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Toxicity and Physiological Effect of Lemongrass Essential Oil Nano-capsules on the Greater Wax Moth Larvae *Galleria mellonella* L. (Lepidoptera: Pyralidae)

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ABSTRACT

The current study focuses on the insecticidal activity of a nanoencapsulated lemongrass essential oil (LEO) *(Cymbopogon citratus*) using chitosan as a natural polymer encapsulate, against the larvae of greater wax moth *(Galleria mellonella)* under laboratory conditions. The study also examines various biological and physiological changes. Gas chromatography-mass spectrometry (GC/MS) analysis of LEO revealed that citral is the primary compound, representing by 70.33% of the total essential oil composition followed by myrcene, geranyl acetate and cis-verbenol. Dynamic light scattering and scanning electron microscopy were used to characterize the preparation of the nanocapsules. Results indicated that treatment over three weeks had satisfactory effects on biological aspects including larval mortality, larval weight, and some morphological deformities. Specifically, 46.59% of the treated larvae were lost during their development to adulthood at the lowest concentration, while the larval loss was recorded at 92.05% at the highest concentration, with an LC_{50} of 49.89 ppm. The study demonstrated a significant reduction in larval weight and various malformations. Treated larvae exhibited a large increase in chitinase activity, while a significant suppression of the enzymatic activity of the protease, acetylcholinesterase, acid, and alkaline phosphatase was observed. These results suggest that LEO-nanocapsules cause biological and physiological disturbances in *G. mellonella* larvae and may be potentially integrated into pest management IPM programs.
Keywords: Biopesticides; Chitosan nano-capsules; *Galleria mellonella*; Lemongrass oil; Pest control; Toxicity assessment; Sustainable agriculture.

INTRODUCTION

Maintaining food sources is one of the world's most important goals. Scientists continuously look for effective ways to ensure food security and control pests. Insects are among the most dangerous pests that affect economic crops and cause direct damage. Conventional agricultural approaches, including integrated pest management, have proven unefficiency. Furthermore, using chemical pesticides not only diminishes soil fertility, undermines pest resistance, and eradicates natural enemies but also contributes to environmental pollution, biodiversity depletion, and risks to human health (Yousef *et al*., 2023).

Honeybees play a vital role in the ecosystem by facilitating pollination, critical for sustaining food production and generating income for the agricultural sector. Honeybee colonies endure significant losses due to infestations by various insects. These adversaries undermine the colony, reduce honey output and pollination (Naz *et al*., 2022). *Galleria mellonella* a member of the Pyralidae family of lepidopterans, is the greater wax moth, one of the world's most destructive and commercially significant wax pests. This pest has wreaked havoc on wax, one of the most valuable and essential byproducts of beekeeping that finds application in the pharmaceutical and cosmetics sectors. The larval stage of *Galleria mellonella*, which has the longest life cycle of all developmental stages, constructs a silk-lined feeding tunnel within the honeycomb and subsists on pollen, wax, and brood cocoons. This results in significant depletion of the honeycomb and, ultimately, the abandonment of honeybees from the compromised colonies. The larvae destroy numerous combs and attack the wax foundation annually. Colonies targeted by this moth may experience malnutrition, disease, the demise of the queen, or widespread worker bee death due to pesticide poisoning. Sixty to seventy percent of the economic losses incurred by beekeepers in developing nations can be attributed to these moths. Among the most significant obstacles to enhancing beekeeping industries, such as royal jelly, honey, and wax, is the identification and implementation of mitigation strategies for honeybee diseases and pests (Komala and Seram, 2020).

Biopesticides are one of the recent procedures used for resisting insects such as natural products extracted from plants that provide crop protection alternatives around the world (Fenibo *et al*., 2022) Lemongrass oil extracted from *Cymbopogan citratus* L. possessed antioxidant and anti-inflammatory activities. Its active components showed insecticidal, herbicidal, and nematicidal properties in addition to fungicides, bactericides, and repellent properties. Citral is the major active agent that has a great potential application in food products and drugs (Silva *et al*., 2023). Essential oils (EOs) possess (such as low solubility in water, rapid evaporation, and degradation of active constituents when exposed to high temperature,

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moisture, and air). These factors pose significant challenges to the investigation of their biological activities and their implementation in crops (Cui *et al.*, 2020). Meanwhile, the greater wax moth, *G. mellonella* is considered one of the best models used in research because it is easily reproduced under lab conditions (temperature and humidity), has simple anatomical structures, a short lifespan, and easy rearing on an artificial diet. It also has the benefit of being cheap to propagate in a laboratory. As a result, it is the perfect model to use in extensive research studies (Singkum *et al.*, 2019).To solve these problems, scientists create alternative formulations using nano-techniques. Nano-insecticides have various advantages that enhanced the formulation's solubility in water and stability to improve insecticidal efficacy. It must be easy to prepare, affordable, less damaging either to the environment or non-target animals, non-toxic, and non-accumulating in the food chain. Moreover, it mustn't affect the flavor, texture, smell of food, and be easily applied (Yadav, 2022). Nano-insecticides increased target specificity, the physicochemical stability and efficacy of the active ingredients are improved, the residual problem is reduced, and the action of the active ingredients is maximized. It also reduces the chemicals used, improves plant protection, and decreases harmful residues (Deka *et al*., 2021). In order to protect these formulations from degradation, encapsulating pesticides in a shell material such as chitosan nanoparticles is used; the thin polymeric layers increased insecticidal activity, provided controlled release of formulations at a slower rate, and could protect active ingredients from degradation caused by environmental factors (Ayyaril *et al*., 2023).

The current study aimed to assess the insecticidal activity of a nanoformulation of lemongrass oil encapsulated in chitosan against larvae of the *G. mellonella* parasite. Additionally, a few biological and biochemical factors were investigated in a laboratory setting, strengthening the notion that employing nanotechnology to manage insect pests is an outstanding and environmentally sustainable method of controlling insects in green agriculture.

MATERIALS AND METHODS

Plant materials and chitosan source

Approximately two kilograms of lemongrass leaves were collected from the Faculty of Pharmacy farm at Cairo University in April 2019. Plant specimens were carefully prepared and deposited in the herbarium of the Desert Research Center (DRC) in Cairo, Egypt. This repository serves as a valuable resource for the verification and further study of the collected plant samples. Medium molecular weight chitosan was obtained from Pratap Chemical Industries Pvt. Ltd., which is derived from shrimp shells and has a minimum of 85% deacetylation (India).

Extraction of lemongrass essential oil (LEO)

Lemongrass essential oil (LEO) was extracted from air-dried lemongrass aerial parts through hydro-distillation (El-Gendy and Sakla,2022, in a specially designed Clevenger apparatus at the central laboratory of the Desert Research Center (DRC) in Cairo, Egypt. The oil was stored at 4°C in glass containers with an amber tint that were hermetically sealed (Meyer-Warnod, 1984).

Identification of LEO chemical components

Using a Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a direct capillary column TG-5MS (length: 30 m; diameter: 0.25 mm; film thickness: 0.25 µm), the chemical composition of the LEO was determined through GC/MS analysis. The column oven temperature was initially set at 50°C, then increased to 230°C at a rate of 5°C/min and held for two minutes. Following this, the temperature was raised to 290°C at a rate of 30°C/min and maintained for an additional two minutes. Temperatures were maintained at a constant 250°C for the injector and 260°C for the MS transfer line. The carrier gas, helium, flowed steadily at a rate of 1 milliliter per minute. After three minutes, one microliter of diluted samples was automatically administered. The temperature of the ion source was then lowered to 200°C. The components were identified by matching their retention times and mass spectra with those stored in the WILEY 09 and NIST 11 mass spectral databases (Adams, 2001).

Preparation of LEO encapsulated in chitosan-nanoparticles

Chitosan-nanoparticles (CNPs) containing essential oils (EO) were prepared according to a procedure of Ferreira *et al.* (2019) with slight modifications. To prepare a solution containing 0.5% chitosan (w/v), chitosan was dissolved in a 2% acetic acid solution and stirred overnight at room temperature using a magnetic stirrer. Polysorbate 80 was then added to the chitosan solution while stirring continuously at 45 ^{o}C for two hrs to achieve a surfactant concentration of 1%. Oil-in-water emulsions were produced by gradually adding varying volumes of EO to the chitosan solution while cooling it to room temperature.

Characterization of LEO-nanocapsules

Dynamic light scattering (DLS)

Particle size spreading of the different nano-composites dispersed in deionized distilled water was examined by DLS (Nicomp N3000, particle size analyzer) at central lab of Desert Research Center. Cairo, Egypt. Polydispersity-Index (PDI) was calculated by the following equation:

$$(PDI) = M_w / M_n$$

Where M_w is the average weight of molar mass; M_n is the number average molar mass.

Scanning electron microscope (SEM)

The Quanta FEG 250 scanning electron microscope (SEM) was used to explore the surfaces of the nanoparticles (FEI Company, Hillsboro, Oregon, USA). Five milliliters of the material were freeze-dried and affixed to SEM stubs. The scanning electron microscope (SEM) was operated under the following

conditions: a working distance of 10.1 mm, an in-lens detector, and an excitation voltage of 20 kV at the Central Lab of the Desert Research Center (DRC) in Cairo, Egypt.

In vitro propagation of *Galleria mellonella*: A model organism in pest control applications

Economically viable *Galleria mellonella* larvae, which recently hatched, were obtained from the Economic Entomology Unit of the Plant Protection Department at the Desert Research Center (DRC). The mass rearing of wax moth larvae was conducted using an artificial diet, as detailed in the report by Metwally *et al*. (2012), which consisted of 130 g of milk powder, 70 g of yeast powder, 350 g of wheat flour, 200 g of corn flour, 100 ml of honey, and 150 ml of glycerin. All dry components were mixed together, after which the honey and glycerin were added to prepare the culture medium fresh. Eggs of *G. mellonella* were placed in glass containers (approximately 3 liters in size) filled with a fresh culture medium to a thickness of 5-7 cm. The containers were then covered with plain paper secured with two rubber bands. The rearing containers were incubated under controlled conditions in complete darkness at a temperature of 28-30°C and a relative humidity of 60-70% (Firacative *et al.*, 2020).

Evaluation of the insecticidal activity of the LEO loaded in CNPS

According to the quantity of lemongrass oil, five formulations of LEO nano-capsules were prepared, and different concentrations of LEO (40, 80, 160, 320, and 640ppm) were loaded in CNPS while maintaining constant chitosan concentration (0.5g). To assess the biological and toxicological impacts, 20g of the synthetic media was weighed and mixed thoroughly with 5ml of each concentration (equivalent to 0.5ml per g of diet). Such 20g medium was placed in ten glass tubes each containing 2g. After allowing each tube to dry for one hour without being covered, five newly hatched larvae were carefully inserted into each tube using a fine toothbrush. Subsequently, the tubes were covered with cotton wool (50 newly hatched larvae were used per concentration). An untreated diet was applied to function as a control. The larval mortality (LC_{50}), and larval weight were determined, and tubes were checked every week to record results till prepupal stage.

Biochemical studies

Possible changes that may occur in the hemolymph of *G. mellonella* larvae as a result of their feeding on the nanocapsules were determined at fifth larval instar. Three replicates of each biochemical test were utilized, and chemical analyses were conducted with a UV-Vis Spectrophotometer (Thermo-UV-Visible Spectrophotometer USA supplied with Thermo Scientific VISION Pro software) at Plant Protection Institute Central Lab.Dokki, Cairo, Egypt.

Preparation of samples for biochemical analysis

For biochemical investigations, number of fresh samples of treated larvae was homogenized in distilled water (1g insect body/5ml water) after 30 days of treatment. This was achieved by pulverizing the homogenates in a mortar for 3 min and centrifuged at 3000 rpm for 15 min using a cooling centrifuge (at Plant Protection Institute Central Lab.Dokki, Cairo, Egypt). For subsequent utilization, the supernatants were transferred to fresh micro-tubes and frozen at 20°C. A control experiment utilizing the supernatant of untreated larvae was established. The inhibition or activation percentage was calculated as described by Adel *et al*., (2010). Inhibition or activation (%) is calculated as following:

$$\textit{Inhibition or activation}\ (\%) = \frac{\textit{Control} - \textit{Treated}}{\textit{Control}} X\ 100$$

Assessment of acid and alkaline phosphatase Activities

The quantification of acid phosphatase (ACP) and alkaline phosphatase (ALP) was conducted following the methodology outlined by Powell and Smith (1954). The absorbance corresponding to the liberation of phenol during the enzymatic hydrolysis of disodium phenyl phosphate was measured spectrophotometrically at 405 nm. This version improves clarity and flow while maintaining the original meaning.

Assessing Acetylcholinesterase (AChE) Activity

Neurotoxicity can be assessed by analyzing Acetylcholine esterase (AchE) activity. The measurement was conducted in accordance with the methodology outlined by Simpson *et al*. (1964), employing acetylcholine bromide (AChBr) as the substrate. The supernatant of the centrifuged mixture was analyzed spectrophotometrically at 515 nanometers.

Determination of protease enzyme activity

The protein activity was evaluated utilizing a modified approach outlined by Tatchell *et al*. (1972). The activity was determined by analyzing the rise of free amino acids isolated from the substrate protein (Albumin) after one hr incubation at 30 ^{o}C.

Determination of chitinase activity

To determine chitinase activity, the procedure described by Ishaaya and Casida (1974) was followed. This method is based on the development of color, which can be measured spectrophotometrically at 550 nm. The free aldehydic groups of hexosamines released during chitin digestion are quantified using 3,5-dinitrosalicylic acid (DNS) reagent. The DNS reagent reacts with the free aldehydic groups, allowing for the measurement of chitinase activity through the absorbance of the resulting colored solution.

Statistical analysis

The mortality data were analyzed using a two-way ANOVA with two factors: Time and concentration. Mortality percentages were adjusted using Abbott's formula (Abbott, 1925). Significant differences between treatments were assessed with Duncan's multiple range test ($p \leq 0.05$) (Duncan, 1955). The median lethal concentration (LC_{50}) values were estimated using the Biostat version 5 Analyst software, employing probit analysis (Finney, 1971). A one-way ANOVA was conducted to evaluate the effect of LEO nano-capsules on larval weight. Enzyme activity data were analyzed using an independent t-test, employing SPSS statistical

software version 11.0 (Dytham, 2011). A *p*-value ≤0.05 was considered indicative of statistical significance.

RESULTS

Chemical composition of LEO

The chemical composition of oil obtained, from extracted lemon grass leaves, was identified using GC/MS. Data represented in Table (1) shows 14 main components. The major components were citral, consisting of α-citral (geranial) at 39.76% and β-citral (neral) at 30.57%, totaling 70.33%. Other significant components included myrcene, geranyl acetate, cis-verbenol, geraniol, neryl acetate, verbenol, linalool, citronella, caryophyllene, D-limonene, nerol, and α-farnesene, each present at percentages lower than one.

Characterization of LEO-nanocapsules

Dynamic light scattering

In photon correlation spectroscopy, the particle size distribution is defined by the parameter polydispersity index (PDI), a dimensionless number derived from the autocorrelation function. As shown in Figure (1) DLS analysis of nanoformulation demonstrated that the nanoparticles' diameter is 253.9 nm, and the variance polydispersity index (PDI) had a value of 0.415.

Scanning electron microscope

The micrograph of the nanocapsules of SEM is shown in Figure (2). The SEM analysis confirmed that most particles were aggregated with semispherical shape structures. The sample was observed at different fields, and the mean particle size ranged between 204 and 230 nm from the acquired micrographs.

Effect of LEO loaded in CNPS on some biological aspects of *G. mellonella*

Different parameters were recorded, such as larval mortality, larval weights, and some physiological traits**.** Results showed that LEO nanocapsules significantly affected larval development and survival; These adverse effects were concentration and time dependent manners. The data tabulated in Table (2) revealed a proportional relationship between LEO nanocapsule concentrations and larval mortality percent. There is a gradual increase in the cumulative larval mortality percent by raising the applied concentrations in the larval diet within 3 weeks.

The minimum mortalities of wax moth larvae after one week of treatment were recorded by the lowest concentration of 40 ppm (13.22±0.33%) and the larval death increased gradually till reached 28.84±0.27% at a higher concentration used. In similar pattern, the second week recorded larval mortalities of 28.73±1.21% and ranged up to 58.19±1.43% among the different tested concentrations. After three weeks of treatment, at the minimum concentration, approximately 46.59% of the individuals died as larvae and only 53.41% succeeded in developing into pupae. Meanwhile, the higher concentrations showed maximum larval mortality, which recorded 82.71±1.98% and 92.05±2.23% at concentrations 320 and 640ppm, respectively. The findings indicated that the lethal concentration (LC_{50}) for larval mortality was determined to be 49.89 ppm. This level signifies a high toxicity associated with the

Table (1): Chemical composition (%) of the lemongrass EOs (*C. Citratus*) as ascertained via GC-MS

Rt: retention time, % = Percentage of the component in total oil.

Peak No.	RT (min)	Compound name	Classification	Molecular Weight	Molecular formula	Area %
1	7.43	D- Limonene (4R)-1-methyl-4-(1-methylethenyl)cyclohexene	Monoterpene	136.23	$C_{10}H_{16}$	**0.23**
2	7.52	Linalool (1,6-Octadien-3-Ol,3,7-Dimethyl)	Terpene alcohol	154.25	$C_{10}H_{18}O$	**0.86**
3	8.73	Myrcene (7-Methyl-3-methylideneocta-1,6-diene)	Monoterpene	136.23	$C_{10}H_{16}$	**8.05**
4	9.22	Verbenol (4,6,6-Trimethylbicyclo[3.1.1]hept-3-en-2-ol)	Monoterpene alcohol.	152.24	$C_{10}H_{16}O$	**1.90**
5	9.71	Cis- Verbenol (4,6,6-Trimethylbicyclo[3.1.1]hept-3-en-2-ol)	Monoterpene alcohol.	152.24	$C_{10}H_{16}O$	**5.04**
6	11.27	α-Citral (Geranial) (3,7-Dimethyl-2,6-octadienal)	Monoterpene aldehyde	152.24	$C_{10}H_{16}O$	**39.76**
7	12.20	Citronellal (3,7-dimethyloct-6-enal)	Monoterpenoid	154.25	$C_{10}H_{18}O$	**0.59**
8	12.66	β- Citral (Neral) (3,7-Dimethyl-2,6-octadienal	Monoterpene aldehyde	152.24	$C_{10}H_{16}O$	**30.57**
9	12.92	Geraniol (E) 3,7-dimethylocta-2,6-dien-1-ol)	Monoterpenoid	152.24	$C_{10}H_{16}O$	**3.73**
10	13.18	Neryl acetal(Z)3,7-Dimethylocta-2,6-dien-1-yl acetate)	Terpenoid	196.29	$C_{12}H_{20}O_2$	**2.06**
11	14.89	Geranyl acetate (2E)3,7-Dimethylocta-2,6-dien-1-yl acetate	Monoterpenoid	196.29	$C_{12}H_{20}O_2$	**6.61**
12	15.17	Nerol (2Z)-3,7-Dimethylocta-2,6-dien-1-ol	Monoterpenoid	154.25	$C_{10}H_{18}O$	**0.12**
13	15.45	Caryophyllene (1R,4E,9S)-4,11,11-Trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene	Sesquiterpenes	204.36	$C_{15}H_{24}$	**0.34**
14	15.88	α- Farnesene (3,7,11-trimethyl-1,3,6,10-dodecatetraene)	Sesquiterpenes	204.36	$C_{15}H_{24}$	**0.14**

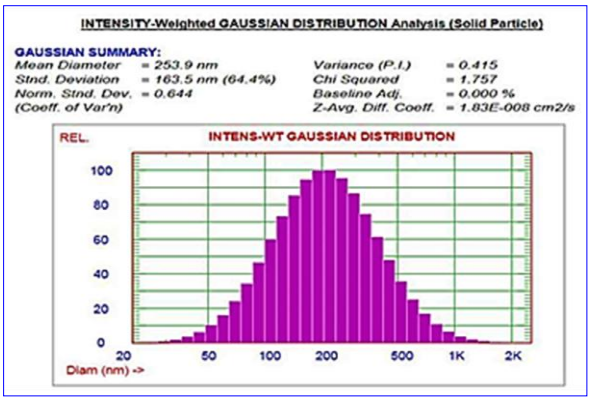

Figure (1): Particle size distribution of the Lemongrass essential oil loaded in chitosan nanoparticles with oil ratio 320ppm in nanocapsule.

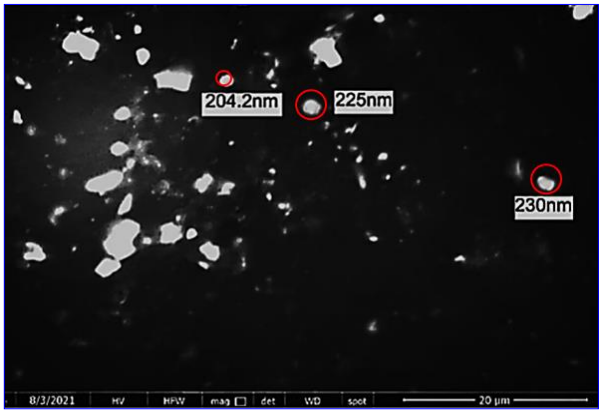

Figure (2): SEM image of Lemongrass essential oil loaded in chitosan-nanoparticles with oil ratio 320 ppm in nanocapsule.

consumption of LEO nano-capsules, as the results showed that these nano-capsule displayed significantly potent insecticidal effects on wax moth larvae, achieving over 90% mortality at elevated concentrations. As illustrated in Table (2), when compared to the control groups, larvae treated with higher concentrations exhibited a notable reduction in weight. Specifically, larvae exposed to 40 ppm measured approximately 0.199 ± 0.46 g. At 80 ppm and 160 ppm, the weights decreased further to about 0.1607 ± 0.32 g and 0.1475 ± 0.68 g, respectively, indicating a decline from the weights recorded in the control group.

The data analysis demonstrated a significant reduction in larval weight that was dependent on the concentration of the treatment. Results from the factorial ANOVA highlighted a significant interaction between the concentration of LEO nanocapsules and the weight of the larvae. Those treated with higher doses (320 - 640 ppm) resulted in smaller larvae (Fig. 3A). Additionally, the data confirmed observations of malformations, including darkening of the cuticle (Fig. 3B). Furthermore, the few larvae that successfully matured into adults were notably smaller, exhibiting clearly deformed wings and were unable to mate or reproduce (Fig. 3C). These records raised the impact value of LEO nanocapsules as nano-insecticide due to their toxic potency, which caused mortality and inhibition of larval development. The reduction in the larval weight may be a consequence of several physiological disturbances.

Biochemical studies

The significant influence of different concentrations on the enzymatic activity of larval specimens reveals the underlying biochemical disturbances. To clarify the mechanisms through which LEO causes toxicity, examination of its effects on a range of enzyme activities were carried out.

Alkaline and acid phosphatase enzymes

The provided table (3) summarizes the results of an experiment investigating the activities of alkaline and acid phosphatase in larvae. The data revealed that the control group exhibited higher mean activities for both alkaline phosphatase (186.34 µg /min/ml) and acid phosphatase (140.79 µg /min/ml) compared to the treated group. Specifically, the treated group showed a marked reduction in alkaline phosphatase activity (to 118.17 µg /min/ml) and acid phosphatase activity (to 74.94 µg /min/ml). This suggests that the treatment applied to the larvae may have a significant inhibitory effect on the enzymatic activities of these phosphatases. Meanwhile, the calculated inhibition percentages indicate that the treatment resulted in a 36.58% decrease in alkaline phosphatase activity and a 46.77% decrease in acid phosphatase activity. The higher inhibition of acid phosphatase suggests that the treatment may have a more pronounced effect on this enzyme compared to alkaline phosphatase. This differential impact could be explored further to understand the biochemical mechanisms involved.

Acetylcholinesterase (AchE) enzyme activity

For the activity of AchE (Table 4), the control group exhibited a mean acetylcholine esterase activity of 69.93 ± 0.85 µg Acetylcholine-bromide/min/ml, while the treated group showed a significantly ($p \leq 0.05$) reduced activity of 25.74 ± 1.59 µg Acetylcholine-bromide/min/ml. The 63.19% inhibition observed in the treated group indicates a substantial decrease in enzyme activity, suggesting that the treatment effectively inhibited acetylcholine esterase function in the larvae.

Protease Activity

For protease activity, the control group had a mean value of 239.56 ± 0.82 µg alanine/min/ml, whereas the treated group displayed a reduced mean of 180.95 ± 1.12 µg alanine/min/ml. The 24.68% inhibition in protease activity suggests that the treatment has a moderate inhibitory effect on this enzyme, which may impact protein digestion and metabolism in the larvae (Table 4).

Chitinase Activity

On the contrary for chitinase activity (Table 4), the control group showed a chitinase activity of 76.92 ± 1.23 µg NAGA/min/ml, while the treated group exhibited an increased activity of 128.71 ± 1.07 µg NAGA/min/ml. The 67.32% increase in chitinase activity in the treated group is notable and may indicate an adaptive response to the treatment, enhancing the

Table (2): Larval mortality rates and weight changes of *Galleria mellonella* in response to varying concentrations of LEO nano-capsules.

Concentration (ppm)	Larval mortality (%)			Weight of larvae (mg)
	1st week	2nd week	3rd week	
Control	0.00±0.00 ^{k}	0.00±0.00 ^{k}	2.00±0.00 ^{k}	226.10±0.25^{f}
40	13.22±0.33 ^{j}	28.73±1.21 ^{gh}	46.59±1.79 ^{ef}	199.20±0.46^{e}
80	18.34±0.35 ^{ij}	35.86±1.15 ^{fg}	60.57±2.65 ^{d}	160.73±0.32^{d}
160	20.21±0.39 ^{i}	42.66±1.09 ^{f}	72.18±2.17 ^{c}	147.50±0.68^{c}
320	25.08±0.38 ^{hi}	51.94±1.37 ^{e}	82.71±1.98 ^{b}	113.07±0.61^{b}
640	28.84±0.27 ^{gh}	58.19±1.43 ^{de}	92.05±2.23 ^{a}	58.37±0.63^{a}
LC_{50}		49.89		
F		137.11		2302.656
p-value		≤0.05		≤ 0.05

Data are in mean ±SE, Mean of three replicates, each set up with 50 larvae. Values followed by the same letter per column are not significantly different at $p \leq 0.05$ according to Duncan Multiple tests.

Figure (3): *G.mellonella* treated with nanocapsules of lemongrass oil (LEO). A, Weight reduction of treated larva with the highest concentration used (640ppm). B, Malformations in treated larvae with conc. 80, 160 and 320 ppm. C, Morphological deformations in adults developed from treated larvae nanocapsules with conc. 80 and 160 ppm.

Table (3): Comparison of enzymatic activity and inhibition rate of phosphatases in treated vs. control Larvae of *G.mellonella.*

Groups of tested larvae	Alkaline phosphatase		Acid phosphatase	
	(µg phosphate /min/ml)	Inhibition (%)	(µg phosphate-/min/ml)	Inhibition (%)
Control	186.34 ± 1.11	--	140.79 ± 0.91	--
Treated	118.17 ± 1.45	36.58	74.94 ± 1.17	46.77
F	3.842		3.843	
t	116.882		111.798	
***p*-value**	0.0001		0.0001	

Data are in mean ±SE of three replicas.

Table (4): Effect of LEO nanocapsule on AchE, protease and chitinase activities in treated larvae of *G. mellonella*.

Groups of tested larvae	Acetylcholine esterase		Protease		Chitinase	
	(µg Acetylcholine-bromide/min/ml)	Inhibition (%)	(ug alanine /min/ml)	Inhibition (%)	(µg NAGA/ min/ml)	Increase (%)
Control	69.93±0.85	--	239.56±0.82	--	76.92±1.23	--
Treated	25.74±1.59	63.19	180.95±1.12	24.68	128.71±1.07	67.32
F	3.843			3.842		3.843
t	75.024			99.506		87.927
***p*-value**	0.0001			0.0001		0.0001

Data are in mean ±SE of three replicas.

larvae's ability to degrade chitin, which could be beneficial for their growth or development.

DISCUSSION

The greater wax moth, *G. mellonella*, a prominent apiary pest, infests the high-value beekeeping enterprise. This encapsulates the difficulty associated with regulating these perilous pests with conventional chemical pesticides; as a result, the quantity of honey produced decreased, and the detrimental effects on the environment and human health were mitigated. Exploring novel substitutes constructed from secure bioactive compounds extracted from organic plant matter is an exceedingly suggested course of action. This study was conducted to determine the toxicity effect of the lemongrass essential oil (LEO) loaded in chitosan nanoparticles (CNPs) as nanocapsule on *Galleria mellonella*. Some physiological studies were examined to evaluate the insecticidal impact of the LEO nanocapsule.

GC-MS analysis revealed that the chemical composition of lemongrass EO varied in accordance with geoclimatic and cultural conditions. The ingredients' abundance varied according to the geographical area of collection, environmental circumstances, and plant age. GC-Ms analysis exhibited that the major compound is citral, representing 70.33% of total EO, which is composed of α- citral (geranial) and β- citral (neral) with percentages of (39.76%) (30.57%) respectively, followed by myrcene, geranyl acetate, cis-verbenol, and geraniol with different ratios (Table 1). Soliman *et al.* (2017) validated this identification by determining that the pale-yellow LEO leaves have an average concentration of 0.25–0.90%. They also found that 79.69% of the total composition of LEO was composed of citral, which includes two major compounds: citral A (geranial) at 42.86% and citral B (neral) at 39.83%. Other major compounds included myrcene (8.05%), geraniol (3.22%), and cis-verbanol (1.84%). A similar observation showed that the primary component was the citral content (73%), which included other components including myrcene (9.6%) and geraniol (4.2%) in addition to geranial (39.8%) and neral (33.2%) (Pino *et al*., 2018).

The PDI value ranges from 0.01 to 0.5–0.7 for monodispersed particles. The PDI of LEO nanocapsules had a value of 0.415, which means that these formulations were nanosized and exhibited a normal distribution pattern (Fig. 1). DLS results demonstrated that the particle size distribution was comparatively uniform and homogenous, which may reflect the overall stability of the capsule (Gomaa *et al*., 2021). The DLS data were consistent with those of SEM. According to SEM data, the morphologies of nanocapsules are approximately spherical with smooth surfaces and densely packed. The particle size distribution is relatively homogeneous. The lack of surface deformations could be explained by the high content of chitosan in wall matrices (Fig .2). These results were in the same trend as those obtained by Oh *et al*. (2019) and Antonioli *et al*. (2020). They are also coordinated with Hadidi *et al.* (2020) results, which clarified that all particle diameters were < 500 nm with regular distribution and spherical shape.

The effect of LEO nanocapsules on larval development was of considerable interest. The toxicological experimental data clarified that LEO nanocapsules have a toxic effect on *G. mellonella* larvae. In order to identify safer substitutes for harmful synthetic insecticides, this study assessed the insecticidal activity of LEO nanocapsules. The experimental findings confirmed a significant larval loss after three weeks of LEO nanocapsules treatment. Its potential as a bio-insecticide, LEO may be indicated by citral substances and secondary metabolites like bioactive cyclic and acyclic terpenes, alkaloids, and flavonoids (Avoseh *et al*., 2015). The potential toxicity of nano chitosan may stem from its pesticidal properties. According to Zhang *et al*. (2003), chitosan at 60mg/ml in a 1% acetic acid solvent has the ability to kill up to 80% of lepidopterous and homopterous insects. Chitosan has been shown by Sayed *et al*. (2014) to have insecticidal action on *Spodoptera littoralis* with LC_{50} 734.923 ppm and detrimental effects on a few key enzymes. This action thereby doubles the effectiveness of nanocapsules as a bio-insecticide. Improved chitosan is now a valuable instrument in IPM programs and a safe way to protect the environment and public health (Alfy *et al*., 2020). Because of its biodegradability, safety, availability, and biocompatibility, chitosan is the perfect biopolymer for EO nanoencapsulation (Kalagatur *et al*., 2018, Yanat and Schroën, 2021). The delayed and continuous release of active chemicals after encapsulation in CNPS demonstrated their effectiveness of CNPS in extending the insecticidal effects (Alshehri *et al*., 2020).

The morphological observations were very important because weight-loss in larvae might cause weakness that did not allow them to reach the adult stage, resist environmental and climatic changes, and be vulnerable to natural predators. Furthermore, the cuticle darkening colour could be attributed to larval weakness and malnutrition which leads to its infection with toxins, pathogens and abiotic stressors (Whitten and Coates, 2017). Many larvae died as malformed larvae throughout the larval stage. Abdel-Hakim *et al*. (2021) reported that treating *Sesamia cretica* larvae with lemongrass oil reduced larval weight from 179 mg for untreated larvae to 29 mg after treatment.

According to Shah *et al*. (2011), LEO includes alkaloids, flavonoids, and glycosides which cause several malformations. Similar observations were also detected by Pinto *et al*. (2015), who concluded that the monoterpene citral and the EO of *Cymbopogon citratus* (Brazil/Cuba) disturbed the development of the treated blowflies, resulting in various morphological abnormalities in adults. The reduction of larval weight could be considered as a direct LEO effect, where its active ingredients may cause wide range of

physiological disturbances leading to such reduction. It may be the reason of inability of insect to digest food as well as absorption of some fractions may led to malnutrition case and influencing the metabolic processes (Loko *et al*., 2021).

Phosphatase, digestive and chitinase enzymes are tightly related to the development of insects and their metamorphic molts. They facilitate the transport of nutrients, permeability, growth and cell differentiation, and protein synthesis, among other metabolic processes (Ram, 1985). Therefore, blocking these enzymes might have an impact on the typical larval development that was seen following LEO nanocapsule therapy. These findings concur with those of Megahed *et al*. (2013), who reported that the fourth larval instar of cotton leafworms showed a significant decrease in ALP activity in response to three bioinsecticides: Proclaim, Romacten, and Tracer. Results are in harmony with those of Pandey and Upadhyay (2023), who demonstrated that the activities of the Indian white termite enzymes ACP and ALP were disrupted by the *Citrus maxima* EO and its combinatorial blends.

Sabry and Abdou, (2016) studied that the decrease in protease enzyme activity is related to larvae feeding behavior; oil components cause a reduction in diet consumption due to the repellent effect of citral, leading to lower protein intake. Because proteins are essential to insect growth, development, metamorphosis, and reproduction, cutting back on their intake is regarded as one of the primary methods for managing insect pests.

Furthermore, consuming LEO nanocapsules may harm the alimentary canal's epithelial cells, slowing the pace at which their enzymes are secreted (Senthil-Nathan, 2013). Tannin chemicals have the potential to function as enzyme activity inhibitors in insect digestion (Rahayu *et al*., 2018).

Ongmali *et al*. (2017) reported results that are similar to these: the hexane extract from lemongrass leaves significantly inhibited alpha-amylase activity, while the ethanol extract significantly inhibited lipase activity and selectively inhibited the digestive system's ability to hydrolyze starch and fat. Chintalchere *et al.* (2021) found that treating houseflies with tea tree and lemongrass EOs significantly decreased their release of digestive enzymes.

Another important enzyme which affects the larval development is the acetylcholine esterase (AchE). It hydrolyses acetylecholine to prevent its accumulation at nerve synapses caused of disruption of nerve transmission. It is a target of many neurotoxic insecticides that inhibit AchE-regulated neuromuscular, parasympathetic, and sympathetic effector junctions and autonomic ganglia physiology (Mladenović *et al*., 2018).

These findings corroborated Abdel-Aziz *et al*. (2013), who claimed that neem and bitter thyme oils inhibited acetylcholine esterase in *Spodoptera littoralis* 4th instar larvae. Similar observations were recorded in houseflies treated with lemongrass and tea tree Eos (Chintalchere *et al*., 2021). Hassan *et al*. (2023) demonstrated that AChE activity significantly decreased after exposure of mealybug nymphs and adults to LEO, which indicates fatal neurological consequences. According to López and Pascual-Villalobos (2010), this may be due to lemongrass extracts contain secondary metabolites like bioactive cyclic and acyclic terpenes, alkaloids, and flavonoids that inhibit acetylcholinesterase and octopamine, paralyzing and killing insects and suggesting that lemongrass may have bioinsecticidal properties.

During the early stages of molting, chitinase typically has an effect. The production of chitin may still be impacted after the formation of a new epidermis if chitinase is consistently produced and active. This could prevent insects from developing normally and possibly result in their death (Liu *et al*., 2017, Xi *et al*., 2015). In insects, chitin content alterations can cause molting and development problems. Additionally, any blockage in the system that facilitates chitin synthesis and breakdown can cause disruptions in the chitin metabolism process (Li *et al*., 2022). Results of chitinase enzyme also agree with those of Jin *et al*. (2022), who suggested that citral and lemongrass EO considerably reduced the ability of *Reticulitermes flaviceps* workers to walk and grip. The analysis findings showed that citral altered the chemical structure of *R. flaviceps* and considerably decreased the amount of chitin.

CONCLUSION

This study concludes that chitosan is an ideal crosslinking material for encapsulating lemongrass oil due to its biodegradability, biocompatibility, non- toxic nature, and controlled release, additionally to its insecticidal impact. The above results indicated that the encapsulation of lemongrass oil ensures high activity as a larvicidal agent against *G. mellonella* and has a negative effect on some important enzymes and physiological functions during its life cycle. LEO nanocapsules proved their bio-insecticidal effect on greater wax moths, encouraging future research on other pests and the extension of nanotechnology for insect pest control. Nanoencapsulation provides the basis for producing novel insecticides based on natural products.

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تأثيرات سمية وفسيولوجية لكبسولة زيت حشيشة الليمون النانونية علي يرقة الفراشات الشمعية *Galleria mellonella*

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الملخـص العــربي

تستهدف الدراسة تقييم سمية المتراكبة النانوية المكونة من زيت حشيشة الليمون (*Cymbopogon citratus*) المغلف بكبسول من النانوكيتوزان علي حشرة دودة شمع العسل (*Galleria mellonella)* فضلا عن دراسة التأثير البيولوجي والفسيولوجي للكبسولات النانوية بعد المعاملة في الظروف المعملية. تم استخلاص الزيت من نبات حشيشة الليمون وتعريف مكوناته عن طريق جهاز GC mass وكان من اهم مكوناته Citral حيث بلغت نسبته (70.33%) ثم تحضير كبسولة نانوية مكونة من زيت حشيشة الليمون مغلف بقشرة من النانوكيتوزان. تم توصيف الكبسولة النانوية المحضرة عن طريق جهاز Dynamic Light Scattering والميكروسكوب الالكتروني الماسح للتأكد من نجاح عملية الكبسلة ضمن الابعاد النانوية.اوضحت النتائج ان معاملة الطور اليرقي الاول بالكبسولة النانوية المحضرة لها تأثير علي موت ووزن اليرقات. ولوحظ خلال الدراسة ان المعاملات ادت الي نقص في وزن اليرقات المعاملة وفي الحجم وظهور بعض التشوهات المورفولوجية. وبدراسة السمية للكبسولة النانوية اوضحت النتائج ان لها تأثير ابادي حيث ان الفقد الكلي في اليرقات المعاملة وصل الي (46.59%) في اقل تركيز و (92.05%) عند اعلي تركيز وبقياس التركيز المميت ل 50% من الحشرات المختبرة (Lc_{50}) كانت 49.89. ppm وفي الدراسات الفسيولوجية لبعض الانزيمات , النتائج اثبتت حدوث تثبيط في نشاط كل من انزيم البروتييز(Protease) والاسيتيل كولين استيريز(AcHE) وانزيمات الفوسفاتيز(Acid & Alkaline Phosphatase) بينما اظهرت النتائج زيادة في نشاط انزيم الكيتينيز(Chitinase) في اليرقات المعاملة. وتشير هذه النتائج الي ان الكبسولة النانوية المحضرة لها تأثير ابادي وتسببت في اضطرابات بيولوجية وفسيولوجية في يرقات دودة شمع العسل ويمكن استخدامها في برامج الادارة المتكاملة لمكافحة الافات.