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Evaluating the toxicity evaluation of nano emulsion derived from sweet Orange fruit Peel against Callosobruchus Chinensis

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Abstract
Citrus fruits produced as essential oils, (EOs), were obtained through the peels, from Citrus sinensis (sweet orange), into hydro distillation, a concentration method that uses a Clevenger-type apparatus for 3 to 4 hours. These EOs and their flavonoids, have various biological activities, such as antioxidant, antimicrobial, and cytotoxic effects, and they are also widely used as food Flavors and cosmetic ingredients. This work aims are to produce as Nano emulsions, through citrus EO from non-used orange peels, and to be study their biological effect as antioxidant, antimicrobial, and cytotoxic agents. The sweet orange peels were detached (removed), air-dried, powdered (mixture), and extracted by hydro distillation. The EOs of the samples were to be analysed by gas chromatography–mass spectrometry (GC-MS), Fourier-transform infrared spectroscopy (FT-IR), dynamic light scattering (DLS), and zeta potential measurements.

Keywords: Citrus nano emulsion, insecticidal activity, callosobruchus chinensis, sweet orange peel

Introduction

Citrous fruit peel is one of the main products of the beginning of citrus and is a valued origin mainly as the dietary fiber, coumarins, vitamins, carotenoids, and flavonoids [1]. Essential oil nanoemulsion for pest control in horticultural carbon-based frog farming. From some plant species, sweet orange peel (C. Sinensis Mexican sunflower giltonia dives folia) ginger rhizome (Zing opticinal) and lemon grass (sim pogon citratus), and these plants are subtitled, they develop vigor, a botanical insecticide [2]. It belongs to the Rutaceae family and other Sapindalska orange tree can be grown in a different leaf variation but the soil should be within pit range between 5.2 to 6.4 and it should be-rich in loam, mix sand, salt, and clay. It should be turned into rich, well-drained humus. So that the roots perforate with the new soil. Orange trees are mostly self-contained [3]. The d-Limonene, a vigorous component present in the essential oil eradicates the wax layer of the insect respirational system so that once applied directly, the insects will suffocate. 4 d-Limonene bio-assay outcomes with the tests were assumed after the action of the fabrics, which is in Bioassay significances can be appearance the toxic action of completed in cotton fabrics treated with the various amounts of the limonene, and also display the effect of experience time in contradiction of mosquitoes. The results illustrate that the repellent, reduced, and killed the action in contradiction of mosquitoes improved with growing absorption of a limonene in over cotton fabrics inside the variety studied (250–1500 mg/m2). Also, results show that the modified gesture (repellence, knockdown, and mortality) is augmented by increasing the contact time [4]. The difficulty of high-pressure elimination establishes a suitable strategy for the elimination of citrus sinensis excess composition permitting full exploitation of their properties possibly original value-added food producers [5]. Citrus peel essential oils were stayed by explored as the source of the active components aimed at developing a new eco-friendly insecticidal activity, because of their new widespread availability, relatively low cost, and efficacy against various series of crop, veterinary, medical, and stored product pests (Campolo *et al*., 2014, 2016) [6]. Oils mainly (EOs) bind through volatile and partial-volatile mixtures. Normally fragments present in oils and produced in some plants are shown as secondary metabolites. (Campolo *et al*., 2018) [7]. The impact of aggressive pests has a worldwide growing leaning mainly due to climate variation and global trade (Seebens *et al*., 2017) [8]. The impact of pests increasing worldwide. In the last two decades, Nano formulated resources and their studies for medicinal purposes. Perhaps to produce nano-size physical & biochemical varieties [9]. In this condition, 18% of citrus fruit was used to divide scientifically in the agriculture sector. Even, In environmental condition problems in citrus production. This is a big huge waste of peel and seeds. Even seed particles are produced with co-products [10]. Citrus Essential Oils were broadly considered for their potential uses in the food industry. The composition of citrus Essential Oil showed that it contains mixtures of hydro-carbons, oxygenated compounds, and non-volatile residues, including terpenes, sesquiterpenes, aldehydes, alcohols, esters, and sterols [11]. Earlier studies have, however, shown that besides being eco-friendly, phytochemical extracts have proved efficacious against many plant pathogens as well as enhancement of plants' productivity variables [12]. Citrus sinensis is one of the most commonly cultivated & evaluated important fruit crops and minimum grown in 160,000 hectares and with an annual production of 17 million [13]. But their citrus-producing countries in Pakistan compared to each unit, the production is low citrus fruit size, and quality can be reduced [14]. The use of citrus fruits as the basis of phytochemicals in the maintainable control of pest vectors would help effectively to clear the unutilized majority and lots of citrus fruits dumped as waste in our indigenous markets and citrus plantations. An evaluation of the literature on the control of different types of species of mosquito showed that the valuation of different

phytochemicals from various plants has been carried out by some researchers on vector control [15]. In a specific, the Taif province of Saudi Arabia has a prosperity of such species, which are commercially and extensively sophisticated in the manufacture of essential oils (EOs). EOs from plant species are low-molecular-weight blends that are produced in huge amounts by a variation of plant families, with the Asteraceae, Apiaceae, Lamiaceae, Rutaceae, Lauraceae, and Myrtaceae [16]. Many studies have been conducted on the use of citrus plants, Bilal *et al*., have used lemon as a larvacide of the Aedes albopictus mosquito with LC50 values at ppm and the value of LT50 at the 31st hour [17]. Prev-Am® is essentially composed of cold-pressed Citrus peel oil, whose main composite is D-Limonene, a monoterpene with high insecticidal actions [18]. Earlier studies have exposed that essential oils (EOs) from many higher plants are known to have better poisonousness and control for the allelopathic activity that decreases the growth of neighboring crops [19]. The genus Citrus, belonging to the family Rutaceae, includes about 140 genera and 1300 species. Citrus sinensis (Orange), Citrus reticulata (tangerine), and Citrus aurantium (sour orange), are some important fruits of the genus Citrus [20]. Citrus is one of the primary crops to spread as a human crop in a minimum of 21000 BC [21]. Of late, these crops have gained deep interest among scientist for their antimicrobial activity. Sweet orange oil is active against some gram-positive and gram-negative bacteria [22]. Orange peel is one of the most imperative marketable fruits cultured in all areas of the world. The position of the orange is accredited to its differentiated use and the farming worldwide and which of the probably stands to the first amongst the cultured fruits. Citrus sinensis (sweet orange) is commonly cultivated in the Nigeria and many of other tropical and semitropical regions [23]. Recently, only a few reports have used performance chromatography liquid electrospray (GC-MS) ionization tandem mass spectrometry (UPLC-ESI-MS/MS) [24]. The natural flavonoids from citrus species (hesperidin, naringin and neohesperidin exhibited anti-fungal activity against the growth of Aspergillus spp., Fusarium semitectum and P. expansum, fungi commonly found in food, and also reduced mycotoxin accumulation [25].

Materials & methods

Test insect

1. *Callosobruchus chinensis*

(Coleoptera: Bruchinidae)

Insects were Collected from PJTSAU (Prof. Jayashankar Telangana State Agriculture University (Rajendra Nagar))

↓

Reared them in the laboratory on stored grains for studying Life cycles

Test plant material

- Sweet orange (Citrus sinensis) fruits were collected from organic farms located in the MAHABUBABAD district of TELANGANA STATE.
- Identification of the composed plant materials was completed through the help of Plant taxonomists after the Department of Botany.
- Voucher specimens were conserved in the Department for future purposes.

Preparation of powder

The peels stayed separated from sweet orange (citrus sinensis) fibres remained removed then peels were dried for one week and the powder remained prepared by using an electric grinder.

Collecting Essential Oils from Peel powders through the Clevenger apparatus

Primary, the sample is weight through a weighting stability/machine then after insertion of the Round Bottom (RB) container use a funnel for placing the sample inside, at that time need to add distilled water in the RB flask, then the percentage of the sample and purified water must be maintained often for dried sample 1:10 ratio is obligatory while adding purified water is level should be above the half line of the RB flask then should blend the sample by shaking the RB flask carefully, in the next stage connect the remaining part of Clevenger apparatus to the RB flask and place it on the electric heater immersion, tightly close knob to avoid the leakage, and temperature is set at 90-100degrees Celsius until it starts to boil. It starts to boil temperature is lowered and set to 40-50◦ Celsius. The condenser is let pipe is connected to the water tap and out of the condenser and the setup is left for 4-5hrs. After 4-5hrs oils were get collected.

Nanoemulsion preparation method

3: 1 (SO (Sweet orange)
(15ml (SO), Tween 80 -5ml)
↓
Magnetic stirrer 30 min
↓
Add 80ml distilled water
↓
Magnetic stirrer 60 min
↓
5min ultra sonification 100w power

Results

1. Life Cycles of the Test Insects

2. Characterization of Test Compounds

a. GC-MS
b. FT-IR
c. DLS
d. Zeta Potential

Life cycles of Test Insects:

Callosobruchus Chinensis (Coleoptera: Bruchinidae)

Callosobruchus chinensis (pulse beetle)

Scientific classification

- Kingdom: Animalia
- Phylum: Arthropoda
- Class: Insecta
- Order: Coleoptera
- Family: Chrysomelidae
- Genus: *Callosobruchus*
- Species: *Chinensis*
- Callosobruchus chinensis is to be mainly, as a common species in beetle originate in the bean weevil, sub-family and is known as, to be a pest to as many stored pulses. Other common names, include the Pulse beetle, Chinese bruchid, and cowpea bruchid.

Life history

- **Distribution:** It is present all over the world.
- **General appearance:** The adult beetles are chocolate-colored and are 3 mm in length.
- They have two white spots near the middle of their body and have comparatively small heads.
- The total life cycle is finalized in 60 days, generally.
- But it may be prolonged due to temperature and humidity.
- The pest hibernates during winter.

Eggs

- The female lays on the upper layer of the stored grains.
- The eggs are white translucent, smooth, and oval
- A single female can lay up to 100 eggs.
- The eggs are laid singly, but the eggs may be present on one grain.
- Grubs: After incubation of 4 to 5 days the grubs hatch out.

Pupa

- The grubs pupate inside the seed.
- The pupa is brown in color.
- The pupation period is of 4 days in summer and about 28 days in winter.

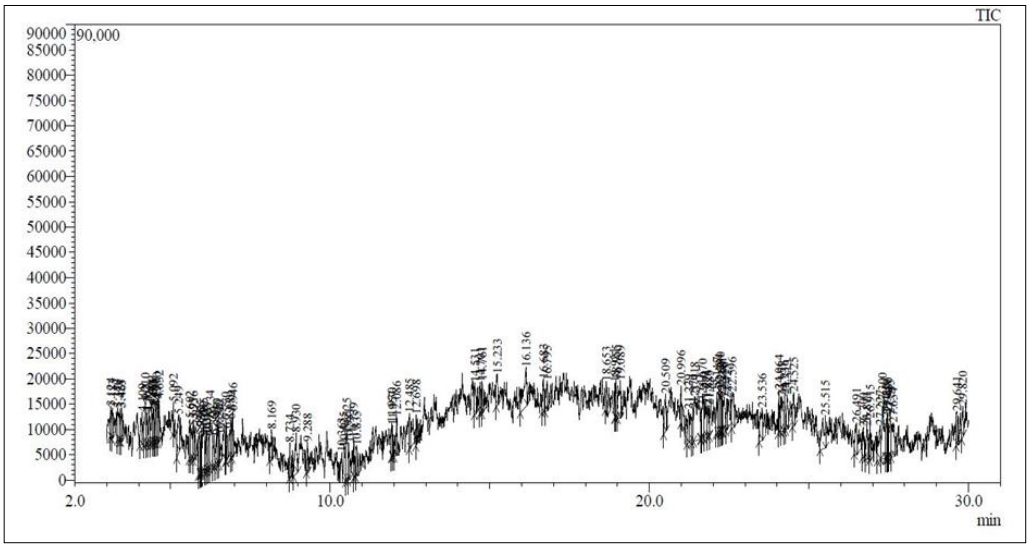
Adults

- The adults come out by removing the cover.
- The beetles shift to the upper layer of the seeds.
- The entire shift to the upper layer of the seeds.
- The entire life span is 8 to 21 days.

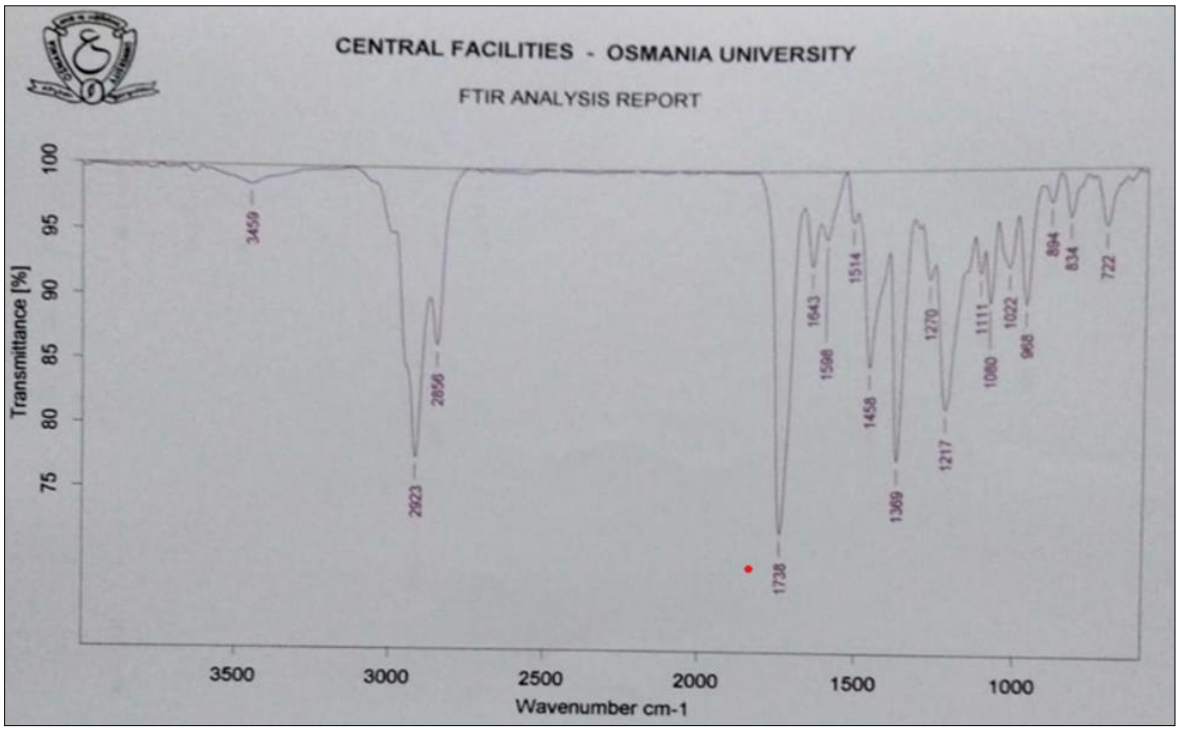
Eggs
↓
Grubs
↓
Adult
↓
Pupa

Results

GC-MS of SOEO-NE



17. Benzoxazole, 2-[2-(4-piperidinyl) pyrimid-5-yl]-
18. 1,3-Cyclohexanedione, 5-[4-methoxyphenyl]-
56. Pentamethylcyclopentadienyl-ethylisonitril
67. Azabicyclo 2.2.2] octan-3-one, 2-[(4-nitrophenyl)
78. Propanoic acid, 3,3'-thiobis-, diethyl ester
93.Thiophene-2-carbonitrile, 3-methyl-4-(3-dimethylamino)

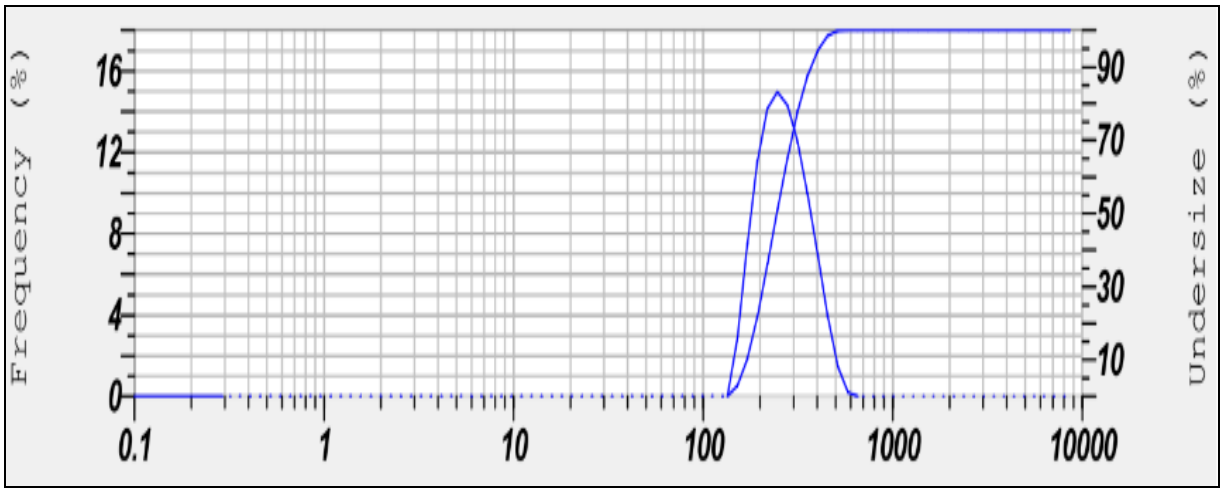


FT-IR of SOEO-NE

3459 OH stretch Hydrated Hydroxy (Aliphatic hydronated sulfate salt or Alcohol)

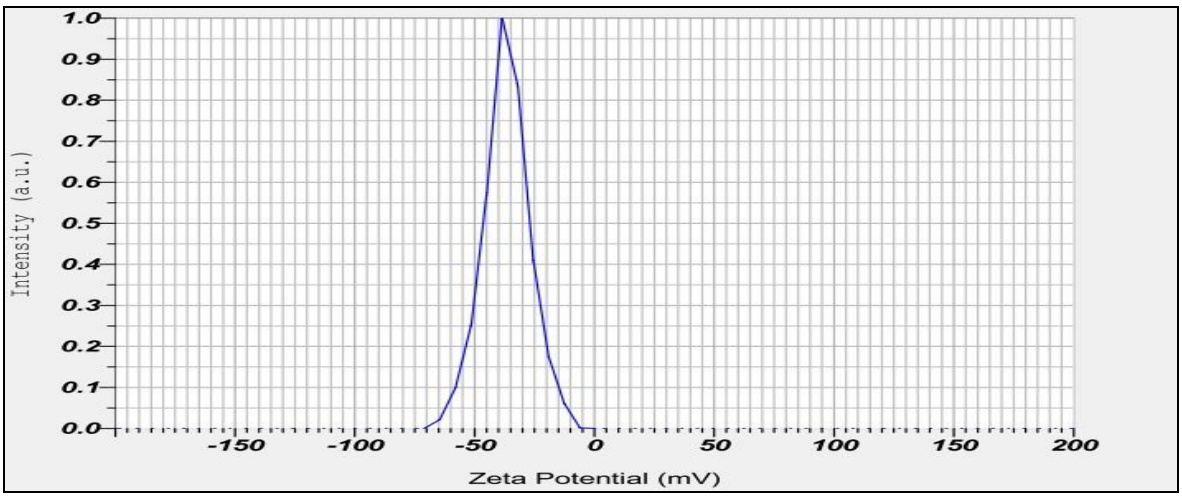
- 2923 CH stretch Alkyl (Aliphatic ether or Sulfonate salt)
- 2856 CH stretch alkyl (Hydroxy compound)
- 1738 CH stretch Alkyl (Aliphatic hydronated sulfate salt or Alcohol)
- 1643 C=O stretch (carbonyl alpha alkyl substituent0
- 1598 OH bend Hydrated (Aliphatic hydronated sulfate salt or Alcohol)
- 1514 C-C stretch ring (Aryl strained ring or Activated Carbonyl)
- 1458 CH bend CH2 (Aliphatic ether or Sulfonate salt)
- 1369 CH bend Ch3 (Aryl strained ring or Activated Carbonyl)
- 1270 Epoxy (epoxy substituent)
- 1217 CO stretch (alkoxy substituent)
- 1111 CO stretch (alkoxy substituent))
- 1080 SO2 Sulfoxy stretch (Aliphatic ether or Sulfonate salt)
- 834 CH bend OOP (Aryl strained ring or Activated Carbonyl)
- 722 CH bend OOP (Aryl strained ring or Activated Carbonyl)

DLS of SOEO-NE



- SOEO-NE particles Average Size =245.3 nm
- RANGE = 157.2(nm) - 372.2 (nm)

Zeta Potential of SOEO-NE



- SOEONE Zeta Potential = -36.6mV

Conclusion

Results to be originate after to this, research by proved that, ethyl & ethanol extracts of sweet orange (SO), juice have variable - degrees of antibacterial activity, in contradiction of the test of creatures. This proposed that extracts of sweet orange. juice can be remain useful in the viable, of a new drugs, which can be used as in the generous of bacterial infections were triggered by the test of organisms in to this study**.**

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