

Custard Apple Seed Oil Anti-Fly Property: A Review

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ABSTRACT- The house fly, *Musca domestica* L, has been identified as a vector for a variety of human and animal illnesses. Eradication of such pests needs the help of insecticides which in turn has led to the growth in insecticides or insect repellents which are not only effective but are also non-toxic. In this paper, the anti-fly property of custard apple (*Annona muricata*) seeds has been shown against *Musca domestica* L. Firstly, oil was extracted from the custard apple seeds. Afterwards, sugar cubes were left untreated or coated with a fresh coat of custard apple seed oil. After 120 hours, the freshly treated sugar samples exhibited anti-fly property against the flies in this study. Moreover, spraying of a solution of custard apple seed oil and water led to death of many flies. It can thus be concluded that custard apple seed oil is a promising natural anti-fly agent and the abundance of custard apple

seeds means that at an industrial scale such insecticide or insect repellent can be produced.

KEYWORDS- Custard apple seeds, Cyto-toxicity, House fly, Insect repellents, insecticide, *Musca domestica* L, MTT assay, sugar cubes.

I. INTRODUCTION

Plants generate a wide range of main (10) metabolism-based compounds, vital for reproductive, physiological and developmental development, and secondary synthesis routes of metabolites. The fact that this is currently used industrially as a means of combating insects hazardous to people, as summarised in Table 1, are crucial to the defence systems of the plant[1][2].

Table 1: Tabulation of sources of insecticides of botanical origin. All these insecticides are now studied for their scalability[3]

Botanical Family	Species	Action	Functional group	Active compound	Organs
Amaranthaceae	<i>Anabasis aphylla</i>	contact	alkaloid	anabasine	Leaves
Asteraceae	<i>Chrysanthemum cinerariaefolium</i>	contact	pyrethrin	Pyrethrin I and II	flowers
Fabaceae	<i>Derris elliptica</i>	contact	rotenoid	rotenone	roots
Meliaceae	<i>Azadirachta indica</i>	contact	limonoid	azadirachtin	seeds
Piperaceae	<i>Piper nigrum</i>	Stomach toxin	alkaloid	piperine	seeds
Salicaceae	<i>Ryania speciosa</i>	Stomach toxin	alkaloid	ryanodine	Bark
Solanaceae	<i>Nicotiana tabacum</i>	Stomach toxin	alkaloid	nicotine	Leaves

Figure 1 shows the molecular formula and structure of Pyrethrin I and Rotenone of *Chrysanthemum cinerariaefolium*, while Figure 2 shows the structures of some common constituents such as Eugenol, menthol, Citronellol, linalol, 1-8, cineol, thymol and limonene. Table

2 shows the main component used in California's agricultural pest management in 2016, while Table 3 shows the ingredients used in botanical pesticides in different nations[4-6].

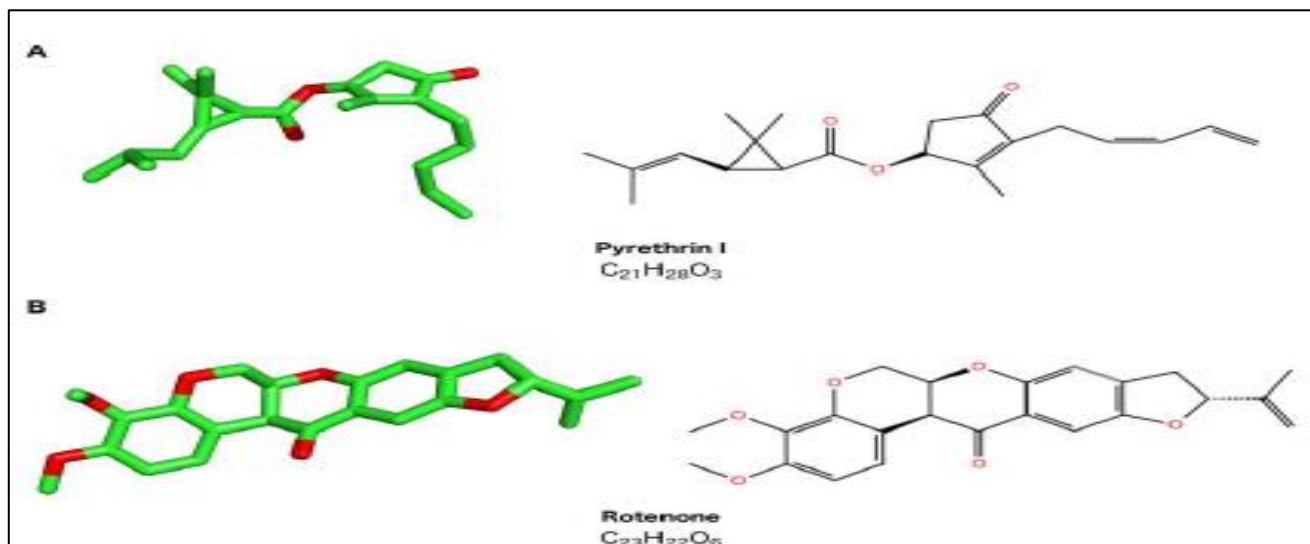


Figure 1: A) Pyrethrin I, a terpenoid isolated from *Chrysanthemum cinerariaefolium*, has a chemical formula and a three-dimensional structure. B) Rotenone, an isoflavone derived from Fabaceae roots that is poisonous to insect mitochondria, and its chemical formula and three-dimensional structure. The molecular formula & the 3D structure of these 2 phyto-molecules would make it easier for the researchers to make targeted anti-insect agents[7-9]

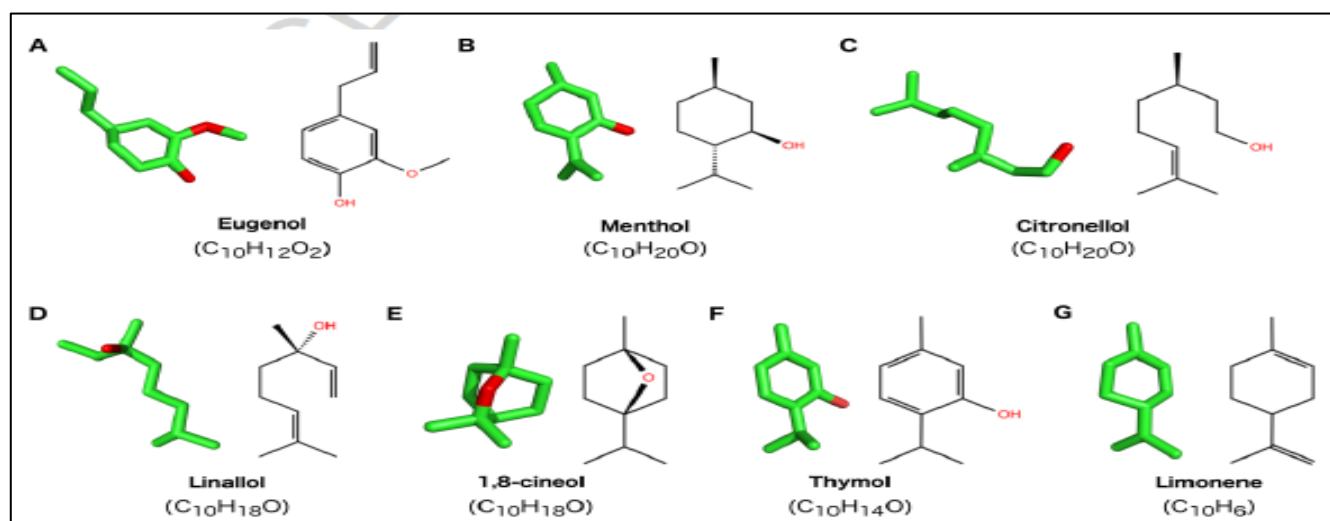


Figure 2: The three-dimensional structure of some of the most prevalent chemicals responsible for the function of essential oils used in pesticides. The elucidation of the 3D structure and the molecular formula associated with these chemicals provide a scope for their further improvements by manipulating their structures Figure courtesy[10-12]

Table 2: Tabulation of various anti-insect agents of botanical origins which had been used in California in the year 2016. The large amount of anti-insect agents of botanical origin being consumed in the state of California indicates that commercialization of such agents is huge[13]

Active ingredient	Kilogram applied
Neem oil	88000
Chenopodium	4600
Pyrethrins	3300
Azadirachtin	2000
Garlic	300
Oil of orange	170
Nerolidol	59
Sabadilla	60
Capsicum oleoresin	60

Table 3: Tabulation of the number of active ingredients of botanical origin that had been approved for use in insect control in various nations. This show the potential of anti-insect agents of botanical origin being recognized the world over[14]

Country	Active Ingredients	Sources & Comments
Australia	5	EOs are AVMPA exempt
Canada	5	non-conventional insecticides
Chile	10	Agricultural ministry
European Union	5	Based on EU pesticide database information
France	5	18 Natural products
India	11	Based on Central government's information
Japan	6	Natural safe products
Kenya	4	Biochemical
South Korea	5	Based on government's information
Mexico	6	Based on government's information
Holland	3	Natural products
United Kingdom	1	Based on safety & health data
United States	10	12 Eos are EPA exempt

APVMA is for Australian Pesticides and Veterinary Medicines Authority; EO stands for essential oil; EPA stands for Environmental Protection Agency; NA stands for not applicable.

Musca domestica L. (Diptera: Muscidae) is an omnipresent organism closely linked with humans, or the common house fly. House fly tends to land and vomit enzymes from

a food source. The flies suck food into it through a proboscis when its food disintegrates. However, this very same flying procedure helps to be a carrier of different infections, some of which have been tabled in Table 4. The purpose of this investigation is to detect an appropriate plant for the isolation of an anti-fly chemical[15].

Table 4: Tabulation of the bacterial species that have been found in house flies, which includes the site of sample collection and the host stage which was infected by the human pathogenic bacteria. This table thus indicates how the common house flies or *Musca domestica* L can potentially spread deadly pathogens among humans and animals[16].

Bacterial genera	Site of sample collection	Host stage infected
Helicobacter	Laboratory reared	Adult
Campylobacter	Piggery, Poultry	Larvae and Adult
Salmonella	Laboratory experiment	Adult
Escherichia	Human habitat	Larvae and Adult
Bacillus	Human habitat	Adult
Staphylococcus	Human habitat	Larvae and Adult

II. LITERATURE REVIEW

Murray B Isman in his paper that shown his study which discloses in this class of pesticides that the standard bearers of pyrethrum and neem (azadirachtin) insecticides still remain, although internationally their growing prevalence is primarily due to new law enforcement. Insecticides made from essential plant oils are becoming more popular as plant protection solutions. Regulatory requirements for some plant extracts and oils have also been loosened, while stiffer limitations have slowed the introduction of new products in North America and the European Union[17]. Julieta Soledad Arena published another review with the goal of determining the chemical properties of essential oils (EOs) from *Dysphania ambrosioides* and *Tagetes minuta*, as well as evaluating their contact toxicity against adults of *A. diaperinus* alone and in conjunction with cypermethrin. In *D. ambrosioides* oil, the key components were ascaridole, p-cymene, and carvacrol, whereas in *T. minuta* oil, the major components were dihydrotagetone, cis-ocimene, trans-tagetone, and trans-ocimene. When applied alone, cypermethrin was marginally toxic to the insect, however(18) the EOs from both plants had a high contact impact. *D. ambrosioides* oil had a six-fold higher

toxicity than *T. minuta* oil and was more than fifty times more effective than cypermethrin. The toxicity of cypermethrin increased significantly when it was sprayed in combination with EOs at low levels. Because the EOs examined have promising anti-*A. diaperinus* properties, their use in novel pest management approaches might be researched[19].

III. DISCUSSION

A. Experimental design

Firstly, custard apple seeds would be collected and oil would be extracted from them. A room having temperature and humidity control would be established. Then house flies, *Musca domestica* L., would be collected and kept in a box placed in this room. Following starvation, flies would be divided into 2 groups; one group would be given only sugar cubes and the second group would be given sugar cubes with freshly coated custard apple seed oil. At the end of 120 minutes, the number of flies near the sugar cubes would be noted. Moreover, MTT assay would be conducted for checking its cyto-toxicity against normal

human cells. The experiment design mirrors report as reported earlier.

Next, another set of flies would be kept in a box and through an aperture, a nozzle of a spray bottle having the custard apple seed oil in water would be sprayed upon for a minute or so. The number of flies dropping dead would then be counted[20].

B. Instruments

- Flask with a round bottom
- Thermostat
- Custard apple seeds
- Stirrer
- Condenser
- Rota-evaporator

C. Extraction of oil from custard apple seeds

The custard apple seeds were collected, and moisture was removed following which the seeds' kernels were separated out which were then grounded to fine particles. The particles were kept in the flask and to which ethanol as solvent was added. The contents were then stirred for 36 h at 45°C. Thereafter the reaction mixture was filtered to remove the solid particles and the solvent and the solvent is removed from the oil[21].

D. Procedure of checking efficiency of the prepared insect repellent or insecticide

The test insects used during investigation of the action and effectiveness of the pure custard apple seed oil were the common housefly, *Musca domestica* L. The flies were confined and deprived for about 24 hours at [(25) °C and (50%) relative humidity]. The flies were divided into 2 groups of which one group was fed untreated sugar cubes

and another was fed sugar cubes laced with custard apple seed oil. Both the groups were observed for 120 minutes.

Also, another set of flies were housed in a box, fed with sugar cubes and then were sprayed with a solution of custard apple seed oil and water and the number of flies dropping dead were counted[7].

E. Cyto-toxicity assay on human cells

Human BJ cell lines of fibroblast origin derived from human foreskin were obtained from the American Type Culture Collection (ATCC) and maintained in Eagle's Minimum Essential Medium (EMEM) with 10% Fetal Bovine Serum (FBS) in normal mammalian cell culture circumstances for the evaluation of cytotoxicity on human cells. After 48 hours, the cells were treated with varied doses of custard apple seed oil (0,100,350,500,750, and 1000 g/ml). The proportion of live cells was determined using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide). Student t test was performed to gauge significance. Procedure was followed as per established protocol[22].

The result of the repellent activity of the custard apple seed oil against house fly, *Musca domestica* L has been shown in table 1 and table 2 respectively. For *Musca domestica* untreated sugar sample had showed nearly negligible repellence whereas the another sample of flies was exposed to the freshly treated samples, only 5 ants were found alive near the sample thereby indicating that custard apple seed oil coated sugar cube samples were most inhibitory for the flies after 120 hours as tabulated in Table 5 and shown in Figure 1[23][24].

Moreover, after spraying the flies with solution of custard apple seed oil and water out of 60 flies nearly 30 flies were counted as dead after a minute of spraying[19].

Table 5: Custard Apple seed oil exhibiting insect repellent and insecticidal activity against house fly. As compared to the untreated samples, the freshly treated samples successfully repelled the flies[14]

Time (mins)	Untreated sample	Newly treated sample
0	0	0
15	30	0
30	38	0
45	40	0
60	46	0
75	49	0
90	60	7
120	66	10

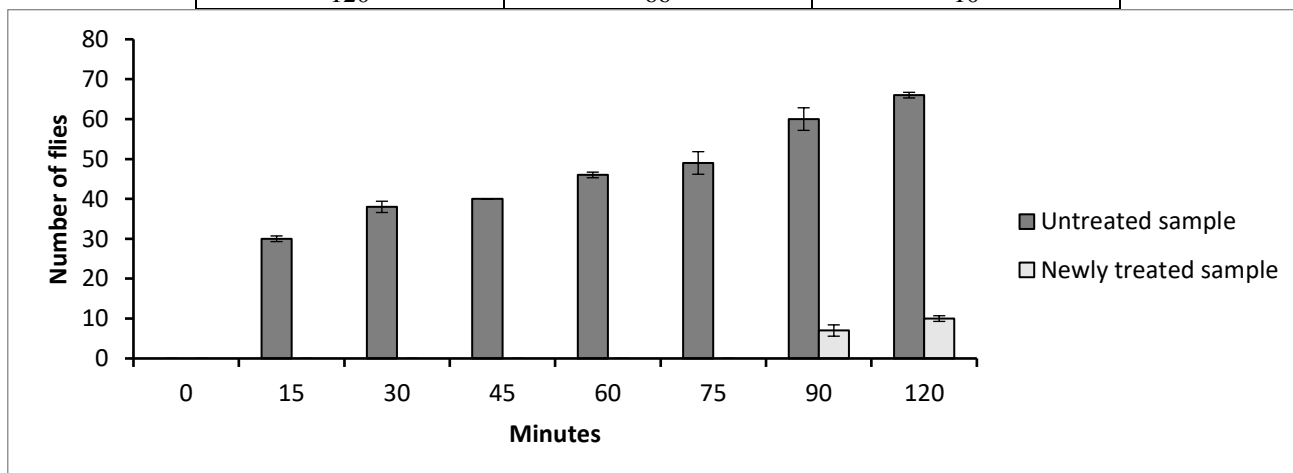


Figure 3: Chart showing number of attracted *Musca domestica* to samples. As compared to the untreated samples, both the depleted treated and the freshly treated samples successfully repelled the ants. All experiments were repeated thrice and $p \leq 0.05$ [13]

F. Cytotoxicity assay

The cytotoxicity of human BJ cells treated with varied doses of custard apple seed oil for 48 hours was low. As a

result, apple seed oil may be assumed to be safe for human consumption (Figure 4)[17].

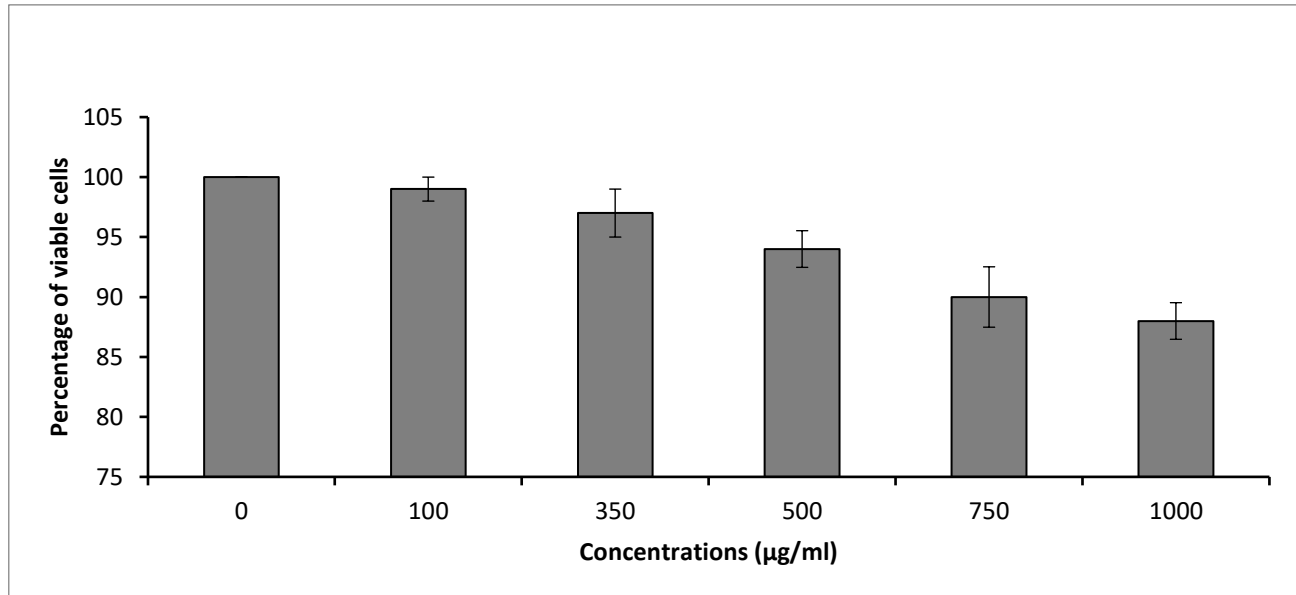


Figure 4: Human BJ cells were used in a cytotoxicity test. After 48 hours, human BJ fibroblast cells were treated with increasing concentrations of custard apple seed oil (0,100, 350, 500, 750, and 1000 g/ml). The custard apple seed oil showed negligible cytotoxicity even after 48 h[21][25]

IV. CONCLUSION

House flies or *Musca domestica* L are known vectors of human pathogens thereby in order to find a solution for this, in this paper, the role of custard apple seed oil has been researched upon its anti-insect role.

In this paper, house flies or members of *Musca domestica* L were kept in a room where temperature and humidity could be controlled. The flies, following starvation were divided into 2 groups; with one group given only sugar cubes, second group given sugar cubes with freshly coated custard apple seed oil. At the end of 120 minutes, the number of flies near the sugar cubes was noted. Moreover, MTT assay was conducted for checking its cyto-toxicity against normal human cells.

After the completion of 120 hours, it was seen that in case of *Musca domestica* the number of flies in the sugar samples with fresh coat of custard apple seed oil as compared to the untreated sugar was quite less. Moreover, MTT assay revealed that the custard apple seed oil has negligible cytotoxicity towards normal human cells.

Going through the results, it can be concluded that custard apple seed oil has acetogenin which is a toxic compound paving way for its use as an effective insecticide against flies. Moreover, its future role as an anti-insect agent could be evaluated on other insects and as custard apple seeds are available in plenty, therefore large amount of oil can be extracted from it on an industrial scale.

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