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Insecticidal and Repellent Activity of PEG - Cardamom Essential Oil Nanoparticles Against *Phoenix dactylifera* Infesting Insect Pest *Oryzaephilus Surinamensis*

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Abstract: The study deals with the EO-NP's encapsulated cardamom essential oil for insecticidal activity. The prepared nanoparticles were subjected to characterization studies using Dynamic Light Scattering (DLS) analysis, Fourier Transform Infra-Red spectroscopy (FTIR) and Transmission Electron Microscopy (TEM) analysis. The DLS analysis revealed that the size of the nanoparticles had a slight increase in diameter after three months of storage. Similarly, the characterization with TEM showed that the prepared nanoparticles were of round or spherical shape with clear morphology without any crack in the external surface. Considerable mortality was observed from the insecticidal bioassay carried out by contact, fumigant toxicity with cardamom essential oil nanoparticles encapsulated with PEG (EO-NPs) against adult Oryzaephilus surinamensis (Linnaeus) (Coleoptra: Silvanidae). Further the Cardamom EO-NP's also produced significant repellency effect on the adult insects. About 85% mortality (Contact Toxicity) and 81% mortality (Fumigant Toxicity) was observed with 0.025 mg x cm2 concentration of EO-NP's. The results indicate that the cardamom essential oil encapsulated with PEG nanoparticles could be an effective alternative to chemical pesticides for the control of stored product grain pests. Further this is the first report on the insecticidal efficacy of PEG encapsulated Cardamom essential oil against Oryzaephilus surinamensis.

Index Terms: Contact toxicity, Elettaria cardamomum, Fumigant toxicity, Insecticide, Oryzaephilus surinamensis and Nano-encapsulation.

INTRODUCTION

Phoenix dactylifera (Dates) is an important crop that provides nutrition in the arid regions where other crop plants cannot be cultivated due to extreme conditions (Al-Shahib and Marshall, 2003). The perennial problem associated with the storage of *P. dactylifera* is the damage and contamination by stored product grain insects. The two most important insects that infect the stored products are *Oryzaephilus surinamensis* and *Tribolium castaneum* (Hussain, 2007). Though, *O. surinamensis* is a secondary pest that does not possess the ability to damage the whole grain, mechanical damage of the grains during harvest and post-harvest handling and storage has led to severe infestation by the insects. Due to its small size and hiding behaviour in the stored grains it becomes difficult in controlling the insects by insecticide application. Moreover, they had also developed resistance to many chemical insecticides (Beckel *et al.*, 2007), (Al-Jabr, 2006). The use of chemical insecticides for the control of stored product pest has led to serious hazards to environment and warm-blooded

animals (Isman, 2000). Hence botanical insecticides could be a better alternative to chemical insecticides (Vinuela *et al.,* 2000).

Essential oils produced by aromatic plants play an important role in plant defence (Pavela, 2015). These secondary metabolites are synthesized by secretory glands present in different organs of the plant, both internally and externally (Svoboda and Greenaway, 2003) and comprise about 20 to70 different organic compounds and in few cases one or two organic compounds might occupy 80%. The major constituents of essential oil are mainly Monoterpenes and Sesquiterpenes (Husnu *et al.,* 2015) and the biological activities of essential oil rely on these organic compounds. The bioavailability, efficacy and applicability of essential oils can be enhanced by encapsulation, a process by which the liquid nature can be converted into solid state by altering the surface and colloidal properties of active components (Bansode *et al.,* 2010). Hence, encapsulation of essential oil results in controlled release kinetics, minimize evaporation and provide protection from external environment there by increasing the shell life of essential oils (Bansode *et al.,* 2010). Though, nanonization of the encapsulate increases the surface area the preparation process is highly challenging due to its small size, large surface area and the requirement of high surface energy (Wang *et al.,* 2004).

Poly Ethylene Glycol (PEG) has a simple, helical molecular structure with remarkable biological and physicochemical properties such as hydrophilicity, nature of solubility both in water and organic solvents and further they also do not possess any toxicity (Yang *et al.,* 2009). Earlier reports states that PEG when used as coating materials for nanocarrier enhances the stability in biological fluids and hence results in effective transport of bioactive macromolecules across the biological membranes and intestinal and nasal epithelia (Tobio *et al.,* 2000; Vila *et al.,* 2002) Hence in the present study, PEG coated *Elettaria cardamomum* essential oil was prepared using melt-dispersion method which is a simple preparation procedure of nanoparticles compared to other methods such as spray drying, interfacial polymerization and complex coacervation (Yang *et al.,* 2009).

Very few literatures report the use of essential oil nanoparticles for the control of stored product pest (Al Qahtani *et al.,* 2010; Ayvaz *et al.,* 2009). Al Qahtani *et al.,* (2012) reported the insecticidal and biochemical effect of *Elettaria cardomomum* dry powder against *O. surinamensis*. In the present study, the cardamom essential oil was encapsulated with Poly Ethylene Glycol (PEG) and further characterization of PEG nanoparticles and its efficacy against the adult *O. surinamensis* was reported.

MATERIALS AND METHODS

A. Solvent extraction of essential oil

The ground powder of Cardamom was extracted with Petroleum Ether (1:2 w/v ratio) for 24 hrs in a Soxhlet apparatus following the procedure of Singh and Ahmed (2015). The extract collected was concentrated in a hot air oven at 40^{o}C.The essential oil that was obtained was weighted and stored at 4^{o}C until further use.

B. Gas Chromatography Mass Spectrometry (GC-MS) analysis

The chemical composition of essential oil obtained from Cardamom was analysed using GC- MS (Agilen HP-5973). Chromatograph equipped with Shimadzu QP-500 Mass Spectrometer as described by Abbasipour *et al.,* (2011). The essential oil was distributed with Acetone (1:25 ratio) and 1µl of the sample was taken for the GC–MS analysis. Fused Silica column coated with polydimethy siloxane (30m/25nm and a film thickness of 0.25 µm) was used. Helium was used as the carrier gas at 1 ml/min flow rate. The temperature of the injector was fixed at 250ºC. The temperature of the oven was set at 60ºC for 1 min, which was then increased to 225ºC steadily at a rate of 2ºC/min. After the steady rise in temperature of the column it was maintained for 5 min. The ionization voltage used was 70ev with 1:25 split rate. The composition of essential oil was identified by comparing the Retention Time (or) its Mass Spectra with that of known compounds (or) from Published data.

C. Insect rearing

Adult O. surinamensis were collected from infested Date Palm Fruits (DPF) from sellers in local market, Thanjavur, Tamilnadu, India. The insects were identified and maintained in the incubators at 29±1º C with 60% relative humidity and 16:8 (L/D) photo period at P.G.& Research Department of Zoology, Rajah Serfoji Government College (Autonomous), Thanjavur, Tamilnadu, India.The insects were reared on rolled oats and brewer's yeast in the ratio 95:5. The adults selected for bioassays were about five days post-eclosion. The adults were separated from the colonies one-week prior to bioassays and were held for a minimum of one hour before being used in bioassays.

D. Contact Bioassay

Different dilutions (2.5, 5, 7.5, 10 and 12.5 µl/cm2) of Cardamom essential oil were prepared using acetone as solvent. The experiment was carried out in glass petridishes (6 cm diameter) with Whatmann No.1 filter paper impregnated into petridish surface and acetone applied to filter paper. The filter paper was allowed to stand for 1 hour for the evaporation of solvent. One ml aliquots of test dilutions were applied uniformly with the help of a sprayer. Twenty adult insects were transferred to petridishes. A control was also maintained with acetone. Five replicates of each test concentrations were maintained. The mortality was observed at different time intervals for a period of 96 hrs (Busvine, 1971).

E. Fumigant Bioassay

The fumigant bioassay was done with the procedure described by Huang and Ho (1997) with slight modification. Twenty adults were transferred to the glass tubes and covered with nylon cloth held tightly with adhesive tape. About 0.25µl of different dilutions (2.5, 5, 7.5, 10 and 12.5 µl/ml) of essential oil were added to separate glass test tubes and allowed to stand for the evaporation of solvent. After evaporation, the glass test tubes, holding the adult insects were placed upside down over the test tubes with essential oils. The glass test tubes saturated with oil vapours were placed in the incubator and mortality was recorded at different time intervals for a period of 96 hrs. Five replicates of each test concentrations were maintained. Similarly, control was also setup with the same procedure but containing only solvent.

F. ***Repellency Bioassay***

The repellency bioassay was carried at using area preference method as described by Jilani and Su (1983). Different concentrations of essential oil (0.005, 0.015, 0.025, 0.035 & 0.045 µl/cm2) were prepared using one ml acetone as solvent. Whatman No.1 Filter paper was used to study the repellent property of essential oil against *O. surinamensis*. The filter paper was cut into two equal halves. One half of the filter paper was used as control which was applied with acetone alone and to the other half of the filter paper the test dose was applied. Both the control and essential oil applied filter paper were allowed to dry for 10 minutes until all the solvent gets evaporated. The two halves of the filter paper were then remade by attaching the treated and control halves with adhesive tape. The remade filter paper disc was then placed tightly on to a petridish. Twenty adult insects were introduced at the centre of the filter paper disc and covered. Then the petridish was placed in incubator at 29±1ºC and 75±5% relative humidity. Five replicates were performed for test concentration and the number of adult present in treated Percentage Repellency (PR) was calculated as follows:

PR = [(Nc - Nt) /Nc] x 100

G. ***Synthesis of Nanoparticles loaded with essential oil***

For the synthesis of nanoparticles loaded with essential oil, the melt dispersion method was followed according to the protocol of Peng *et al.,* (2008) with slight modification. Poly Ethylene Glycol (PEG) was taken in several parts (100g/part) and was heated to 65ºC to melt. Essential oils of volume 5.0, 7.5 and 10ml were added separately to each part of PEG. The melted PEG along with essential oil was mixed thoroughly with a glass rod to obtain uniform distribution. The mixture was then allowed to cool naturally at 25ºC and ground to fine powder using a mortar box. The ground powdered was sieved with sieve mesh 200 and the powder was stored in an airtight container and stored at 25ºC for further analysis.

H. ***Encapsulation and oil loading efficiency***

The synthesized nanoparticles loaded with essential oil was weighed and heated to 60oC for 48 hrs in a petri plate until dryness to remove all the trapped essential oil. The encapsulation and oil loading efficiency were determined using the formula;

Encapsulation Efficiency % = $(W_m\text{-}W_0)/(W_1)\times100$

Oil loading % = $(W_m\text{-}W_0)/(W_2)\times100$

W_m= weight of nanoparticles after preparation

W_0 = weight of nanoparticles after drying

W_1 = amount of E0 introduced into nanoparticles

W_2 = Total amount of PEG used

I. ***Oil release study***

The release of oil from the nanoparticles was experimentally determined by a slightly modified procedure of Yang *et al.,* (2005). About 0.1 g of nanoparticles were dissolved in 10 ml of absolute ethanol. The mixture was then heated to 60ºC in a boiling water bath till all the nanoparticles were completely dissolved. An aliquot of this mixture was filtered and absorbance was read at 313 nm spectrophotometrically every 30 minute for three hrs. The

experiment was carried out with several sets of mixture and each set was replicated three times. The oil release from the nanoparticles was calculated using the formula;

Oil Release % = (Amount of oil released from X (g)of NP at time 't')/(Total amount of oil present in X (g) of NP)×100

J. Characterization of Nanoparticles

Nanoparticles prepared with 10% optional ratio of essential oil and PEG were selected for characterization studies. Dynamic Light Scattering (DLS) analysis, Fourier Transform Infra-Red Spectroscopy (FTIR), and Transmission Electron Microscopy (TEM) analysis were carried out for the characterization of nanoparticles. Dynamic Light Scattering analysis was done to determine the particle size. About 0.01% of diluted samples were prepared using freshly prepared double distilled water in order to avoid multiple scattering effects. Particle size was reported as normalized intensity distribution that was defined as average emulsion diameter. Two samples were analyzed in 3 replicates. The identification of chemical bonds between the essential oil and the polymer was observed by Fourier Transform Infra-Red Spectrometer (FTIR). The sample was read in wavelength ranging from 650- 4000cm.-1About 2 mg of sample was analysed with DSC cell containing aluminium crucible. The experiment was conducted under dynamic air atmosphere of 100ml/min and a heat rate of 20 ºC / min at temperature ranging from 50 to 300 ºC. The calibration of DSC cell was done with Indium and Zinc, and Purge gas used was of high purity Ar grade. A small quantity of nanoparticles was dispersed in absolute ethanol to form a homogenized solution. The dispersion of nanoparticles was carried out for 10 min in a Sonicator and then a drop of this homogenized solution was placed in a carbon-coated copper grid for negative staining with 2% Phosphotungstic acid solution (pH = 6.7). After drying at room temperature the image was obtained by Transmission Electron Microscope (TEM) operated at 80 kv (Hitachi H - 7650, Japan).

RESULTS AND DISCUSSION

K. Essential oil characterization

The insecticidal efficacy of the PEG encapsulated cardamom essential oil was evaluated for its contact toxicity, fumigant toxicity and the repellency effect against adult *O. surinamensis*. GC-MS results of the essential oil was presented in the Table 1. The Cardamom EO was found to be rich in terpenoids and saturated long chain fatty acids. The dihydroterpinyl acetate (24.32%) and linalyl acetate (10.80%) were found to be the in the higher proportions among other compounds (Figure 1). Besides these two compounds,17 more compounds that were found to be occupied less than 10%. Al Dawsari Mona *et al.,* (2020) reported the presence of trans-sabinene hydrate, α-terpinyl acetate was found to be in higher concentration as revealed by the GC-MS analysis. The presence of Terpinyl acetate (36.61%), 1, 8-Cineole (30.42%), Linalyl Acetate (5.79%) and Sabinene (4.5%) in the essential oil of E.cardamomum was reported by Abbasipour et al (2016). Xin Chao Liu *et al.,* (2013) reported the presence of α-Terpinyl acetate, Spathulenol, α- Terpineol and Linalool. These results of the present study was found in complete agreement with the present findings.

L. PEG encapsulated EO

The PEG coated cardamom essential oil was prepared using melt-dispersion method. Three preparations of essential oils containing 5, 7.5 and 10 ml were used for encapsulation with 100g of PEG. These nanoparticles preparations were designated as A, B and C respectively with respect to the amount of essential oil introduced. The prepared PEG nanoparticles were evaluated for its encapsulation efficiency, oil load and oil release profile. The percentage of oil load and the encapsulation efficiency of PEG were presented in Table 2.

The oil loading efficiency of PEG was found to be positively correlated to the amount of essential oil introduced. An increase in the amount of essential oil resulted in increased encapsulation by PEG. The mean percentage of oil loading with 5 ml of essential to 100g of PEG was found to be 3.79±002. Similarly, the nanopreparation with 7.5 ml of essential oil reported an oil load of 6.07±0.06. The results indicate that 83.73% encapsulation of essential oil was achieved with 10% optimal ratio of essential oil to PEG. The percentage of oil load and the encapsulation of essential oil by PEG was found in the order A < B < C. The oil release pattern of the three nanoparticle preparations (A, B and C) was presented in Figure 2. An initial burst of oil release from the nanoparticles was observed with nanoparticle preparation A accounting 30%, B with 33% and C recorded 38% of oil release in initial 30 min. Beyond 30 min, the release of oil from the nanoparticles were slow and sustained with increase in time.

Encapsulation of bioactive compounds or drug molecules by polymeric nanoparticles such as Poly Ethylene Glycol (PEG) offers protection against adverse environmental conditions as well as preserves the odour and prevents volatility of enclosed core material. These features increase the effectiveness of core material. In the present study, encapsulation of cardamom essential oil by PEG was carried out to evaluate its insecticidal potential against the stored grain product pest, *O. surinamensis*. The Nano particles prepared in this study were of matrix type rather than reservoir type. The active agents were dispersed in the matrix encapsulates (Zuidam *et al.,* 2010). Encapsulation efficiency of 78.2 to 83.4 % was reported by Kumar *et al.,* (2014) from the preparation of PEG–Mentha oil nanoparticles. High encapsulation efficiency was reported with lipid nanoparticles. About 80-90% encapsulation with Curcuminoids (Nayak *et al.,* 2010) and 92.99% with Geranium oil are reported with Lipid nanoparticles. The results from the present study showed that 10% of Cardamom essential oil had maximum encapsulation efficiency of 83% coincided with the results of Kumar *et al.,* (2014) with PEG Nanoparticles coated Mentha oil.

The pattern of oil release from Nanoparticle depends upon variety of factors that include composition of coating material and oil load (Gomes and Moreira, 2011). In the present investigation the oil release was found to have a rapid burst during initial period followed by a steady flow. This initial rapid flow might be due to diffusion of essential oil that has been adsorbed on the external surface of nanoparticles. The percentage release of oil was found 28, 31 and 35% in A, B and C nanoparticles respectively within the 30 min. Beyond the initial period of 30 min, a constant oil flow from the nanoparticles was observed.

In this present study the nanoparticles designated “C” had maximum oil release followed by ‘B’ and ‘A’ nanoparticle, depending on the amount of oil load. The possible reason for this increased oil release with high oil load was due to less effective dispersive force of the stirrer caused by low rotational energy. Further the large size of oil vesicles caused due to

less effective vs dispersive force results in less thick encapsulates by the polymer. Hence, in the present study, the slow release of oil during toxicity application is desirable due to long time persistence.

M. Characterization Studies of EO-NP's

The size of the nanoparticles was examined using Dynamic Light Scattering (DLS) analysis (Table 3). The size of nanoparticles at 0 months varied in range between 166 to 226 nm. A slight increase in particle size was observed after 3 months of storage ranged between 176 to 275 nm. This indicates the stability of the nanoparticles between storage periods. Similarly, the PEP-Mentho oil nanoparticles varied in size between 226-331 nm (Kumar, 2014). But in contrary, Yang *et al.,* (2009) reported very slight increase in particle size by 2 nm after 5 months of storage (233 to 235 nm). The variation in particle size in the present study may be due to matrix type nanoparticles prepared by melt-dispersion method.

The presence of various functional groups was identified by FTIR analysis (Figure 3). The FTIR spectrum revealed absorption peaks at 3851 cm-1,3697.66 cm-1, 3025.97 cm-1, 1557.12 cm-1, 1491.43 cm-1, 1428.30 cm-1, 1259.55 cm-1, 1050.91 cm-1, 947.85 cm-1, 796.17 cm-1, 617.03 cm-1 and 571.95 cm-1. The possible function groups and compound present in the Cardamom essential oil was identified using FTIR analysis. The absorption spectrum revealed bands at 1491.43 cm-1, could be probably related to aromatic rings, where the vibrations might be due to stretching of aromatics (Mot *et al.,* 2011), (Silva *et al.,* 2014). Similarly, the bands at 1557 cm-1 were due to aromatic ring stretch (stretching of N-H band) (Franca *et al.,* 2014). Methyl C-H-bend (Symmetrical/ Asymmetrical) bend was revealed with adsorption peak at 1428 cm-1. The band at 1259.55cm-1 was due to the primary or secondary OH in plane bend (Silva *et al.,* 2014). The presence of cyclic ether's, large rings and C-O stretch was attributed with a band at 1050.91 cm-1 (Tobio *et al.,* 2000). The C-H out of plane blend of alkenes was identified with the presence of band at 947.85 cm-1. The C-CL stretch of aliphatic chloro compounds were identified with the band at 796.17 cm-1 (Tobio *et al.,* 2000) and disulphide S-S stretch was represented with peak at 617.03cm-1. The band at 571.95 was not identified. The morphology of the essential oil encapsulated PEG nanoparticles was examined using TEM analysis (Figure 4). The nanoparticles were round or spherical in shape with good dispersion. Further, the nanoparticles had no apparent cracks or porosity in their external surface indicating the effective protection or encapsulation of the core material, the essential oil.

N. Toxicity Bioassay

The percentage mortality due to contact toxicity against adult insects that were exposed to different concentration of cardamom EO-NPs varied between 20-85% (Table 4). About 12hrs treatment of EO-NPs exhibited 20 to 85% mortality in *O. surinamensis*. The Lethal Concentration (LC_{50}) was observed to be 10.32 µl/cm^2 and LC_{90} value recorded was 66.93 µl/cm^2. With increase in exposure time of EO-NPs the mortality rate also increased. About 96 hrs of exposure of EO-NPs showed a LC_{50} value of 2.63 µl/cm^2 and LC_{90} value of 21.35 µl/cm^2 (Table 5). Table 5 presents the mortality percentage and lethal concentration value due to fumigant toxicity. High mortality percentage (81%) was recorded with 12.5 µl/mL air after 96 hrs of treatment. The LC_{50} value was 3.10 µl/mL air and LC_{90} value was 27.88 µl/mL air.

The cardamom essential oil showed significant repellent activity attaining 96% at 0.045 µl/cm^2 concentration after 4 hrs exposure (Figure 5). The lowest concentration (0.005 µl/cm^2) post two hrs of exposure attracted the adult insects, but the Percentage Repellency (PR) value reached 42% after another two hrs of exposure. The maximum of 84% PR value was obtained with 0.045 µl/cm^2 concentration after two hrs of exposure.Generally, the pesticide nano preparation is aimed in controlled release of products for a desired period of time to achieve maximum biological efficacy (Ghormade *et al.,* 2011). The most important advantage of using EO nano formulation is that the nanoparticles are soluble in water and do not require any additional organic solvents. The usage of organic solvents in case of chemical insecticides application poses a potential threat to ecology. The cardamom EO encapsulated with PEG 6000 matrix showed effective insecticidal activity. Werdin Gonzalez *et al.,* (2013) evaluated the Geranium and bergamot essential oil encapsulated PEG nanoparticles for the control of *T. castaneum* and *R. dominica* and reported that Geranium nanoparticles were effective against *T. castaneum* comparatively to Bergamot nanoparticles. The possible reason for the differential toxicity effect among stored product insect pest might be due to the size of insects, development of detoxifying metabolic pathways and the composition and thickness of cuticle (Korunic, 1998), (Stefanazzi *et al.,* 2011), (Hashemi and Safavi, 2012). Cuticles are secreted by epidermal cells that cover the entire body of the insects and extend into trachea, fore gut and hind gut and genital system. The cuticle is composed of several layers such as cement, wax, epicuticle, exo and endo cuticle. The presence of wax externally in the cuticle layer of insects enables the Essential Oil loaded NanoParticles (EO-NPs) horizontal and vertical diffusion across the solid hydrophilic endocuticle due to the amphiphilic nature of PEG 6000. Moreover, the large specific surface exhibited by the nanoparticles causes high adhesiveness of EO-NPs with insect body. This interaction of EO-NPs with insect cuticle increases the exposure time of the active molecules.

Contact toxicity of EO-NPs is highly effective compared to fumigant toxicity as observed in the present study. Since the majority of toxicity inducing compounds in EO of cardamom was found to be Terpenes, the nano formulation reduces its volatility. The solid–lipid nanoparticles produced with *Artemisia arborescens* EO exhibited controlled release and reduced rapid evaporation (Lai *et al.,* 2006). The toxicological properties of NPs are the result of its smaller size, chemical composition, aggregation and solubility (Nel *et al.,* 2006 and 2009). The other most important factor for the effectiveness of contact toxicity to that of fumigant toxicity is the direct contact of nanoparticles with the cuticle as the insects moves through the stored products. Further, the low EO-NP respiratory uptake might also have contributed to its effectiveness. The maximum percentage of repellency (70%) was reported to be achieved after 70 hrs of treatment (Roya Amiri *et al.,* 2016) with 5µl/ml concentration. Treatment with 10 µl/ml of dust extract has a repellency effect of 31.11% after 12hrs and 96% after 24hrs of exposure. Fumigant toxicity of *E. cardamomum* essential oil showed significant mortality against the leaf miner, *Tuta absolulta* exhibiting a LC_{50} value of 1.88 µl/l and LC_{90} value of 2.84 µl/l (Abbasipour *et al.,* 2016).

CONCLUSION

Nanotechnology besides its applications in wide variety of fields can also be an effective alternative in pest control strategy. The nanoparticles possess high chemical activities

compared to their bulk counterparts. The PEG nanoparticles are further known to increase the bioavailability of drugs by increasing its pharmacokinetic properties. They are effective in drug delivery and stability. Due to its size in nano ranges, they are highly mobile, enabling quick penetration into tissues and thereby enhancing the insecticidal activity. The mode of entry of nano particles can take place either by direct contact through the cuticle and by ingestion and subsequent penetration into the digestive tract. The important observation in the present study is the enhanced efficacy of EO-NP's, insecticidal property was due to higher surface area, sustained and controlled release of NP's, high mobility of EO-NP's due to its size and ecofriendly nature.

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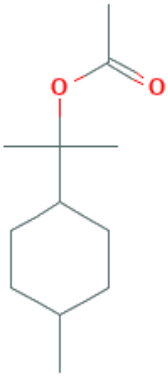
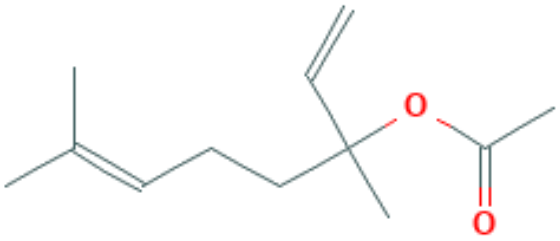
Figure 1: Major compounds identified from Cardamom essential oil



Dihydroterpinyl acetate



Linalyl acetate

Table 1: C**ompounds identified from GC-MS analysis of acetone extract of cardamom essential oil**

Sl.No	Compound Name	Peak Area (%)	Molecular Formula	Retention Time
1	Trans-sabinene hydrate acetate	2.56	$C_{12}H_{20}O_2$	5.153
2	Linalyl acetate	10.80	$C_{12}H_{20}O_2$	5.516
3	2-Propenal,3-phenyl-(CAS) Cinnamaldehyde	5.74	C_9H_8O	5.961
4	Geraniol formate	1.19	$C_{11}H_{18}O_2$	6.129
5	Myrcenylacetate	2.42	$C_{12}H_{20}O_2$	6.430
6	Dihydroterpinyl acetate	24.32	$C_{12}H_{22}O_2$	6.896
7	Neryl acetate	5.84	$C_{12}H_{20}O_2$	7.158
8	Geranyl acetate	1.64	$C_{12}H_{20}O_2$	8.309
9	7-Isopropenyl-4 a - methyl-1-methylene Deca hydro naphthalene	2.31	$C_{15}H_{24}$	8.829
10	alpha.-selinene	1.16	$C_{15}H_{24}$	8.925
11	d-Nerolidol	7.66	$C_{15}H_{26}O$	9.549
12	(S)-(+)-5-(1-(Acetoxy)-1-methylethyl)-2-methyl-2-cyclohexen-1-one	1.65	$C_{12}H_{18}O_3$	9.707
13	Farnesol isomer A	4.20	$C_{15}H_{26}O$	11.454
14	Farnesyl acetate 3	3.48	$C_{17}H_{28}O_2$	12.728
15	Pentadecanoic acid	4.77	$C_{18}H_{30}O_2$	14.021
16	Octadec-9-enoic acid	8.74	$C_{20}H_{36}O_2$	15.914
17	Ethyl Linoleolate	2.99	$C_{12}H_{20}O_2$	15.983
18	Retinal, 9-cis- (CAS) 9-cis-Retinal	7.26	$C_{20}H_{28}O$	16.200

19	Ambrox	1.27	$C_{16}H_{28}O$	17.729

Table 2: Encapsulation efficiency and oil load of PEG encapsulated Cardamom essential oil nanoparticles

Parameters	Nanoparticles		
	A	B	C
Amount oil introduced (ml)	5	7.5	10
Oil load (%, mean ± S.D.)	3.79±0.02	6.07±0.06	8.3±0.04
Encapsulation efficiency (%, mean ± S.D.)	76.23±0.40	81.27±0.69	83.73±0.52

Table 3: Characterization of PEG encapsulated Cardamom essential oil nanoparticles

Ratio of essential oil to PEG	Mean size of Nanoparticles (nm)	
	0 month	3rd month
5 ml essential oil/100g PEG (A)	166.60±6.54	176.00±4.84
7.5 ml essential oil/100g PEG (B)	197.20±10.23	201.20±7.91
10 ml essential oil/100g PEG (C)	266.40±15.53	275.80±8.87

* (%, mean ± SD, n = 3).

Figure 2: Oil release profile of PEG encapsulated nanoparticles with increase in time

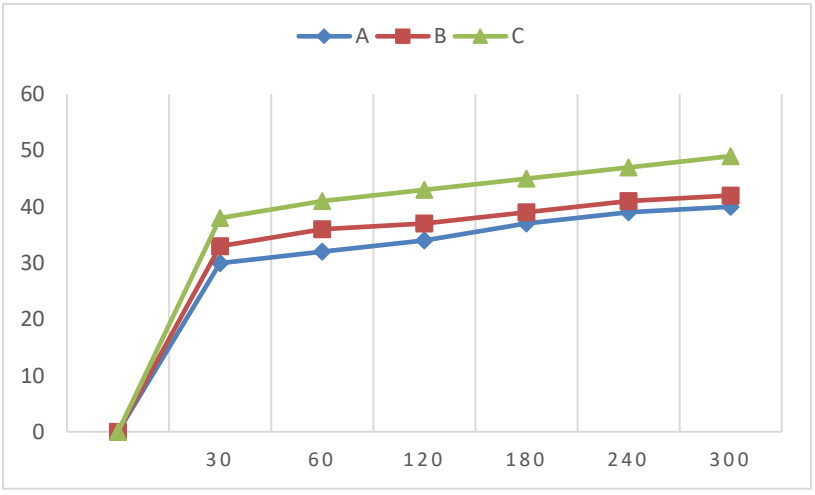


Figure 4: FTIR spectra of Cardamom essential oil

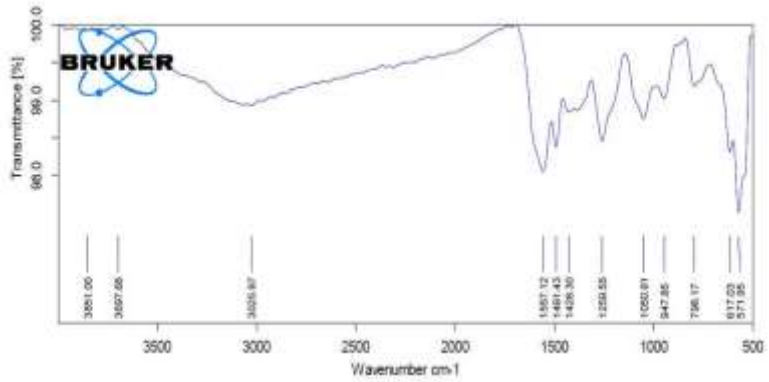


Figure 5: TEM images of PEG encapsulated Cardamom essential oil

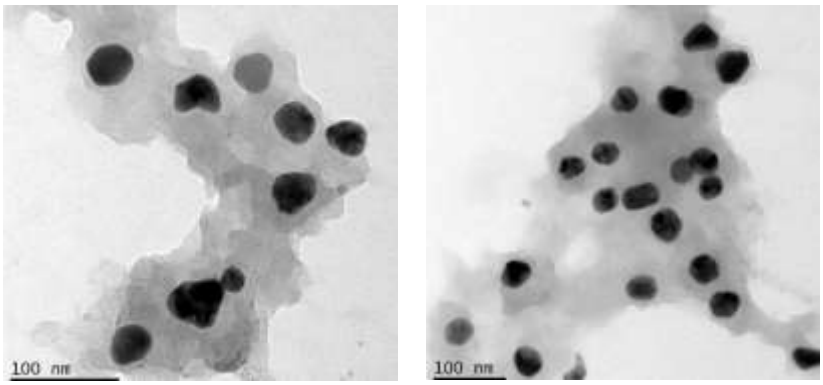


Table 4 : PEG encapsulated Cardamom essential oil induced contact toxicity against adult *O. surinamensis*

Duration of treatment (h)	% Mortality after exposure to PEG encapsulated cardamom essential oil (µl/cm^2)					LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	χ^2	Regression Equation
	2.5	5	7.5	10	12.5				
12	20 ± 6.12	26 ± 5.48	38 ± 5.48	50 ± 7.91	59 ± 4.18	10.318 (8.614-13.454)	66.932 (38.359-189.260)	3.079	Y = 8.000 X + 4.08
24	27 ± 8.37	40 ± 6.12	52 ± 10.37	59 ± 9.62	63 ± 12.55	7.093 (5.864-8.722)	59.056 (33.350-181.738)	0.175	Y= 20.900 X + 3.64
48	34 ± 4.18	48 ± 5.70	58 ± 7.58	64 ± 15.57	69 ± 12.45	5.282 (4.085-6.442)	50.903 (28.961-160.778)	0.360	Y = 28.800 X + 3.44
72	40 ± 7.19	52 ± 10.37	64 ± 10.84	70 ± 6.12	71 ± 5.70	4.096 (3.005-5.016)	33.761 (21.601-79.541)	0.672	Y = 33.000 X + 3.68
96	53 ± 5.70	58 ± 9.75	73 ± 11.51	81 ± 8.22	85 ± 11.73	2.634 (1.623-3.454)	21.353 (14.911-42.177)	3.567	Y = 43.900 X + 3.48

Table 5: PEG encapsulated Cardamom essential oil induced fumigant toxicity against adult *O. surinamensis*

Duration of treatment (h)	% Mortality after exposure to PEG encapsulated cardamom essential oil (µl/mL air)					LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	χ^2	Regression Equation
	2.5	5	7.5	10	12.5				
12	16 ± 4.18	22 ± 4.47	34 ± 4.47	46 ± 5.48	55 ± 3.54	11.834 (9.803-15.852)	69.078 (39.922-139.556)	2.792	Y= 4.000 X + 4.08
24	23 ± 5.70	36 ± 4.18	48 ± 7.58	55 ± 6.12	59 ± 4.18	8.421 (7.015-10.679)	66.857 (36.930-214.004)	0.157	Y= 16.900 X + 3.64
48	30 ± 3.54	51 ± 2.24	56 ± 6.52	60 ± 3.54	65 ± 3.54	5.831 (4.556-7.193)	62.592 (33.170-241.734)	1.149	Y= 28.700 X + 3.16
72	36 ± 4.18	56 ± 6.52	60 ± 3.54	66 ± 2.24	73 ± 2.74	4.457 (3.265-5.483)	43.016 (25.421-125.301)	0.798	Y= 33.000 X + 3.36
96	49 ± 5.48	54 ± 4.18	69 ± 6.52	77 ± 2.71	81 ± 6.52	3.099 (1.995-3.983)	27.882 (18.243-64.336)	3.072	Y= 39.900 X + 3.48

Figure 4: PEG encapsulated Cardamom essential oil induced repellency effect against adult *O. surinamensis*