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Toxicity Evaluation of Punica Granatum Fruit Peel Nanoemulsion against Tribolium Castaneum (Coleoptera: Tenebrionidae)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In this work, the essential oils were extracted from the peels of Punica granatum (Pomegranate). Insects carry natural ecosystem service area as biological controller of pests specifically the biodiversity-rich countries. Insects one of the maximum common pest stored grains and identified to infest 233 different types of pests. The study is to be synthesised the Essential oils and nano emulsion from the waste of Mosambi fruit peel. The nano emulsion were prepared and before, double distilled water added to mixture (4:1 respectively) and stirred for 60min to attain a homologous Emulsified phase.

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Keywords: Tribolium castaneum; pomegranate peel; nanoemulsion; Insecticidal activity.

1. INTRODUCTION

"The world population will reach 10 billion by 2050. To feed this increasing population more food is to be produced. One method to secure high food reserves is reducing losses by applying crop protection chemicals" [1]. "Post-harvest losses by insect pests account for 25–50%. Insects are known to infest about 233 different types of stored grains" [2]. "These insect pests are associated with a loss of more than one billion US dollars per year worldwide" [3]. "Synthetic pesticides are very important in the cropping system to minimize food losses caused by insects, weeds, microbial diseases, and other pests" [4]. "Therefore, the use of such natural pesticides in the management of different pests should be taken into account. Although different researches on the effect of different acaricide on two-spotted spider mite have been done" [5], "According to the FAO estimate, 10 to 25% of the world's harvested food is destroyed annually by insects and rodent pests" [6]. "No wonder that different chemical constituents have been extracted from different parts of pomegranate like triterpenoids, steroids, alkaloids, flavonoids, tannins, polyphenols and some anthocyanins which have been isolated from peels" [7]. "The combination of biological principles with different chemical and physical methods is called bio-nanotechnology producing nanoparticles with specific functions. The chemical synthesis of nanoparticles is a very costly method, and it is considered toxic with low productivity. Subsequently, the biological synthesis methods from plants or microbes have attractive application because they are costless, safe, rapid, and eco-friendly" [8]. "Insect pests inflict heavy losses on stored food grains especially in tropical and semitropical environments. There is need for use on stored food grains. Certain essential oils, on account of their volatile nature and other traditional uses, offer possibilities for their use as effective repellents against stored grain insect species" [9]. "Even though recently, alternative molecular mechanisms were hypothesized to explain the virus's entry into the cells" [10], "The binding of SARS-CoV-2 S protein to human ACE2 remains the main route of the virus's access to the cells and more directly related to the subsequent levels of infectivity" [11]. "An alternative management strategy that is receiving increased interest is fumigation process which defined as a process that occurs when volatile compounds with pesticidal properties are released during decomposition of plant materials or animal products" [12].

2. MATERIALS AND METHODS

2.1 Test Insect

1. Tribolium castaneum

(Coleoptera: Tenebrionidae)

Collected insects From PJTSAU (Prof. Jayashankar Telangana State Agriculture University (Rajendra Nagar))
↓
Reared them in the laboratory on stored grains for studying Life cycles

Fig. 1. Lab analysis

2.2 Test Plant Material

- *Punica granatum (Pomegranate) fruits* were collected from organic farms located in MAHABUBABAD district TELANGANA STATE.
- Identification of the collected plant materials was done with the help of Plant taxonomists from the Department of Botany.
- Voucher specimens were preserved in the Department for future purposes.

2.2.1 Preparation of powder

The peels were separated from Pomegranate fruit fibers were removed then peels were dried for one week and the powder was prepared by using an electric grinder.

2.2.2 Collecting essential oils from peel powders through the clevenger apparatus

At first, the sample weight using a weighting balance/machine then after placing the Round Bottom (RB) can use a funnel for placing the sample inside, then need to add distilled water in the RB flask, the ratio of the sample and distilled water must be maintained usually for dried sample 1:10 ratio is required while adding distilled water is level should be above the half line of the RB flask and should mix the sample by shaking the RB flask carefully, in the next step connect the remaining part of Clevenger apparatus to the RB flask and place it on the electric heater bath, tightly close knob to prevent 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 degrees Celsius. The condenser is let pipe is connected to the water tap and out of the condenser and the setup is left for 3-4 hours. After 3-4 hours oils get collected.

2.2.3 Preparation of essential oil-nanoemulsion

- The EO nano-emulsion was prepared following Bouchemal et al. (2004), with modifications.
- A mixture (3:1 v:v) of PG-EO and Tween 80® [Polyoxyethylene (20) sorbitan monooleate], was stirred for 30min...
- Then, double-distilled water was added to this mixture (4:1 respectively) and stirred for 60 min to attain a homogeneous emulsified phase.
- This coarse emulsion was sonicated for 5 min using a UP200ST ultrasonic immersion homogenizer at 100 W power to optimize its physical characteristics and reduce micellar dimension.
- The obtained oil in water nano-emulsion was composed of 5% Tween 80®, 15% PG-EO, and 80% water.
- Lastly, the SO-EO nano-emulsion was stored at 25 ± 0.5°C in an airtight glass bottle and used for the bioassays within the following 5 days.

2.3 Nanoemulsion Preparation Method

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

3. RESULTS AND DISCUSSION

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:

1. *Tribolium castaneum* (Coleoptera: tenebrionidae)

2. *Tribolium castaneum* (Red flour beetle)

Scientific classification:

Kingdom : Animalia
Phylum : Arthropoda
Class : Insecta
Order : Coleoptera
Family : Tenebrionidae
Genus : *Tribolium*
Species : *castaneum*

Fig. 2. Samples for analysis

- **EGGS**

The egg duration ranges from 9-14 days

- **First instar larva**

The duration of the first instar ranged from 16-18 days**.**

- **2nd larval stage**

The second larval duration ranges from 10-14 days.

- **Larva**

The larval stage is in 8-10 days. The 7th larval stage duration is 9-11 days.

- **Pre pupal stage**
- **Post pupal stage**

Post pupal stage ventral view of male and female

The male and female pupal period ranged from 6-7 days for males and 7-9 days for females.

- Initial stage of adult
- Adult male and female
- The adult duration of males is 45-50 days and females 70-80 days.
- Ventral view of adult females and males the total life cycle of a beetle was 164 to 195 days.
- The red flour beetle (*Tribolium castaneum*) is a worldwide pest of stored products, particularly food grains.
- It is the most common pest of wheat flour., and a model organism for ethological and food safety research.
- It also causes serious damage to dried fruits, pulses, and prepared cereal foods, such as cornflakes, pasta, biscuits, beans nuts, etc.
- Both larvae and adults feed on grain dust and broken grain, but not the undamaged whole grains, and spend their entire life cycle outside and grain.

GC-MS: PGEO-NE:

5. Isonipecotic acid, N-(3-fluorobenzoyl)-, heptadecyl
27. Thiophosphoric acid, o-(2-benzylthio-1-phenylethenyl)
28. Glycine, N-[N-[N-[3-[(2-decanamidoethyl) thio]-
62. 20.675 20.755 40309 1.95 3,8,13,18-Tetraethyl-2,7,12,17-tetramethyl-7,8-(diacetyl)
63. Silane, (16.beta.,17.beta.) -3-methoxyestra -1,3,5(10)-triene-16,17-diyl] bis(oxy)] bis [trimethyl-
99. Sebacic acid monomethyl ester

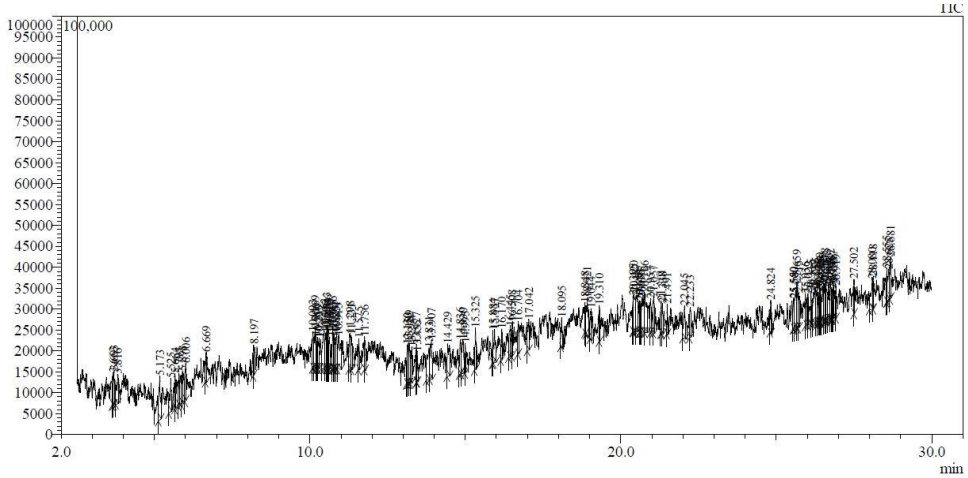

Fig. 3. GC-MS result

FT-IR of PGEO-NE:

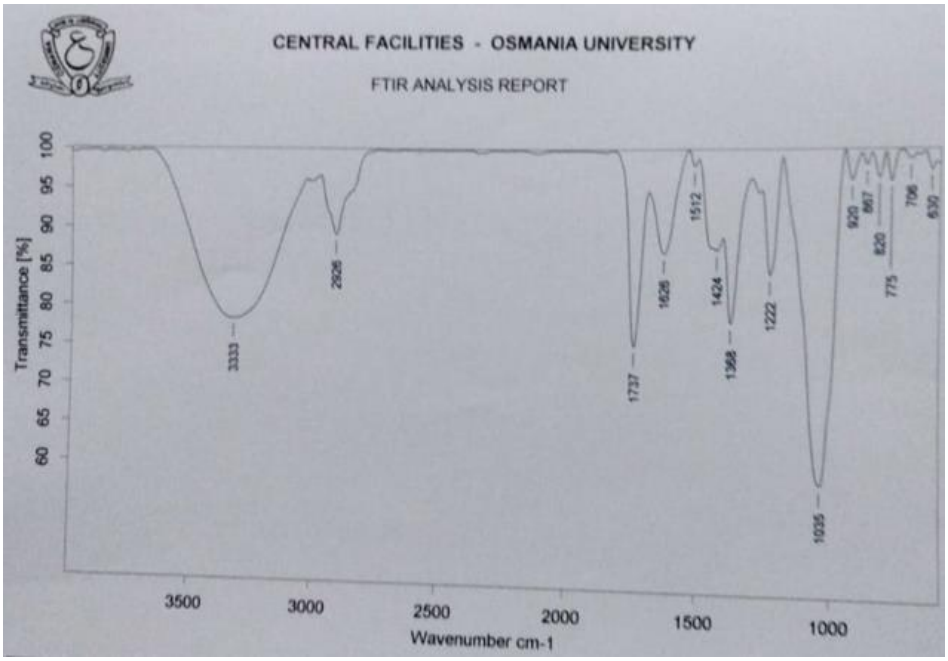

Fig. 4. FTIR analysis report

3333 O-H stretch Hydroxy or Hydrate (Aliphatic hydrated sulfonyl or alcohol)
2926 OH stretch (Hydroxy compound)
1737 C-H stretch Alkyl (Aliphatic ether orsulfonate salt)
1626 C-O stretch Carbonyl Alpha alkyl substituent
1512 O-H bend Hydrate (Aliphatic Hydrated sulfonate salt)
1424 C-H bend CH2 (Aliphatic ether or sulfonate salt)
1368 CH bend CH3 Alkoxy Substituent
1222 C-C CH2 bend (Aliphatic mercapto group)
1035 SO2 Sulfoxy bend (Aliphatic ether or sulfonate salt)
920 Epoxy substituent

DLS of PGEO-NE:

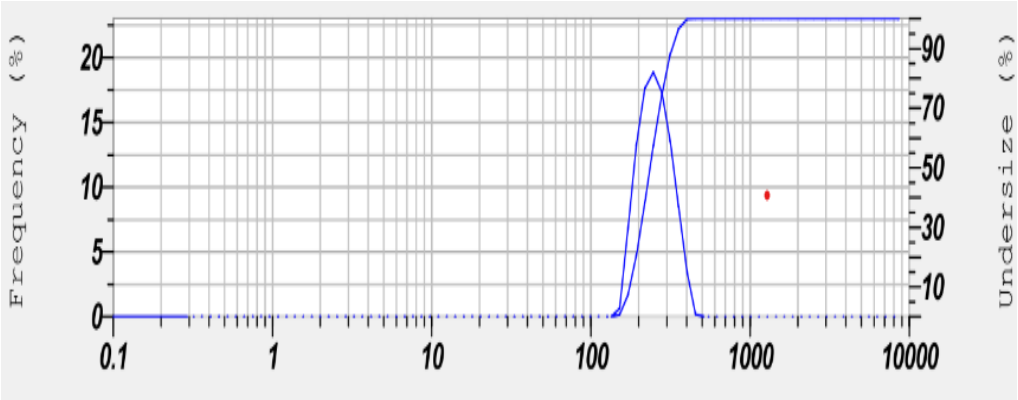

Fig. 5. Graph showing DLS of PGEO-NE

PGEO-NE Particles Average Size = 251.8 nm
RANGE = 164.2(nm) - 324.7 (nm)

Zeta Potential of PGEO-NE:

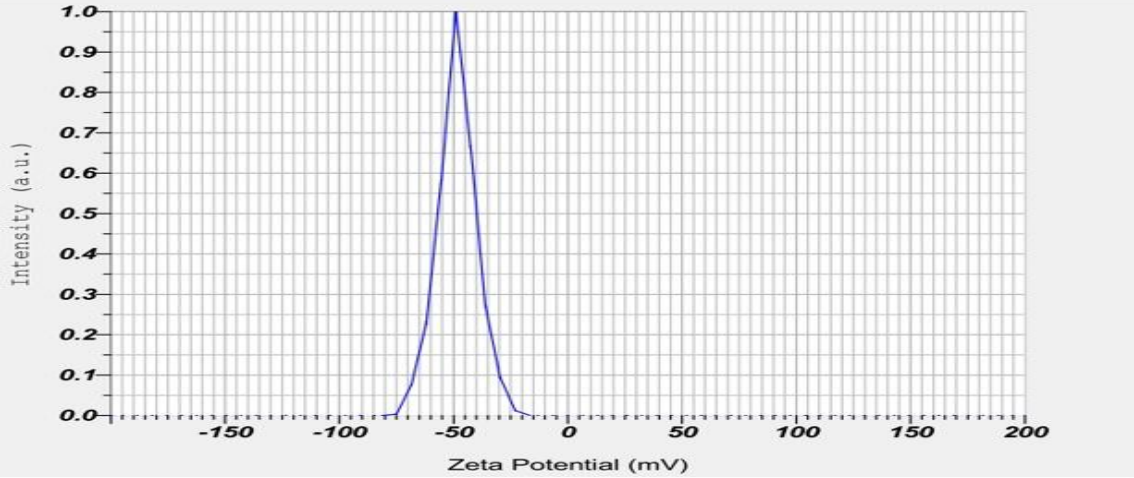

Fig. 6. Graph showing Zeta Potential of PGEO-NE
PGEONE Zeta potential = -48.4 mV

4. CONCLUSION

Pomegranate is a popular fruit with sweet taste but hard pericarp. The yield of juice is less than half of the total weight of the fruit, which leads to a large amount of peel waste. The study is to be synthesised the Essential oils and nano emulsion from the waste of Mosambi fruit peel.
Abbreviation:

GCMS :Gas chromatography/ mass spectrometry
PGEO : Pomegranate Essential oil
NE : Nano Emulsion
FTIR : Fourier transform infrared
DLS : Dynamic light scattering

Work to be done:

1. Studies on contact & fumigation activities of the test compounds.
2 Studies of repellent activities of the test compunds.
3. Statistical analysis
4. Comparison of the efficacy of test compunds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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