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INSECTICIDAL ACTIVITIES OF ZANTHOXYULUM RHETSA ROXB DC CARPELS OIL EXTRACT ON DROSOPHILA MELANOGASTER.

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1. INTRODUCTION

Since there is a continual increase in the human population worldwide, one of the most challenging situations is to provide enough food to the ever growing human population. There are two possibilities to reach such endeavor, first is to increase the area under agricultural and secondly to optimize the production of the already cultivated fields. Insect pests are one of the most important threats to the cultivated crops thereby causing a serious reduction in the global production (E.C Oerke, 2006).

To control these insect pests there is an immense use of synthetic insecticides but the chemical properties of these products make them dangerous and highly unsafe for both human and the environment (D. G. Heckel, 2012). The plasticity of these insect pests makes them much prone to develop resistance to many of these compounds (P. M. Mendonca, et.al. 2011). There is a search for new insecticides that offer no or low risks and that are easily decomposed to safe compounds after its action. Plant derived insecticides can be a suitable alternative, since plant species have evolved molecular mechanisms that protect them against herbivorous insects and other animal species (S. Sujatha, 2010). The essential oils from plant species have been reported as acting on digestive and neurological enzymes as well as with insects tegument (M. B. Isman, 2006) which is probably due to the secondary metabolites as terpenoids and phenylpropanoids (E. Ennan et.al., 1998.). Insecticidal activity of some monoterpenes as α-pinene, β-pinene, 3-carene, limonene, myrcene, α-terpinene, and camphene had been demonstrated in literature (C. Viegas Jr., 2003).

In the last decade, *Drosophila melanogaster* has became a model for testing toxicity in vivo as this species has many homologous genes with humans and can be easily kept at the laboratory allowing many assays to be performed (P. B. Bagatini, L. et al., 2011.).

Recently the essential oils have received much attention as resources of potentially useful bioactive compounds. Particular emphasis has been placed on their antimicrobial, antifugal, antitumor, and insecticidal activity.(Franzios et al., 1997) Essential oils are plant secondary metabolites mainly composed of terpenoid compounds and play an important role in the interactions between plants and insects. The effects of the essential oils on insects range from an attraction or repellence to that of toxicity or even lethality. Even though the use of "insecticidal" plants is known from antiquity, only a few of these are commercially available (Balandrin and Klocke, 1988).

1.2 *Drosophila melanogaster*

a) Classification

Kingdom	**Animalia**
Phyulum	Arthropoda
C;lass	Insecta
Order	Diptera
Family	Drosophilidae
Genus	*Sophophora*
Species	*melanogaster*
Species:	*Drosophila melanogaster*

b) Identification

Drosophila melanogaster is commonly called as the fruit fly, it is a little insect about 3mm long and 2 mm in width. Its eggs are about half a millimeter long. The body is covered in a chitinous exoskeleton; it has three main body segments; and has three pairs of segmented it has a single pair of wings on its thorax middle section. The last segment of the thorax has a pair of rudimentary wings called haltere's that serve as "knobby" balancing organs (Miller. C, 2008).

c) Geographical distribution

Originally native to the Old World tropical regions, man has been successful in introducing *Drosophila melanogaster* to every continent of the world except Antarctica. It can survive in a wide range of habitats. It is limited by cold temperatures and the lack of water. The preference for moist environments is indicated by its scientific name, *Drosophila*, which means "lover of dew." (Miller. C, 2008).

d) *Drosophila*'s behaviour

Drosophila melanogaster has simplistic behaviour. They are easily drawn towards light or the smell of any food source, and will almost indiscriminately mate with any individual of the opposite sex. (Miller. C, 2008).

The adults thrive on rotting plants, spoiled fruit and fermenting liquids. The female looks for fruit that is green or only slightly ripened fruit. Her objective is to find fruit that will be just starting to rot when her eggs hatch. (Miller. C, 2008).

e) Life Cycle Fruit Fly

Drosophila melanogaster life cycle begins when the female lays her eggs on a piece of fermenting fruit or decaying, sweet organic material. She lays up to 500 eggs, making it difficult to control the population. After eggs hatch into small white larvae, they eat from their nesting site for about four days, absorbing the nutrients to transform into adults.

The larvae then locate dark, dry places for pupation. During this stage, the legless larvae grow six legs and a pair of wings before emerging as adults. Full pupation takes approximately four days.

The average natural life span of fruit fly adults in optimal temperatures is 40 to 50 days. Female fruit flies are capable of mating and laying several batches of eggs at a time, allowing the fruit fly population in a home to multiply quickly. The life span of the fruit fly is heavily influenced by temperature.

2. MATERIALS AND METHODS

2.1. Insects tested

Cultures of *Drosophila melanogaster* were maintained in the laboratory without exposure to any insecticide on bananas in a fruit basket.

2.2 Culturing *Drosophila melanogaster*

Drosophila thrive on fermenting soft fruits. A very suitable culture medium, crushed banana. It provides all the necessary nutrients for both the larval and adult stages. The banana was kept along with the flies in sterile pint jars with cotton plugs. Another standard medium, that was used. Mix and boil 420 ml water and 4.5 gm agar 3–5 minutes. Add 60 ml unsulfured molasses and heat to boiling again Mix together49 gm cornmeal, 6.5 gm brewer's yeast, and 145 ml cold water in a separate container until all lumps are removed. Add cornmeal-yeast mixture to molasses-agar mixture. Boil 5 minutes, stirring constantly. Cool mixture to 60°C. Add 3.4 ml propionic acid (as mold inhibitor). Pour the culture medium 1-inch deep into sterile culture jars with sterile plugs. Pint milk bottles work well, but any wide mouthed jar fitted with a plug made of cotton covered with cheesecloth or foam rubber should work well. Add a sprinkle of active baker's yeast (from a salt shaker) to each jar before adding flies.

Note: It is important when maintaining cultures not to overcrowd (about 100 flies per pint culture jar) and to subculture approximately every other day. This keeps the flies healthy, large, and mold-free.

2.2 Sample collection

The carpels of *Zanthoxylum rhetsa* Roxb *(*DC) were collected from Sindhudurg Dist, Western Ghats of Maharashtra, India and authenticated at Blatter's herbaria, Mumbai (accession no. 9046 /2010).

2.3. Oil extraction: Oil extraction carried out at Kelkar Scientific Research Centre, Mulund, from fresh green carpels.

3. RESULTS

3.1: Extraction of Oil

Moisture content 36.2% at 100 degrees Celsius till constant weight

Distillation Parameters

Oil Yield: 1.75 gm %

Yield (dry weight basis) 2.742 gm %

3.2. Contact activity with treated filter paper

The toxicity of plant extracts and essential oils to *Drosophila melanogaster* adults exposed to direct contact with the oil is recorded in table 1.

Table 1: Insecticidal activities of essential oils against *Drosophila melanogaster* adults, using the filter paper diffusion method in closed containers along with a piece of 20 grams banana.

Time in hours	100ul	200ul	400ul	600ul	800ul	Control
5	3	7	8	12	14	20
10	4	11	18	19	20	20
15	13	18	20	20	-	20
20	20	20	-	-	-	20

Each datum represents the mean of six replicates, each set up with 20 adults (n=120).

4. DISCUSSION

Chemical pesticides that are used for insect control may be dangerous to humans and wild life. These compounds have induce insect resistance and other adverse effects, which have motivated the search for alternative forms of control (P. M. Mendonça, et.al., 2011). In the present study we demonstrate the toxicity of the essential oil *Zanthoxylum rhetsa* Roxb (DC) in *Drosophila melanogaster*. Plant derived compounds are reported to induce toxicity to a wide range of insects and may interfere directly with all developmental stages of fruit fly, *Drosophila melanogaster* (K. Ravi et. Al., 2007).

Compounds such as terpenes, flavonoids, alkaloids, steroids, and saponins are important phytochemicals when considering the insecticide activity of plant extracts (M. Belo et.al. 2009). In addition to acute toxicity and mortality, terpenoids and flavonoids have been also studied for their insect repellent activity (R. Ndemah, et.al. 2002). There are a variety of chemical compounds present in the *Zanthoxylum rhetsa* Roxb (DC) essential oil as pinene, Phellendrene, Benzene, 1- methyl-3-methylethyl , 3- cyclohexane-1-ol, 4 – mehyl-1-(1-methylethyl) and Cyclohexane, 1-ethenyl-1-methyl 2-4bis (1 – methylethenyl).

5. CONCLUSION

According to our results, the essential oil of *Zanthoxylum rhetsa* Roxb (DC) a fumigant action by compromising survivorship and locomotor activity of *D. melanogaster*. Therefore, our results point out to the potential application of *Zanthoxylum rhetsa* Roxb (DC) essential oil and/or its compounds as an alternative to the synthetic insecticides in agricultural and pest control practices. Additional experiments are necessary to clarify the exact mechanisms of toxicity induced by *Zanthoxylum rhetsa* Roxb (DC) oil in insects and to identify candidate compounds derived from this oil.

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