# “EUCALYPTUS GLOBULES”: NATURAL PESTICIDES

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# "EUCALYPTUS GLOBULES": NATURAL PESTICIDES

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**Abstract:** The objective of this research work was to investigate antifungal activity, insecticidal activity and chemical composition of eucalyptus globules (eucalyptus oil) against grain bugs originated in stored grains. In this study, essential oil of Eucalyptus globules was obtained by hydro-distillation using Clevenger apparatus. Cineole was major chemical constituent isolated from eucalyptus oil. Presence of cineole in eucalyptus oil was determined and confirmed by GC and FT-IR analysis. Organoleptic and Physio-chemical properties of eucalyptus oil were also analyzed. Apart from cineole other compounds present in eucalyptus oil were α-pinene, β-pinene, and terpenene-4-ol. 78.77 % of cineole was quantified in Eucalyptus oil.



**Keywords:** Insecticidal activity, grain bugs, eucalyptus globules, cineole, GC, FT-IR, organoleptic, physio-chemical, α-pinene, β-pinene, terpenene-4-ol.

**Introduction:** The food damage causing due to insects is thoroughly subject of concern for scientists in recent years. Grain bug alone bring about majority of reduction in the yield of stored grain products. Thus to fight against grain bugs, pesticides are used that kill or destroy pests (including Bacteria, Fungi, Insects, Weeds, Termites etc), that damages fruits, vegetables, grains etc. Greater part of country utilized synthetic pesticides for inhibiting growth of insects in stored grains. Synthetic pesticides do give protection to products (vegetables, fruits, grains etc) but at the same time excessive usage of this pesticides result in damage to same product. Thus synthetic pesticides play dual nature of acting both as protector and destroyer. As synthetic pesticides are produced from harmful chemical they are hazardous and highly toxic to human health. Synthetic pesticides are not biodegradable in nature forming residues that remain in soil, water and air for month to month affecting public

health and environment. Moreover, synthetic pesticides are less resistant to pests due to which they take long time to inhibit insects and so there is need for alternative tool to replace them.

To overcome this drawback, scientists thought about replacing synthetic pesticides with natural pesticides originating from plant source. Natural pesticides has tendency to overcome all the limitation of synthetic pesticides. They are neither toxic nor hazardous to mankind but they are highly toxic to pest. Natural pesticides do not leave any of its traces in soil, water or air as they are bio degradable in nature. These pesticides are not even toxic to kids as they have very less immunity to fight against harmful chemicals. In the present work fair efforts have been made to show a eucalyptus oil derived from plant extracts i.e. essential oil possessing pesticidal (insecticidal) property. Eucalyptus tree is a traditional tree grown in India for more than 100 years. Eucalyptus trees are well known for their greenery nature growing up to 50 to 60 m. They belong to family Myrtaceae[1] and kingdom Plantae[1]. The Myrtaceae family consists of almost 130 genera and 3000 species[2]. Eucalyptus oil is mostly used for medicinal purposes in pharmaceutical industries and to limited extent in flavor, fragrances and cosmetics industry. Eucalyptus oil can be extracted from both leaves[3] and stems of eucalyptus globules trees. Eucalyptus oil shows good anti bacterial[2,4], anti fungal[4,5] and antiviral[4] activities. Thus they are considered as green[6] and natural pesticides[7]. Moreover they also show high insecticidal[8,9] and antimicrobial[9,10] activities. Eucalyptus oil extracted from different origin has different chemical constituents[4]. But this paper is totally concerned with chemical composition of eucalyptus trees grown in Mumbai, India.



## Materials and methods

**Test insects:** Insects were collected from local market, found adhering in stored grains. It was known that these insects remain alive in closed system for month to month. Thus were stored in container under observation at room temperature until applying for insecticidal tests.

**Extraction materials:**

The fresh stems and leaves of Eucalyptus trees were collected from Maharashtra, Mumbai. India. The stems and leaves of eucalyptus were sunlight dried prior to extraction for 48 hour. After drying, leaves and stems were stored away from moisture until extraction of oil. The essential oil of different plants possesses high pesticides property. Similar is the case with

essential oils like Eucalyptus oil. Hence, these essential oils were selected as natural pesticides for the further research work.

**Methods:**

**Isolation of essential oil:** The isolation of Eucalyptus oil was done by using hydro distillation method. Cleaning of leaves and stems was done to remove the external foreign material such as soil particles, dust, etc. Raw materials were ground to ease the operation of hydro-distillation and to increase the yield. Further 100 grams of ground raw materials were subjected to distillation i.e. hydro distillation by using Clevenger apparatus. After completion of extraction, immediately heating was stopped to avoid excessive heating. Then sufficient cooling time was provided to avoid the loss of essential oil in the form of vapors. Oil and water formed separate two layer in Clevenger tube based on density difference and accordingly separation of oil was carried out. Collected oil was further dried using anhydrous sodium sulphate to remove traces of moisture. Batches were carried out in atmospheric pressure. The ratio of sample to water was maintained at 1:10 w/w for stems and 1:14 w/w for leaves. The total volatile oil was determined by Clevenger type apparatus. Storage of essential oil was done in refrigeration until analysis.



**Analysis of Eucalyptus oil:**

1. Physio-chemical analysis

Oil obtained from hydro-distillation was subjected to physio-chemical analysis such as refractive index, specific gravity, acid value, moisture.

- **Moisture:** Moisture content of all the samples were carried out by constant oven drying method.
- **Refractive index:** The abbes refractometer is convenient for measurement of refractive index. To achieve accuracy apparatus should be calibrated against distilled water, which has refractive index of 1.3325 at 25°C. After calibration samples refractive index was measured.
- **Specific gravity:** Weight per milligram of a liquid is weight in gram of 1 ml of a liquid when weighed in air at 25° C, unless otherwise specified. Procedure: Thoroughly clean and dry pycnometer was selected. Specific gravity of liquid was obtained by dividing the weight of liquid contained in the pycnometer by the weight of water contained, both determined at 25°C.

- **Acid value:** Procedure: 2.5 g. of the oil was taken into a100 ml. saponification flask. Then Addition of 15 ml of neutral 95 per cent alcohol and 3 drops of a 1% phenolphthalein solution was done. Titration of the free acids with a standardized 0.1 N aqueous sodium hydroxide solution was carried out by adding the alkali drop wise at a uniform rate of about 30 drops per min. The first appearance of a red coloration that does not fade within 10 sec is considered the end point.

2. Organoleptic evaluation

Organoleptic properties of oil such as color, physical appearance, odor and solubility were determined.

- **Color:** Color of all the four essential oil was done by visual observation.
- **Physical appearance:** Physical appearance of all the four isolated essential oil was done by visual observation.
- **Odor:** Odor of oil was determined by sensory evaluation
- **Solubility:** Solubility of all isolated essential oil was checked in water as well in solvents.



**Table 1: Physio chemical and organoleptic analysis of Eucalyptus oil**

| Test | Eucalyptus oil |
|---|---|
| Moisture | 0.02% |
| Odour | Aromatic |
| Solubility | Soluble in solvent |
| Physical state | Liquid |
| Colour | White |
| Refractive index at 25 °C | 1.466 |
| Specific gravity at 25 °C | 0.91 |
| Acid value | 5.654 |

3. Phyto-chemical analysis

This analysis does not give any brief information about compounds of oil but it do show group of phyto-chemical compound present in oil. Phyto-chemical screenings were performed using standard procedures.

- **Test for anthraquinones:** 0.5 g of the extract was boiled with 10 ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

- **Test for terpenoids (Salkowski test):** To 0.5 g each of the extract was added 2 ml of chloroform. 3ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

- **Test for flavonoids:** 5 ml of dilute ammonia was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappears on standing indicates the presence of flavonoids.



- **Test for tannins:** About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration

- **Test for saponins:** To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

- **Test for cardiac glycosides (Keller-Killianitest):** To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a

greenish ring may form just above the brown ring and gradually spread throughout this layer.

**Table 2: Phyto-chemical analysis of Eucalyptus oil**

| Phtyo-Chemical | Eucalyptus Oil |
|---|---|
| Anthraquinones | Absent |
| Terpenoids | Present |
| Flavonoids | Absent |
| Saponin | Absent |
| Tannins | Absent |
| Cardiac glycosides | Present |

4. Confirmatory test for presence of compounds

This test confirms the presence of organic compound present in isolated oil.



- **Test for aldehyde:** 0.05 g or 1-2 drop of compounds + 3 $cm^3$ of 2, 4 – dinitrophenyl hydrazine solution, shake well.
- **Test for ketone:** 0.01 g or 2-3 drops of compound + 1 $cm^3$ of sodium nitroprusside solution + 2 drop of NaOH solution.
- **Test for phenol:**
  i. Phthalein test: 0.01 g of compound + 0.01 g of phthalic anhydride + 2 drops of conc. $H_2SO_4$. Heat it gently until the mixture fuses, cool and pour it in 20 $cm^3$ of very dilute NaOH solution.
  ii. Liebermann test: 0.01 g of compound + 1 $cm^3$ of conc. $H_2SO_4$ + 2 crystals of $NaNO_2$. Heat it gently. Dilute it with water, add 20% NaOH solution.
- **Test for ester:** Dissolve 0.01 g or 0.5 $cm^3$ of compound in 1 $cm^3$ of ethyl alcohol + a drop of phenolphthalein + 2 drops of very dilute NaOH solution. Heat on water bath.
- **Test for alcohol:** Take a small piece of dry Na metal in a fusion tube and add a few drops of compound.

**Table 3: Confirmatory test of Eucalyptus oil**

| Tests | Eucalyptus oil |
|---|---|
| Aldehyde | Present |

| | |
|---|---|
| Ketone | Present |
| Phenol | Absent |
| Ester | Present |
| Alcohol | Present |

5. GC analysis

The gas chromatography (GC) analysis of the essential oil was carried out on capillary column (HP-5 Agilent 19091J-413; 325 C 30 m X 320 µm X 0.25 µm). Temperature programming was 90 -190°C with 10 °C ramp/min and 190 – 290 °C with 5 °C ramp/min. Inlet temperature and detector temperature was 180 °C and 210 °C. Nitrogen was used as carrier gas at 1ml/min. FID section was used. The sample injection volume was 1.0 µl/min diluted in n-hexane, with split ratio of 100:1.

**Table 4: Chemical constituents of Eucalyptus oil**

| Sr. no | RT | Constituents |
|---|---|---|
| 1 | 8.946 | α-pinene |
| 2 | 10.146 | β-pinene |
| 3 | 11.821 | Cineole |
| 4 | 13.380 | - |
| 5 | 15.939 | Terpenene-4-ol |
| 6 | 16.311 | - |



6. FTIR spectrometry analysis

FTIR analysis does not determine quantitative determinations of different compound present in the extracted sample. But it helps to known the functional group of different constituents present in samples. Infrared Spectra of different samples were recorded in a spectrophotometer shimadzu FTIR model happ-genzel in a frequency range from 4500 $cm^{-1}$ to 500 $cm^{-1}$. The specimens having exposure area of 1 $cm^2$ per sample were prepared as it mentioned above. The liquid sample was directly place on platform provided for sampling and sampling was done. Before and after sampling the specimen were properly clean by

using n- acetone and again washed with distilled water and then dried. Subsequently, part of the surface of material was corresponding to obtain the infrared spectrum of specimen.

**Table 5: FT-IR results of Eucalyptus oil**

**For cineole**

| Sr. No | Bond | Functional group | Frequency, $cm^{-1}$ | Compound confirmed |
|---|---|---|---|---|
| 1 | C-H Substituted | para disubstitute aromatic | 842.89 | Cineole |
| 2 | C- bonding, C-H bonding | methyl, methylene | 983.7 | Cineole |
| 3 | C-H bonding | iso-carbon (ring substituent) | 1375.25 | Cineole |
| 4 | C-C stretch | aromatic ring | 1465.9 | Cineole |
| 5 | C-H stretch | aromatic ring | 2966.52 | Cineole |
| 6 | C-O stretch | aromatic ether linkage | 1080.14 | Cineole |
| 7 | C-O stretch | aromatic ether linkage | 1215.15 | Cineole |



**Identification of chemical constituents**: Identification of cineole and other constituents of eucalyptus oil occurring in GC graph shown below were done by comparing both retention time and area of samples, of extracted oil and standard cineole. Both standard and extracted samples were diluted in same concentration followed by same method of programming for analyzing on GC. Few other compounds, whose standards were not available, were identified by comparing literature data from library. Calibration curve was used to quantify cineole in oil. Confirmation of cineole present in eucalyptus oil was done by FT-IR analysis. In FT-IR analysis cineole was identified by confirming functional group of peaks adhering in FT-IR spectrum with standard peaks of cineole.

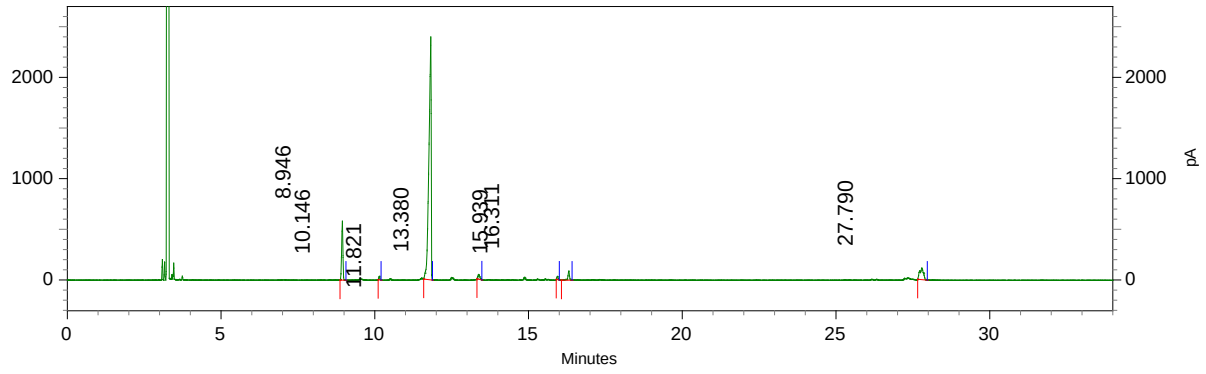


**Figure 1: GC graph of extracted "Eucalyptus oil"**

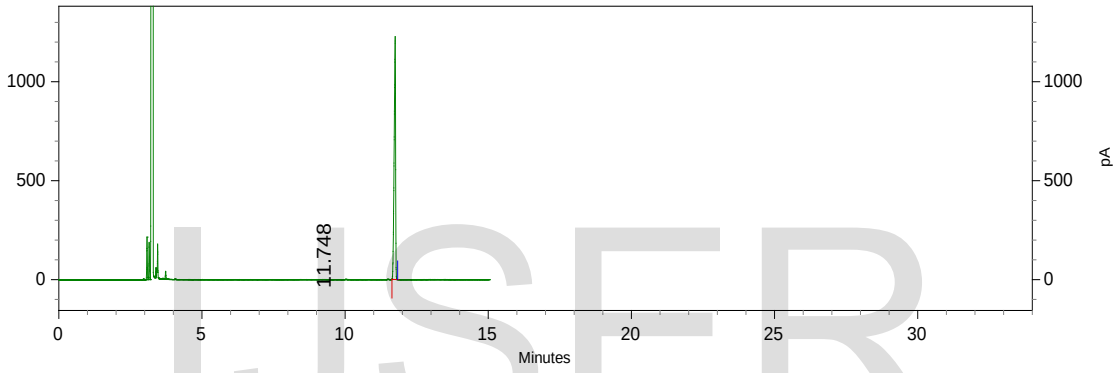




**Figure 2: GC graph of standard cineole**

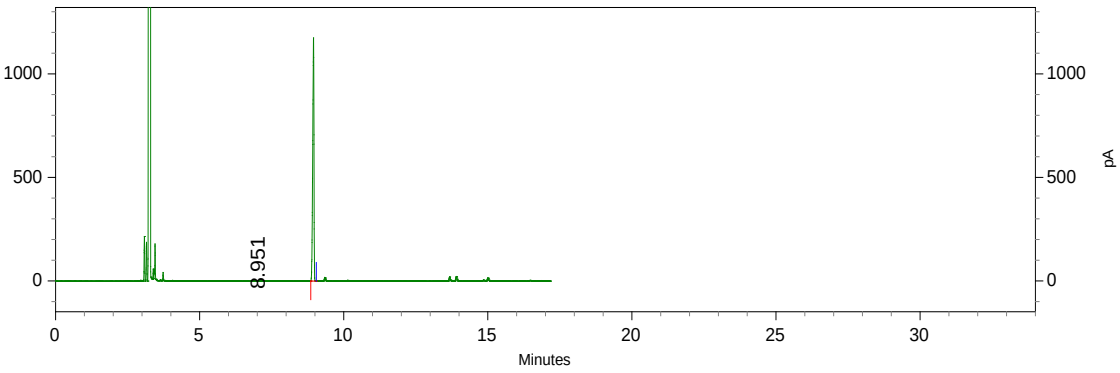


**Figure 3: GC graph of standard α - pinene**

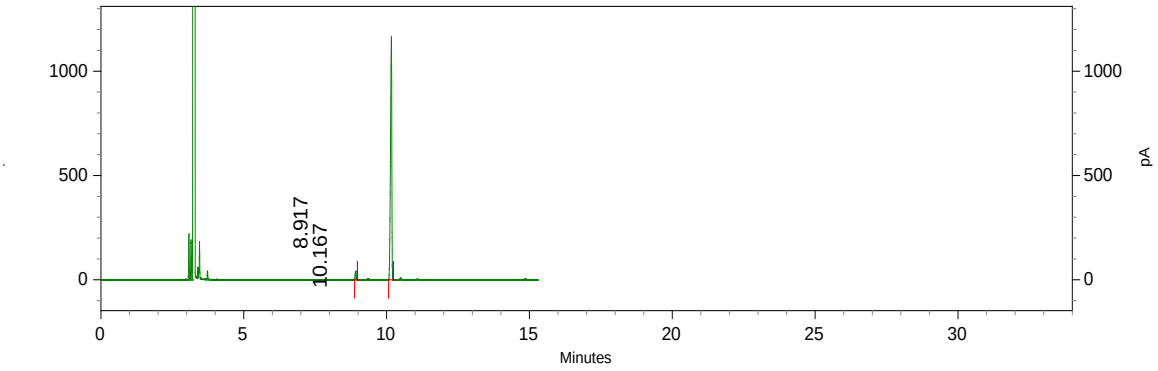


**Figure 4: GC graph of standard β – pinene**

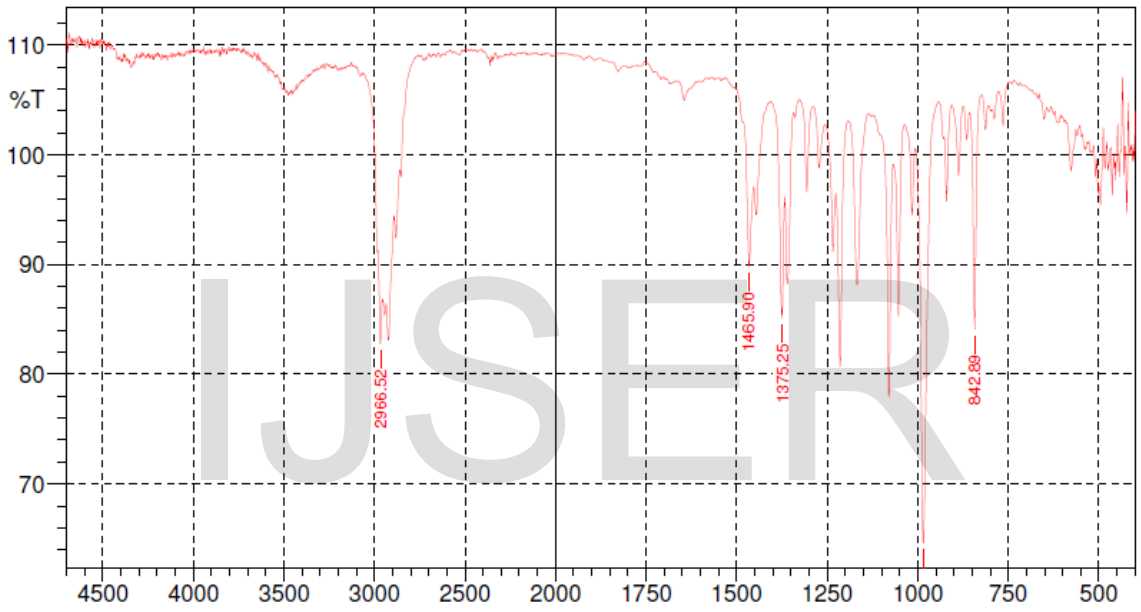


**Figure 5: FT-IR spectrum of Eucalyptus oil**

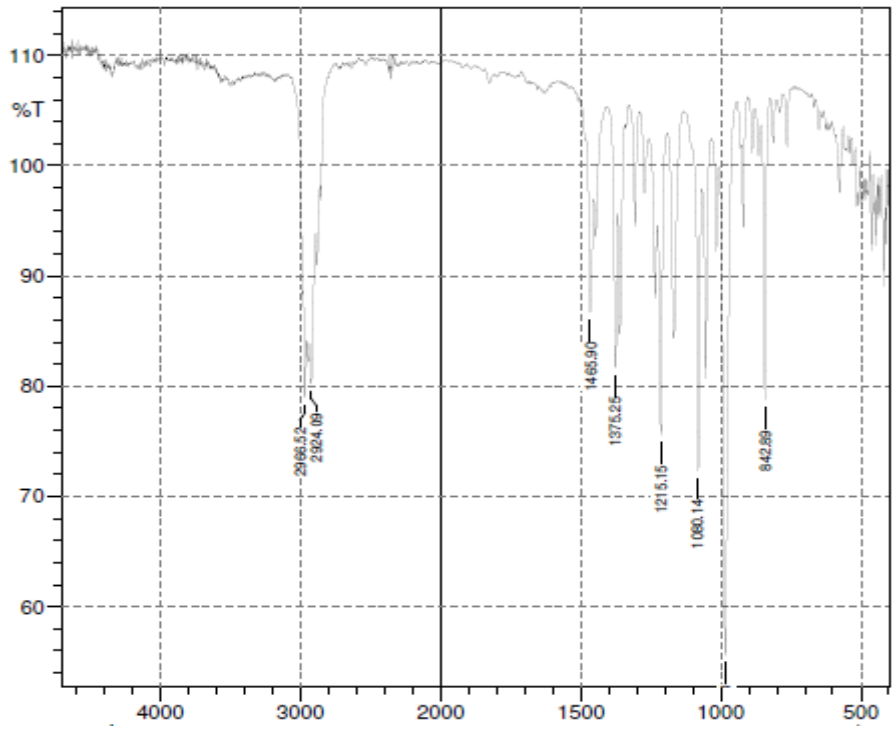


**Figure 6: FT-IR spectrum of standard Cineole**



**Insecticides tests:**

**Test insects:** Insects were collected from local market, found adhering in stored grains. It was known that these insects remain alive in closed system for month to month (almost 6 months). Thus, they were stored in container under observation at room temperature until applying for insecticide tests.

Insecticides test of pure essential oil was performed using two toxicity tests: **Fumigant toxicity test and Contact toxicity test**

**Fumigant toxicity test:** Sterilized Petri dish was used for performing this test to avoid any contamination of oil and insects. Eucalyptus oil being tested to check insecticidal activity was accurately weight and applied at exactly centre of Petri dish. Surrounding to the Eucalyptus oil placed at centre of Petri dish and away from their boundary; five samples of insects were introduced in Petri dish. This prevented direct contact of insects with the eucalyptus oil and all the insects were surrounding the essential oil placed at centre. Sufficient care was taken to avoid direct contact of oil and insects in Petri dish. The insects kept in dish along with oil

were covered from top with another dish. Before doing this it was checked that this insects remain alive for months in closed system without any contact of air. As soon as the Petri dish was covered with another Petri dish from top, stop watch was started until they were dead. Time was noted when all the insects were found dead. Their mortality rates were noted in terms of time and concentration of oil required for their death. These insects were considered dead if their appendages did not move when prodded with a brush. This treatment was carried out three times to known exact parameter of insect mortalities. Also minimum inhibition concentration of oil required for insects mortalities in minimum time intervals were noted.

Standard sample was prepared wherein ten insects sample were kept in empty Petri dish free of essential oil. The Petri dish containing insects were covered from top with another dish and their mortality time was noted. These standard samples were compared with one containing essential oil.

**Table 6: Minimum inhibitory concentration of Eucalyptus oil against grain bugs**

| Sr. no | MIC (grams) | Essential oil | Mortality time (In mints) |
|---|---|---|---|
| 1 | 0.03 | Eucalyptus oil | 19 |
| 2 | 0.06 | Eucalyptus oil | 19 |
| 3 | 0.1 | Eucalyptus oil | 17 |
| 4 | 0.2 | Eucalyptus oil | 13 |

**Table 7: Fumigant toxicity results of eucalyptus oil**

| Sr. No | Oil | MIC of oil (grams) | Mortality of grain bugs (In min) | Blank sample |
|---|---|---|---|---|
| 1 | Eucalyptus oil | 0.03 | 19 | 4 months |
| 2 | Eucalyptus oil | 0.03 | 23 | 4 months |
| 3 | Eucalyptus oil | 0.03 | 22 | 4 months |

**Contact toxicity test:** Sterilized Petri dishes were used for performing this test to avoid contamination of oil and insects. This test was carried out in three different ways:

1. Eucalyptus oil being tested to check insecticidal activity was accurately weight and spread on Petri dish using cotton. On this spreaded essential oil, insects were placed or made to walk to known the mortality rate of insects on oil in terms of time.
2. Insects were kept in Petri dish on which oil to be tested was poured. Their mortality rates were considered in terms of time.

**Table 8: contact toxicity results of eucalyptus oil**

| Sr. No | Oil | MIC of oil (grams) | A | B |
|---|---|---|---|---|
| | | | Sample 1 Mortality time of grain bugs (In secs) | |
| 1 | Eucalyptus oil | 0.05 | 45 sec | 19sec |
| 2 | Eucalyptus oil | 0.05 | 39 sec | 21 sec |

A. Oil was applied in centre of Petri dish and insects were allowed to walk on it.
B. Oil was poured on insects.



**Results and discussions**:

**Analysis of essential oil composition:** Physio-chemical and organoleptic analysis of eucalyptus oil are very much similar to standard oil samples. Confirmatory test of eucalyptus oil confirm presence of aldehyde, ketone, ether, alcohol and ester group. GC result of eucalyptus oil show presence of many different compounds out of which cineole is major compound followed by α-pinene, β-pinene, terpene-4-ol etc. **Table 4** indicates different compound present in eucalyptus oil including cineole based on their retention time. Also, presence of cineole in eucalyptus was confirmed by FT-IR analysis **(Table 5)**. The entire bonds present in structure of cineole were confirmed with standard frequency and functional group adhering in their spectrum.

**Table 6** shows minimum inhibitory concentration of eucalyptus oil against bugs. At 0.03 grams, eucalyptus oil was 100% effective against bugs and its mortality time was 19 minutes. But as concentration increased from 0.03 to 0.2 grams mortality time of bugs was reduced to 13 minutes.

**Table 7** shows fumigant effect of eucalyptus oil against grain bugs. Five samples of insects were tested against eucalyptus oil at same concentration (0.03 grams). This test was conducted thrice at same concentration to confirm their mortality time. Average time required for eucalyptus oil to kill grain bugs was 21 minutes.

**Table 8** shows contact toxicity effect of eucalyptus oil against grain bugs. Five samples of insects were tested against eucalyptus oil at same concentration. This test was conducted twice at same concentration to confirm their mortality time. Minimum inhibitory concentrations of individual essential oil were 0.05 grams. Average time taken by Eucalyptus oil was 42 sec and 20 sec.

**Conclusion**: From the above work it can be concluded that eucalyptus oil is good source to utilize it as natural pesticide. Moreover it is efficient oil to isolate natural cineole considering yield, purity and aroma of oil.

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