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Exploring aqueous floral extracts as mosquito larvicides: Histopathological, Morphological and FT-IR Analysis

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Abstract- Mosquito-borne diseases pose significant global health threats, underscoring the need for eco-friendly mosquito control strategies. This study investigates the larvicidal efficacy of aqueous floral extracts from *Tagetes erecta* and *Plumeria rubra* against *Culex* mosquito larvae. The phytochemical-rich extracts exhibited concentration-dependent larvicidal activity, with LC_{50} values indicating higher efficacy of hot extracts. Histopathological analysis revealed substantial tissue damage, while morphological observations showed developmental abnormalities in treated larvae. FT-IR analysis identified bio-active compounds responsible for larvicidal activity. Our findings demonstrate the potential of aqueous floral extracts as sustainable, non-toxic mosquito larvicides, aligning with integrated pest management principles.

Key words: Larvicide, toxicity, Floral extracts, *Culex*, phytochemicals, *Tagetes erecta* and *Plumeria rubra*

INTRODUCTION

Mosquitoes pose significant health risks worldwide as vectors of various infectious diseases, including malaria, dengue fever, Zika virus, and others. Effective mosquito control strategies are essential for mitigating these risks and protecting public health. Traditional methods such as insecticides have been widely used, but their indiscriminate application raises concerns about environmental impact and resistance development among mosquito populations. As a result, there is growing interest in exploring alternative, eco-friendly approaches to mosquito control.

Herbal flower extracts have emerged as promising alternative for mosquito larvicides due to their perceived safety, biodegradability, and potential efficacy against mosquito larvae. These extracts are derived from various plant species known for their insecticidal properties and have been traditionally used in folk medicine and pest control practices.[1-13] Flower extracts demonstrated both insecticidal and antibacterial properties.[8] Research into their effectiveness has intensified, driven by the need for sustainable and non-toxic alternatives to conventional chemical insecticides.

This introduction sets the stage for understanding the rationale behind exploring herbal flower extracts as larvicides. It emphasizes the need for effective mosquito control measures while highlighting the potential benefits of utilizing natural plant-based solutions in combating mosquito-borne diseases.

The practice of using flowers in temples, homes, and as decorative materials, followed by their utilization for the production of floral extracts, presents an intriguing

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opportunity that intertwines cultural practices with environmental and public health concerns, particularly in the realm of mosquito control.

In many cultures, flowers hold religious significance and are offered in temples as a symbol of devotion. After their ceremonial use, these flowers are often discarded, creating significant organic waste. This waste can pose environmental challenges if not managed properly, contributing to landfill accumulation or polluting water bodies if disposed of irresponsibly.

However, the concept of reusing these flowers for the production of floral extracts offers a sustainable solution. By extracting bio-active compounds from these spent flowers, such as essential oils or other active constituents with insecticidal properties, communities can mitigate environmental impact by reducing waste and harnessing valuable natural resources for beneficial purposes.

From a mosquito control perspective, floral extracts obtained from these discarded flowers can be evaluated for their effectiveness against mosquito larvae. Research has shown that certain plant-derived compounds exhibit larvicidal properties, capable of disrupting the life cycle of mosquitoes without harming non-target organisms or causing significant environmental harm. This approach aligns with the principles of integrated pest management (IPM), which emphasizes using environmentally friendly methods to control pests while minimizing adverse effects on ecosystems and human health.

Moreover, utilizing floral extracts for mosquito control not only addresses environmental concerns but also promotes sustainable practices rooted in cultural traditions. By reusing flowers that would otherwise be waste, communities can harness their natural properties to contribute positively to public health initiatives, reducing reliance on synthetic chemicals that may have broader environmental implications.

In conclusion, the convergence of cultural practices involving flowers with modern environmental and public health needs highlights a promising avenue for sustainable mosquito control. By reusing flowers for the production of larvicidal extracts, communities can foster environmental stewardship, preserve cultural traditions, and promote effective, eco-friendly solutions to combat mosquito-borne diseases.

MATERIAL & METHODS

Collection of flowers - The fresh flowers of *Tagetes erecta* and *Plumeria rubra* were collected from the various temples of Nashik.

A. *Tagetes erecta*-

Kingdom	-	Plantae
Order	-	Asterales
Family	-	Asteraceae
Genus	-	*Tagetes*
Species	-	*erecta*

Tagetes erecta is commonly called as 'Zendu' in marathi. It is commonly known as the African marigold. This plant grows to a height of 20-90 cm (7.9-35.4 in). People in some parts of the world gathered wild plants and cultivated them for medicinal, ceremonial, and decorative purposes. The flowers are grouped in small heads or solitary inflorescence, on peduncles up to 15 cm long, and range in colour from yellow to red. The disc flowers have 150 to 250 simple heads, while the doubles have varying degrees of transformation in ligules, yellow to orange corollas that are 8 to 10 mm long.

The flowers of this plant are either used in preparation of garland or offered as it is in temples. These flowers are available in two colours like yellow and orange with two sizes, large and small. The flowers have a strong smell which may be due to the presence of certain phytochemicals. Farmers from India cultivate the plants for getting flowers.

Since prehistorian times, this plant has been used medicinally. The pigments in *Tagetes* are caused by the presence of carotenoids, the most prominent of which is lutein, which has been linked to the prevention of age-related eye diseases such as cataracts and macular degeneration. The flowers contain carotenoids, specifically xanthophyll. According to some studies, the latter can help prevent coronary artery disease, heart attacks, immune response, old age, and cancer. In some parts of Mexico, it is used to treat digestive issues such as stomach pain, diarrhoea, colic, liver problems, bile, vomiting, and indigestion.[14-16]

B. *Plumeria rubra*-

Kingdom	-	Plantae
Order	-	Gentianales
Family	-	Apocynaceae
Genus	-	*Plumeria*
Species	-	*rubra*

Plumeria rubra is commonly called as 'Chafa' in marathi. *Plumeria rubra* is a plant that grows as a spreading shrub or small tree to a height of 2-8 m (5-25 ft) and a similar width. The flowers are terminal and appear at the ends of branches throughout the summer. They are often profuse and prominent, with five petals and a strong fragrance. The flowers release their fragrance in the morning and evening. This fragrance is similar to rose, citrus, and cinnamon. The colours range from pink to white, with shades of yellow in the centre of the flower. The flowers are initially tubular before opening out and have a diameter of 5-7.5 cm (2-3 in). Its flowers and bark are also used in traditional Chinese medicine to treat fever, bacillary dysentery, and pertussis, among other conditions. *P. rubra* contains fulvoplumierin, an antibiotic that inhibits the growth of *Mycobacterium tuberculosis.* The plant has been shown to have anti-fungal, antiviral, analgesic, antispasmodic, and hypoglycemic properties.[17] The plant has been shown to improve digestion, excretion, respiratory, and immune functions. The plant's sap acts as a laxative and treats bloating and stomachaches. The bark is said to have purgative properties and can be used to treat venereal sores. The flowers can be boiled in water or juice and mixed into a salad to help with bowel movement, urine flow and gas control.[17,18] In India the flowers of this plant are either used in preparation of garland or offered as it is in temples. The flowers have the smell which may be due to the presence of certain phytochemicals.

Aqueous extract preparation- After collection, the flowers were dried in shade at environmental temperature. The dried flowers were powdered mechanically using grinder and boil in the water till decolourization of powder takes place.

Hot extract preparation- Total 100 mg of powder of flower was boiled in 100 ml of distilled water then filter it properly. The filtrate was stored in refrigerator at 4^{o}C in airtight container separately. The filtrate was act as a stock solution and used for preparation of further larvicidal concentrations. Same procedure followed for both the type of flower powder.

Cold extract preparation- Total 100 mg powder was taken and added 100 ml of distilled water and kept in shaker for 24 hours and then filtered with cotton cloth. Allow the excess water to evaporate at room temperature. Extract was stored in the refrigerator at 4^{o}C till further used. Same procedure followed for both the type of flower powder.

Mosquito larvae rearing- Extracts of various plant parts in a variety of solvents, including aqueous extract[13,19,20], were prepared and tested against three major mosquito species[7,9], in the current experiment, we chose the *Culex* species. Larvae of *Culex* mosquito were collected from NCL, Pune. The larvae were reared in the laboratory under control conditions. The larvae were kept in glass tub containing tap water. The temperature was maintained at 30^{o}C, 75-85% relative humidity. The larvae were fed with mixture of dog biscuits and yeast. 4th instar larvae were used for larvicidal activity.

Toxicity assay- Floral extracts were tested for their larvicidal activity against the 4th instar *Culex* larvae. The results of previous researchers also showed that the older larval stages were more resistant to the extract and compound than younger larval stage.[21] Since we selected the 4th instar larvae to carried out toxicity assay. Both the hot and cold extracts were tested. 20 larvae into each test beaker and control beaker of capacity 250 ml were used for the assay. The assay was conducted in triplicates for each concentration. The larvae were checked for mortality after 24 hrs. A larva was considered as dead, if it not moves, when gently probed with an object. The number of dead larvae were recorded for each concentration of every set. The percentage of dead larvae for each concentration were calculated. If control mortality exceeds 5%, Abbott's formula was applied to adjust the mortality in treated groups.

Developmental abnormalities- The larvae were examined for abnormalities following treatment with hot and cold extracts. The developmental abnormalities were reported and photographed.

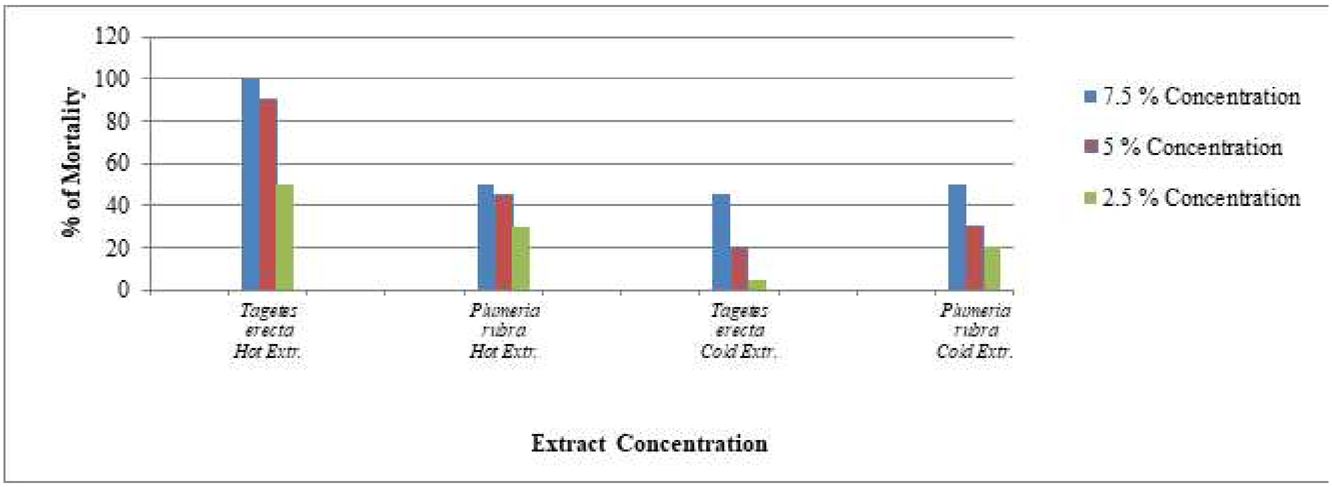
Histological changes- The living larvae were used to assess histopathological changes after the treatment of floral extracts. The midgut plays a vital role in mosquito larvae's survival, as it serves as the main center for nutrient processing, defense, and energy production. Since the histology of midgut, for normal and treated larvae was conducted. The photographs were taken.

FT-IR analysis of flower extracts - FT-IR analysis of each extract was performed, and the presence of phytochemicals was determined for all extracts.

RESULTS & DISCUSSION

Toxicity assay

The effect of hot and cold floral aqueous extracts of both the plants on 4th larval instar are shown in Graph 1. The hot extracts are more effective than the cold extracts.[22] Hot extracts have higher efficacy than cold extracts as they contain phytochemicals with more larvicidal activity. The extracts demonstrate larvicidal activity due to the presence of phytochemicals such as Alkaloids[1,13,22,23], Saponins[13,22], Phenols[3,22], Terpenoids[13,22], Terpenes, Flavonoids[1,3] and Tannins[1,3,22]. Phyto-chemicals are responsible not only for larvicidal activity, but also for histological and morphological abnormalities. The hot extracts exhibit not only greater larvicidal activity, but also increased histological and developmental abnormalities. It indicates that hot extracts are more effective, but cold extracts are also effective at controlling mosquito larvae in the wild. Cold extracts can control mosquito larvae by leading to histological or morphological changes. It is best to control mosquito larvae with cold extracts instead of boiling the flowers for producing hot extracts. The flower extracts showed the highest larvicidal activitythan other plant parts.[24] Bouabid *et al.* (2020)[19] proved that the antioxidant test for *C. ternatea* flowers extract revealed that aqueous extract had the highest antioxidant activity when compared to other extraction methods. The aqueous extract was discovered to have a high antioxidant because water extract can absorb antioxidant compounds better than chemicals.[19] Since in the present study the extracts are prepared in cold and hot water. We got the positive results in both the aqueous extracts. Hot water extraction was significantly more effective in enhancing the larvicidal properties of both plants, particularly *Tagetes erecta*, which achieved complete larval mortality (100%) at the highest concentration (7.5%). Cold water extracts were less effective, with lower mortality rates at all concentrations. Present finding in this study was supported by many earlier research work.[22,25] Larvae of *Anopheles gambiae*, a malaria vector was obtained from the wild, and a 24-hour bioassay showed that the LC_{50} values for cold water, hot water, and boiled extracts were 316.96 ppm, 176.20 ppm, and 136.14 ppm, respectively, indicating the order of effectiveness as boiled extracts > hot water extracts > cold water extracts.[25]



Graph 1- Mortality of hot and cold floral aqueous extracts plants

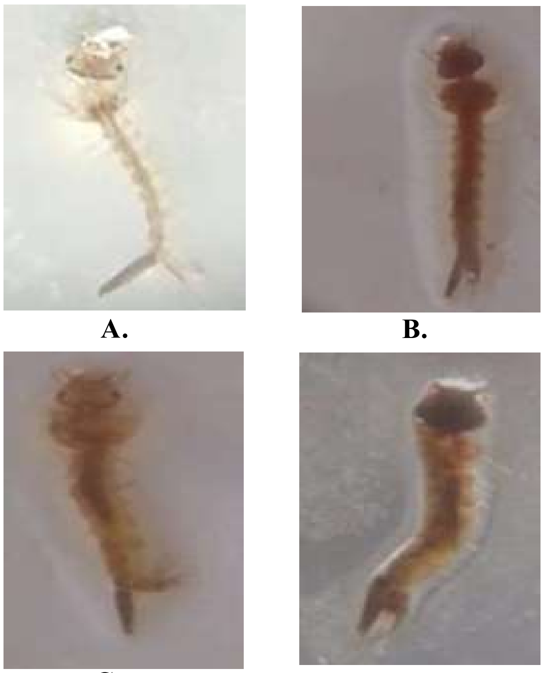
The study compared the larvicidal activity of *Tagetes erecta* and *Plumeria rubra* petals extracted using hot and cold-water methods. The results indicate that the hot water extracts were significantly more effective than the cold-water extracts at all tested concentrations.

For hot water *Tagetes erecta* floral extract demonstrated the highest larvicidal activity, achieving 100% mortality at a concentration of 7.5%, 90% mortality at 5%, and 50% mortality at 2.5%. The *Plumeria rubra* floral extract was less effective, showing a maximum of 50% mortality at 7.5%, 45% mortality at 5%, and 30% mortality at 2.5%.

For cold water extracts of Tagetes erecta floral extract showed moderate larvicidal activity, with a maximum of 45% mortality at 7.5%, 20% mortality at 5%, and 5% mortality at 2.5%. The Plumeria rubra floral extract showed a maximum of 50% mortality at 7.5%, 30% mortality at 5%, and 20% mortality at 2.5%.

Developmental abnormalities-

The normal larva (Fig. 1 A) has a well-defined, elongated, and segmented body structure. The larva's body is divided into three main regions head, thorax and abdomen. The head is large, rounded, and heavily sclerotized. The body appears to be symmetrical, and the tail (siphon) is intact. The thorax is relatively wider than the head and abdomen, without legs but covered with fine hairs or setae. The abdomen is elongated and segmented, typically consisting of 8 segments. The body is mostly light with a brownish tint, especially in the head region. There are no discolorations or abnormal dark spots on the body. Normal larvae are usually active and respond to stimuli. The larvae when treated with the flower extracts showed the developmental abnormalities.[1,2,22]



A- 4th instar normal larva, **B-** larva treated with hot water *Tagetes erecta* floral extract, **C-** larva treated with hot water *Plumeria rubra* floral extract, **D-** larva treated with cold water *Plumeria rubra* floral extract

Fig. 1- Normal larval structure and Developmental abnormalities observed after treatment of floral extracts

The larva when treated with hot water *Tagetes erecta* floral extract (Fig.1 B) shows more darker colour compared to the normal larva. Some darkening may occur in parts of the body as a result of necrosis or the effect of the larvicide (phytochemicals act as larvicides). Elongation of neck and swelling of thoracic region also observed. The larva when treated with hot water *Plumeria rubra* floral extract (Fig.1 C) shows a curled or distorted body, which is often a result of exposure to toxic substances. The swelling or bending of the body segments, indicating stress or damage. The color of the treated larva is darker compared to the normal larva, possibly due to loss of internal structural integrity or tissue breakdown. The larvae when treated with cold water *Plumeria rubra* floral extract (Fig.1 D) shows bending and swelling of the body segments and pigmentation in abdominal region. These visual and structural changes indicate the toxic effects of the floral larvicide (phytochemicals) on the treated larvae compared to the healthy, untreated control group.

Histological changes-

Culex 4th instar larvae have four main midgut regions: anterior, posterior, cardia, and gastric caeca Fig. 2. Each region's epithelium comprises a single layer of cuboidal digestive cells with apical microvilli, facilitating nutrient absorption. The cytoplasm contains numerous mitochondria, and the nuclei hold polytene chromosomes, while regenerative cells are located at the base of the digestive cells. A robust peritrophic membrane barrier separates the midgut epithelium from digested food, providing structural support.[26] The presence of microvilli within the midgut accelerates nutrient uptake.[27]

The 4th instar larvae when treated with floral extracts showed various histological changes[2,28] which are similar to few of the results in present work.

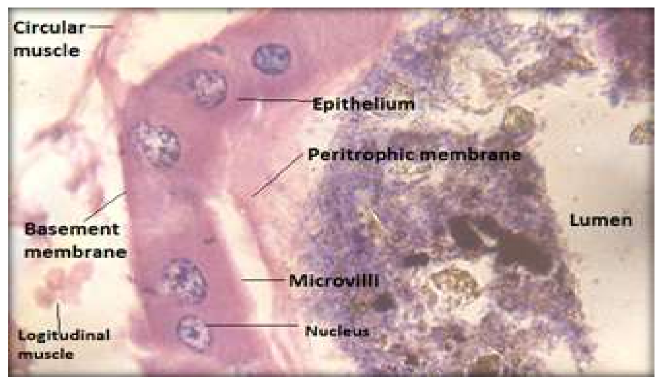


Fig. 2- Normal T.S. of midgut of mosquito larvae

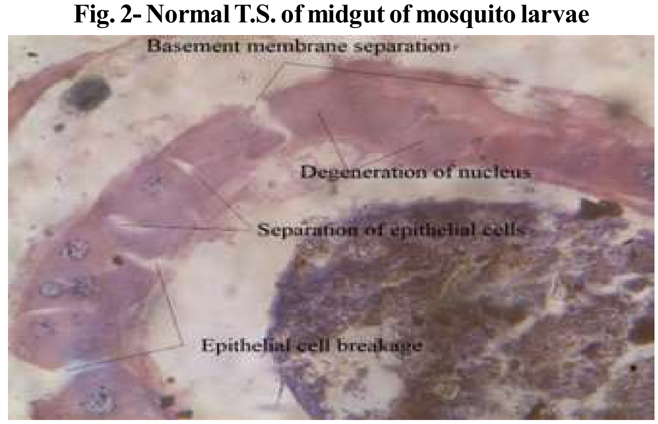


Fig. 3- T.S. of midgut showing effect of *T. erecta* hot flower extract

The larvae treated with hot *T. erecta* flower extract shows the effects like degeneration of nucleus, separation of epithelial cells[29], epithelial cell breakage and basement membrane separation shown in fig. 3.

The larvae treated with cold *T.erecta* flower extract shows the effects like detachment of peritrophic membrane[30], separation of epithelial cells and enucleated cell shown in fig. 4.

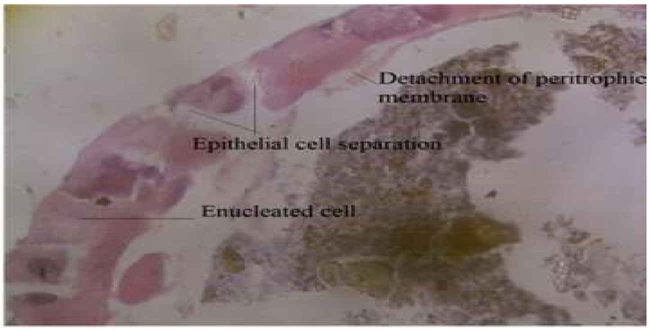


Fig. 4- T.S. of midgut showing effect of *T. erecta* cold flower extract

The effects like separation of epithelial cells, damaged epithelial layer[31,32], separation of peritrophic membrane[33] and shrinkage of epithelial cells shown in fig. 5 are due to the larvae treated with hot *P. rubra* flower extract.

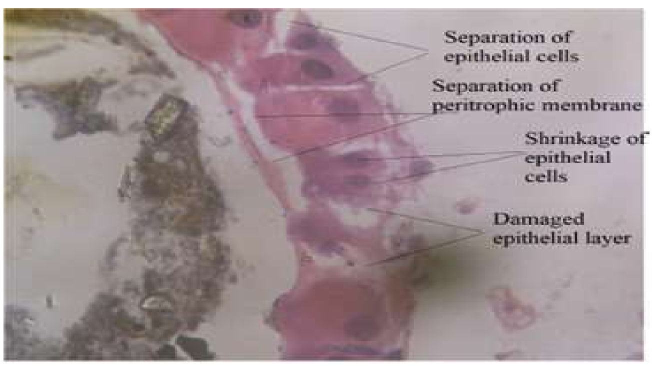


Fig. 5- T.S. of midgut showing effect of *Plumeria rubra* hot flower extract

The effects like separation of epithelial cells, enucleated cell, separation of crypt cell and basement membrane separation shown in fig. 6 are due to the larvae treated with cold *P. rubra* flower extract.

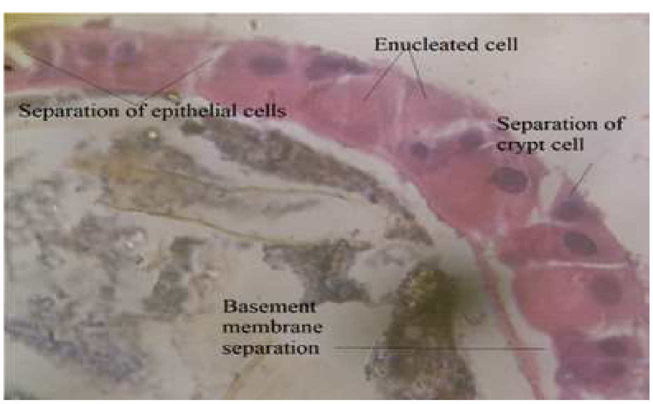


Fig. 6- T.S. of midgut showing *P. rubra* cold flower extract

FT-IR analysis

Fourier Transform Infrared Spectroscopy, also known as FTIR analysis or FTIR Spectroscopy. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties. Phytochemicals present in flower extracts were detected from FT-IR analysis.

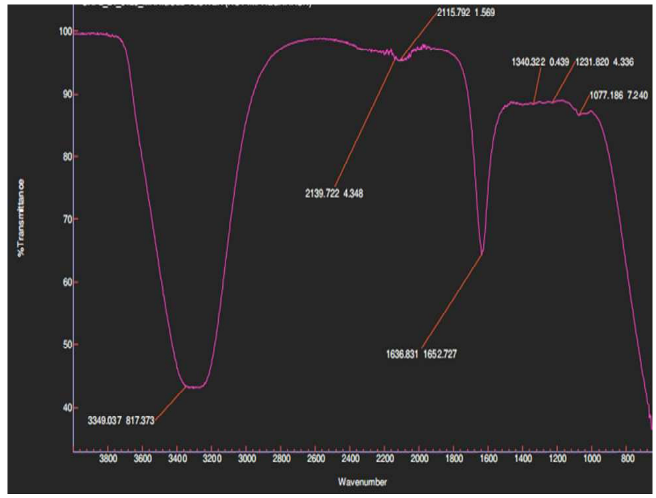


Fig. 7- FT-IR of *T. erecta* hot flower extract

This spectrum (Fig. 7) of hot extract of *T. erecta* flower suggests the presence of a complex mixture of phytochemicals like phenols (Ar-OH), flavonoids, aromatic compounds, and possibly some nitrile (-C≡N) or alkyne (-C≡C-) containing molecules.

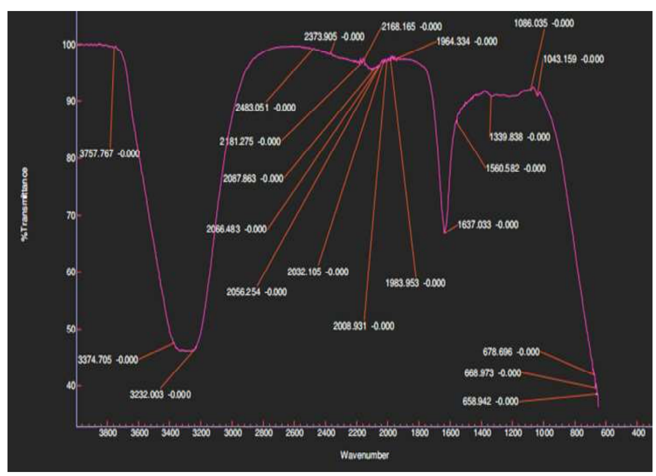


Fig. 8- FT-IR of *T. erecta* cold flower extract

The *T. erecta* flowers cold extract likely contains phenols, flavonoids, aromatic compounds, nitriles(-C≡N), alkynes(-C≡C-), alcohols(-OH), carbohydrates, and carbonyl-containing compounds (R-CO-R). These indicate a rich composition of bioactive phytochemicals with potential medicinal or pesticidal properties shown in fig.8.

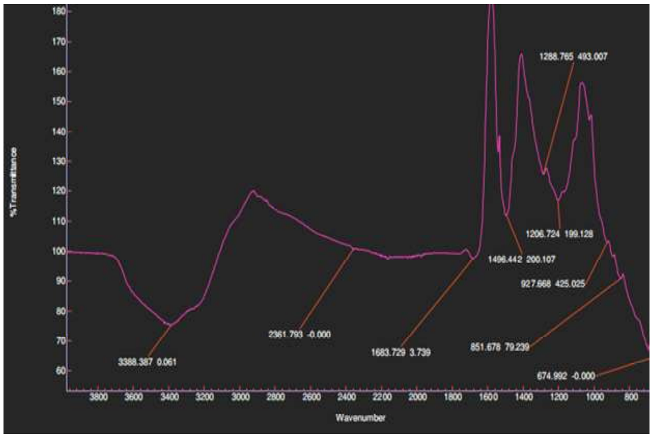


Fig. 9- FT-IR of *P. rubra* hot flower extract

The hot extract of *P. rubra* flowers (Fig. 9) likely contains a complex mixture of phenols, flavonoids, tannins, aromatic hydrocarbons, nitrile compounds (-C≡N), carbonyl compounds, ethers (R-O-R), and esters (R-CO-OR). These phytochemicals are bioactive and could contribute to the extract's medicinal or pesticidal properties.

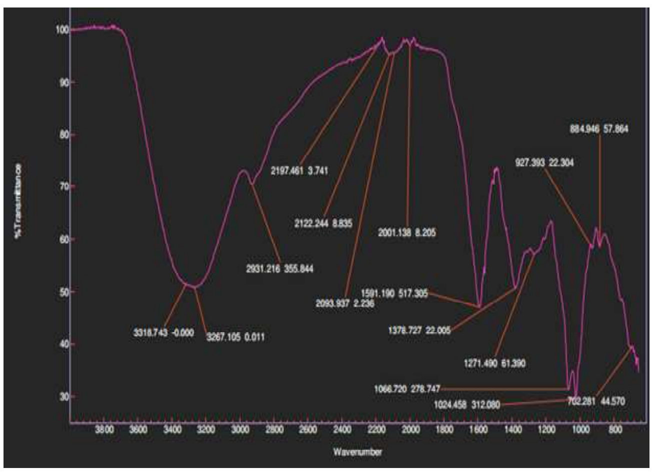


Fig. 10- FT-IR of *P. rubra* cold flower extract

Fig. 10 shows the cold extract of *P. rubra* flowers likely contains phenols, flavonoids, aromatic compounds (Ar-R), nitriles(-C≡N), alkynes(-C≡N), aliphatic hydrocarbons, and possibly nitro or carboxylic acid derivatives. These phytochemicals contribute to the extract's potential bioactive and therapeutic properties.

The hot extract of both the floral extracts has a higher concentration of phenols, flavonoids, and aromatic compounds, which are known for their potent larvicidal and antioxidant properties. These compounds are often the primary drivers of insecticidal activity. Even though the cold extract of both the floral extracts contains a wider range of phytochemicals including nitriles, alkynes, and alcohols, the specific larvicidal activity of these compounds may not be as strong as the phenolic compounds found in higher concentrations in the hot extract.

It is clear from the result got in the present study that the Cold Extract of *T. erecta* contains a broader range of phytochemicals, including nitriles, alkynes, alcohols, carbohydrates, and carbonyl-containing compounds along with phenols, flavonoids, and aromatic compounds. Suggests a more diverse chemical profile, possibly due to gentle extraction conditions preserving heat-sensitive compounds. Hot Extract of *T. erecta* contains primarily phenols, flavonoids, and aromatic compounds, with fewer mentions of nitriles, alkynes, alcohols, or carbohydrates. Indicates the absence or degradation of some heat-sensitive compounds during high-temperature extraction. Since the cold floral extract is more effective than hot floral extract. Phenols and Flavonoids present in both cold and hot extracts of *P. rubra*. Known for their antioxidant[34,35] and insecticidal activity[36,37], disrupting insect metabolism and growth. Aromatic Compounds found in both extracts and may serve as deterrents or toxic agents against insects.[38,39] Nitriles present in both extracts and can act as metabolic disruptors in insects.[40-42] Alkynes (Cold Extract) known for their strong bioactivity and toxicity to insects but are less common in hot extracts which provide cold extract with unique insecticidal potential.[43]

Tannins (Hot Extract) present only in the hot extract known for their insecticidal effects by binding to proteins in the insect gut, inhibiting digestion.[44,45] Carbonyl Compounds (Hot Extract) found only in the hot extract and often toxic to insects, interfering with their enzymatic functions.[46] Ethers and Esters[47] (Hot Extract) present only in the hot extract. They can disrupt the nervous system of insects[48] and act as repellents. Nitro Compounds (Cold Extract) are potentially toxic to insects by disrupting cellular respiration.[49]

The finding in the present study, are similar like other results shown by many researchers in their research papers. The aqueous extract of *C.procera* showed the presence of many phytochemicals like carbohydrates, alkaloids and saponins, while the ethanolic extracts showed the occurrence of carbohydrates, alkaloids, saponins, phenols, tannins, terpenoids and flavonoids. Many of these phytochemicals screened are known to show medicinal as well as physiological activity.[22] Flavonoids are widely distributed in plants and have many functions including producing pigmentation in flowers and protection from microbial and insect attack.[50] Terpenoids have different functions like growth regulation, colour, odour and antimicrobial activity[51] and the presence of terpenoids has also been reported from many other plants like clove, chillies, turmeric etc.[52] Tannins and other polyphenols have been identified in cinnamon, eucalyptus, lemon balm and neem and may be responsible for its broad spectrum anti-microbial activity against viruses, bacteria and fungi.[53] The saponins are naturally occurring surface-active glycosides mainly produced by the plants. Saponins are known to be anti-microbial and to inhibit moulds and protect plants from insect attacks.[54]

CONCLUSION

This study emphasizes the critical need for environmentally friendly and sustainable mosquito control strategies to address the global health threat posed by mosquito-borne diseases. The aqueous floral extracts of *Tagetes erecta* and *Plumeria rubra* showed significant larvicidal efficacy against *Culex* mosquito larvae, with hot extracts having higher potency as indicated by LC_{50} values. The observed larvicidal activity was attributed to phytochemical compounds identified using FT-IR analysis. Histopathological and morphological studies revealed significant tissue damage and developmental abnormalities in treated larvae, confirming the extracts' negative effects on larval growth and survival. These findings highlight the potential for using phytochemical-rich, non-toxic herbal extracts as effective alternatives to chemical insecticides, reducing environmental impact and ensuring the safety of humans and other organisms.

In short, the use of *Tagetes erecta* and *Plumeria rubra* floral extracts represents a promising approach to integrated pest management, addressing the dual goals of ecological safety and mosquito population control in a sustainable manner.

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