatty Acids [oleic acid (50%–60%), palmitic acid (13%–15%), stearic acid (14%–19%), linoleic acid (8%–16%), and (1%–3%) arachidic acid.], Limonoids [azadirachtin (azadirachtin A), salannin, salannol, nimbin, nimbinin, nimbidin, nimbidiol, nimolicinol, gedunin, 3-tigloylazadirachtol (azadirachtin B), epoxyazadiradione, 17β-hydroxyazadiradione, 1-tigloyl-3-acetyl-11-hydroxymeliacarpin (azadirachtin D), 1α,2α-epoxy-17β-hydroxyazadiradione, 1α,2α-epoxynimolicinol, and 7-deacetylnimolicinol] and Sterols (β-sitosterol, stigmasterol, campesterol, and fucosterol) [23, 24, 25].

Castor oil (ricinus oil) is natural oil extracted from castor seeds by cold pressing (for pharmacological use). Castor is a wide plant in the spurge (Family Euphorbiaceae) and the only member of the genus Ricinus and the *Ricininae subtribe*, which is commercially grown for its oil, which is in high demand for industrial (biodiesel, paints, coatings, inks, lubricants, dyes, etc.) and food uses [26, 27]. It is also known for its anticancer, antidiabetic, antiprotozoal, insecticidal, larvicidal, and adult emergence inhibition activities [28].

Castor oil contains the fatty acids palmitic acid (0.00%–0.41%), stearic acid (0.04%–1.58%), oleic acid (0.32%–2.08%), linoleic acid (1.9%–21.69%), linolenic acid (0.05%–0.86%), and ricinoleic acid (74.68%–95.49%) [29, 30]. Although the castor bean's toxicity has been known since antiquity, castor oil is not toxic because ricin, a toxic protein found in the seeds, is not lipid soluble, limiting the toxic component to the castor bean. Ricin inactivates eukaryotic ribosomes in an irreversible manner, preventing protein synthesis [31]. The essential oil showed toxic, repellent, and antifeedant effects on stored product insects [32, 33, 34, 35].

Despite these promising properties, issues such as essential oil volatility, poor water solubility, and degradation of some active components before they are used in pest control systems [36], have been identified to overcome this problem, formulating essential oils into nanoemulsions reduces volatility, hydrophobicity, and reactivity of the bioactive molecules constituting the essential oils [37]. Nano formulations of plant extracts or essential oils are considered to be safe for humans and their ecosystems [38].

The development of controlled release formulations of insecticides using polymers for pest management programs is presently being given major attention, keeping in mind the protection of the photo-liable active ingredients [39]. Polymer-based delivery systems increase the dispersion of active ingredients in aqueous media and act as a protective reservoir covering that leads to a controlled release of active ingredients [40]. Slow release of active ingredients depends on the nanocarrier's degradation properties, bonding between active ingredients and the carrier, and weather factors [41]. Nowadays, there has been an increased trend toward using eco-friendly and biodegradable natural materials such as lecithin [42,43]. The materials used to produce SLN are low-cost. Moreover, there is the possibility of production on a large scale [44]. Solid lipid nanoparticles loaded with essential oil of *Ziziphora clinopodioides* have been prepared and characterized by [45], and their insecticidal activity was evaluated against flour

beetles. In another study, the insecticidal activity of polyethylene glycol (PEG) nanoparticles loaded with garlic essential oil was evaluated against flour beetles [46].

To our knowledge, no studies have been prepared neem oil or castor oil incorporated into solid lipid nanoparticles (SLNs) formulations and investigated their effects against *S. oryzae, T. castaneum*, and *O. surinamensis* in Egypt. The aim of this work is to incorporate two natural oils, neem oil and castor oil, into solid lipid nanoparticles (SLNs) to prepare a nanoformulation and choose as carrier material for the two oils. Evaluate contact and residual toxicities of the two natural oils pre and post loaded-SLNs against the adults of three stored product pests, and their progeny production. The potential insecticidal properties of using a new generation of formulations for these oils might provide an effective alternative to conventional synthetic insecticides, encourage large-scale use of these natural oils, and improve pest management strategies.

2. Materials and methods

2.1. Compounds

The two natural oils, neem and castor oil, are obtained as pure (crude) oils from the oil extrac-tion unit at the National Research Centre (NRC), Dokki, Cairo, Egypt. .Stearic acid, the surfactant soybean lecithin, surfactant Tween-80, and Dichloromethane were obtained from Biodiagnostic Co., 29 Tahrir Street, Dokki, Giza, Egypt.

2.2. Commodity

The wheat grains (*Triticum vulgare*) var. Misr 2 was obtained from the farm of the Faculty of Agriculture, Alexandria University.

2.3. Insect Culture (Rearing)

The rice weevil, *S. oryzae*, the red flour beetle *T. castaneum* and the saw-toothed grain beetle *O. surinamensis* used in this study were obtained from stored products and grains pests' department research institute, al-Sabahia, Alexandria *S. oryzae* and *O. surinamensis* were reared on sterilized wheat grains, *T. castaneum* was reared on wheat flour mixed with yeast (10: 1 w/w). The three pests were maintained at room temperature in laboratory reared colonies at 30 ±2 0C and relative humidity 75 ±5% in continuous darkness. The adults used in all experiments were two weeks post emergence of unknown sex and mating status.

2.4. Neem and Castor oil Solid lipid nanoparticles (SLNs) preparation.

Solid lipid nanoparticles (SLNS) were prepared using ultrasonic solvent emulsification technique according to [47,48]. Oil phase consists of 1% (w/w) stearic acid, which acts as a lipid, and a 10% concentration of neem or castor oil mixed with dichloromethane (50 ml) and heated to 50°C. The water phase consists of 2.5% (w/w) soy bean lecithin and tween-80, which act as emulsifier were dispersed in 50 ml of distilled water with magnetic stirring at the same temperature. This combination of emulsifiers helps to prevent particle agglomeration after evaporating most of the solvents. The water phase was added to the oil phase drop-by-drop at 50 °C followed by magnetic stirring for l0 min.

The coarse emulsion was subjected to 55 w of ultrasonic treatment for 5 min using a high power ultra (sonication probe Sonics -Vibra cell, Ningbo Haishu Ultrasonic Equipment Co., Ltd, China) with a water bath (0 °C). The cold nanoemulsion then dispersed into cold water using a homogenizer (CAT Unidrive X 1000 homogenizer). The cold water prevented lipid aggregation. This process is followed by magnetic stirring to remove any traces of organic solvents, if any. After the solvents had completely evaporated, the neem or castor oil loaded (SLNs) suspension was filtered through a 0.45 um membrane in order to remove the impurity materials (e.g. metals) and then stored at 4°C for further bioassays.

2.5. Transmission Electron Microscope (TEM).

Structural characterization and the morphology of neem or castor oil SLNs were observed with JEOL JEM-2100 transmission electron microscopy (TEM). Samples were placed on carbon-coated TEM grids after a suitable dilution was created, then a drop of 2% phosphotungstic acid was added. The excess liquid was removed by blotting with a filter paper for two minutes. The sample was allowed to dry for 10 minutes at room temperature before observation.

2.6. Contact toxicity bioassay.

The insecticidal activity of the free natural oil pre/post nano-encapsulation was assessed by the contact toxicity method to demonstrate the toxic effect of neem or castor oil against the three selected pests according to [49]. A series of concentrations of free oil (2500, 5000, 7500, and 10000 ppm) and oil-loaded solid lipid nanoparticles (SLNs) (250, 500, 750 and 1000 ppm) were prepared in acetone was applied inside glass petri dish (9cm dim.) spread uniformly along the whole surface of the dishes. The solvent was allowed to evaporate, leaving a thin film on the floor of the dishes. After evaporation of the solvent, then 20 adults of each insect species were separately introduced to each petri dish. Three replicates of each treatment were used. The numbers of dead insects were counted after 72 hours of treatment and the mortality percentage was estimated. LC_{50} values were calculated according to [50].

2.7. Exposure of Adults to Treated Wheat Grains.

Sixty grams of wheat grains were treated with different concentrations of neem or castor free oil at 5, 15, 30 and 45 % (v/v) and neem or castor (SLNs) at 0.5, 1.5, 3, and 4.5 % (v/v) concentrations. Each concentration is mixed manually with grains and then divided into three equal replicates in glass jars (0.4 liter capacity). After evaporation of acetone, the

treated grains were infested with 10 pairs of newly emerged adult insects. Mortality was recorded every week for two weeks. The number of progeny for each pest was recorded after 6 weeks and 3 months from infestation. Control treated with and without solvent was used. Three replicates of each treatment were used.

2.8. Progeny production counts.

After two weeks, the mortality count and all adults (dead and alive) were removed from the jars and left under the same conditions for another 6 weeks and 3 months post-treatment to assess progeny production. The number of emerged individuals (adults and immature) was then counted in the controls and in the treated commodities and they were introduced to [51], was adopted for the estimation of the percentage reduction in progeny production.

$$\text{Reduction in progeny \% (IR)} = \frac{\text{Cn - Tn}}{\text{Cn}} \text{ X } 100$$

Where:
Cn:-number of newly emerged untreated (control)
Tn: number of newly emerged insect treatment.

2.9. Statistical analysis

The lethal effect was evaluated as % of cumulative daily mortality, corrected for mortality in the control variant according to Abbott's formula [52]. As follows:

$$\text{Corrected (M)} = \frac{\text{M\% (t) - M\% (c)}}{\text{100- M\% (c)}} \text{ X100}$$

Where:
t= treated insect **c**=Control insect

A median lethal concentration (LC_{50}) was calculated using a computerized software program (Ld-p line) Copy right by Ehab, M.Bakr, plant Research Institute, ARC, Giza, Egypt.

3- Results and Discussion

This study found that the incorporation of neem or castor oil into solid controlled release nanoformulations using stearic acid as a coating material prevents their rapped degradation enhance their stability insecticidal activities by contact and ingestion.

3.1. Neem and Castor oil solid lipid nanoparticles and its characterization

The morphological appearance and size of nano sized colloids were investigated using transmission electron microscopy (TEM). Figures 1 and 2 show a TEM scan of a neem (SLNS) and castor (SLNS) in the studied sample, with the average particle size determined from the TEM images being 33.34 and 48.66 nm, respectively. This result demonstrates that all compounds were successfully nanometrically prepared. Our findings are consistent with those of others who have reported solid lipid nanoparticles (SLNS) sizes ranging from 50–1,000 nm [53,54]. The morphology and characterization of neem and castor oil-loaded solid lipid nanoparticles (SLNs) were visualized using transmission electron microscopy (TEM). Figures (1 & 2) show the particles appearing round, spherical in shape, and with good dispersion. These findings are consistent with those of [47, 55, 56], showed that the particles of geranium and garlic after loading appeared round in shape.

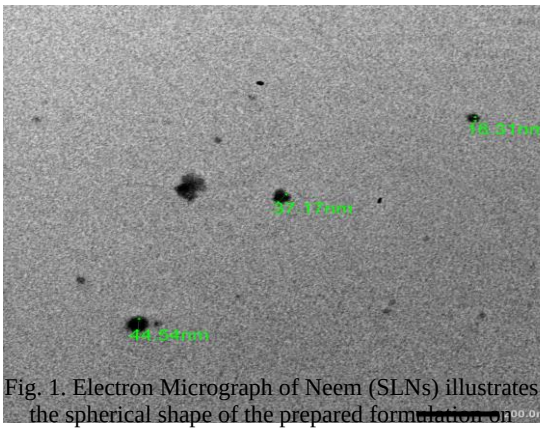

Fig. 1. Electron Micrograph of Neem (SLNs) illustrates the spherical shape of the prepared formulation on TEM.

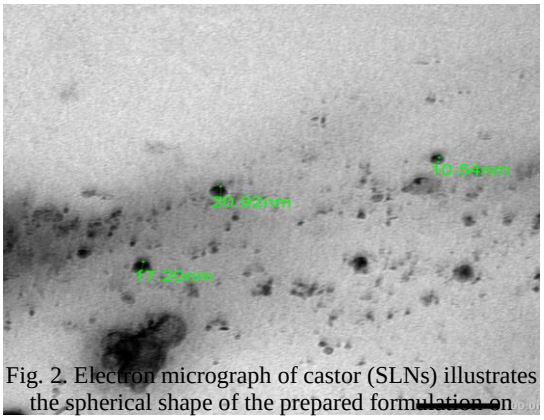

Fig. 2. Electron micrograph of castor (SLNs) illustrates the spherical shape of the prepared formulation on TEM.

loaded-SLNs against Sitophilus oryzae, Tribalium castaneum and Oryzaeaephilus surinamensis.

Data in (Table 1) showed that the neem-bulk oil was very toxic against the pests. The LC_{50} of the test oil after 72 h of exposure was (7995 and 540 ppm) against *T. castaneum* and *O. surinamensis*, respectively, whereas *S. oryzae* shows no value for (LC_{50}) because all concentrations used gave 100% mortality. On the other hand, the potential activities of the oil neem loaded (SLNs) formulation against the three pests were recorded in (Table 2). The estimated LC_{50} was obviously different and could be screened in the following three categories exhibited high mortality rate with a low LC_{50} (157.5 ppm) against *O. surinamensis* adult and moderately effective against *S. oryzae*, the LC_{50} was (785 ppm) and complete mortality against *T. castaneum*, and the LC_{50} was no value.

B- Contact toxicity of castor oil pre and post loaded-SLNs against Sitophilus oryzae, Tribalium castaneum and Oryzaeaephilus surinamensis.

The LC_{50} values obtained from contact toxicity using thin-film residue and after 72 hours of exposure of the three selected pests to natural castor bulk oil are shown in Table 3 and 4. The results pointed to the direct contact toxicity of castor oil loaded-SLNs against *S. oryzae*, *T. castaneum*, and *O. surinamensis* adults (405.5, 660 and 356.5 ppm) respectively, was higher than bulk oil (845, 7932 and 359 ppm) respectively.

It is noticeable from the results of the current study that neem bulk oil had the greatest effect on S. oryzae, while neem-loaded SLN_S formulation had the greatest effect on *T. castaneum* and *O. surinamemsis*. Also, the toxicity of castor oil was increased during the experiment in *S. oryzae* and *O. Surinamensis*, and found to be more susceptible than *T. castaneam*. Similar effects were observed in the case of the treatment with castor oil loaded (SLNs), but the values of LC_{5o} were significantly less toxicity than castor bulk oil against the three pests.

Our findings showed that neem and castor (SLNs) had higher insecticidal toxicity than bulk oil against *S. oryzae*, *T. castaneum*, and *O. surinamensis* adults. These results are in line with [45], who demonstrated that SLN had higher toxicity effects on red flour beetles. Likewise, Jesser et al. [57], reported that in a contact toxicity bioassay, the insecticidal effects of

Table 1: Toxicity of Neem bulk oil against *Tribolium castaneum* and *Oryzaephilus surinamensis* adults using thin film residue method after 72 h post-exposure.

Insects	LC_{50} ppm	Confidence limits		Slope	X^2
		Lower	Upper		
Sitophilus oryzae	785	559.3	1844	0.842±0.283	3.988
Oryzaephilus surinamensis	157.5	22.26	266.7	0.935±0.29	1.702

Table 2: Toxicity of Neem oil loaded-SLNs on *Sitophilus oryzae* and *Oryzaephilus surinamensis* adults using thin film residue method after 72 h post-exposure.

Insects	LC_{50} ppm	Confidence limits		Slope	X^2
		Lower	Upper		
Tribolium castaneum	7995	5231	9523	4.494±0.495	8.523
Oryzaephilus surinamensis	540	347	689	2.785±1.44	0.52

the oils were increased by all polyethylene glycol PEG 6000 polymeric nanoparticles loaded with essential oil (EOPN), with palmarosa oil being the most toxic against *Plodia interpunctella*. Nanoparticles encapsulated with *Artemisia haussknechtii* essential oil showed 100% mortality at 166 ppm [58]. Also, Massoud et al. [59] studied the effect of *Mentha piperita* essential oil nanoemulsion on *S. oryzae* and showed that the highest and fastest toxic effect was observed in the case of *M. piperita* (4%) nanoemulsion via thin film residue method against *S. oryzae* and treatment with the wheat grain method. Werdin-González et al. [60] showed that nano-particles loaded with geranium and bergamot EO increased the contact toxicity between 8 and 10 times on adults of *Blattela germanica.* Adel et al. [48] investigated the insecticidal efficacy of *M. piperita* essential oil and its nanoemulsion formulation against adult *T. castaneum* after 72 hours of exposure; the direct contact toxicity of *M. piperita* nanoemulsion was higher than bulk essential oil, with lower LC_{50} values concentration than bulk essential oil, according to contact toxicity using the thin film residue method and contact toxicity using the treatment with wheat grain method.

3.3. Effect of neem oil pre and post loaded-SLNs on mortality of Sitophilus oryzae, Tribolium castaneum and Oryzaephilus surinamensis adults and its progeny production.

The results presented in Table 5 show that the mortality was significant for the three selected pests and affected by the exposure intervals, the stage of pest, and the different concentrations of neem oil pre/post loaded. After one week and two weeks of exposure, the adult mortality of *S. oryzae* was more than 50%. At the highest application rate (4.5% v/w) of neem oil loaded, the mortality was 61.67 and 75%, respectively. Similarly, the mortality in case of exposure to bulk neem oil after one and two weeks was high regardless to the concentration (45% v/w) was given 80 and 90% mortality, respectively.

In addition, the mortality as shown in Table 6, adults of *T. castaneum* adults reached 75.00 and 91.67% at the same concentration (4.5% v/w) and the same exposure interval to neem oil post-load. While the mortality after one week of exposure to bulk neem oil was low, it did not exceed 8.33%. Two weeks post-application, mortality levels increased slightly and reached two times (16.67%) after exposure to the highest application rate (45% v/w). At the same time, and related to *O. surinamensis* as shown in Table 7, mortality remained < 50% after one and two weeks (6.67%) exposed to the highest application (4.5% v/w) of neem post loaded. Also, the mortality levels of *O. surinamensis* were significantly not affected by different concentrations of neem bulk oil and exposure intervals. The mortality of *O. surinamensis* adults ranged between (3.33¬ 6.67%) respectively, after one-week exposure to wheat grains treated with neem-free oil at 5, 15, 30

Table 3: Toxicity of Castor bulk oil on *Sitophilus oryzae*, *Tribolium castaneum* and *Oryzaephilus surinamensis* adults using thin film residue method after 72 h post-exposure.

Insects	LC_{50} ppm	Confidence limits		Slope	X^2
		Lower	Upper		
Sitophilus oryzae	405.5	322	547	11.041±0.879	28.716
Tribolium castaneum	660	518	711	9.482±0.763	36.928
Oryzaephilus surinamensis	356.5	203	498	12.193±1.265	9.75

Table 4: Toxicity of Castor oil loaded-SLNs on *Sitophilus oryzae*, *Tribolium castaneum* and *Oryzaephilus surinamensis* adults using thin film residue method after 72 h post-exposure.

Insects	LC_{50} ppm	Confidence limits		Slope	X^2
		Lower	Upper		
Sitophilus oryzae	845	800	890	1.866±0.48	33.817
Tribolium castaneum	7932	7235	8007	2.631±0.338	13.658
Oryzaephilus surinamensis	359	315	1012	1.294±0.428	0.585

and 45 % (v/w). It did not exceed 6.67% even after two weeks of exposure to the highest application rate (45% v/w) of bulk neem oil.

Data presented in Table 5 indicated that the emergence of *S. oryzae* adults was remarkably affected by neem oil-loaded treated wheat grains at all concentrations, particularly at the highest application rate (4.5% v/w) after 6 weeks and 3 months. The progeny production counts were significantly very low and became dead individuals' vessel inhibition rate (IR) l00% after 6 weeks and 3 months. Similarly, when treatment with bulk neem oil at the highest concentration rates of 15, 30 and 45 % (v/w), achieved high inhibition of reproduction (zero adults ‘emergence) and IR became 100% after 6 weeks and 3 months, but the IR at concentration of 15% (v/w) after 3 months became 58.91%. Concerning the sensitivity of adult *T. castaneum* to neem oil pre/post loaded evaluated in terms of progeny production, as shown in Table 6, there was no significant effect by neem oil and loaded, particularly at low concentrations (0.5 and 1.5%) v/w). Progeny counts were 90.74 and 93.16% after six weeks and three months of exposure, respectively, and the inhibition rate (IR) was very small (l.41 and 20.38%) while at the highest application rate (4.5% v/w) the (IR) became 100% after three months.

Likewise, in the case of the treatment with neem-bulk oil, progeny reduction rates ranged from 76.08–90.91% after 6 weeks and from 00.00–99.15% after three months at the concentrations of 5, 15, 30 and 45 % (v/w) respectively. With regard to O. surinamensis, the reduction in progeny shown in (Table 6) adults exposed to wheat grains treated with neem oil pre and post loaded at the highest application rate (4.5 and 45% v/w) was significantly lower and achieved a reduction in progeny (100%) after six weeks, Three months post-exposure of parental, offspring emergence increased slightly with neem oil pre/post and became 51.72 and 65.52 % respectively at the same high concentration.

3.4. Effect of castor oil pre and post loaded-SLNs on mortality of the three selected pests and its progeny production.

Mortality levels of the three selected pests were presented in Tables 8, 9 and 10. The results from Table 8 showed that, after one week of exposure, the highest mortality was recorded for *S. oryzae*, exposed to 4.5 % (v/w) of castor oil loaded-SLNs, where all exposed adults were dead. After two weeks of exposure, 4.5 % (v/w) of the tested castor oil loaded-SLNs formulations caused 91.67% mortality of exposed adults. Among castor bulk oils, the highest mortality was recorded after one week for S. oryzae exposed to 45 % (v/w) and after two weeks of exposure, 30% (v/w). So our results showed that the castor oil post-loaded recorded the highest percentage of mortality, more than 50%, with lower concentrations compared to the castor bulk oil.

Table 5: Residual toxicity of Neem bulk oil and loaded-SLNs on mortality and emergence of *Sitophilus oryzae* adults up to 12 weeks post-treatment of wheat grains.

Essential oil	Conc %	Mortality (%) after		Mean of emerged adults after			
		1week (M±SE)	2 week (M±SE)	6week (M±SE)	IR% After 6 weeks	3Months (M±SE)	IR% After 3 months
Neem oil loaded- SLNs	0.5	10.00±0.81	15.00±0.58	76.25±0.54	4.68	72.05±2.3	47.02
	1.5	11.67±0.39	21.67±0.48	48.75±2.83	39.06	71.32±2.3	47.56
	3	11.67±0.39	25.00±0.57	25.00±0.57	68.75	63.23±1.7	53.51
	4.5	61.67±1.26	75.00±3.52	0.00±0.0	100	00.0±0.00	100
Neem bulk oil	5	13.33±0.95	38.33±1.45	62.50±1.28	21.87	94.12±1.4	30.79
	15	38.33±1.45	63.33±2.29	00.00±0.0	100	55.88±1.3	58.91
	30	73.33±3.21	75.00±3.52	00.00±0.0	100	00.0±0.00	100
	45	80.00±2.31	90.00±1.53	00.00±0.0	100	00.0±0.00	100
Control	0	00.00	00.00	80.00	-	136.0	-

Table 6: Residual toxicity of Neem bulk oil and loaded-SLNs on mortality and emergence of Tribolium castaneum adults up to 12 weeks post-treatment of wheat grains.

Essential oil	Conc %	Mortality (%) after		Mean of emerged adults after			
		1week (M±SE)	2 week (M±SE)	6week (M±SE)	IR% After 6 weeks	3Months (M±SE)	IR% After 3 months
Neem oil loaded- SLNs	0.5	1.67±0.39	3.33±0.54	73.68±3.21	22	93.79±1.34	35.32
	1.5	1.67±0.39	3.33±0.54	37.89±1.42	60.1	79.31±1.86	45.30
	3	3.33±0.54	5.00±0.58	28.42±1.20	70.1	68.97±0.71	52.43
	4.5	6.67±0.33	6.67±0.39	00.00±0.0	100	65.52±1.53	54.81
Neem bulk oil	5	00.00±0.0	1.67±0.39	76.09±1.26	14.9	95.86±1.35	33.89
	15	3.33±0.54	5.00±0.58	00.00±0.0	100	95.17±1.36	34.37
	30	5.00±0.58	5.00±0.58	00.00±0.0	100	90.34±1.53	37.69
	45	6.67±0.33	6.67±0.33	00.00±0.0	100	51.72±1.34	64.33
Control	0	00.00	00.00	95.00	-	145.0	-

Table 7: Residual toxicity of Neem bulk oil and loaded-SLNs on mortality and emergence of Oryzaephilus surinamensis adults up to 12 weeks post-treatment of wheat grains..

Essential oil	Conc %	Mortality (%) after		Mean of emerged adults after			
		1week (M±SE)	2 week (M±SE)	6week (M±SE)	IR% After 6 weeks	3Months (M±SE)	IR% After 3 months
Neem oil loaded- SLNs	0.5	1.67±0.58	1.67±0.58	90.74±1.53	1.36	93.16±1.53	20.37
	1.5	3.33±0.54	10.00±0.81	88.68±2.31	3.60	92.31±1.53	21.10
	3	50.00±1.3	50.00±1.3	85.26±2.33	7.32	00.00±0.00	100
	4.5	75.00±3.21	91.67±1.53	00.00±0.00	100	00.00±0.00	100
Neem bulk oil	5	3.33±0.54	3.33±0.54	90.91±1.53	1.18	99.15±1.36	15.25
	15	5.00±0.33	5.00±0.33	88.30±2.31	4.02	98.29±1.36	15.99
	30	6.67±0.33	11.67±0.81	86.96±2.33	5.47	38.46±0.57	67.12
	45	8.3±0.393	16.67±0.58	76.08±3.52	17.30	00.00±0.00	100
Control	0	00.00	00.00	92.00	-	117.0	-

The mortality levels of *T. castaneum* were not significantly affected by different concentrations of castor oil pre/post loaded after one week, as shown in Table 9, and did not exceed 3.33% and 10.0%, respectively, while the two weeks post-application mortality levels were slightly increased and reached 5.00 and 16.67% at the highest application rate of 4.5% (v/w).The mortality levels of *O. surinamensis* adults, as shown in Table 10, were also not significantly affected by different concentrations and exposure intervals, mortality of the adult insect ranged between 0.00–5.00% after one week and 0.00–45.0% after two weeks of exposure to wheat grains treated with 0.5–4.5% (v/w) of castor oil loaded respectively, whereas mortality levels reached less than 50% (3.33 and 11.67%) after one and two weeks, respectively, from the application of the highest rate (45% v/w) of castor bulk oil.

The effect of castor oil pre/post loaded on insect progeny production in the F1 generation with regard to *S. oryzae* is determined in Table 8. Progeny reproduction none occurred in all cases examined after 6 weeks and 3 months of exposure and achieved a high inhibition rate (100%) except at the lowest application rate of castor bulk oil (75.00 and 86.76%) respectively at the same intervals.

Data presented in Table 9 illustrated that the emergence of *T. castaneum* adults was remarkably not affected by castor oil pre or post-loaded treated wheat grains. The progeny production counts were significantly moderated at the lowest concentration of castor oil loaded. While at the highest application rates (3 and 4.5% v/w), no reproduction occurred and did not give any adult emergence after 6 weeks and 3 months. On the other hand, the results shown in Table 11 showed that the emergence of *O. surinamensis* adults was affected by castor oil after loaded treated wheat grains for all concentrations used, particularly at the highest application rate (4.5% v/w). It gave a 100% reduction in the offspring at the two lifetime periods (6 weeks and 3 months) exposure interval. Concerning castor bulk oil treatment at the highest concentration, it gave the minimum number of adults (offspring).found in the wheat grains, it possessed 52.11 and 73.79% reduction in the number of the offspring compared with untreated wheat grains, 95.00 and 145.00 during the two periods. Based on the results of this study, it was indicated that examined natural neem or castor oil after loaded in nanoparticles possessed persistence insecticidal properties, against the three selected insects' mortality percentages, obtained after one week and remained until two weeks. Previous reports showed that the main monoterpenes loaded in nanoparticles of oil have much higher chemical activity than the bulk oil produced the lethal and sub-lethal against stored product pests [61,62]. According to Yang et al. [46], the control efficacy of nanoparticles containing garlic oil was superior to that of bulk garlic (free) against *T. castaneum*, and the mortality rate remained above 80% after five months, presumably due to the slow and persistent release of active components from the nanoparticles. In vitro experiments done by Lai et al. [63], showed

Table 8: Residual toxicity of castor bulk oil and loaded- SLNs on mortality and emergence of *Sitophilus oryzae* adults up to 12 weeks post-treatment of wheat grains.

Essential oil	Conc %	Mortality (%) after		Mean of emerged adults after			
		1week (M±SE)	2 week (M±SE)	6week (M±SE)	IR% After 6 weeks	3Months (M±SE)	IR% After 3 months
Castor oil loaded- SLNs	0.5	41.67±1.20	60.00±1.26	00.00±0.0	100	99.26±0.42	27.01
	1.5	58.33±1.74	73.33±3.21	00.00±0.0	100	00.00±0.0	100
	3	58.33±1.74	88.33±1.13	00.00±0.0	100	00.00±0.0	100
	4.5	81.67±1.00	91.67±1.52	00.00±0.0	100	00.00±0.0	100
Castor bulk oil	5	13.33±0.95	20.00±0.47	75.00±3.52	6.25	86.76±1.20	36.21
	15	25.00±0.57	55.00±1.00	00.00±0.0	40	00.00±0.0	100
	30	83.33±1.77	100±0.58	00.00±0.0	100	00.00±0.0	100
	45	98.33±0.33	100±0.58	00.00±0.0	100	00.00±0.0	100
Control	0	00.00	00.00	80.00	-	136.00	-

Table 9: Residual toxicity of castor bulk oil and loaded- SLNs on mortality and emergence of *Tribolium castaneum* adults up to 12 weeks post-treatment of wheat grains.

Essential oil	Conc %	Mortality (%) after		Mean of emerged adults after			
		1week (M±SE)	2 week (M±SE)	6week (M±SE)	IR% After 6 weeks	3Months (M±SE)	IR% After 3 months
Castor oil loaded- SLNs	0.5	00.00±0.0	00.00±0.0	87.82±1.21	4.54	99.15±0.42	15.26
	1.5	00.00±0.0	3.33±0.54	86.73±1.20	5.728	00.00±0.0	100
	3	5.00±0.58	5.00±0.58	00.00±0.0	100	00.00±0.0	100
	4.5	10.00±0.81	16.67±0.88	00.00±0.0	100	00.00±0.0	100
Castor bulk oil	5	00.00±0.0	3.33±0.54	80.21±1.00	12.82	98.29±0.33	15.99
	15	1.67±0.39	3.33±0.54	74.78±1.52	18.72	97.43±0.32	16.72
	30	3.33±0.54	5.00±0.58	72.61±3.21	21.07	96.58±0.67	17.45
	45	3.33±0.54	5.00±0.58	72.61±3.21	21.07	95.73±1.35	18.18
Control	0	00.00	00.00	92.00	-	117.00	-

Table 10: Residual toxicity of castor bulk oil and loaded- SLNs on mortality and emergence of *Oryzaephilus surinamensis* adults up to 12 weeks post-treatment of wheat grains.

Essential oil	Conc %	Mortality (%) after		Mean of emerged adults after			
		1week (M±SE)	2 week (M±SE)	6week (M±SE)	IR% After 6 weeks	3Months (M±SE)	IR% After 3 months
Castor oil loaded- SLNs	0.5	00.00±0.0	00.00±0.0	88.95±1.13	6.37	00.00±0.0	100
	1.5	00.00±0.0	11.67±0.39	88.95±1.13	6.37	00.00±0.0	100
	3	3.33±0.54	16.67±0.88	00.00±0.0	100	00.00±0.0	100
	4.5	5.00±0.58	45.00±0.41	00.00±0.0	100	00.00±0.0	100
Castor bulk oil	5	00.00±0.0	1.67±0.39	75.26±1.52	20.29	97.94±0.32	32.46
	15	1.67±0.39	5.00±0.58	75.26±1.52	20.29	84.83±1.22	41.49
	30	1.67±0.39	5.00±0.58	64.74±1.53	31.85	81.37±1.00	43.88
	45	3.33±0.54	11.67±0.39	52.11±1.35	45.15	73.79±3.21	49.11
Control	0	00.00	00.00	95.00	-	145.00	-

that essential oil-loaded solid lipid nanoparticles (SLNs) were able to protect the essential oil active ingredient and remain effective for up to 14 days. Also, in another experiment focused on the testing of the insecticidal activity of purslane, mustard, and castor oils used in a nano formulation against the granary weevil, *Sitophilus granarius*, under laboratory and stored conditions, it was found that the nanopurslane exhibited the highest sterilizing effect after 125 days of storage [64].

According to the application rate and the period of exposure (6 weeks), neem or a castor oil loaded produced no development and gave 100% progeny mortality against *O. surinamesis*, *S. oryzae*, and *T. castaneum*, [65]. Treatment of cowpea seeds with castor oil resulted in increased mortality in adults after 3 days and prevented oviposition when compared to control. Furthermore, it decreased egg viability and F1 progeny, according to Bhargava and Meena [66]. Castor oil at higher concentrations provided protection for up to 280 days with considerable mortality in *Callosobruchus chinensis* grubs [67]. Castor oil protected chickpeas from *Callosobruchus maculatus* for 150 days and bean beetles, *Callosobruchus phaseoli*, for 90 days in chickpeas [68].

The control efficacy of nanoparticles containing garlic essential oil was superior to that of bulk garlic oil against the stored-product adult *T. castaneum* and remained over 80% after five months, [46,15] presumably due to the slow and persistence release of the active components, particularly terpenes, from the nanocapsule. Harish et al. [69], found that adult emergence was not observed in groundnut pods treated with castor oil at a concentration of 10% (v/w).

Nanomaterials can modify the bulk materials, and change their chemical and physical characteristics. Furthermore, they have contributed to the development of botanical pesticides based on essential oils [70]. Nanoparticles are also more mobile, allowing for improved penetration into tissues and increased insecticidal action. This can be accomplished by either direct contact through the insect's cuticle or ingestion and penetration through the digestive tract [71, 72].

Obviously, when the essential oil is synthesized as a nanoemulsion, the particle size decreases and the biological activity increases due to increased surface area [73, 59], giving the formulation more opportunities to come into touch with the insect pest. While the fewer mortality caused by castor bulk oil with the greatest particle size indicated that the smaller particle size, the greater the possibility of higher efficacy.

On the other hand, several researches have been done to assess the lethal potential of nanopesticides against a wide range of insect pests. Nevertheless, detailed information on their mode of action against insects is absent [74, 75]. Because nanopesticides are relatively novel materials that have not been well researched, only a few studies have been conducted to determine their toxicokinetics or toxicodynamics against storage grain insect pests. Toxicokinetics refers to the movement and changes that an insecticide goes through inside an organism, including absorption, distribution, metabolism, and excretion, whereas toxicodynamics refers to the compounds' physiological, biochemical, and molecular effects, as well as the mechanisms by which they work [76].

4. Conclusions

From the foregoing results, it could be concluded that the castor and neem oil were enhanced by incorporating the oil into controlled release nanoformulation. To protect the active constituents of

oil from degradation and undesirable environmental conditions, it could be successfully formulated in the form of solid lipid nanoparticles (SLNS) using an ultrasonic-solvent emulsification method with a mean droplet size of 48.66 nm for castor (SLNS) and neem (SLNS) of 33.34 nm, and characterized by TEM that shows a spherical shape in the formulation. The prepared nanocapsules exhibited toxicity against *S. oryzae*, *T. castaneum*, and *O. surinamensis* more than that of bulk oil at low concentrations under laboratory conditions. The castor (SLNs) was the most efficient one for controlling *O. surinamensis*, *S. oryzae*, and *T. castaneum* adults compared to the neem (SLNs), and showed high insecticidal efficacy and protection from emerging adults to the wheat grains until the storage period of 3 months. The results indicated that these novel systems could be used successfully as an alternative to synthetic chemical insecticides in stored wheat as a protectant and can be used in integrated pest management programs for control of different stored product insect pests.

5. Conflicts of interest

The authors declared no potential conflicts of interest.

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7. References.

[1] Grenier, A. M., Pintareau, B. and Nardo, P. (1994).Enzymatic variability in three species of Sitophilus (Coleoptera : Curculionidae). J. Stored Product Res., 30: 201-213.

[2] Hagstrum, D., Klejdysz, T., Subramanyam, B. and Nawrot, J. (2013). Ciate of Cereal Chemists International, Inc. USA, St. Paul, Minnesota, MN 3– 198.

[3] Akhtar M., Raza A.M, Iram N., Chaudhry M.I, Azeem W. (2015). Effect of infestation of *Sitophilus oryzae* L. (Coleoptera: Curculionidae) on protein quality of rice under storage conditions. Int .J. Agric. Appl. Sci.,7(1):43–45.

[4] EL-Kashap, A. M. Y. (1996). Effect of some pesticides on some stored grain pests and on the quality of some grain products. M.Sc. Thesis, Fac. Agric.Mansoura Univ. Egypt.

[5] Skourti, A., Kavallieratos, N. G., and Papanikolaou, N. E. (2020).How is fitness of *Tribolium castaneum* (Herbst)(Coleoptera: Tenebrionidae) affected when different developmental stages are exposed to chlorfenapyr?. Insects, 11(8), 542.

[6] Edde, E. and IAmatobi C. (2003). Seed coat has no value in protecting cowpea seed against attack by *Callosobruchus maculatus* (F.). 39: (1), 1-10.

[7] Đukic, N., Andric, G., Ninkovic, V., Golic, M.P., Kljajic, P., Radonjic, A. (2020).Behavioural responses of *Tribolium castaneum* (Herbst) to different types ofuninfested and infested feed. Bull. Entomol. Res. 110, 550–557.

[8] Hashem, M.Y., Ahmed, S.S., ElMohandes, M.A, and Gharib M.A. (2012).Susceptibility of different life stages of saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) to modified atmospheres enriched with carbon dioxide. J . Stored Product Res., 48: 46-51.

[9] Nawrot, J. (2002). Owady – szkodniki magazynowe. Wyd. Themar, Warszawa, 149 pp.

[10] Laszczak-Dawid, A., Kosewska A., Nietupski M., and Ciepielewska D. (2008). Rozwój spichrzela surynamskiego (*Oryzaephilus surinamensis* L.) na ziarnie pszenicy ozimej. Prog. Plant Prot./Post. Ochr. Roślin 48 (3): 908–912.

[11] Klys, M. (2012).Emigration activity of the Saw-toothed grain beetle *Oryzaephilus surinamensis* L.(Coleoptera: Silvanidae) in various environmental temperatures. J. Plant Protection Res., 52(4).

[12] Mowery, S. V., Mullen, M. A. Campbell, J. F. and Broce, A. B. (2002). Mechanisms underlying sawtoothed grain beetle (Oryzaephilus surinamensis [L.]) (Coleoptera: Silvanidae) infestation of consumer food packaging materials. J. Econo. Entomo., 95: 1333–1336.

[13] Tapondjou, A., Adler, C., Fontem, D., Bouda, H. and Reichmuth, C. J. R. (2005). Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum*. J. Stored Prod. Res., 41(1):91-102.

[14] Abdel-Aziz, N.F., El-Bakry, A.M., Metwally, N.S., Sammour, E.A. and Farrag, A.R.H. (2018).Insecticidal efficiency of some green-based formulations on *Spodoptera littoralis* and their side effects on albino rats. Asian J. Crop Sci., 10 (4): 198–206.

[15] Werdin-Gonzalez, J.O., Gutiérrez, M.M., Ferrero, A.A. and Band, B.F. (2014). Essential oils nanoformulations for stored-product pest control-Characterization and biological properties. Chemosphere, 100:130–138.

[16] Norten E. (1999). Neem: India's miraculous healing plant. Inner Traditions/Bear and Co, www.Inner Traditions.com.

[17] Forim, M. R., D. S. M. F. Fernandes, J. B. Fernandes, and P. C. Vieira. (2014). Processo de obtenção de nanopartículas biopoliméricas contendo óleo e extratos de Azadirachta indica a. juss (neem), nanopartículas biopoliméricas e micropartículas em pó Google Patents. http://www.google.co.ve/patents/WO2014113860A1

[18] Ilesanmi, J.O. and Gungula, D.T. (2013).Quality attributes of cowpea seeds stored with neem and Moringa seed oils. World J. Agric. Sci., 9:155-160.

[19] Guet, G. (2002).Applications de nim comme insecticide (nématicide en agriculture biologique). Alternative Agriculture, 54, 4-6.

[20] Ricci, F., Berardi, V. and Risuleo, G. (2009). Differential cytotoxicity of MEX: A component of neem oil whose action is exerted at the cell membrane level. Molecules, 14(1), 122–132.

[21] Gossé, B., Amissa, A. A., Adjé, F. A., Niamké, F. B., Ollivier, D. and Ito, Y. (2005).Analysis of components of neem (Azadirachta indica) oil by diverse chromatographic techniques. Journal of Liquid Chromatography and Related Technologies, 28(14), 2225–2233.

[22] Benelli, G., Bedini, S., Cosci, F., Toniolo, C., Conti, B. and Nicoletti, M. (2015).Larvicidal and ovideterrent properties of neem oil and fractions against the filariasis vector *Aedes albopictus* (Diptera: Culicidae): A bioactivity survey across production sites. Parasitology Research, 114(1), 227–236.

[23] Hallur, G., Sivramakrishnan, A. and Bhat, S. V. (2002).Three new tetranortriterpenoids from neem seed oil. J. Natural Products, 65(8), 1177–1179.

[24] Ismadji, S., Kurniawan, A., Ju, Y. H., Soetaredjo, F. E., Ayucitra, A. and Ong, L. K. (2012).Solubility of azadirachtin and several triterpenoid compounds extracted from neem seed kernel in supercritical CO2. Fluid Phase Equilibria, 336, 9–15.

[25] Momchilova, S., Antonova, D., Marekov, I., Kuleva, L., Nikolova-Damyanova, B. and Jham, G. (2007). Fatty acids, triacylglycerols, and sterols in neem oil (*Azadirachta Indica* A. Juss) as determined by a combination of chromatographic and spectral techniques. J. Liquid Chromatography and Related Technologies, 30(1), 11–25.

[26] Antwi, S., Martey, O.N.K., Donkor, K. and Nii-Ayitey Okine, L.K. (2009). Anti-diarrhoeal activity of Blighia sapida (Sapindaceae) in rats and mice. J Pharm Toxicol 4:117–125.

[27] Yeganeh, H. and Mehdizadeh M.R. (2004).Synthesis and properties of isocyanate curable mill able polyurethane

elastomers based on castor oil as a renewable resource polyol. Eur Polym. J.40(6):1233–1238.
[28] Manpreet, R.; Dhamija, H.; Prashar, B.; Sharma, S. (2012). Ricinus Communis L.–A Review. Int. J. Pharm.Tech. Res. 4 (4), 1706–1711.
[29] Perdomo, F. A., Acosta-Osorio, A. A., Herrera, G., Vasco-Leal, J. F., Mosquera-Artamonov, J. D., Millan-Malo, B., and Rodriguez-Garcia, M. E. (2013). Physicochemical characterization of seven Mexican *Ricinus communis* L. seeds & oil contents. Biomass and Bioenergy, 48, 17-24. http://dx.doi.org/10.1016/j.biombioe.2012.10.020.
[30] Velasco, L., Rojas-Barros, P. and Fernández-Martínez, J. M. (2005). Fatty acid and tocopherol accumulation in the seeds of a high oleic acid castor mutant. Industrial Crops and Products, 22(3), 201-206. http:// dx.doi.org/10.1016/j.indcrop .2004.11.002.
[31] Dorman, H.J.D. and Deans S.G. (2000).Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol., 88:308–316.
[32] Regnault-roger, C. (2013). Essential oils in insect control. In: Ramawat, K.G., Mérillon, J.-M. (Eds.), Natural Products. Springer, Berlin, Heidelberg, pp. 4087–4107.
[33] Saroukolai T. A., Nouri-Ganbalani G., RafieeDastjerdi H., and Hadian J. (2014). Antifeedant activity and toxicity of some plant essential oils to Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). Plant Protection Science 50, 207-216..
[34] Tatun, N., Vajarasathira, B., Tungjitwityakul, J., and Sakurai, S. (2014). Inhibitory effects of plant extracts on growth, development and α-amylase activity in the red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae). European Journal of Entomology, 111.
[35] Haridasan, P., Gokuldas, M. and Ajaykumar, A.P. (2017). Antifeedant effects of Vitex negundo L. leaf extracts on the stored product pest, *Tribolium castaneum* H. (Coleoptera: Tenebrionidae). International Journal of Pharmacy and Pharmaceutical Sciences 9(3):17-22.
[36] Moretti, M.D.L.; Sanna-Passino, G.; Demontis, S. and Bazzoni, E. (2002). Essential oil formulations useful as a new tool for insect pest control. AAPS. Pharm.Sci.Tech., 3 (2), article 13:1–11.
[37] Huang, Q., Yu, H. and Ru, Q. (2010).Bioavailability and delivery of nutraceuticals using nanotechnology. J. Sci., 75(1): 50-57.
[38] Mossa, A.T. H., Afia S. I., Mohafrash S. M. and Abou-Awad B.A. (2018).Formulation and characterization of garlic Allium sativum (L.) essential oil nanoemulsion and its acaricidal activity on erio- phyid olive mites (Acari: eriophyidae). Enviro. Sci. Poll. Res. 25:10526 – 10537.
[39] Neri-Badang, M. C., and Chakraborty, S. (2019). Carbohydrate Polymers as Controlled Release Devices for Pesticides. J. Carbohydr. Chem. 38 (1), 67–85.
[40] Kalia, A., Sharma, S. P., Kaur, H., and Kaur, H. (2020). Novel Nanocomposite-Based Controlled-Release Fertilizer and Pesticide Formulations: Prospects and Challenges, in Multifunctional Hybrid Nanomaterials for Sustainable Agri-Food and Ecosystems (Elsevier), 99–134.
[41] Kumar, S., Nehra, M., Dilbaghi, N., Marrazza, G., Hassan, A. A., and Kim, K. H. (2019). Nano-based Smart Pesticide Formulations: Emerging Opportunities for Agriculture. J. Controlled Release 294, 131–153. doi:10.1016/j .jconrel.2018.12.012.
[42] Nguyen, H. M., Hwang, I. C., Park, J. W., and Park, H. J. (2012). Photo protection for Deltamethrin Using Chitosan-coated Beeswax Solid Lipid Nanoparticles. Pest Manag. Sci. 68 (7), 1062–1068. doi:10.1002/ps.3268.
[43] Kashyap, P. L., Xiang, X., and Heiden, P. (2015). Chitosan Nanoparticle Based Delivery Systems for Sustainable Agriculture. Int. J. Biol. macromolecules 77,36–51. oi:10.1016/j.ijbiomac.2015.02.039
[44] Muller, R., Dingle,r A., Schneppe, T. and Gohla, S. (2000).Large scale production of solid lipid nanoparticles (SLNTM) and nanosuspensions (DissoCubesTM). In: Wise D, editor. Handbook of pharmaceutical controlled release technology. Boca Raton (FL): CRC Press. p. 359–376.
[45] Hosseinpour Jajarm, F., Moravvej, G., Modarres Awal, M., and Golmohammadzadeh, S. (2020). Insecticidal activity of solid lipid nanoparticle loaded by *Ziziphora clinopodioides* Lam. against *Tribolium castaneum* (Herbst, 1797)(Coleoptera: Tenebrionidae). Int. J. . Pest Management, 67(2), 147-154.
[46] Yang, F. L., Li, S. G., Zhu, F. and Lei, C. L. (2009). Structural characterization of nanoparticles loaded with garlic essential oil and their insecticidal activity against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), J. Agric. Food Chem., 57 :10156-10162.
[47] Asnawi, S.; Abd Aziz, A.; Abd Aziz, R. and Khamis, A.K. (2008). Formulation of geranium oil loaded solid lipid nanoparticles for mosquito repellent application. J. Chemi. A Natural Resources Engineering, 2: 90-99.
[48] Adel, M.M., Massoud, M.A., Mohamed, M.I., Abdel-Rheim, K.H. and El- Naby, S.S.A. (2018). New nano-biopesticide formulation of Mentha piperita L. (Lamiaceae) essential oil against stored product red flour beetle *Tribolium castaneum* herbs and its effect on storage. AdvCrop Sci Technol. 6(6):409–415.
[49] Broussalis, A. M., Ferraro G. E., Martino V. S., Pinzon R., Coussio J. D. and Alvarez J. C. (1999).Argentine plants as potential source of insecticidal compounds. J. Ethnopharmacol 67:219–223.
[50] Finney. D.J. (1971). Probit analysis. Cambridge University Press, UK. Cambridge, 333.
[51] Tapondjou, L.A., Adler,C., Bouda, H. and Fontem D. A. (2002). Efficacy of powder and 17 essential oil from Chenopodium ambrosioides leaves as post-harvest grain protectants 18 against six-stored product beetles. J. St. Prod. Res. 38, 395–402.
[52] Abbott, W. S., A method of computing the effectiveness, of an insecticide. J. Econ. Entomol, 18: 265-267(1925).
[53] Shidhaye, S.S., Vaidya, R., Sutar, S., Patwardhan, and A. Kadam, V.J. (2008). Solid lipid nanoparticles and nanostructured lipid carriers-innovative generations of solid lipid carriers. Current drug delivery, 5(4), 324-331.
[54] Haider, M., Abdin, S. M., Kamal, L., and Orive, G. (2020). Nanostructured lipid carriers for delivery of chemotherapeutics: A review. Pharmaceutics, 12(3), 288.
[55] Adel, M.M.; Atwa, W.A.; Hassan, M.L.; Salem, N.Y.; Farghaly, D.S. and Ibrahim, S.S. (2015). Biological Activity and Field Persistence of Pelargonium graveolens (Geraniales: Geraniaceae) loaded Solid Lipid Nanoparticles (SLNs) on Phthorimaea operculella (Zeller) (PTM) (Lepidoptera: Gelechiidae). International Journal of Science and Research, 4 (11): 514-520.
[56] Ibrahim, S. S., Salem, N. Y., Abd ElNaby, S. and Adel, M. M. (2021).Characterization of Nanoparticles Loaded with Garlic Essential Oil and Their Insecticidal Activity Against *Phthorimaea Operculella* (Zeller)(PTM)(Lepidoptera: Gelechiidae). International Journal of Nanoscience and Nanotechnology, 17(3), 147-160.
[57] Jesser, E., Yeguerman, C., Stefanazzi, N., Gomez, R., Murray, A. P. and Ferrero, A. A.,(2020).Ecofriendly Approach for the Control of a Common Insect Pest in the Food Industry, Combining Polymeric Nanoparticles and Post-application Temperatures. J. Agric. Food Chem. 68 (21), 5951–5958.
[58] Khanahmadi, M., Pakravan, P., Hemati, A., Azandaryani, M. N., and Ghamari, E. (2017).Fumigant Toxicity of *Artemisia haussknechtii* Essential Oil and its Nano-Encapsulated Form. Pharma 2, 1776–1783.
[59] Massoud, M.A., Adel, M.M., Zaghloul, O.A., Mohamed, M.I.E. and Abdel-Rheim, K.H. (2018). Eco-friendly nano-emulsion formulation of *Mentha piperita* against stored product pest *Sitophilus oryzae* . Adv. Crop Sci. Tech., 6: 404.
[60] Werdin-González, J. O., Stefanazzi, N., Murray, A. P., Ferrero, A.A. and Fernández Band, B. (2015). Novel nanoinsecticides based on essential oilsto control the German cockroach. J. Pest Sci. 88, 393−404.

[61] Margulis-Goshen, K. and Magdassi, S. (2012). Nanotechnology: An Advanced Approach to the Development of Potent Insecticides. In: Ishaaya I, Horowit,z AR, Palli SR. (eds.) Advanced Technologies for Managing Insect Pests. Dordrecht: Springer;. 295-314.
[62] Dimetry, N.Z. and Hussein H.M. (2016). Role of nanotechnology in agriculture with special reference to pest control. Int. J. Pharm.Tech .Res., 9:121–144.
[63] Lai, F., Wissing, S.A., Muller, R.H. and Fadda, A.M. (2006). *Artemisia arborescens* L essential oil-loaded solid lipid nanoparticles for potential agricultural application: preparation and characterization. Aaps Pharmscitech. 7(1): 1–10.
[64] Sabbour, M.M. and Abd-El-Aziz, S.E. (2016). Efficacy of three essential oils and their nano-particles against *Sitophilus granarius* under laboratory and store conditions. J. Entomol. Res., 40(3): 229 –234.
[65] Campolo, O., Malacrinò A. and Zappalà L., Fumigant bioactivity of five Citrus essential oils against *Tribolium confusum*," Phytoparasitica, 42(2), 223–233(2014).
[66] Bhargava, M.C. and Meena, B.L. (2002). Efficacy of some vegetable oils against pulse beetle, *Callosobruchus chinensis* (Linn.) on cowpea, *Vigna unguiculata* (L.). Ind. J. Plant Prot., 30(1): 46–50.
[67] Singh, V. and Yadav, D.S. (2003). Efficacy of different oils against pulse beetle, *Callosobruchus chinensis* in green gram, *Vigna radiata* and their effect on germination. Indian J. Entomol. 65(2): 281–86.
[68] Paceco, I.A., de Castro, M.F.P.P.M., de Paula, D.C., Lourencao, A.L., Bolonhezi, S. and Barbieri, M.K. (1995).Efficacy of soybean and castor oils in the control of *Callosobruchus maculatus* (F.) and *Callosobruchus phaseoli* (Gyllenhal) in stored chick peas. J. Biol. Sci. 9(2): 175–79.
[69] Harish, G., Nataraja, M.V., Holajjer, P., Thirumalaisamy, P.P., Jadon, K.S., Savaliya, S.D., Padavi, R.D., Koradia, V.G. and Gedia, M.V. (2014). Efficacy and insecticidal properties of some essential oils against *Caryedon serratus* (Oliver)-a storage pest of groundnut. J. Food Sci. Technol., 51(11): 3505–3509.
[70] Luiz de Oliveira, J., Ramos Campos, E. V. and Fraceto, L. F. (2018). Recent Developments and Challenges for Nanoscale Formulation of Botanical Pesticides for Use in Sustainable Agriculture. J. Agric. Food Chem.,66:8898−8913.
[71] Souguir, S.; Chaieb, I.; Ben Cheikh, Z. and Laarif, A. (2013). Insecticidal activities of essential oils from some cultivated aromatic plants against *Spodoptera littoralis* (Boisd.). J. Plant Prot. Res., 53 (4): 388-391.
[72] Khandelwal, N., Barbole, R. S., Banerjee, S. S., Chate, G. P.;Biradar, A. V.; Khandare, J. J. and Giri, A. P. (2016). Budding trends in integrated pest management using advanced micro-and nano-materials: Chal-lenges and perspectives. J. Environ. Manage., 184:157−169.
[73] Mossa, A.T.H. (2016). Green pesticides: Essential oils as biopesticides in insect-pest management. J. Environ. Sci. Technol. 9: 354–378.
[74] Rai, M., Kon, K., Ingle, A., Duran, N., Galdiero, S., and Galdiero, M. (2014).Broad-spectrum Bioactivities of Silver Nanoparticles: the Emerging Trends and Future Prospects. Appl. Microbiol. Biotechnol. 98 (5), 1951–1961.
[75] Athanassiou, C. G., Arthur, F. H., & Hartzer, K. L. (2018). Efficacy of low temperatures for the control of all life stages of *Plodia interpunctella* and *Liposcelis bostrychophila*. J. Pest Sci., 91(4), 1363– 1369.
[76] Alzogaray, R. A., and Zerba, E. N. (2017). Rhodnius prolixus Intoxicated. J. Insect Physiol. 97, 93–113.