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# Biopesticide Activity of *Costus Igneus* -A Preliminary Study

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**ABSTRACT**

*Costus igneus* plant was tested for potential biopesticide use against *D. melanogaster* larvae as a model organism. Aqueous and methanol extracts were prepared using fresh leaves of *C. igneus*. Methanol extracts experiential more effective with $LC_{50}$ (50% lethal concentration) at 2.71% whereas aqueous extract recorded 1.25 folds higher than methanol with $LC_{50}$ at 3.37%. The $LC_{50}$ values were statistically significant ($p < 0.05$). Results explain 92.4% and 83.2% competence of methanol and aqueous extracts of *C. igneus* against *D. melanogaster* larvae correspondingly. The preliminary results proposes *C. igneus* plant as a potent source of biopesticide.

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## Introduction

*Costus igneus*, commonly known as insulin plant, is indigenous to South and Central America. Recent introduce to India from America as a herbal cure for diabetes. A widespread wild plant and a potential ornamental plant that extensively grown in backyards in southern India [1]. It is known to be efficient in diabetic patients. Tribal people of Kolli hills, Namakkal district, Tamil Nadu was reported to practice leaves of *C. igneus* to combat diabetes. The plant belongs to Costaceae family consists of four genera and approximately 200 species. The genus Costus is the largest in the family with about 150 species that are mainly tropical in distribution [1-4]. Phytochemical study reveals that C. igneus posses protein, iron, and antioxidant components such as ascorbic acid, α-tocopherol, β-carotene, terpinoids, steroids, and flavonoids [5]. The methanol extract of the plant reported containing a high number of Phytochemicals such as carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, and flavonoids [6]. Beside this nutrient profile and anti-diabetic activity, the plant also possesses hypolipidemic, antioxidant, antibacterial, antifungal and anticancer activity [7-9]. Presence of such bioactive compounds in this plant, urged us to take up this study where the potential use of *C. igneus* as a biopesticide has been studied. The presence of larvicidal compounds viz., fatty acids- palmitic acid, oleic acid, linoleic acid, terepenoids-lupeol, carene in *C. igneus* supports the study [5, 6, 10]. Hence the purpose of the study is to prove the impending use of *C. igneus* as a biopesticide. Being an invertebrate model and a common household pest, *Drosophila melanogaster* used as a model organism in the present study.

## Materials and Methods

### Capturing and Rearing Fruit Fly

*D. melanogaster* or fruit flies were captured using natural media i.e. banana in an open glass bottle by keeping it in the environment. After capturing of enough individuals bottles were transferred to the lab where further generations of fruit flies were rare at room temperature (22-30 °C) either in banana media or artificial lab media. Artificial lab media was chemically defined media consist of yeast, sucrose, dextrose, agar in a particular quantity. Orthophospharic acid and propionic acid were used as an anti-fungal and anti-bacterial agent respectively.

### Plant Collection and Identification

*C. igneus* plant was obtained from the botanical garden of our college. The specimen was confirmed by Dr S. Agarwal, Head of Dept. of Biotechnology and the gardener of the botanical garden. Daily fresh leaves were collected for the experiment between January to April 2016.

### Experimental Design

Two types of extracts were made using leaves of the plant viz., aqueous and methanol. One gram of chopped leaf was taken, crushed and grind it fine in pistil mortal followed by 24 hours deepen into distilled water and methanol at 24°C (chill treatment for better extract). Both were labelled as *C. igneus*- aqueous and *C. igneus*- methanol. This is kept as a stock solution. Before experiment different concentrations of both extracts were made in percentage using stock solutions by diluting in distilled water i.e. 0.5, 1, 2, 3, 4 & 5%. Extracts were tested on the larval stage of *D. melanogaster* by preparing a set of seven test-tubes in a row. One for the control and rest testing concentration of extracts prepared. Two such sets were prepared for each type of extracts. A constant batch of larvae was released into all test-tubes with a smooth brush and the respective percent concentration of both extracts were added to respective test-tubes keeping control as without extract. The set of test-tubes were then left for 24 hours of treatment. After the treatment duration mortality caused by the two extracts were noted. Several replicates were taken for the same experiment and mean percent mortality was then recorded for both extracts i.e. C. igneus aqueous and methanol. 50% lethal concentration ($LC_{50}$) was calculated.

**Statistical Analysis**

To find $LC_{50}$ from percent mean mortality data probit analysis was done using SPSS for Windows (*version 16.0*) developed by SPSS Inc.

**Results**

The prepared concentration of aqueous and methanol extracts of *C. igneus* was tested on larvae of *D. melanogaster* to find out 50% lethal concentration ($LC_{50}$). Mean mortality recorded after 24 hours of treatment with several experiments. Being a control there is no mortality recorded in the control test tube. Test tube with 0.5% concentration observed to be nil in mortality for both the aqueous and methanol extracts of *C. igneus*. In 1% concentration, there is 15% and 14% mean mortality for aqueous and methanol extracts of the plant respectively. Further, the mean percent mortality increases with increasing percent concentration of both type of plant extracts with maximum mortality at 5% concentration, 80% and 87% mean mortality for aqueous and methanol extracts of *C. igneus* respectively. *C. igneus*- methanol extract found to be effective at $LC_{50}$ value 2.71%, statistically significance ($\chi^2 = 11.884$, $p = 0.018$) refer Table 1.1. Whereas the toxicity for C. igneus- aqueous extract was recorded to be 1.25 fold higher than C. igneus- methanol extract, $LC_{50}$ value at 3.37% with significance value ($\chi^2 = 21.736$, $p < 0.001$) see Table 1.1. A relation between mean percent mortality and extract concentration was plotted using simple linear regression for both types of extracts, showed a positive relation (Figure 1.1 and 1.2).

**Table 1.1: Probit equation model for Costus igneus extracts**

| Parameter | β ± Std. Error | Z | Sig. | 95% Confidence Interval | | $\chi^2$ | *p* | $R^2$ |
|---|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | | | |
| *Costus igneus - methanol* | | | | | | | | |
| Concentration | 3.012±0.241 | 12.485 | < 0.001 | 2.539 | 3.485 | 11.884 | 0.018 | 0.924 |
| Intercept | -1.302±0.118 | -11.052 | < 0.001 | -1.420 | -1.184 | | | |
| *Costus igneus - aqueous* | | | | | | | | |
| Concentration | 2.724±0.242 | 11.238 | < 0.001 | 2.249 | 3.199 | 21.736 | < 0.001 | 0.832 |
| Intercept | -1.437±0.122 | -11.759 | < 0.001 | -1.559 | -1.314 | | | |

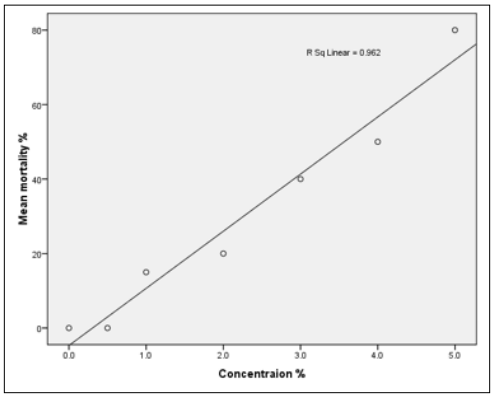


**Figure 1.1:** Linear regression between percent concentrations of *C. igneus*- aqueous extract and mean percent mortality of *D. melanogaster*

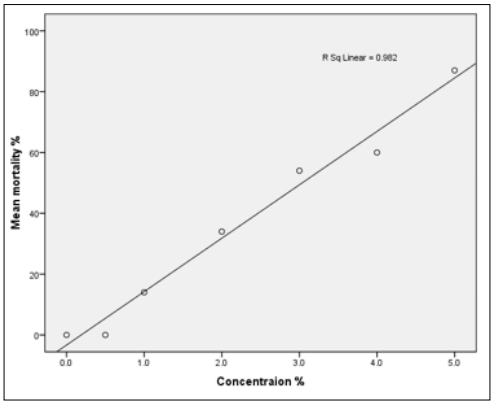


**Figure 1.2:** Linear regression between percent concentrations of *C. igneus*- methanol extract and mean percent mortality of *D. melanogaster*

**Discussions**

Prior study with Neem plant extracts as biopesticide inflicts considerable mortality in larvae of *Musca domestica* and *D. melanogaster* [11]. Present study corroborates the study where *C. igneus* is shown to impose mortality in *D. melanogaster* larvae. Further fatty acids viz., oleic acid and linoleic acid were revealed promising larvicidal compound against the mosquito larvae [12]. Similarly, gluanol acetate; a terapenoids possess larvicidal activity against four instars larvae of *Aedes aegypti L* [13]. These compounds fatty acids, palmitic acid and terapenoids reported to be present in *C. igneus* plant could also possess larvicidal activity [5, 6]. Therefore, the present study undertaken to investigate the larvicidal effect of *C. igneus* plant extract on D. melanogaster also showed the larvicidal activity of *C. igneus* extracts. The methanol extract prove to inflicts effective mortality at low concentration than aqueous extract perhaps because methanol is a better solvent than water and consequently the dissolved concentration of a bioactive compound in methanol is more as compared to aqueous or water, therefore, methanol extract of the plant is more effective. The tested extracts are of natural origin i.e. Plant extracts.

Hence this preliminary study proposes *C. igneus* as a prospective biopesticide against the common insect pest of the society.

Both the extracts of *C. igneus* plant found to be effective against the larvae of common fruit flies consequently methanol extract 92.4% and aqueous extract 83.2% efficient. In comparison methanol extract inflicts the mortality at low doses ($LC_{50} = 2.71\%$) than the aqueous extract ($LC_{50} = 3.37\%$). Despite being a crude extracts *C. igneus* considerably showed the larvicidal activity, it is suggested that more polar solvent should be used in extract preparation to lower the $LC_{50}$ value to a minimum; further mortality can be checked at different exposure time and toxicity level can be compared for the sensitivity of compound. The study concludes that *C. igneus* is indisputably a source of potential biopesticide.

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