

**VOLUME 9**

**ISSUE 2**

**2023**

**ISSN 2454 – 3055**



**INTERNATIONAL  
JOURNAL OF  
ZOOLOGICAL  
INVESTIGATIONS**

***Forum for Biological and  
Environmental Sciences***

**Published by Saran Publications, India**



## International Journal of Zoological Investigations

Contents available at Journals Home Page: [www.ijzi.net](http://www.ijzi.net)

Editor-in-Chief: Prof. Ajai Kumar Srivastav

Published by: Saran Publications, Gorakhpur, India



ISSN: 2454-3055

### Larvicidal Bioefficacy of *Euphorbia cyathophora* Leaf Extracts Against Bhindi Fruit Borer *Earias vittella*, Fabricius (Lepidoptera: Noctuidae)

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Received: 2<sup>nd</sup> August, 2023; Accepted: 24<sup>th</sup> August, 2023; Published online: 30<sup>th</sup> August, 2023

<https://doi.org/10.33745/ijzi.2023.v09i02.052>

**Abstract:** Agriculture insect pests can cause significant damage to crops and stored products, either directly or indirectly. *Abelmoschus esculentus* (bhindi/bhendi) is an essential nutrient source and economically important vegetable crop grown in the world's tropical and subtropical parts. The major agriculture and bhindi shoot and fruit borer insect pest is *Earias vittella* (Fab.). Chemical pesticides always have adverse environmental effects, but plant products could be used effectively in well-being as an ecologically acceptable pest management system. The *Euphorbia cyathophora* is a medicinal plant used for numerous health-beneficial activities. Hence, the present investigation is focused on evaluating the larvicidal bioefficacy of *E. cyathophora* leaf extract treated against larvae of *E. vittella* under laboratory conditions. In this study we analysed the functional groups present in *E. cyathophora* leaves extract of chloroform, acetone and methanol through FTIR spectroscopy. FTIR analyses revealed that significant functional groups were present in all the leaves extract of *E. cyathophora*. In laboratory conditions, larvicidal toxicity of chloroform, acetone and methanol leaf extracts of *E. cyathophora* were assessed against *E. vittella*. The larvae mortality was observed after 24 h of exposure. However, the highest larvicidal mortality rate was found in methanol extract compared to the acetone and chloroform extract. The mortality rate was directly proportional to the concentration of extracts. The LC<sub>50</sub> values of *E. cyathophora* leaf extracts (chloroform, acetone, and methanol) against I, II, III, and V instar larvae of *E. vittella* were determined. The chloroform leaf extracts of *E. cyathophora* were toxic against larval instars (I, II, III, V) of *E. vittella*; LC<sub>50</sub> values were 303.757 ppm (I instar), 332.212 ppm (II instar), 374.893 ppm (III instar), 475.721 ppm (V instar). Acetone leaf extracts of *E. cyathophora* were highly effective, with LC<sub>50</sub> of 273.990 ppm (I instar), 302.374 ppm (II instar), 340.105 ppm (III instar) and 423.568 ppm (V instar). Methanol leaf extracts of *E. cyathophora* were highly effective, with LC<sub>50</sub> of 232.710 ppm (I instar), 263.909 ppm (II instar), 287.877 (III instar), and 351.255 ppm (V instar), respectively. To our knowledge, this is the first report of the use of *E. cyathophora* to evaluate toxicity against *E. vittella* larvae. Overall, this study suggests that the methanol leaf extract of *E. cyathophora* has the highest larvicidal activity.

**Keywords:** *Euphorbia cyathophora*, FTIR analysis, *Earias vittella*, larvicidal toxicity, LC<sub>50</sub>, *Abelmoschus esculentus*

**Citation:** Kannan Moorthi, Kanagaraju Natesan, Chandramohan Balamurugan and Madhiyazhagan Pari: Larvicidal bioefficacy of *Euphorbia cyathophora* leaf extracts against bhindi fruit borer *Earias vittella*, Fabricius (Lepidoptera: Noctuidae). Intern. J. Zool. Invest. 9(2): 459-470, 2023.

<https://doi.org/10.33745/ijzi.2023.v09i02.052>



## Introduction

*Abelmoschus esculentus* (L) is one of the most popular and widespread vegetables throughout India. Fruits have high nutritional value as they contain minerals, calcium, phosphorus, iron, and vitamins A, B, and C (Singh, 1970). *A. esculentus* is commonly known as okra, bhindi, or lady's finger. A critical analysis of quantitative and qualitative crop losses has shown that the majority of the fruit produced is damaged by some dreaded insect pests. *Earias vittella* Fabricius, 1794 (Lepidoptera: Noctuidae) is the world's most serious, polyphagous, and noxious pest of European corn borer and European corn borer (Gajmer *et al.*, 2002; Syed *et al.*, 2011). According to the season, the affected plants exhibit symptoms of wither, drop, and drying of shoots due to the caterpillars' larvae feeding on vegetative fruiting bolls, shoots, buds, and flowers. These symptoms range from 8.4 to 73.2% infestation on okra fruit (Aziz *et al.*, 2012; Rahman *et al.*, 2013). Pest control is necessary to prevent crop failure and crop damage. The use of chemical pesticides has an adverse impact on human and animal health. The growth of insect species has become more resistant to pesticides due to this practice. Natural enemies of pests and pollinators are two types of beneficial organisms essential for maintaining a healthy environment. However, over time, the indiscriminate use of chemical pesticides has negatively impacted human health, non-target organisms, significant groundwater contamination, and the environment. Biopesticides have served as adequate substitutes for synthetic insecticides in this situation. Because biopesticides are readily biodegradable, they have no adverse effects on the environment or plants (Ignacimuthu, 2004). Rotating pest control strategies are becoming increasingly important with the development of biopesticides. There is an urgent need to develop new, more environmentally friendly, biodegradable, insect-specific

insecticides to control pests and insect vectors. Therefore, the ongoing studies focus on the insecticidal properties of natural products such as plant extracts. Medicinal plants are an essential part of herbal wealth and these plants, which contain beneficial phytochemicals, can also satisfy the human body's cravings by acting as herbal antioxidants. However, plants have been healing human diseases for hundreds of years. It has a large and diverse collection of natural compounds that can induce specific physiological movements in the human body (Nayak and Krishna, 2007; Boots *et al.*, 2008). Many of these benefits point to the likely role of phytochemicals in preventing and treating disease. Today, the daily array of chemicals derived from plant life is used as key pills in several countries around the world (Debnath *et al.*, 2006; Gangola *et al.*, 2017).

The annual herb *Euphorbia cyathophora* is used for numerous healing activities, which include dwarf poinsettia, fire-on-the-mountain and painted leaf; it is local to North and South America and naturalized elsewhere. This plant has been used for wound healing, antimicrobial anticancer activities, and ayurveda remedies. Secondary metabolites from plant are known as phytochemicals that might be taking place and biologically lively compounds that can save you from diseases (De-Fatima *et al.*, 2006; Banso and Adeyemo, 2007). FT-IR is undoubtedly considered one of the broadly used strategies to perceive the chemical components and has been used as a needful technique for drug treatments in the pharmacopeia of many countries (Liu *et al.*, 2006). In this study, the functional groups present in *E. cyathophora* leaf extract from chloroform, acetone and methanol were evaluated using FTIR spectroscopy. In addition, we investigated the larvicidal toxicity of *E. cyathophora* leaf extract against *E. vittella* larvae.

## Materials and Methods

### *Collection and identification of plant material:*

Fresh leaves of *Euphorbia cyathophora* were collected from in and around Namakkal district, Tamil Nadu, India. The plant was taxonomically identified and authenticated by Dr. K. Raju, PG and Research Department of Botany, Kandaswami Kandar's College, Velur-Namakkal Tamil Nadu, India. The voucher (Voucher Number: BSI/KKC/4/11/2017/Tech/212) specimen was retained in our laboratory for further reference.

### *Preparation of plant extract:*

The leaves (250 g) were rinsed under running water and then with distilled water. Thereafter leaves were dried in the shade for 5 to 10 days at  $28 \pm 2^\circ\text{C}$ . Because certain compounds denature when exposed to sunlight, it is dried in the shade to prevent decomposition. The dried leaves were put in a Soxhlet apparatus (Borosil Glass Workers Ltd, Mumbai, India) and extracts were prepared using chloroform, acetone and methanol [(Loba Chemie Pvt. Ltd., Mumbai, India. 99% purity) (concentration of chloroform, acetone and methanol is 100%, extraction period 72 h and the temperature were maintained at  $30\text{-}40^\circ\text{C}$ )]. The yielded extract was dried in a rotary vacuum evaporator to obtain the dried residues, which were then kept in airtight bottles in the refrigerator for future use.

### *Fourier Transform Infrared Spectrophotometer (FTIR) analysis:*

The chloroform, acetone and methanol extracts of *E. cyathophora* leaves were used for FTIR analysis. Translucent sample discs were prepared by encapsulating 10 mg of the dried extract powder within a 100 mg KBr pellet. Each specimen's powdered sample was loaded into a Japanese Shimadzu FTIR spectroscope (IR Affinity 1) with a scan range of  $400$  to  $4000\text{ cm}^{-1}$  and a resolution of  $\text{cm}^{-1}$ . The annotated spectrum shows that the wavelength of light that is absorbed is indicative of the chemical bond. The infrared absorption spectrum can be used to identify chemical bonds in a molecule (Cakmak *et al.*, 2006).

### *Collection and mass culture of test insects:*

The egg mass and larvae of *E.vittella* were collected from the Bhindi fruit field at Namakkal, Tamil Nadu, India. The *E. vittella* was taxonomically identified and authenticated by Dr. K. Murugan, Department of Zoology, Bharathiar University, Coimbatore, Tamil Nadu. The culture of *E. vittella* was maintained under laboratory conditions. Fresh Bhindi was provided daily to the larvae.

### *Larvicidal bioassay:*

The sliced bhindi pods were treated with different concentrations of leaves extract (150, 300, 450, 600 and 750 ppm) of *E. cyathophora*. Control bhindi pods were treated with distilled water alone. The pods were allowed to dry at room temperature for 10 min and placed on a wet filter paper disc in 10 cm diameter plastic petri dishes. The experiments were carried out with newly moulted, 3 h starved I, II, III, and V instar larvae using one larvae per dish in five replicates (30 larvae/concentration). After 4 days, the larvae were transferred to fresh untreated sliced bhindi pods and maintained until they moulted into adults or died. A total number of normal larvae that survived was noted. The larvae were observed for mortality and morphological changes associated with growth-disrupting effects. After corrections, the per cent mortality data were subjected to probit analysis for calculating mean lethal concentration ( $\text{LC}_{50}$ ) (Finney, 1971). Results were corrected for control mortality by using Abbott's (1925) formula:

$$P_t = (P_o - P_c / 100 - P_c) \times 100$$

Where,  $P_t$  = Corrected per cent mortality;  $P_o$  = Observed mortality;  $P_c$  = Observed mortality in the control

From the mean lethal concentration ( $\text{LC}_{50}$ ), the physiological doses were selected for biological, nutritional and biochemical studies.

### *Statistical analysis:*

The average larvae mortality data were subjected to probit analysis for calculating  $\text{LC}_{50}$ ,  $\text{LC}_{90}$  and

Table 1: FTIR spectrum of chloroform leaf extract of *E. cyathophora*

| S. No. | Wave number $\text{cm}^{-1}$ | Stretching      | Functional group  |
|--------|------------------------------|-----------------|-------------------|
| 1.     | 2916.37                      | C-H             | Alkane            |
| 2.     | 2854.65                      | C-H             | Alkane            |
| 3.     | 1712.79                      | C=O             | Carboxylic acid   |
| 4.     | 1627.92                      | C=C             | Alkenes           |
| 5.     | 1550.77                      | NO <sub>2</sub> | Nitro compounds   |
| 6.     | 1450.47                      | C-H bend        | Alkanes           |
| 7.     | 1373.32                      | O-H bending     | Phenol            |
| 8.     | 1165.00                      | S=O             | Sulfonic acid     |
| 9.     | 1033.85                      | S=O             | Sulfoxide         |
| 10.    | 887.26                       | C=C bending     | Alkene            |
| 11.    | 833.25                       | C=C bending     | Alkene            |
| 12.    | 756.10                       | C-Cl stretch    | Alkyl halides     |
| 13.    | 725.23                       | C=C bending     | Alkene            |
| 14.    | 671.23                       | C=C bending     | Alkene            |
| 15.    | 601.79                       | C-Br            | Alkyl halides     |
| 16.    | 547.78                       | C-I             | Halo compound     |
| 17.    | 470.63                       | S-S stretch     | Aryl disulfides   |
| 18.    | 416.62                       | C-halogen       | Halogen compounds |

other statistics at 95 % fiducial limits of upper fiducial limit and lower fiducial limit, and Chi-square values were calculated using the SPSS Statistical software package 16.0 version was used. Results with  $P < 0.05$  were considered to be statistically significant.

## Results

### *FTIR analysis of E. cyathophora leaf extract:*

In the present study, FTIR spectrum analysis was used to identify major peaks associated with functional groups present in the chloroform extract of *E. cyathophora* leaves (Table 1, Fig. 1). The results of FTIR spectrum observed the different peaks at 2916.37  $\text{cm}^{-1}$ , 2854.65  $\text{cm}^{-1}$ , 1712.79  $\text{cm}^{-1}$ , 1627.92  $\text{cm}^{-1}$ , 1550.77  $\text{cm}^{-1}$ , 1450.47  $\text{cm}^{-1}$ , 1373.32  $\text{cm}^{-1}$ , 1165.00  $\text{cm}^{-1}$ , 1033.85  $\text{cm}^{-1}$ , 887.26  $\text{cm}^{-1}$ , 833.25  $\text{cm}^{-1}$ , 756.10  $\text{cm}^{-1}$ , 725.23  $\text{cm}^{-1}$ ,

671.23  $\text{cm}^{-1}$ , 601.79  $\text{cm}^{-1}$ , 547.78  $\text{cm}^{-1}$ , 470.63  $\text{cm}^{-1}$  and 416.62  $\text{cm}^{-1}$  which correspond to the presence of an alkane, alkane, carboxylic acid, alkenes, nitro compounds, alkanes, phenol, sulfonic acid, sulfoxide, alkene, alkene, alkyl halides, alkene, alkene, alkyl halides, halo compound, aryl disulfides and halogen compounds. The FTIR spectrum obtained a total of 16 different functional groups of active components of acetone extract of *E. cyathophora* leaves, (Table 2, Fig. 2). The results indicated intense peak values of 2924.09  $\text{cm}^{-1}$  and 2854.65  $\text{cm}^{-1}$  which are attributed to the C-H stretch. Also, the peaks at 833.25  $\text{cm}^{-1}$ , 725.23  $\text{cm}^{-1}$  and 671.23  $\text{cm}^{-1}$  are assigned to the C=C bending, followed by 478.35  $\text{cm}^{-1}$  and 447.49  $\text{cm}^{-1}$  corresponding to the S-S stretch. Additionally, other functional groups such as esters-saturated aliphatic,  $\alpha$ ,  $\beta$ -unsaturated

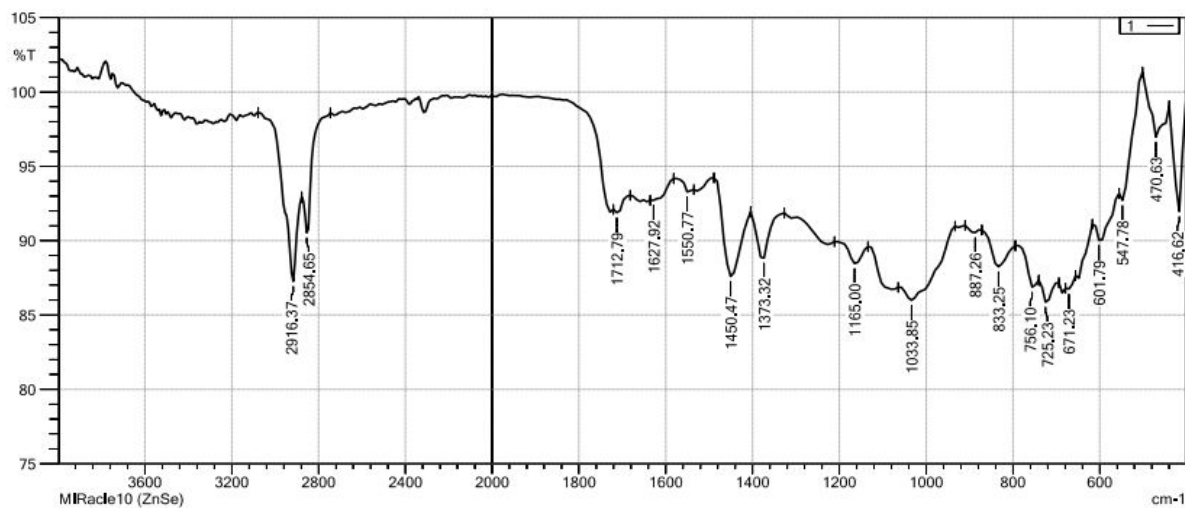


Fig 1: FTIR spectrum compounds of chloroform leaf extract of *E. cyathophora*.

Table 2: FTIR spectrum of acetone leaf extract of *E. cyathophora*

| S. No. | Wave number $\text{cm}^{-1}$ | Stretching  | Functional group                       |
|--------|------------------------------|-------------|----------------------------------------|
| 1.     | 2924.09                      | C-H         | Alkane                                 |
| 2.     | 2854.65                      | C-H         | Alkane                                 |
| 3.     | 1735.93                      | C=O         | Esters, saturated aliphatic            |
| 4.     | 1612.49                      | C=C         | $\alpha$ , $\beta$ -unsaturated ketone |
| 5.     | 1450.47                      | C-H bend    | Alkanes                                |
| 6.     | 1373.32                      | O-H bending | Phenol                                 |
| 7.     | 1157.29                      | C-O         | Tertiary alcohol                       |
| 8.     | 1033.85                      | S=O         | Sulfoxide                              |
| 9.     | 833.25                       | C=C bending | Alkene                                 |
| 10.    | 725.23                       | C=C bending | Alkene                                 |
| 11.    | 671.23                       | C=C bending | Alkene                                 |
| 12.    | 601.79                       | C-Br        | Halo compound                          |
| 13.    | 555.50                       | C-I         | Halo compound                          |
| 14.    | 478.35                       | S-S stretch | Aryl disulfides                        |
| 15.    | 447.49                       | S-S stretch | Aryl disulfides                        |
| 16.    | 408.91                       | C-halogen   | Halogen compounds                      |

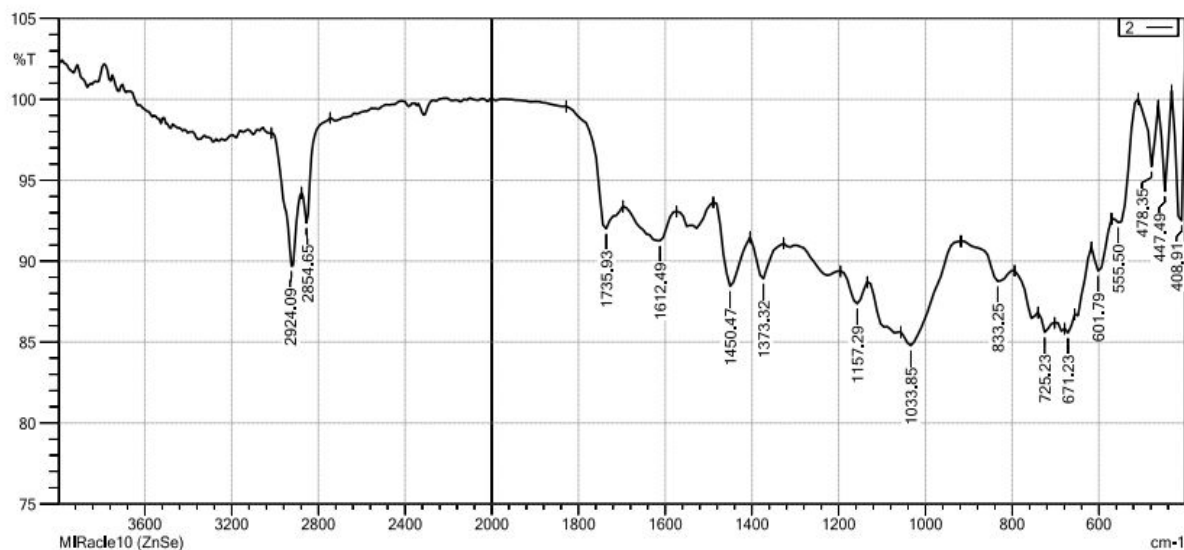


Fig. 2: FTIR spectrum of acetone leaf extract of *E. cyathophora*.

ketone, alkanes, phenol, a tertiary alcohol, sulfoxide, halo compound and halo compound are absorbed at 1735.93  $\text{cm}^{-1}$ , 1612.49  $\text{cm}^{-1}$ , 1450.47  $\text{cm}^{-1}$ , 1373.32  $\text{cm}^{-1}$ , 1157.29  $\text{cm}^{-1}$ , 1033.85  $\text{cm}^{-1}$ , 601.79  $\text{cm}^{-1}$  and 555.50  $\text{cm}^{-1}$ . The characteristic absorption bands of methanol leaves extract of *E. cyathophora* were exhibited at 2916.37  $\text{cm}^{-1}$  and 2854.65  $\text{cm}^{-1}$  for C-H stretching, followed by 493.78  $\text{cm}^{-1}$ , 470.63  $\text{cm}^{-1}$  and 447.49  $\text{cm}^{-1}$  for aryl disulfides, respectively. IR spectrum of methanol extract displayed peaks at 1604.77  $\text{cm}^{-1}$ , 1450.47  $\text{cm}^{-1}$ , 1373.32  $\text{cm}^{-1}$  and 1033.85  $\text{cm}^{-1}$  for C=C, C-H bend, O-H bending and S=O stretch showed the presence of alkenes, alkanes, phenol and sulfoxide. Absorption bands at 756.10  $\text{cm}^{-1}$ , 671.23  $\text{cm}^{-1}$ , 601.79  $\text{cm}^{-1}$  and 570.93  $\text{cm}^{-1}$  and 424.34  $\text{cm}^{-1}$  indicate the presence of Alkyl halides, Alkene, Halo compound, Halo compound and Halogen compounds (Table 3, Fig. 3). Among the leaves extract of *E. cyathophora*, the chloroform leaves extract of *E. cyathophora* possesses more functional groups when compared to acetone and methanol leaves extract and those bio-active compounds are of pharmaceutical importance.

#### *Larvicidal toxicity of E. cyathophora leaf extract:*

The results of the larvicidal activity of chloroform,

acetone, and methanol leaf extract of *E. cyathophora* against larvae of *E. vittella* are presented in Tables 4-6. Among the different larval instars of *E. vittella* examined, the highest larval activity was observed in the early developmental stages of I instar larvae, then in II, III and V instar. No mortality was observed in the control. All plant extracts showed significant toxicity to *E. vittella* after 24-h exposure to the highest concentration of 750 ppm. However, the highest mortality was observed in the methanol extract of *E. cyathophora* leaves compared to acetone and chloroform. The rate of mortality was directly proportional to the concentration of the extracts. The lethal effect of *E. cyathophora* leaf extract on the *E. vittella* larvae may be due to the presence of bioactive compounds that enter into the larvae organism, delay normal physiological processes, and inhibit the activity of metabolic processes. In our laboratory assays, larvicidal toxicity of chloroform leaf extracts of *E. cyathophora* showed a considerable mortality rate against I, II, III, and V instar larvae of *E. vittella*.  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values of chloroform leaf extracts against *E. vittella* were 303.757 and 754.001 ppm (I instar), 332.212 and 830.366 ppm (II instar), 374.893 and 890.917 ppm (III instar) and 475.721

Table 3: FTIR spectrum of methanol leaf extract of *E. cyathophora*

| S. No. | Wave number $\text{cm}^{-1}$ | Stretching   | Functional group  |
|--------|------------------------------|--------------|-------------------|
| 1.     | 2916.37                      | C-H          | Alkane            |
| 2.     | 2854.65                      | C-H          | Alkane            |
| 3.     | 1604.77                      | C=C          | Alkenes           |
| 4.     | 1450.47                      | C-H bend     | Alkanes           |
| 5.     | 1373.32                      | O-H bending  | Phenol            |
| 6.     | 1033.85                      | S=O          | Sulfoxide         |
| 7.     | 756.10                       | C-Cl stretch | Alkyl halides     |
| 8.     | 671.23                       | C=C bending  | Alkene            |
| 9.     | 601.79                       | C-Br         | Halo compound     |
| 10.    | 570.93                       | C-I          | Halo compound     |
| 11.    | 493.78                       | S-S stretch  | Aryl disulfides   |
| 12.    | 470.63                       | S-S stretch  | Aryl disulfides   |
| 13.    | 447.49                       | S-S stretch  | Aryl disulfides   |
| 14.    | 424.34                       | C-halogen    | Halogen compounds |

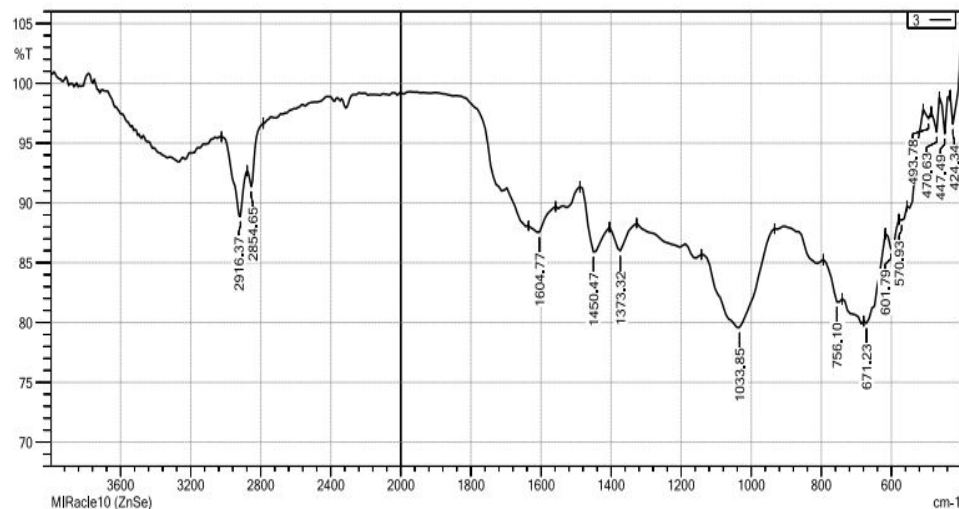


Fig. 3: FTIR spectrum of methanol leaf extract of *E. cyathophora*.

and 977.639 ppm (V instar), respectively (Table 4). Acetone leaf extract (Table 5) of *E. cyathophora* was moderately toxic against larvae of *E. vittella*.  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values were 273.990 and 696.540 ppm (I instar), (II), 302.374 and 756.758 ppm (II instar), 340.105 and 836.527 ppm (III instar) and 423.568 and 916.218 ppm (V instar), respectively.

In laboratory experiments, *E. cyathophora* methanol leaf extract was highly toxic against larvae of *E. vittella*.  $\text{LC}_{50}$  and  $\text{LC}_{90}$  were 232.710 and 572.147 ppm (I instar), 263.909 and 666.641 ppm (II instar), 287.877 and 693.432 ppm (III instar) and 351.255 and 793.995 (V instar), respectively (Tables 6).

Table 4: Larvicidal toxicity of chloroform leaf extract of *E.cyathophora* against *E. vittella*

| Target | Larval and pupal mortality (%) |                |                |                |                | LC <sub>50</sub><br>(LC <sub>90</sub> ) | 95% confidence Limit                 |                       | Regression equation   | $\chi^2$ |
|--------|--------------------------------|----------------|----------------|----------------|----------------|-----------------------------------------|--------------------------------------|-----------------------|-----------------------|----------|
|        | Concentration (ppm)            |                |                |                |                |                                         | LC <sub>50</sub> (LC <sub>90</sub> ) |                       |                       |          |
|        | 150                            | 300            | 450            | 600            | 750            |                                         | LCL                                  | UCL                   |                       |          |
| I      | 36.12±<br>2.27                 | 48.37±<br>2.12 | 62.20±<br>1.18 | 79.32±<br>1.32 | 92.19±<br>2.28 | 303.757<br>(754.001)                    | 249.279<br>(682.752)                 | 348.052<br>(857.458)  | Y=-0.865<br>+0.003 x  | 1.825*   |
| II     | 34.72±<br>2.08                 | 46.22±<br>2.22 | 57.58±<br>1.58 | 74.36±<br>2.16 | 88.63±<br>1.17 | 332.212<br>(830.366)                    | 275.601<br>(744.799)                 | 378.963<br>(959.546)  | y=-0.855<br>+0.003x   | 1.842*   |
| III    | 31.18±<br>2.16                 | 43.17±<br>1.19 | 52.64±<br>1.02 | 69.36±<br>2.16 | 85.72±<br>1.20 | 374.893<br>(890.917)                    | 321.552<br>(795.635)                 | 421.680<br>(1037.042) | y= -0.931<br>+ 0.002x | 2.123*   |
| V      | 22.35±<br>2.07                 | 32.62±<br>1.92 | 43.18±<br>1.30 | 62.0±<br>1.58  | 78.12±<br>2.02 | 475.721<br>(977.639)                    | 430.066<br>(873.847)                 | 523.661<br>(1135.863) | y=-1.215<br>+0.003x   | 1.272*   |

The larval mortalities are expressed as mean±SD of five replicates. Nil mortality was observed in the control. LFL - Lower Fiducidal Limit; UFL - Upper Fiducidal Limit.  $\chi^2$ , Chi-square value. \*Significant at P< 0.05 level.

Table 5: Larvicidal toxicity of acetone leaf extract of *E.cyathophora* against *E.vittella*

| Target | Larval and pupal mortality (%) |                |                |                |                | LC <sub>50</sub><br>(LC <sub>90</sub> ) | 95% confidence Limit                 |                       | Regression equation  | $\chi^2$ |
|--------|--------------------------------|----------------|----------------|----------------|----------------|-----------------------------------------|--------------------------------------|-----------------------|----------------------|----------|
|        | Concentration (ppm)            |                |                |                |                |                                         | LC <sub>50</sub> (LC <sub>90</sub> ) |                       |                      |          |
|        | 150                            | 300            | 450            | 600            | 750            |                                         | LCL                                  | UCL                   |                      |          |
| I      | 39.62±<br>1.04                 | 51.40±<br>2.07 | 64.38±<br>1.21 | 84.0±<br>1.48  | 95.22±<br>1.93 | 273.990<br>(696.540)                    | 218.966<br>(633.206)                 | 317.805<br>(786.701)  | Y=-0.831<br>+ 0.003x | 3.647*   |
| II     | 37.0±<br>1.18                  | 48.14±<br>1.93 | 61.62±<br>1.06 | 79.16±<br>2.18 | 92.42±<br>1.98 | 302.374<br>(756.758)                    | 247.183<br>(684.619)                 | 347.087<br>(861.898)  | y=-0.853<br>+0.003x  | 2.467*   |
| III    | 34.28±<br>1.92                 | 45.52±<br>2.18 | 55.22±<br>1.94 | 75.16±<br>2.58 | 88.0±<br>2.07  | 340.105<br>(836.527)                    | 284.815<br>(750.569)                 | 386.350<br>(966.102)  | y=-0.878<br>+0.003x  | 2.450*   |
| V      | 26.19±<br>2.48                 | 36.58±<br>2.16 | 49.21±<br>1.40 | 67.17±<br>1.20 | 82.37±<br>1.18 | 423.568<br>(916.218)                    | 376.663<br>(821.971)                 | 468.515<br>(1058.181) | y=- 1.102<br>+0.004x | 1.140*   |

The larval mortalities are expressed as mean±SD of five replicates. Nil mortality was observed in the control. LFL - Lower Fiducidal Limit; UFL - Upper Fiducidal Limit.  $\chi^2$ , Chi-square value. \*Significant at P< 0.05 level.

Table 6: Larvicidal toxicity of methanol leaf extract of *E.cyathophora* against *E.vittella*

| Target | Larval and pupal mortality (%) |                |                |                |                | LC <sub>50</sub><br>(LC <sub>90</sub> ) | 95% confidence Limit                 |                      | Regression equation  | $\chi^2$ |
|--------|--------------------------------|----------------|----------------|----------------|----------------|-----------------------------------------|--------------------------------------|----------------------|----------------------|----------|
|        | Concentration (ppm)            |                |                |                |                |                                         | LC <sub>50</sub> (LC <sub>90</sub> ) |                      |                      |          |
|        | 150                            | 300            | 450            | 600            | 750            |                                         | LCL                                  | UCL                  |                      |          |
| I      | 41.27±<br>2.02                 | 55.23±<br>1.78 | 71.45±<br>1.06 | 89.72±<br>1.65 | 100±00         | 232.710<br>(572.147)                    | 183.418<br>(524.576)                 | 271.831<br>(636.588) | Y=-0.004<br>+0.879 x | 4.892*   |
| II     | 39.67±<br>2.18                 | 53.0±<br>2.34  | 67.43±<br>2.40 | 85.24±<br>1.26 | 96.48±<br>1.78 | 263.909<br>(666.641)                    | 210.134<br>(607.738)                 | 306.684<br>(749.301) | y=-0.840<br>+0.003x  | 3.106*   |
| III    | 37.55±<br>2.38                 | 49.0±<br>1.30  | 64.29±<br>1.02 | 84.24±<br>1.30 | 95.12±<br>2.08 | 287.877<br>(693.432)                    | 237.243<br>(632.566)                 | 329.233<br>(778.904) | y=-0.910<br>+0.003x  | 3.275*   |
| V      | 30.72±<br>1.02                 | 42.86±<br>2.38 | 56.47±<br>2.12 | 78.35±<br>1.32 | 88.58±<br>1.54 | 351.255<br>(793.995)                    | 303.471<br>(720.598)                 | 392.747<br>(899.813) | y=-1.017<br>+0.003x  | 1.687*   |

The larval mortalities are expressed as mean±SD of five replicates. Nil mortality was observed in the control. LFL - Lower Fiducial Limit; UFL - Upper Fiducial Limit.  $\chi^2$ , Chi-square value. \*Significant at P< 0.05 level.

## Discussion

The present study compared to numerous earlier research findings has shown that various plants belong to different functional groups. FTIR is a technique that uses infrared radiation beams to identify the functional groups in gaseous, liquid, and solid materials (Khan *et al.*, 2018). Fourier transform infrared spectroscopy was used by Tuapattinaya *et al.* (2019) to analyse a methanol extract of *E. acoroides* leaves from the coastal waters of Ambon, Indonesia, and discovered the presence of flavonoids. Hemmalakshmi *et al.* (2017) studied that the *Erythrina variegata* leaves, flowers, and bark contain a variety of functional groups, including aliphatic, alkynes, alcohols, amides, aromatics, carboxylic acids, hydroxy group, ketones, metal carbonyl, nitrile, and phenols. Kavipriya and Chandran (2018) reported that FTIR spectrum confirmed the presence of alkanes, alkenes, alkynes, aldehyde, alcohol, alkyl halides, amide, aromatic, acetylene, ester, nitrile, phosphine, isocyanates, ketenes, sulfonyl chlorides, sulfates, sulfones, sulphonamides in *Cassia alata*. Nandagopalan and Kavitha (2021) found functional compounds such as alcohol, aldehyde, alkane, alkenes, alkyl halide, alkene,

aromatic compound, cyclic alkene, carboxylic acid, and isothiocyanate. In addition, Khatri *et al.* (2017) discovered alcohol, alkanes, and phenols in *E. cardamomum* leaves. Rajiv *et al.* (2017) also noted that different functional groups, including carboxylic acids, aromatics, alkanes, alcohols, phenols, aliphatic amines, alkenes, and amine groups, are present in the fruit extracts. Sithara *et al.* (2017) assessed the FTIR analysis of *A. paniculata* by using various solvent extracts, including chloroform, ethyl acetate, ethanol, and methanolic, revealing different compound compositions. Dhivya and Kalaichelvi (2017) showed that the FT-IR spectrum verified the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, aromatics, nitro compounds, and amines.

Chemical pesticide use causes resistance to insects, re-emergence of small pests, impact on human, animal and other organism health, destruction of natural enemies, and environmental pollution. Biopesticides could be a viable alternative to chemical insecticides as they are relatively safe, cheap and available worldwide (Murugan *et al.*, 2012). Plants defend themselves against insects by producing secondary

metabolites. Phytochemicals are produced by extracting different plant materials with various organic solvents in different proportions. These secondary plant metabolites exhibit a variety of biological activities that help protect plants from insects (Sharma and Bhist, 2008). Plant extracts alter the protein content and midgut histology of *Spodoptera litura* and the protein content of *Helicoverpa armigera* (Baskar *et al.*, 2015). Natural preparations have shown genotoxicity against *Helicoverpa armigera* (Packiam *et al.*, 2015). Also, Pavunraj *et al.* (2017) noted that *Catharanthus roseus* has antifeedant effects on *Earias vittella*. The present study in continuation to our previous report Kannan *et al.* (2023) demonstrated the toxic effects of *E. cyathophora* leaf extracts on larvae and pupae of *E. vittella*, the highest larval and pupal mortality rates in the methanol extract was recorded compared to any other extract. Recently, Villavan *et al.* (2022) showed significant mortality against *Earias vittella* by *M. esculenta*'s larvicidal activity. *M. esculenta* methanolic seed extract had LC<sub>50</sub> (LC<sub>90</sub>) values of 1.294% (5.268%), 1.398% (5.539%), 1.546% (5.827%), 1.719% (6.164%), and 1.948% (6.548%) for I, II, III, IV, and V instar larvae of *E. vittella*, respectively. The present results agreed with those of Muthu *et al.* (2010), who found significant mortality (85.33%) of *A. monophylla* larvae after hexane extract exposure, followed by ethyl acetate extract (78.67%), and chloroform extract (69.33%), all at 5% concentration. Reena and Singh (2007) studied the efficacy of methanolic extracts of Karanj (*Pongamia pinnata*) seeds against *E. vittella* (Fabricius). Jeyasankar *et al.* (2012)<sup>1</sup> examined that *Solanum pseudocapsicum* may have insecticidal, antifeedant, and growth-inhibiting effects on the field pests *Helicoverpa armigera* and *Spodoptera litura*. Bandeira *et al.* (2013) found that ethanol flowers and fruits extracts of *Muntingia calabura* were the most toxic against first instar *P. xylostella* larvae with LC<sub>50</sub> values of 0.61 µg ml<sup>-1</sup> and 1.63 µg ml<sup>-1</sup> respectively, followed by the hexane extract of the fruits (LC<sub>50</sub>= 5.5 µg ml<sup>-1</sup>) and flowers (LC<sub>50</sub>=18.9 µg ml<sup>-1</sup>).

Selvaraj *et al.* (2005) reported that *Pteridium aquilinum*'s a- and b-ecdysone exhibited effective insecticidal activity against *H. armigera* and *S. litura*. Furthermore, Kumari *et al.* (2003) isolated six neo-clerodane diterpenoids from *Clerodendrum* species and found that all the compounds increased the duration of *E. vittella* larval and pupal stages compared to control. The most promising substance was cleroderdrin B, which decreased adult emergence while extending the duration of the larval and pupal stages.

## Conclusion

In this study the FTIR analysis of various leaf extracts of *E. cyathophora* confirmed presence of potential functional groups in all of the extracts with critical medicinal properties and can be used for medical importance. They also exhibited the significant larvicidal activity of all the leaf solvent extracts of *E. cyathophora*. However, since methanol leaf extracts showed high toxicity to *E. vittella*, therefore we selected these extracts for further investigation. The present study concluded that the methanol leaf extract of *E. cyathophora* represents potential sources for the development of botanical formulations to control the agricultural pest *E. vittella*, which can be considered as alternative biological management techniques that are suitable, inexpensive, and safe for the environment and eco-friendly approaches.

## Acknowledgements

The authors would like to acknowledge authorities at the PG and Research Department of Zoology, Kandaswami Kandar's College, Namakkal, for providing the required research facilities.

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