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EVALUATION OF LARVICIDAL ACTIVITY OF FIVE ASTERACEAE PLANT EXTRACTS AGAINST AEDES AEGYPTI AND IT' S CYTOTOXIC EFFECT ON THE ROOT TIPS OFALLIUM CEPA LINN.

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ABSTRACT

The Mosquitoes belong to the family Culicidae. Aedes aegypti (Linn) is one of the mosquito species that acts as the carrier responsible for the transmission of these vector borne diseases like malaria, chikun kunya, Dengue Haemorrhagic Fever (DHF), yellow fever. The larvicidal effects of leaf, root and flower extracts (aqueous, acetone, and ethanol) of Tridax procumbensis, Heliopsis helianthoides, Tagetes erecta (yellow and orange), Chrysanthemum indicum, of Asteraceae family were tested against the dengue vector Aedes aegypti. Larvicidal bioassay was done and monitored for the first six hours and the final reading was noted after 24 hours. Five extracts showed 100% mortality of the mosquito larva. Among those Tagetes erecta yellow flower (ethanol) showed 100% mortality within 5 minutes. The five extracts which showed 100% mortality were selected for the further study of the cytotoxic effect to analyse the chromosomal aberrations in the root tips of Allium cepa (Onion). Mitotic index % and the % of aberrant cells were calculated. Very lower % of aberrant cells and higher mitotic index was noted in the ethanolic extract of Tagetes erecta flower (yellow). So, the present findings indicate that components from Tagetes erecta yellow flower possess high toxicity to mosquito larva, and low toxicity to the non-target organisms.

KEYWORDS: Asteraceae, Larvicidal activity, *Aedes aegypti*, Cytotoxic effect, *Allium cepa*.

INTRODUCTION

Mosquitoes belong to the family Culicidae. Mosquitoes are responsible for more diseases than any other group of arthropods (Cepleanu., 1993). The females of many species of mosquitoes are blood-eating insects. Mosquitoes serve as vector for various tropical and subtropical diseases which cause destructive effects to human. They do not only transmit parasites and pathogens but they also source of allergic reactions that includes local skin and systemic sensitivity (Cheng *et. al.,* 2003). Mosquitoes spread many diseases like malaria, chikun kunya, lymphatic filariasis, Dengue Haemorrhagic Fever(DHF) etc. The malarial fever, and the dengue haemorrhagic fever (DHF) is endemic to South East Asia, the pacific islands area, Africa and America (Maillard *et al*., 1993). *Aedes aegypti* (Linn.) is one of the mosquito species responsible for the transmission of these vector borne diseases (Kovendan, 2011).These mosquitoes prefer to breed in areas of stagnant water, such as flower vases, uncovered barrels, buckets, and discarded tyres, but the most dangerous areas are wet shower floors and toilet tanks, as they allow the mosquitos to breed in the residence. Research has shown that certain chemicals emanating from bacteria in water containers stimulate the female mosquitoes to lay their eggs. They are particularly motivated to lay eggs in water containers that have the correct amounts of specific fatty acids associated with bacteria involved in the degradation of leaves and other organic matter in water. The chemicals associated with the microbial stew are far more stimulating to discerning female mosquitoes than plain or filtered water in which the bacteria once lived. (Newswise, Inc.2008.).

Scientific classification of mosquito by linnaeus.

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Diptera
Family	Culicidae
Genus	*Aedes*
Subgenus	*Stegomyia*
Species	a*egypti*

Plate -1: Larva of *aedes aegypti* linn.

DHF and yellow fever are the diseases found majorly in the tropics and subtropics regions of the world. Recently, mosquito spreads a new virus called giga virus which is again harmful to the human beings. More than 95% of cases, suffer from the DHF and DSS. DHF is a major cause of child morbidity and hospitalization in Thailand and other countries (Yasui, 1993).The yellow fever mosquito belongs to the tribe Aedini of the dipteran family Culicidae and to the genus *Aedes* and subgenus *Stegomyia (*Catherine Zettel & Phillip Kaufman,2010).Every year, about 300 million people are estimated to be affected by malaria, a major killer disease which threatens 2,400 million (40%) of the world's population (Sharma, 1999; Snow *et. al.*, 2005).About 20 million people are infected every year by dengue virus transmitted by *Aedes* mosquito with about 24,000 deaths (Poopathi.,2012). WHO, 1982 stated that about 2/5 of the global human population are currently threatened of dengue and the best way to control the transmission of dengue virus is fight the mosquitoes that cause the disease and an another strategy is to destroy their vectors or intermediate hosts.

The technique in controlling mosquitoes depends on the larval stages (egg, larvae, pupae, and adult) on target. Mosquito control includes targeting the adult mosquito through spraying chemical insecticides or by killing the mosquito larvae before they emerge into adults via using synthetic larvicides or botanical extracts as an alternative larvicide (Tiwary., 2007). Instead of using synthetic larvicides, the plant derived compounds are used in controlling mosquito larvae is inexpensive and environment-friendly (Das *et.al*., 2007). Plants offer an alternative source of insect-control agents because they contain a range of bioactive

chemicals, which have little or no harmful effect on non-target organisms and the environment. Aware of this effect, mankind has used plant parts or extracts to control insects since ancient times. Population turns to plant derived medicines as first line of defence for maintaining health and combating diseases. The use of synthetic larvicides imposes threats not only to human health but also to the ecosystem because when they are applied into the environment; they may stay on for a very long time or even remain there without end (Mathivanan., 2000.).

The mosquito control programs mainly focus on the larval stages (II and III) of its life cycle. (Hag *et. al.,* 1999) Current research trends use plant extracts as alternative larvicides because they contain various phytochemicals that are specific in killing mosquito larvae without harming other organisms and the environment. Some medicinal plants containing natural toxins were effective against mosquito larvae. Medicinal plant extracts are not only effective but also greatly reduce the risk of adverse effects and do not induce pesticide resistance in mosquitoes. Chemicals extracted from medicinal plants, are very effective as they are expected to have low human toxicity and a high degree of biodegradation.. (Choochote *et. al*., 1999).

The plants (some species) of Asteraceae family contains the PYRETHRIN compound which is used in the production of many synthetic larvicides along with some hazard causing chemical compounds. The pyrethrins are a class of organic compounds normally derived from *Chrysanthemum cinerariifolium* that have potent insecticidal activity by targeting the nervous systems of insects. Pyrethrin is synthetically made by industrial methods, but it also naturally occurs in chrysanthemum flowers, thus is often considered an organic insecticide, or at least when is not combined with piperonyl butoxide or other synthetic adjuvants (Mader, *et. al.,* 2015) Their insecticidal and insect-repellent properties have been known and used for thousands of years.

Pyrethrins are gradually replacing organophosphates and organochlorides as the pesticides of choice, since these other compounds have been shown to have significant and persistent toxic effects to humans. Because they are biodegradable compounds, pyrethrins are now widely regarded as being preferable to pyrethroides. Pyrethrins are considered to be low-toxicity pesticides from a human health standpoint.

Cytotoxicity is a major subject in pharmaceutical studies particularly in the area of cancer research. Low cytotoxicity to healthy cells and high cytotoxicity to cancerous cells is the ultimate goal of many chemotherapy drugs. Medicinal plants are a common, cheap and renewable source of pharmacological active substances and because of this it is extremely important that genotoxicity tests are applied to the active ingredients of these preparations in order to assess their mutagenic potential (Apostolides et al., 1996). Treating cells with the cytotoxic compound can result in a variety of cell fates. The cells may undergo necrosis in which they lose membrane integrity and die rapidly as a result of cell lysis. The cells can stop actively growing and dividing (a decrease in cell viability), or the cells can activate a genetic program of controlled cell death apoptosis.

In view of the above I have chosen five species of Asteraceae and tested against the mosquito larva. These five species are *Tridax procumbensis*, *Heliopsis helianthoides*, *Tagetes erecta* (yellow, orange), and *Chrysanthemum indicum.*

Taxonomical characters of the selected samples.

1.) *Tridax procumbensis* Linn

Scientific classification:

- Kingdom Plantae
- Order Asterales
- Family Asteraceae
- Tribe Heliantheae
- Genus ***Tridax***
- Species ***procumbens***

Distribution

Tridax procumbensis is native to tropical America and spread to tropical, sub-tropical and mild temperate regions.

Common Names

Coatbuttons and tridax daisy, jayanthi, ghamra, gaddi chemmanti, Vettukaaya pondu.

Botanical description

It is a small weed and pest plant The stem is hairy, leaves dark green, arrow-head shaped, and margins toothed.. The plant bears daisylike yellow-centered white or yellow flowers with

three-toothed ray florets. Its fruit is hard achene covered with stiff hairs and having a feathery plume like white pappus at one end. Calyx is represented by scales or reduced to pappus. The plant is invasive in part because it produces more achenes nearly 1500 per plant and each achene is wind dispersed. This weed is found in croplands, distributed areas, fields, meadows, and lawns, and in the areas with tropical and semitropical climates.

Chemical constituents

A flavonoid called procumbenetin was isolated from the aerial parts. Other constituents were alkyl esters, sterols, pentacyclic triterpenes, fatty acids and polysaccharides.

Medicinal Uses

The plant is used for several therapeutic activities like antiviral, antibiotic efficacies, antioxidant, wound healing, insecticidal and anti-inflammatory activity.

Traditionally it is used in India as anticoagulant, anti-fungal, insect repellent. It is also used in diarrhoea and dysentry. It is a well known ayurvedic medicine for liver disorders.

Plate-2: *Tridax procumbens* linn.

2.) *Heliopsis helianthoides* L(Sweet)

Scientific classification

- Kingdom Plantae
- Order Asterales
- Family Asteraceae
- Genus ***Heliopsis***
- Species ***helianthoides***

Distribution

It is native to eastern and central North America from Saskatchewan east to Newfoundland and south as far as Texas, New Mexico, and Georgia.

Common Names

Rough ox eye, smooth ox eye, false sunflower, Oxeye daisy, Heliopsis sunflower, Sunflower Heliopsis.

Botanical description

Heliopsis helianthoides is a rhizomatous herbaceous perennial growing 40–150 cm (16–59 in) tall. The toothed leaf blades are dark green, oval to triangular or lance shaped and may be smooth or hairy or rough in texture. The stems are light green to reddish green, variably pubescent or hairy, and terete to slightly angular. The flowers are produced from midsummer to early autumn (fall). The inflorescence contains one to many composite flower heads. Each head contains yellow ray florets which are generally 2–4 cm (0.8-1.6 inches) long. At the center are many yellow to brownish disc florets. The fruit is an achene about 5 mm long.

Chemical constituents

Heliopsin, an unsaturated isobutylamide.

Medicinal Uses

American Indians made a tea from the flower to treat lung ailments and malaria. A tea made from the leaf for high fevers, also used as an astringent. A leaf poultice was applied to snake bites and spider bites. The seed and leaves considered to be a diuretic and an expectorant.

Plate-3: *Heliopsis helianthoides* L.(Sweet)

3.) *Tagetes erecta* Linn. – yellow coloured flower

Scientific classification:

- Kingdom Plantae
- Order Asterales
- Family Asteraceae
- Sub family Asteroideae
- Tribe Tageteae
- Genus ***Tagetes***
- Species ***erecta***

Distribution

The genus is native to North and South America, but some species have become naturalized around the world.

C**ommon Names**

Marigold, hundred- leafed flower.

Description

Tagetes is a genus of annual or perennial, mostly herbaceous plant in the sunflower family.It vary in size from 0.1 to 2.2 m tall. It has pinnate green leaves. The stem is green, succulent, Blooms naturally occur in golden orange, often with maroon highlights. Floral heads are typically (1-) to 4–6 cm diameter, generally with both ray florets and disc florets.

Chemical constituents

β-**S**itosterol, β- Daucosterol, Erythrodiol 3 palmitate, lupeol, kaempferol, erythrodiol, quercetagetin.

Medicinal Uses

The plant has insecticidal, Nematicidal, and Fungicidal properties.

Plate-4: *Tagetes erecta* linn. (yellow)

4.) *Tagetes erecta* Linn – orange coloured flower*:*

Scientific classification:

- Kingdom Plantae
- Order Asterales
- Family Asteraceae
- Sub family Asteroideae
- Tribe Tageteae
- Genus ***Tagetes***
- Species ***erecta***

Distribution

The genus is native to North and South America, but some species have become naturalized around the world.

Common Names

Marigold, hundred- leafed flower.

Description

Tagetes is a genus of annual or perennial, mostly herbaceous plant in the sunflower family. It vary in size from 0.1 to 2.2 m tall. It has pinnate green leaves. The stem is green, succulent, Blooms naturally occur in golden orange, often with maroon highlights. Floral heads are typically (1-) to 4–6 cm diameter, generally with both ray florets and disc florets.

Chemical constituents

β-**S**itosterol, β- Daucosterol, Erythrodiol 3 palmitate, lupeol, kaempferol, erythrodiol, quercetagetin.

Medicinal Uses

It has insecticidal, Nematicidal, and Fungicidal uses.

Plate -5: *Tagetes erecta* linn. (Orange)

5.) *Chrysanthemum indicum* Linn

Scientific classification:

- Kingdom Plantae
- Order Asterales
- Family Asteraceae
- Sub family Asteroideae
- Genus ***Chrysanthemum***
- Species ***indicum***

Distribution

They are native to Asia and northeastern Europe. Most species originate from East Asia and the centre of diversity is in China.

Common Names

Mums and chrysanths.

Description

Chrysanthemum indicum is a herbaceous perennial plant or subshrubs. They have alternately arranged leaves with toothed or occasionally smooth edges. The compound inflorescence is an array of several flower heads, or sometimes a solitary head. The head has a base covered in layers of phyllaries. The simple row of ray florets are white, yellow or red; many horticultural specimens have been bred to bear many rows of ray florets in a great variety of colors. The disc florets of wild taxa are yellow. The fruit is a ribbed achene.

Chemical constituents

Pyrethroid, Pyrethrin, Allethrin, Esbiothrin

Medicinal Uses

It is used as larvicide and insecticide.

PLATE-6. *CHRYSANTHEMUM INDICUM* LINN.

MATERIALS AND METHODS

1. Collection of five species of asteraceae

- The root, shoot and flower samples of five species of Asteraceae family (*Tridax, Heliopsis*, *Tagetes* [Orange and Yellow], *Chrysanthemum*) were washed with distilled water and shade dried to evaporate all the water contents in those samples.
- After drying, these samples were ground in an electric blender into fine powder.

2. Preparation of the extracts

- The method of plant sample extraction was modified from those of Satoto (1993) and Choochote (1999).
- 10g of each sample was measured and taken in 15 separate beakers.

- 50 ml of aqueous, Ethanol, Acetone, was measured and added to each beaker containing sample.
- These suspensions were stirred with a magnetic stirrer and kept aside undisturbed for 48 hours.
- The suspensions were filtered through Whatmann no.1 filter paper and the filtered extract was evaporated using rotary evaporator.
- About 5mg of the condensed extract was taken and dissolved in 10ml of distilled water and kept air tight in the screw cap bottles.
- These were then stored in the refrigerator until needed for tests.

PLATE-7: Extraction preparation through filter paper.

3. Collection of larva of *aedes aegypti*

- Mosquito larvae were collected from the stagnant water present in the coconut shells in Maduravoyal.
- The target mosquito larva of *Aedes aegypti* were identified by the morphological appearance of a single hair, a three branch air tufts on each side of the air tube or the siphon.
- The identified larva were separated from other mosquito species and were placed in a water - filled plastic container.

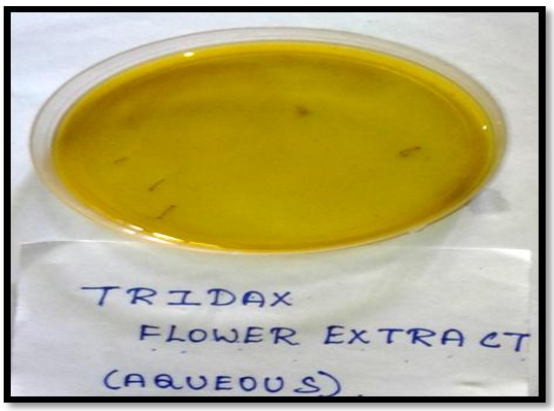
Plate-8: *Aedes aegypti* larva in a petriplate containing water.

4. LARVICIDAL BIOASSAY OF MOSQUITO: (W.H.O. Guidelines, 2005)

- The efficacy of the plant extracts as larvicide against the dengue-vector *Aedes aegypti* (third instar larvae) mosquito was evaluated in accordance with the guidelines of World Health Organisation with slight modifications adopted for the study (W.H.O. Guidelines, 2005).
- 5mg of the mosquito coil was measured, ground and dissolved in 10ml of distilled water. Distilled water was used as negative control. Mosquito control was used as positive control.
- Tests were conducted in petriplates. Five healthy larvae were released into each petriplates and 5ml of each of the prepared extracts (Aqueous, Acetone, Ethanol) were poured into these petriplates.
- The mortality rate of the mosquitoes was observed for first six hours and the final observation was made after 24 hrs by counting the dead larva.
- The percentage mortality was calculated using the following formula.

$$\text{Percentage mortality} = \frac{\text{Number of larvae dead}}{\text{Number of larvae introduced}} \times 100$$

Plate-9: Larvicidal bioassay in a petriplate.



6. Cytotoxic effect in the root tips of onion

- The sample which showed 100% mortality was tested for the cytotoxic effects using *Allium cepa* (onion) root tips.
- The onion bulbs were planted and watered regularly. After two days small roots were emerged.
- The root tips were harvested between 9AM- 10AM. The mitotic division is active during these hours.
- The harvested root tips were boiled in a water bath and then with acetocarmine stain until root tips become dark red.
- It is then washed with distilled water and squashed in a slides. The prepared slides were observed under the microscope to check for chromosomal aberrations.

RESULTS

Leaf, root and flower samples of *Tridax, Heliopsis, Tagetes [yellow and orange]*, and *Chrysanthemum* with 100% mortality of the *Aedes aegypti* mosquito larva was noted. *Tridax* flower (ethanol), *Heliopsis* flower (ethanol), *Tagetes* yellow flower(ethanol), *Tagetes* orange flower (acetone and ethanol) shows 100% mortality within short duration. The results are tabulated in Table-1, Table-2, Table-3, Table-4, Table-5, Table-6.

Table 1: Showing mortality rate of the extracts of *Tridax procumbensis* Linn.

Sr.No.	Solvent	Number of samples (5mg/10ml)	Mortality % of three replicates	Time for mortality (0 to 24hr.)
1.	Aqueous	Leaf	46.66±11.54	24h
2.		Root	0 ± 0	-
3.		Flower	53.33± 11.54	1hr.
4.	Ethanol	Leaf	73.33 ± 11.54	5hr
5.		Root	6.66 ± 11.54	24hr
6.		Flower	100± 0	10min.
7.	Acetone	Leaf	73.33± 11.54	6h
8.		Root	13.33± 11.54	24h
9.		Flower	80± 0	30min.
10.	Positive control	Good knight.	80±0.	20min.
11.	Negative control	Distilled water.	0±0	-

The ethanol extract of the flowernwas found to show 100% mortality in 10minutes. This showed better results than the positive control (Good Knight).

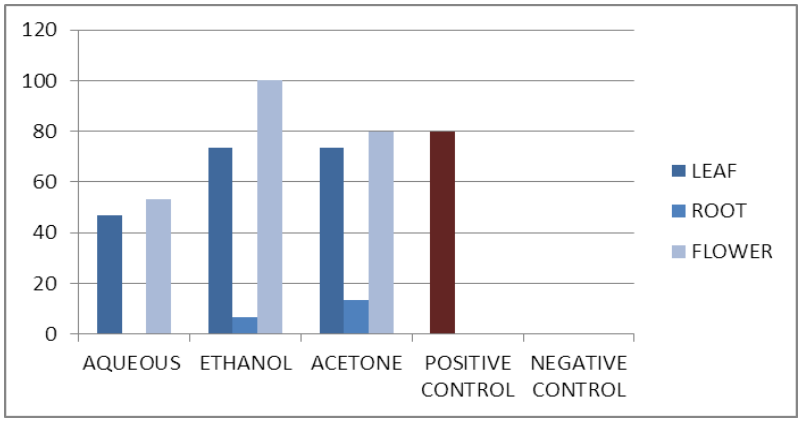


Figure 1: Percentage mortality of *Aedes aegypti* larvae treated with *Tridax procumbens.*

Table 2: showing mortality rate of the extracts of *Heliopsis helianthoides* L (Sweet).

Sr.No.	Solvent	Number of samples (5mg/10ml)	Mortality % of three replicates.	Time for mortality (0 to 24hr.)
1.	Aqueous	Leaf	60±20	24hr
2.		Root	0 ±0	-
3.		Flower	40±20	24hr
4.	Ethanol	Leaf	80±0	6hr
5.		Root	20±0	24hr
6.		Flower	100±0	3hr
7.	Acetone	Leaf	80±0	5hr
8.		Root	6.66±11.54	24hr
9.		Flower	80±20	5hr
10.	Positive control	Good knight	80±0	20min
11.	Negative ccontrol	Distilled water	0±0	-

The ethanol extract of flower showed 100% mortality in 3 hrs after application. The negative control had no effect while Good Knight took only 20 minutes for 80% mortality.

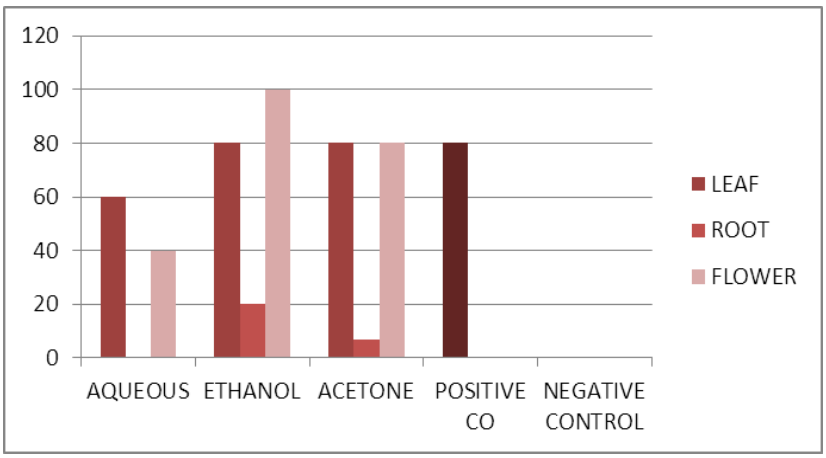


Figure 2: Percentage mortality of *Aedes aegypti* larvae treated with *Heliopsis helianthoides.*

Table 3: showing mortality rate of the extracts of *Tagetes erecta* Linn. (yellow coloured flower)

Sr.No.	Solvent	Number of samples (5mg/10ml)	Mortality % of three replicates.	Time for mortality (0 to 24hr.)
1.	Aqueous	Leaf	73.33±11.54	5hr
2.		Root	0±0	-
3.		Flower	80±20	60min.
4.	Ethanol	Leaf	60±20	4hr 20min.
5.		Root	20±20	24hr
6.		Flower	100±0	5min.
7.	Acetone	Leaf	46.66±11.54	4hr 30min.
8.		Root	6.66±11.54	24hr
9.		Flower	80±0	15min.
10.	Positive control	Good knight	80±0	20min.
11.	Negative control	Distilled water	0±0	-

Tagetes erecta (yellow colour) shows 100% mortality of *Aedes aegypti* mosquito larva in 5 minutes was observed in ethanol flower extract. These results were much faster and reliable than the positive control (Good Knight).

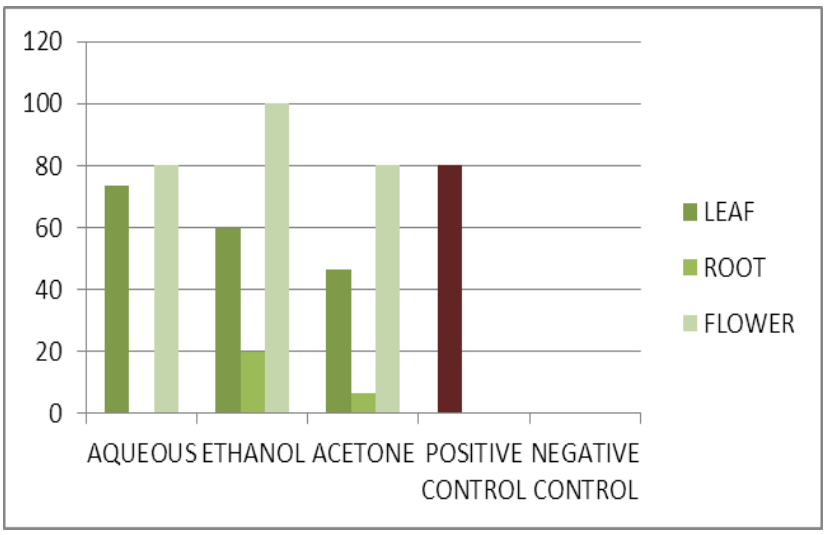


Figure 3: Percentage mortality of *Aedes aegypti* larvae treated with *Tagetes erecta* (yellow).

Table 4: showing mortality rate of the extracts of *Tagetes erecta* Linn (orange coloured flower)

Sr.No.	Solvent	Number of samples (5mg/10ml)	Mortality % of three replicates	Time for mortality (0 to 24hr.)
1.	Aqueous	Leaf	53.33±11.54	2hr
2.		Root	0±0	-
3.		Flower	66.66±11.54	1hr 30min.
4.	Ethanol	Leaf	80±20	1hr
5.		Root	20±20	24hr
6.		Flower	100±0	10min.
7.	Acetone	Leaf	80±0.	1hr 25min.
8.		Root	13.33±11.54	24hr
9.		Flower	100±0.	20min.
10.	Positive control	Good knight	80±0	20min.
11.	Negative control	Distilled water	0±0	-

Flower of *Tagetes erecta* (orange colour) shows 100% mortality in 10 minutes (ethanol) and 20 minutes (acetone). The negative control had no effect and the positive control had 80% mortality.

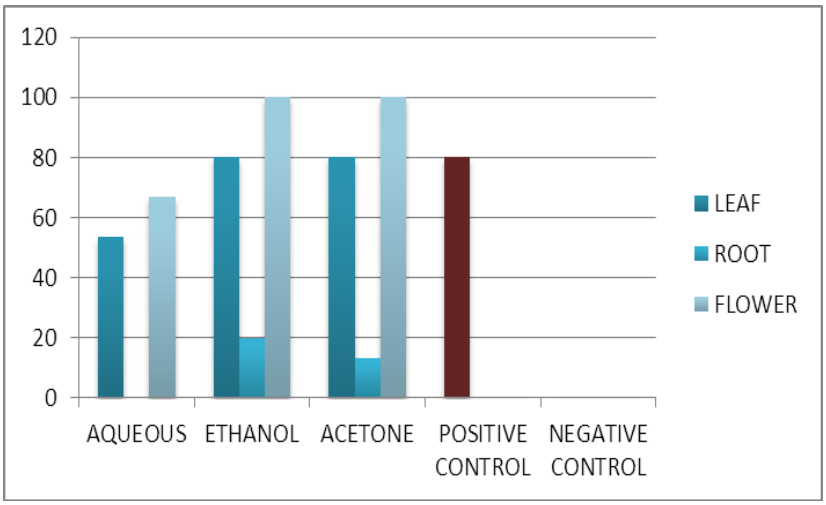


Figure 4: Percentage mortality of *Aedes aegypti* larvae treated with *Tagetes erecta* (orange).

Table 5: showing mortality rate of the extracts of *Chrysanthemum indicum* Linn.

Sr.No.	Solvent	Number of samples (5mg/10ml)	Mortality % of three replicates	Time for mortality (0 to 24hr.).
1.	Aqueous	Leaf	60±20	6hr
2.		Root	0±0	-
3.		Flower	73.33±11.54	5hr
4.	Ethanol	Leaf	80±20	3hr
5.		Root	13.33±11.54	24hr
6.		Flower	73.33±11.54	2hr 45 min.

7.	Acetone	Leaf	60±20	4hr
8.		Root	6.66±11.54	24hr
9.		Flower	80±0	10min.
10.	Positive control.	Good knight	80±0	20min.
11.	Negative control	Distilled water	0±0	-

The acetone extract of *Chrysanthemum indicum* shows 80% mortality in 10 minutes. The positive control (Good Knight) also shows 80% mortality but in 20 minutes.

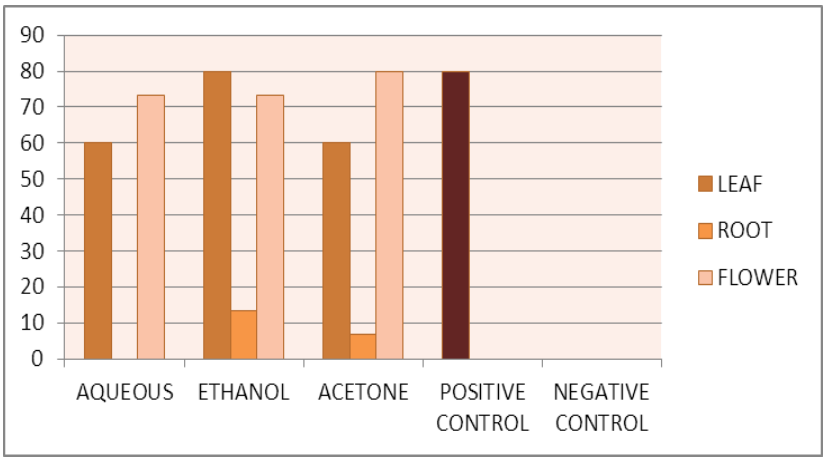


Figure-5. Percentage mortality of *Aedes aegypti* larvae treated with *Chrysanthemum indicum*.

Table 6: showing mitotic index and % of aberrant cells.

S.NO.	SAMPLE	SOLVENT	NO. OF CELLS OBSERVED	NO. OF DIVIDING CELLS.	MITOTIC INDEX %.	% OF ABBERRANT CELLS.
1.	Tridax flower	Ethanol	500	233	46.6	6.6
2.	Heliopsis flower.	Ethanol	500	240	48	4.3
3.	Tagetes (Yellow) flower.	Ethanol	500	380	76	1.4
4.	Tagetes (Orange) flower	Ethanol	500	335	65	1.8
5.	Tagetes (Orange) flower	Acetone	500	305	61.2	3.1

The sample which showed 100% mortality alone was chosen for cytotoxic study using *Allium cepa* (Onion) root tips.

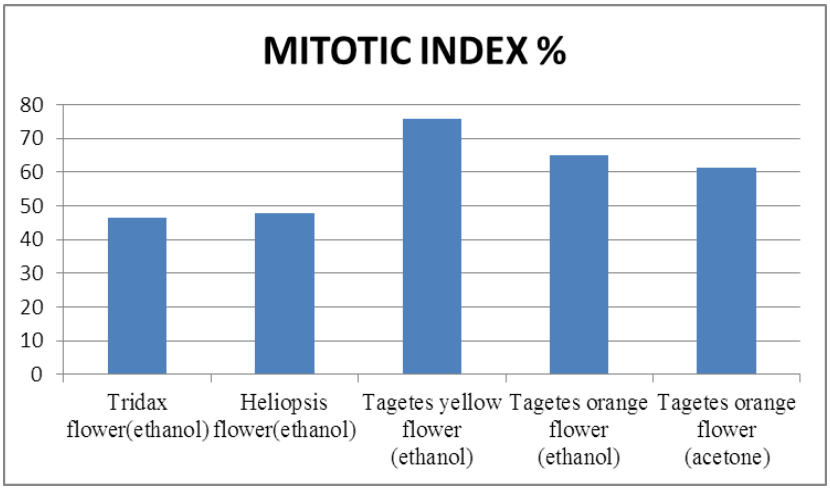
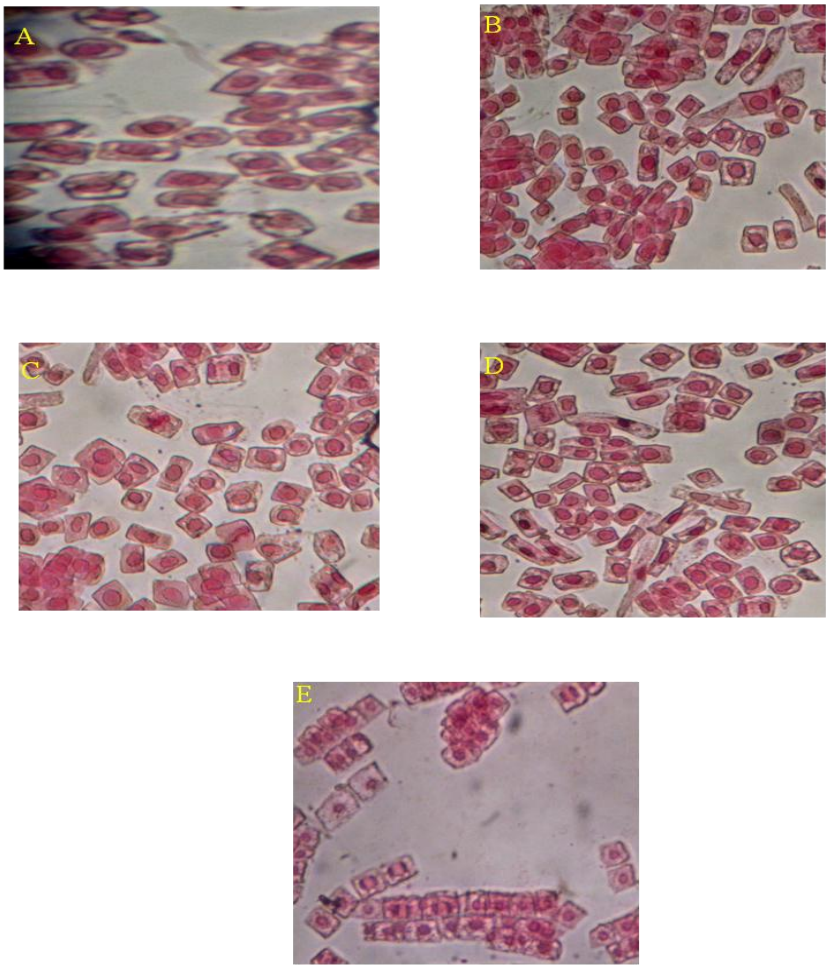


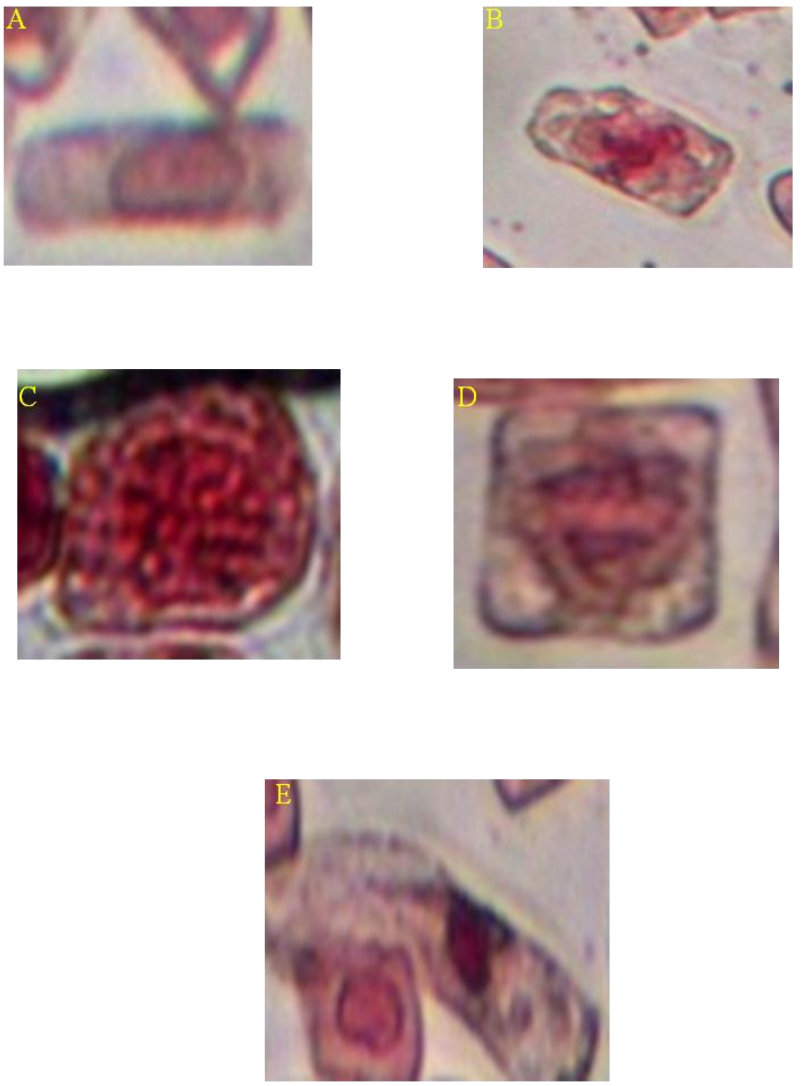
Figure - 6 Mitotic index in *Allium cepa* root tip cells.

PLATE – 10 DIVIDING CELLS OF *ALLIUM CEPA* TREATED WITH PLANT EXTRACTS



A) TRIDAX PROCUMBENS FLOWER (ethanol),
B) HELIOPSIS HELIANTHOIDES FLOWER (ethanol),
C) TAGETES ERECTA YELLOW FLOWER (ethanol),
D) TAGETES *ERECTA* ORANGE FLOWER (ethanol),
E) TAGETES ERECTA ORANGE FLOWER (acetone).

PLATE-11 *ALLIUM CEPA* CELLS WITH ABBERRATIONS TREATED WITH PLANT EXTRACTS



A) STICKY PROPHASE, B) STICKY METAPHASE, C) NUCLEAR LESION, D) TELOPHASE STICKYNESS, E) GIANT CELL.

DISCUSSION

Tagetes erecta Linn- yellow shows 100% mortality in ethanolic flower extracts of *Aedes aegypti* within 5 mins in 5mg/10ml. Studies of Maradufu *et. al.,* 1978 revealed that 1.5 1.0mg/ml of ethanolic extract of *Tagetes* showed 50% mortality of *Aedes aegypti*. Similar study reported by Peneremmal Amrutha *et. al.,* 2013 showed that 4.73mg/ml of ethanolic extracts of *Tagetes erecta* (yellow) also showed 50% mortality.

Tagetes erecta Linn. - orange colour shows 100% mortality in the ethanolic extract within 10mins in5mg/10ml. Likewise, ethanolic flower extracts of *Tagetes erecta* (orange) showed 50% mprtality rate of 4.11mg/ml in *Aedes aegypti*. (Peneremmal Amrutha *et. al.,* 2013).

Tridax procumbens Linn. ethanolic flower extract shows 100% mortality within 10mins in 5mg/10ml in *Aedes aegypti*. Similarly, C.Kamaraj *et. al.,* 2011 in acetone leaf extracts of *Tridax procumbens showed* high mortality rate of 51.57 against the mosquito larvae - *Anopheles subpictus*. Another study revealed by Anitha Rajasekaran, *et. al.,* 2012 determined that petroleum ether extract of *Tridax procumbens* has 60% mortality rate in 219µg/ml, *Lantana camara* has 80% morality rate in 251 µg/ml, *Datura stramonium* has 100% mortality rate in 288 µg/ml. Recently, another study done by Syed Mohammed Imthiyaz Begum *et. al.,* 2014 showed 54% mortality in 125ppm acetone extracts of Tridax procumbens in a duration of 24 hr.

Chrysanthemum indicum Linn. Acetone flower extracts shows 80% mortality of *Aedes aegypti* in 20 mins in 5mg/10ml. According to Kamaraj *et. al.,* 2011 ethyl acetate leaf extract of *Chrysanthemum indicum* shows 42.29 larval mortality against *Culex tritaeniorhynchus*.

The ethanolic extract of *Tridax procumbens* (5mg/10ml) shows chromosomal aberrations like c-metaphase, nuclear lesion, in the root tips of *Allium cepa* etc. Mondal *et. al.,* 2006, investigated few anomalies like c- metaphase, nuclear lesion, and micronucleus, in the aqueous extracts of different *Ipomoea* species(10%) on the root tips of *Allium cepa* (Onion).

Heliopsis helianthoides L(Sweet) (ethanolic extract) shows sticky prophase and sticky metaphase in 5mg/10ml in *Allium cepa* root tips. In a similar manner, Selestian Rathnasamy *et. al.,* 2013 reported the aqueous and ethanolic extract of *Clinacanthus nutans*, *Adhatoda vasica*, *Carica papaya,* shows more abnormalities like sticky prophase, sticky metaphase, micronucleus, cytoplasmic vacuolization, and more chromosomal aberrations in *Allium cepa* root tip cells. Another study by Daniel *et. al.,* 2011 conducted the cytotoxic effect of squeezed extracts from toasted cassava (10%) showed sticky chromosomes.

The ethanolic extract of *Tagetes erecta* Linn. yellow flower, (5mg/10ml) shows sticky chromosomes, Nuclear lesion, giant cell in *Allium cepa*. Similarly, Asito *et. al.*, 2013 reported *Allium cepa* root tip cells showed sticky chromosomes, c-metaphase, chromosomes bridges by the application of pesticides like permethrin. Mercaptothion.

CONCLUSION

From the results, it is concluded that, *Tagetes* yellow flower (ethanol) was very effective among the samples. So the present study revealed that the ethanolic extract of *Tagetes* yellow flower was identified as the best natural larvicide and it caused very low cytotoxic effect in the root tips of Onion (*Allium cepa*).Hence, the larvicidal activity of the above sample is very potent and persistent. Further investigations are needed to elucidate this activity against all stages of mosquito species and also to identify the active components of the extract responsible for larvicidal activity.

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