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Research Article

SYNTHESIS AND CHARACTERIZATION OF PEG-CINNAMON ESSENTIAL OIL NANOPARTICLES AND THEIR APPLICATION AS AN INSECTICIDAL AGENT

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ABSTRACT

The study deals with the EO-NPs encapsulated cinnamon 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 with cinnamon essential oil nanoparticles encapsulated with PEG (EO-NPs) against adult *Oryzaephilus surinamensis* (Linnaeus) (Coleoptra : Silvanidae). Further, the cinnamon EO-NPs also produced significant repellency effect on the adult insects. About 85% mortality (Contact Toxicity) was observed with 0.025 mg x cm^2 concentration of EO-NPs. The results indicate that the cinnamon 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 cinnamon essential oil against *Oryzaephilus surinamensis*.

Keywords: *Cinnamon zeylanicum Blume,* Contact toxicity, Insecticide, *Oryzaephilus surinamensis*, Nano-encapsulation.

1. INTRODUCTION

Nanotechnology has developed very rapidly in various fields during recent years and also received considerable attention in the field of medicine particularly in the development of therapeutically drugs. The polymeric nanoparticles are highly effective in drug delivery due to its controlled and sustainable release of drugs [1]. Cinnamon (*Cinnamon zeylanicum Blume*) has strong antioxidant, antibacterial, antipyretic [2] anti-inflammatory [3] and insecticidal properties [4]. Essential oils of cinnamon have been reported for its acaricidal and repellent activity against house dust mites [5]. Dates have an excellent nutrition profile and high in fibres. Getting enough fibre is important for our overall health. *Phoenix dactylifera*, commonly known as date or date palm, is a flowering plant species in the palm family, Arecaceae, cultivated for its edible sweet fruit. Dates have been a staple food of the Middle East and the Indus Valley for thousands of years. They are believed to have originated around Iraq. *Oryzaephilus surinamensis*, the saw toothed grain beetle, in the super family Cucujoidea. It is a common, worldwide pest of grain and grain products as well as chocolate, drugs, dates and tobacco. The species binomial name, meaning rice-lover from Surinam, was coined by Carl Linnaeus, who received specimens of the beetle from Surinam. *O. surinamensis* can be found worldwide. The beetle is one of the most commonly encountered stored product pests [6] and is widespread within the food industry and can be found in food manufacturing, storage, and retail facilities as well as in home pantries [7]. 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 cinnamon essential oil nanoparticles PEG (EO-NPs) by PEG was done to evaluate its insecticidal potential against the stored date palm product pest, *O. surinamensis*.

2. MATERIAL AND METHODS

2.1. Solvent extraction of essential oil

The ground powder of cinnamon 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°C.The essential oil that was obtained was weighted and stored at 4°C until further use.

2.2. Synthesis of cinnamon essential oil nanoparticles

Melt dispersion method was followed for the synthesis of nanoparticles [8]. Preparation of Poly Ethylene Glycol nanoparticle for encapsulation of Mentha oil by melt dispersion method was reported by Peeyush Kumar *et al.* [9]. Characterization of the prepared nanoparticles was carried out using DLS, Zeta potential, FTIR and DSC. The nanoparticles were effective against *O. surinamensis*. For the synthesis of nanoparticles loaded with essential oil, the melt dispersion method was followed according to the protocol of Peng *et al.,* [10] with slight modification. Poly Ethylene Glycol (PEG) was taken in several parts (100g/part) and was heated to 65°C to melt. Essential oils of volume 5.0, 7.5 and 10 ml 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 25°C 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 air tight container and stored at 25°C for further analysis.

2.3. Encapsulation and oil loading efficiency

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

Encapsulation efficiency (%) = (Wm-W0)/W1

Oil loading (%) = (Wm-Wo)/W2

Wm= weight of nanoparticles after preparation

W0 = weight of nanoparticles after drying

W1 = amount of E0 introduced into nanoparticles

W2 = Total amount of PEG used

2.4. Oil release study

The release of oil from the nanoparticles was experimentally determined by a slightly modified procedure of Yang *et al.* [8]. About 0.1 g of nanoparticles was dissolved in 10 ml of absolute ethanol. The mixture was then heated to 60°C 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 minutes 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

2.5. Insecticidal Activity

2.5.1. 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±1°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.

2.5.2. Contact Bioassay

Different dilutions (2.5, 5, 7.5, 10 and 12.5 µl/cm^2) of cinnamon 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 one hour for the evaporation of solvent. One ml aliquots of test dilution so 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 [11].

2.5.3. Repellency Bioassay

The repellency bioassay was carried out using area preference method as described by Jilani and Su [13]. Different concentrations of essential oil (0.005, 0.015, 0.025, 0.035 & 0.045 µl/cm^2) 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 the test dilution was applied to the other half of the filter paper. 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±1°C 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.

3. RESULTS AND DISCUSSION

The percentage of oil load and the encapsulation efficiency of cinnamon essential oil by PEG were presented in table 1. The mean percentage of oil loading with 5 ml of essential oil to 100g of PEG was found to be 3.62±0.36. Similarly, the nanopreparation with 7.5 ml of essential oil reported an oil load of 5.75±0.63 and with 10 ml of essential oil reported an oil load of 7.81±0.68. The results indicate that 68.00 % 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.

Table 1: Encapsulation efficiency and oil load of PEG encapsulated Cinnamon essential oil nano-particles

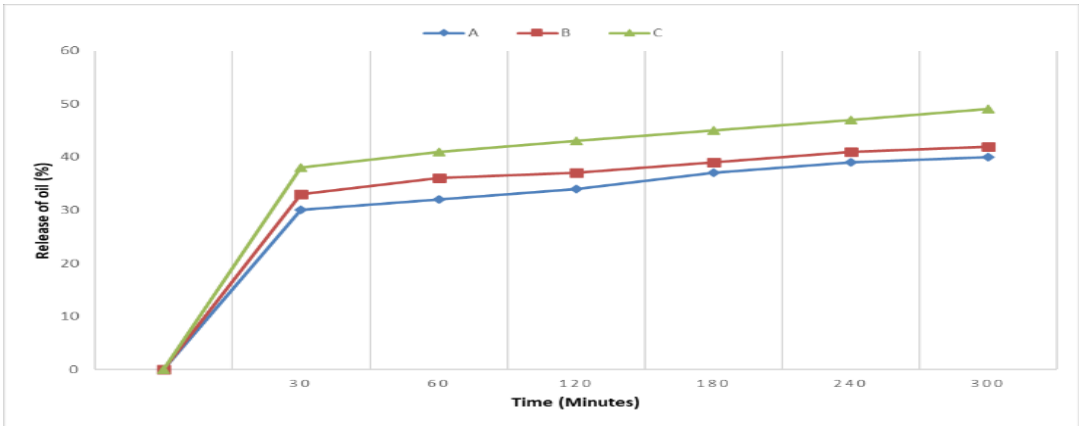
Parameters	Nanoparticles		
	A	B	C
Amount oil introduced (ml)	5	7.5	10
Oil load (%, mean ± S.D.)	3.62±0.36	5.75±0.63	7.81±0.68
Encapsulation efficiency (%, mean ± S.D.)	61.6±0.50	64.27±1.43	68.00±0.53

The insecticidal efficacy of the PEG encapsulated cinnamon essential oil was evaluated. The size of the nanoparticles stored for 0 to 3 months was examined using Dynamic Light Scattering (DLS) analysis. The size of nanoparticles at 0 months varied in range between 205.2 to 282 nm. A slight increase in particle size was observed after 3 months of storage ranged between 222.4 to 319 nm. This indicates the stability of the nanoparticles between storage periods. After 3 month of storage the size of essential oil encapsulated PEG nanoparticles increased. The nanoparticle preparation "A" was to found to possess an increased diameter of 222.4 nm. Similarly, the average particle size of "B" and "C" measured an increased particle size of 243.2 and 319.0 nm respectively.

Dynamic light scattering (DLS) was used to measure the size of the nanoparticles. The particle size varied with the amount of essential oil introduced. The particle size of cinnamon essential encapsulated PEG nanoparticles was observed with size of 205 nm (5ml EO), 232 nm (7.5 ml EO) and 282 nm (10 ml EO). However, on storage for a period of 3 months the EO-NP showed a slight increase in the size. The encapsulation efficiency of PEG with cinnamon essential oil was found to be 61.6% with 5 ml of EO, 64.27% with 7.5 ml of EO and 68.00% with 10 ml of EO. But cinnamon essential encapsulated PEG nanoparticles had low encapsulation efficiency when compared with cardamom essential PEG nanoparticles. The encapsulation efficiency was in range between 61 to 68%. The oil release pattern of the three nanoparticle preparations (A, B and C) was presented in Fig. 1. An initial burst of oil release from the nanoparticles was observed with nanoparticle preparation A accounting 42%, B with 48% and C recorded 52% 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.

The FTIR analysis of cinnamon bark essential oil exhibited absorption peaks at 2990.26cm^{-1} was assigned to C-O stretching, the peak at 1727.75 cm^{-1} denoted -C=O stretching. The peak at 1483.89 cm^{-1} signifies the presence of C-C stretching vibrations of aromatic ring [14]. The peak at 1039.44 cm^{-1} is due to the presence of C-O stretching [15]. The peak in the range between 950-780 cm^{-1} represents C-H bending vibrations [16]. The C=C vibrations of benzene ring is represented by 690.17 cm^{-1} [17]. The alcohol OH-out of blend is represented by the group frequency wave length of 720-590 cm^{-1}. The presence of various function groups was identified by FTIR analysis (Fig. 2). The FTIR spectrum revealed absorption peaks at 2990.26, 1727.75, 1483.89, 1039.44, 778.00, 690.17 and 571.97 cm^{-1}.

The morphology of the essential oil encapsulated PEG nanoparticles were examined using TEM analysis. The morphology of the nanoparticles prepared using 10% optimal ratio of cinnamon essential oil to PEG was examined using TEM. The images displayed in fig. 3 clearly show that the synthesized nanoparticles were spherical in shape with good dispersion. A similar observation of spherical morphology of nanoparticles in this study was reported by Yang *et al*. [10] with garlic essential oil loaded PEG nanoparticles.



(%, mean ± SD, n = 3)

Fig. 1: Oil release profile of Cinnamon essential oil encapsulated PEG nanoparticles with increase in time

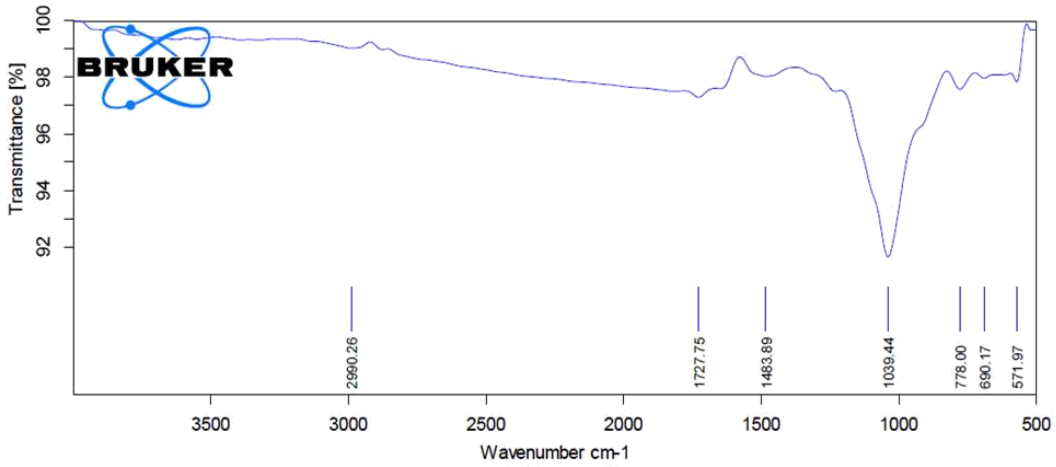


Fig. 2: FTIR spectra of Cinnamon essential oil

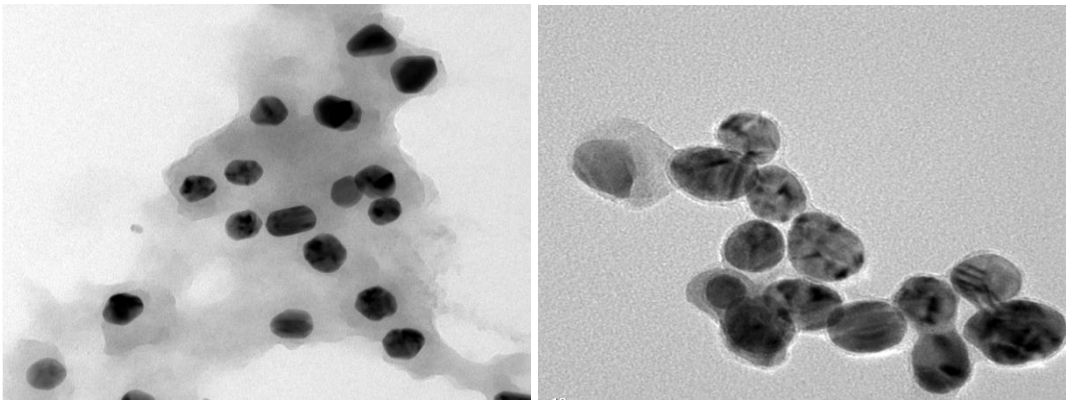


Fig. 3: TEM images of PEG encapsulated Cinnamon essential oil

The contact bioassay of cinnamon essential oil coated with PEG nanoparticles were found to be effective in inducing toxicity even after 96 h of exposure. With increase in concentration of test dosage the percentage of mortality was also found increasing. A clear dose dependent mortality was evident from the regression equation which clearly shows the positive correlation between the rate of mortality (Y) and the concentration (X) of essential oil encapsulated PEG nanoparticles. The LC_{50} and LC_{90} values were found decreasing with increase in the exposure time.

Fig. 4 portrays the repellency activity of cinnamon essential oil encapsulated with PEG nanoparticles against the adult *O.surinamensis* at 0 month of preparation. The percentage of repellency exhibited after 2 h of exposure was found to be -34%, 11%, 27%, 45% and 64% with 0.005, 0.015, 0.025, 0.035 and 0.045 µl/cm^2 respectively. Similarly, 4 h of exposure to 0.005, 0.015, 0.025, 0.035 and 0.045 µl/cm^2 concentration of EO-NPs produced 21%, 37%, 49%, 58% and 73% repellency respectively.

Fig. 5 portrays the repellency activity of cinnamon essential oil encapsulated with PEG nanoparticles against the adult *O. surinamensis* at 3rd month of preparation. The percentage of repellency exhibited after 2 h of exposure was found to be -54%, 8%, 19%, 29% and 43% with 0.005, 0.015, 0.025, 0.035 and 0.045 µl/cm^2 respectively. Similarly, 4 h of exposure to 0.005, 0.015, 0.025, 0.035 and 0.045 µl/cm^2 concentration of EO-NPs produced 17%, 23%, 34%, 47% and 67% repellency respectively.

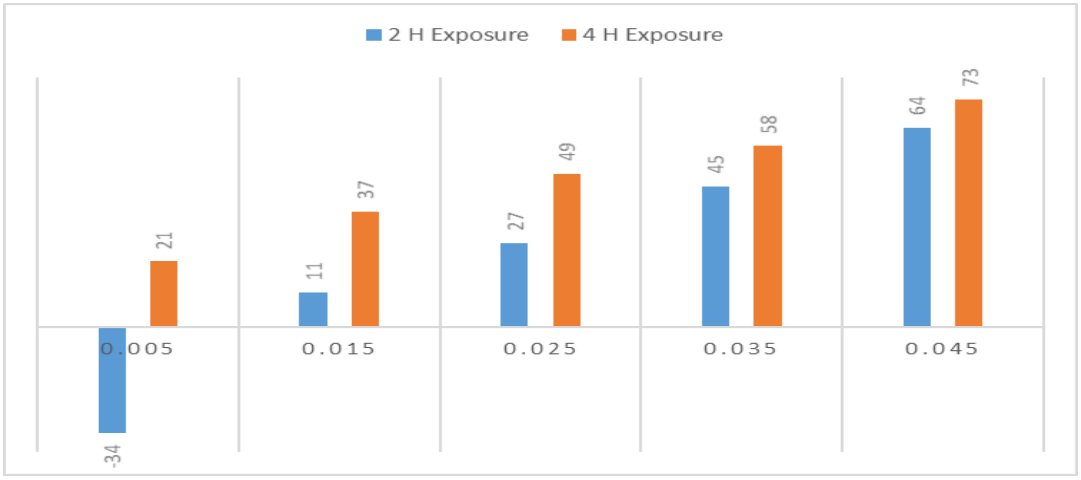


Fig. 4: PEG encapsulated cinnamon essential oil induced repellency effect (%) against adult *O. surinamensis*. (0th month)

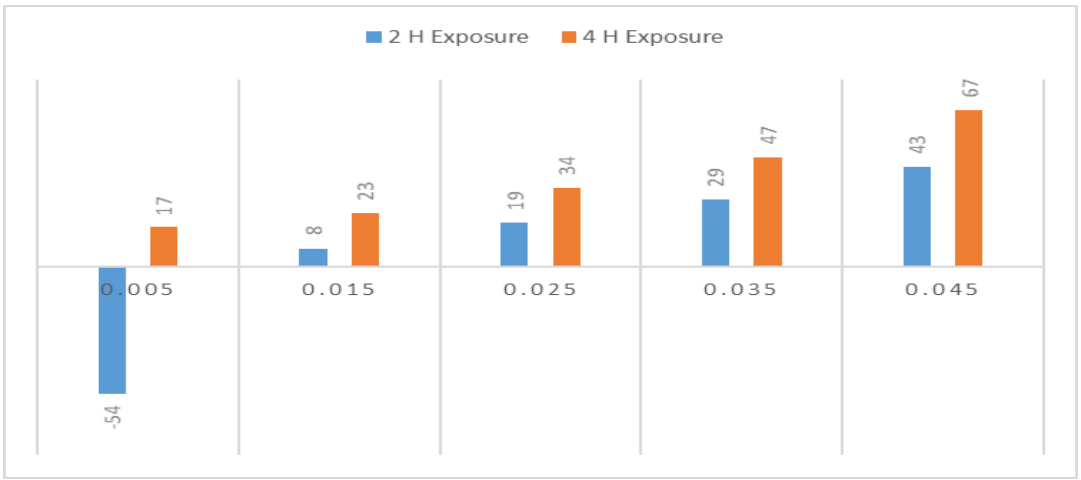


Fig. 5: PEG encapsulated cinnamon essential oil induced repellency effect (%) against adult *O. surinamensis* (3rd month)

The contact bioassay with essential oil of *Artemisia orgyi* showed high toxicity and repellent activity against *O. surinamensis*. The essential oil produced 97% of repellency at 0.4µl/cm^2 [2]. The plant powder of cinnamon exhibited 68.4% mortality at 4% concentration against *O. surinamensis* [18]. 100% mortality was observed with 0.5% concentration of *C. camphora* essential oil against adult *O. surinamensis*. 49.8 % mortality was observed in 24 h exposure. Similarly, 48 h and 72 h exposure produced 67.7 and 80.3% mortality respectively [19]. Adult *O. surinamensis* on exposure to 10 µl/cm^2 of cardamom essential oil for 12h developed 31.11% repellency. The observed repellency was found to be time dependent and thus with increased exposure period the percentage of repellency also increased to 96% after 24 h [20]. Similarly, essential oil of *Cinnamomum camphora* exhibited strong repellent activity against *O.surinamensis*. The percentage of repellency after 12 h of treatment was 83.75. But the essential oil had a reduced percentage of repellency (75%) after 24 h of treatment. Al-Jabr *et al*. [19] reported 39.46% percentage of repellency with 1% concentration of *C. camphora* essential oil.

Werdin Gonzalez *et al* [21] reported the lethal and sub lethal activity of essential oil encapsulated PEG nanoparticles against *Tribolium castaneum* and *Rhizopertha dominca*. The results showed that 10% EO-PEG nanoparticles had high oil loading efficiency. The average size of nanoparticles was found to be < 235 nm and these nanoparticles induced effective contact toxicity against the stored product insect pest. Our results are found to be complete agreement with the findings of Werdin Gonzalez *et al* [21].

4. 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 pharma-cokinetic 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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Conflict of interest

None declared

6. REFERENCES

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