International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 20-05-2024, Accepted: 10-06-2024, Published: 01-07-2024
Volume 9, Issue 7, 2024, Page No. 1-8

Chemical profiling and assessment of egg hatchability with different solvent extracts of selected invasive weeds

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Abstract
A revolutionary change is utmost important to overcome the problems created due to overuse of chemical pesticides. Phyto-pesticides will be economically available at an affordable price, environmentally safe and reduce the cost of synthetic chemicals. Plant families such as Asteraceae, Lamiaceae, Meliaceae, Rutaceae and Solanaceae are widely known to possess various types of larvicidal, adulticidal, repellent, oviposition deterrent and ovicidal/egg hatchability activities against different species of mosquitoes studied till today. Weeds are nowadays preferable plants for research because of their easy availability and undesirability. Botanical pesticides can be in the form of essential oils, plant extracts or secondary metabolites like alkaloids, polyphenols, steroids, terpenes or fatty acids. Alkaloids along with tannins, steroids, glycosides, triterpenoids and saponins present in plants possess insecticidal properties. β-sitosterol in plants, animals, microbes are well known to possess insecticidal/ toxic larvicidal property. These compounds also possess adulticide, repellent and oviposition-deterrent property. Studies conducted have revealed that these compounds affect the autonomous nervous system, constrict the blood vessels at very low concentrations and are reported to be toxic to larvae of *Aedes aegypti, Culex* and *Anopheles* mosquito species. Ovicidal effects of phytochemicals have not been studied to greater extent hence undertaken for study. The present investigations were carried out to assess the ovicidal/ egg hatchability efficiency of three weeds *Euphorbia heterophylla* L (Euphorbiaceae), *Cosmos sulphureus* Cav (Asteraceae) and *Alternanthera tenella* Colla (Amaranthaceae) against *Aedes aegypti* L (Diptera: Culicidae). In present study petroleum ether, chloroform, ethyl acetate and methanolic extracts of all 3 weeds exhibited variable ovicidal effects. Zero hatchability was observed at 1000 ppm with ethyl acetate extracts of *Cosmos* and *Euphorbia*. Chloroform and ethyl acetate extracts of *Alternanthera* showed similar results at 1000 ppm concentration whereas petroleum ether extracts showed significant results at lower concentration (400 ppm).

Keywords: *Alternanthera tenella*, biochemical analytical tests, *cosmos sulphureus*, *euphorbia heterophylla*, GC-MS analysis, FTIR spectroscopy, total egg hatchability

Introduction

Weeds are nowadays preferable plants for research because of their easy availability, cultivability, undesirability, utmost need of management and a reason for eradication. Members of the plant families Asteraceae, Labiatae/ Lamiaceae, Meliaceae, Rutaceae and Solanaceae with few other families like Apiaceae, Myrtaceae, Piperaceae, Verbenaceae, Zingiberaceae and Poaceae that are studied by other researchers possess various types of larval, adulticidal or repellent activities against different species of mosquitoes. A drastic change is necessary to overcome problems of overuse of synthetic chemical compounds in controlling mosquitoes. Considering a holistic approach, development of an environmentally safe, eco-friendly botanical pesticide is not only of utmost importance but also a need of today's research. The importance of use of phyto-pesticides as an alternative solution for chemical pesticides has been emphasized by the authors in their present study. Botanical insecticides can be in the form of essential oils, plant extracts or secondary metabolites (Maira *et al*., 2020). Secondary metabolites produced by plants acts as antifeedants, moulting hormones, oviposition deterrents, repellents, growth hormones which are involved in activity of target species. Ghosh *et al.* (2012) [10] mentions about 1% of world's pesticide market is product prepared from phytochemicals.

Indian Medicinal plants-based formulations have been used in traditional practices effective against pests for generations with promising results from the observations on plant-insect interactions. Based on these observations it can be concluded that plants possess repellents, deterrents, fecundity (fertile), suppressants, ovicidal and insecticidal properties (Dua, 2009; Sogan *et al*., 2020) [5]. According to Bowers (1992) [3] plant-based formulations are pest specific, eco-friendly and bio-degradable.

There is meagre research carried out on ovicidal effects of phytochemicals (Shaalan *et al*., 2005) [32]. The present investigations were carried out to assess the ovicidal/ egg hatchability efficiency of three weeds *Euphorbia heterophylla* L (Euphorbiaceae), *Cosmos sulphureus* Cav (Asteraceae) and *Alternanthera tenella* Colla (Amaranthaceae) against *Aedes aegypti* L (Diptera: Culicidae).

Alternanthera tenella Colla (syn *A. ficoidea* (L.) P Beau is a pioneer species (Family: Amaranthaceae) growing on disturbed areas commonly found along roadsides and on wetland habitats, wastelands, agricultural fields, preferring shady, wet, warmer and swampy areas with bright sunlight. It is a perennial prostate herb native to tropical and sub-tropical regions of South America, Australia and Brazil. It is used in traditional folk medicines and consumed as vegetable by local tribal communities. *Amaranthaceae* family is characterised by its diversity in secondary metabolites: essential oils, sesquiterpenes, diterpenes, triterpenes, phenolic acids, flavonoids and betalains (Miguel, 2018) [22]. The genera are known to

possess many medicinal properties like ant-oxidant, anti-inflammatory, anti-malarial (Rodrigues *et al*., 2014; Rathinam *et al*., 2017) [29, 28]. *A. tenella* aqueous extracts were evaluated for allelopathic effects on germination of agricultural crops by several researchers (Dhole *et al*., 2011; Gharpure *et al*., 2023a; Gharpure *et al*., 2023 b) [4, 8, 9]. Previous studies have revealed the presence of biologically important compounds as gallic acid, flavonoids fractions such as formononetin, myricetin, quercetin, kaemferol, acacetin, vitexin, 8-C- [α-L rhamnopyranosyl-(1-2)- β-D-glucopyranoside-], 2”-O-α-L-rhamnopyranosyl-vitexin from ethanolic extracts of *A. tenella* (Salvador *et al*., 2006; Ramani and Poonguzhali, 2015) [30, 27].

Cosmos sulphureus Cav an ornamental native from Mexico now widely distributed in Central and South America up to northern Argentina, Carribbean islands, Thailand with vernacular name as Mirasol -Amarillo in Mexico. *C. sulphureus* is used as a traditional medicine against *Plasmodium vivax*, a malarial parasite. It is a potential herbicide due to presence of sesquiterpene from leaves and it also possesses larvicidal activity against *Aedes aegypti* larvae (Bansod *et al*., 2024) [2].

Euphorbia heterophylla L (syn *E. geniculata* Ort) with vernacular name as Dudhani in Maharashtra state has been originated from South America and now present in all tropical regions with worldwide distributions in Fiji, Philippines, Indonesia, California, Africa and Thailand. It was first reported in Poona, India in 1948. It is used in ethno-medicines as anti-malarial and as insecticide.

Aedes aegypti L. (Dipteria: Culicidae) is mainly originated from Ethiopian regions of Africa and is now widely distributed in tropical and sub-tropical country like India. It is a fresh water breeding mosquito which mainly breeds by onset of monsoon at fresh water puddles, ponds, lakes and proliferates rapidly under given favorable conditions. It is a tropical temperature tolerant species ranging from 14℃-30℃with low temperature as the major limiting factor. *Aedes aegypti* is well known for vector borne diseases such as Dengue, Chikungunya, Zika virus that affect human health. The mosquito species has been selected for study as it can be easily colonized in laboratory as it is a fresh water breeding mosquito, less susceptible to insecticides, robust than other mosquito species as *Culex* and *Anopheles* (Raveen *et al*., 2017). Refer fig 1.

Fig 1: *Aedes aegypti* L

Materials and Methods

1. Identification and Authentication of plant sample

Alternanthera tenella Colla (Auth no: 19-167) was identified and authenticated from Department of Botany, Agharkar Research Institute, Pune. It was collected at its vegetative and reproductive stages from Paud- Pirangut area, Tal: Mulshi (18º 30’42.30” N, 73º 40’49.28” E). Plant samples were collected during the month of Aug-Sept.

Cosmos sulphureus (Auth no: Bot/ BGC-05/AUTH/2021-22) has also invaded the area of about 30 kms from Bhugaon upto Mulshi covering the whole area along road sides, along with *Alternanthera tenella* (syn *A. ficoidea*) which is another dominant species occupying the fallow lands of rice crop. *C. sulphureus* was collected post-monsoon at its flowering stage in the month of Oct-Nov from Dehu Road (18º 67’4.024” N, 73º 76’3.434” E) cantonment area.

Euphorbia heterophylla L. (Auth no: 23-48) was collected from Dive Ghat area (18° 29' 47.292"N, 73° 56' 36.564" E). Anthropogenic activities like road side constructions, wild fires, wastelands, puddles due to accumulation of rain water in monsoon and bright sunlight are the most suitable factors for gregarious growth of these weeds. *A. tenella* (composite) whole plant and leaves of *C. sulphureus* and *E. heterophylla* were pulverised and stored in airtight containers at room temperature till further use.

2. Solvent Extraction method

Accurately weighed (1kg) dried powder of each weed was soaked in 2.5 liters of organic solvents in sequential order with increasing polarity (Pet ether > chloroform > ethyl acetate > methanol) (AR) for 72 hours at room temperature. Bio-guided cold percolation method was used for extraction to obtain solvent extracts. The solutions were filtered through muslin cloth and original stock solutions were subjected to Rotary evaporator for complete evaporation of solvents. Thick sticky greenish extracts were obtained from each solvent and stored at 4℃ till further analyses.

3. Ovicidal Bioassays

Ovicidal bioassays were conducted as per Govindarajan *et al*., (2011) [13]. Egg rafts were procured from insectary Ross Life sciences, Bhosari, Pune. Dimethyl sulphoxide (DMSO) was added to 1mg extract and a homogeneous solution was prepared in aqueous medium. 25 eggs were exposed to concentrations 100-1000 ppm for 48 and 72 hours. Total number of hatched eggs were observed under stereo-microscope after 48 hours (Fig 2). Experiment was arranged in triplicates along with control and treated control and mean of cumulative 6 data sets were calculated.

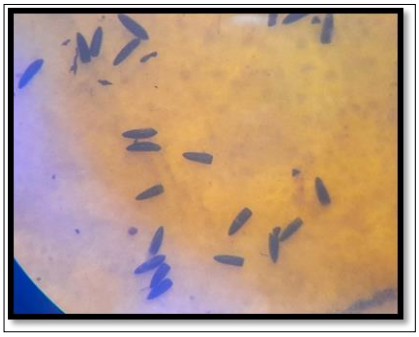


Fig 2: Hatched or unhatched eggs under field

4. Fourier-transform Infra-red spectroscopy
Approximately 1mgs of solvent extracts were analysed on Bunker analytical instrument. The peaks corresponding with the functional groups were further interpreted with the available database.

5. GC-MS analyses
Gas chromatography-Mass spectroscopy (GC-MS) analysis was performed to evaluate and quantify the volatile compounds with other details as retention time, area %, height of eluted compound for further comparison of indices with those of stored data of National Institute Standard and Technology (NIST 4) and Wiley 9 (Vandendool and Kratz 1963) online library.

6. Statistical analyses
Data were analysed by software Microsoft Excel for calculating standard deviation with mean and Standard error.

Results
The ovicidal effects of phytochemicals is least studied (Shaalan *et al*., 2005) [32]. There is paucity of no records of ovicidal or egg hatchability of three weeds *Euphorbia heterophylla* L (Euphorbiaceae), *Cosmos sulphureus* Cav (Asteraceae) and *Alternanthera tenella* Colla (Amaranthaceae). The present investigations hence were undertaken against *Aedes aegypti* L (Diptera: Culicidae) to study ovicidal effects of these phytochemicals.

1. Ovicidal/ Egg hatchability
During our research petroleum ether, chloroform, ethyl acetate and methanol extracts of composite plant extracts of *A. tenella* and leaves of *C. sulphureus* and *E. heterophylla* exhibited variable ovicidal effects. The egg rafts were directly exposed to different concentrations *viz*: 100 ppm-1000 ppm and egg hatchability was recorded after 48 hrs and 72 hrs or sometimes even after 96 hrs due to seasonal variations in temperature, light and moisture. The hatched eggs were observed under stereo-microscope.
Ethyl acetate fractions of *Cosmos* and *Euphorbia* showed no hatchability at 1000 ppm. Chloroform and ethyl acetate extracts of *Alternanthera* resulted similar 100% egg mortality at 1000 ppm concentration whereas petroleum ether extracts showed significant results at lower concentration (400 ppm). No mortality was observed in untreated and treated control. The results are mentioned as averages of total egg hatchability in each concentration (ppm) that are summarised in Table 1. The insecticidal effects of plant extracts depend upon many factors such as plant species, its geographical location, mosquito species, plant parts used for extraction and different solvents. Plant extracts produce different results with change in solvent polarity during fractionation. The chemical components of plant crude extracts thus play role in egg hatchability (Tehri *et al*., 2014) [38]. It can be thus concluded that different responses are due to polarity of solvents which play an important role in extraction of compounds responsible for the toxicity to the test species.

Table 1: Comparative analysis of solvent extracts on egg hatchability of *Aedes aegypti*

Plant sample	Solvent	Egg hatchability in different Concentration (ppm)					
		Control	200	400	600	800	1000
	Pet ether	25 ± 0	6 ± 2.6	3 ± 1.6	2 ± 0.8	1 ± 0.7	1 ± 0.8
Cosmos sulphureus	CHCl3	25 ± 0	11 ± 3.2	8 ± 2.4	4 ± 1.9	6 ± 2.5	6 ± 3.8
	Ethyl acetate	25 ± 0	7 ± 2.3	9 ± 2.9	4 ± 1.2	1 ± 0.6	0±0.1
	Methanol	25 ± 0	13 ± 3.9	11 ± 1.5	11 ± 3.1	16 ± 3.5	8 ± 3.2
	Pet ether	25 ± 0	5 ± 0.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Alternanthera tenella	CHCl3	25 ± 0	4 ± 3.1	7 ± 3.8	8 ± 4.1	8 ± 3.9	0 ± 0
	Ethyl acetate	25 ± 0	12 ± 3.5	7±3.6	4 ± 1.6	2 ± 0.8	0 ± 0
	Methanol	25 ± 0	14 ± 3.2	14 ± 2.4	11 ± 3.1	12 ± 2.7	10 ± 3.1
	Pet ether	25 ± 0	4 ± 2.3	3 ± 2.4	2 ± 2	2 ± 1.5	1 ± 0.5
Euphorbia heterophylla	CHCl3	25 ± 0	14 ± 4.5	9 ± 3.1	7 ± 2.3	7 ± 2.4	1 ± 0.6
	Ethyl acetate	25 ± 0	3 ± 0.4	3 ± 0.4	1 ± 0.4	1 ± 0.3	0 ± 0.2
	Methanol	25 ± 0	15 ± 3.6	14 ± 3.3	14 ± 2.9	17 ± 3.3	12 ± 4.4

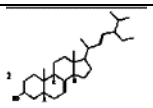
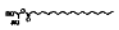
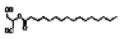
Values are mean ± Standard error (n =6), NH= No hatchability

2. FTIR spectroscopy results
In present investigation the obtained results of zero hatchability or low to moderate response could be corelated to the compounds present in the plants. Therefore, to derive the probable co-relation between percentage of egg hatchability with the compounds present in the plants, FTIR and gas chromatography analytical tests were further conducted.
The prominent peaks in *A. tenella* are observed at 3340.29 cm^{-1} due to –OH stretching, Peak observed at 2918.37 $^{cm-1}$ & 2850.17 is due to -CHO to carbonyl and C=C bond. 1735.62 &1633.70^{cm-1} peaks are due to C=O stretching vibrations. 1459.82^{cm-1}and 1407.78 $^{cm-1}$ represent aromatic region. Presence of ether compounds is by 1080^{cm-1} peak.779.08 $^{cm-1}$ and 719.93 $^{cm-1}$ are due to aromatic C-H peaks.
The IR spectroscopy results of *C. sulphureus* are 3397.1^{cmcm-1} peak represents O-H stretching vibrations.2919.15^{cm-1} and 2850.99 are stretching vibrations due to -CHO group or C=C or C-H bonds.1708.28^{cm-1} peak is due to C=O group.1622.70^{cm-1} and 1458.68^{cm-1}`peaks may be contributing due to $=CH_2$ or aromatic region.1377.30^{cm-1} is also may be due to aromatic region. 1243.28^{cm-1} peaks due to stretching vibrations related to alcohols, phenols, esters. 1030.54^{cm-1} represents the presence of ether (C-O-C) linkage. Peaks at .819.94^{cm-1} and 722.51 $^{cm-1}$ shows the presence of aromatic C-H bonds.
E. heterophylla infra-red spectroscopy results shows 3394.75 ^{cm} $^{cm-1}$ peak represents O-H stretching vibrations.2917.53^{cm-1} and 2849.71 are stretching vibrations due to -CHO group or C=C or C-H bonds.1731.89 $^{cm-1}$ peak is due to C=O group.1646.71^{cm-1} and 1460.34^{cm-1}`peaks may be contributing due to $=CH_2$ or aromatic region.1375 $^{cm-1}$ is also may be due to aromatic region.1026.03 $^{cm-1}$ represents the presence of Ether (C-O-C) linkage Peaks at .824.36^{cm-1} and 722.06 $^{cm-1}$ shows the presence of aromatic C-H bonds.

3. GC-MS analyses

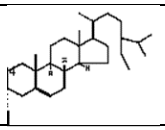
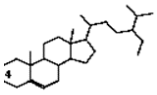
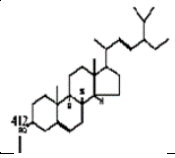
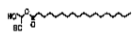
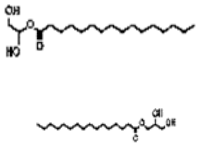
GC-MS analyses of *Alternanthera tenella* revealed the presence of 3 major compounds which are as Chondrilasterol, Octadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester and hexadecenoic acid 2-(hydroxy-1-hydroxy methyl) ethyl ester as summarized in Table 2.

Table 2: Chromatogram of GC-MS of methanolic extract of *Alternanthera tenella*

Sr No	Retention Time	Area %	IUPAC name	Molecular Formula	Structure	Nature of compound	Biological activity
1	19.364	9.32	Chondrilasterol	$C_{29}H_{48}O$		Stigmastanes or sterol lipids	Anti-bacterial (Soodabeh *et al*., 2014)
2	13.842	12.86	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{21}H_{42}O_4$		Stearic acid	Toxic (Kumari, 2023)
3	11.889	15.90	Hexadecanoic acid 2-(hydroxy-1-hydroxy methyl) ethyl ester	C19H38O		Monoacylglycerol	Nematicide, Insecticide, Anti-microbial, Toxic (Kumari, 2023

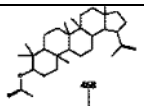
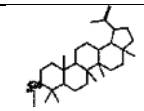
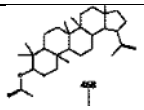
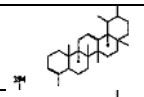
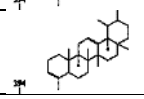
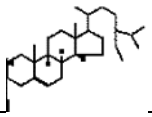
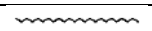
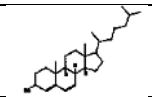
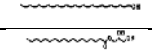
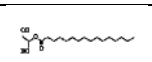
GC-MS analyses of methanolic leaf extract of *C. sulphureus* in our present study revealed the presence of 5 major compounds as γ-sitosterol, stigmasterol, Octadecanoic acid 2-hydroxy-1-hydroxy methyl ethyl ester, hexadecenoic acid hydroxyl-1-(hydroxy methyl) ethyl ester or Glycerol 1-palmiate as presented in Table 3.

Table 3: Chromatogram of GC-MS of methanolic leaf extract of *Cosmos sulphurous*

Sr No	Retention Time	Area %	IUPAC name	Molecular Formula	Structure	Nature of compound	Biological activity
1	19.384	3.23	gamma-sitosterol	$C_{29}H_{50}O$		Phytosterol	Anti-diabetic (Kumari, 2023)
2	19.385	3.23	β-sitosterol	$C_{29}H_{50}O$		Phytosterol	Larvicidal insecticidal (Rahuman *et al*., 2008)
3	18.827	2.71	Stigmasterol	$C_{29}H_{48}O$		Phytosterol	Trypanocidal, larvicidal (Soodabeh *et al*., 2014)
4	13.840	14.21	Octadecanoic acid 2-hydroxy-1-hydroxy methyl ethyl ester	$C_{21}H_{42}O_4$		Stearic acid	Toxic (Kumari, 2023)
5	11.888	12.12	Hexadecanoic acid, 2-hydroxyl-1-(hydroxy methyl) ethyl ester or Glycerol 1-palmiate	$C_{19}H_{38}O_4$		Monoacylglycerols	Toxic, nematicide, insecticide, anti-microbial (Kumari, 2023)

GC-MS of *Euphorbia heterophylla* revealed 13 major compounds as Lup-20 (29)-en-3-ol, acetate 3(beta), lupeol, Lup-20(29)-en-3-ol, acetate 3(beta), 24- Norusa 3,12-diene, 24- Norusa 3,12-diene, gamma-sitosterol. 1-Pentacosanol, Tetracosane, Cholesta-4-6-dien-3-ol (3, beta), Heptacosanal, 1-Pentacosanol, Octadecanoic acid 2,3-dihydroxyl propyl ester, Hexadecenoic acid 2-hydroxy 1-(hydroxymethyl) ethyl-ester which are summarised in table 4. Amongst these hexadecenoic acid, octadecanoic acid and β- sitosterol are known to possess toxicity and pesticidal property (Dr Duke's phytochemical database, 2016).

Table 4: Chromatogram of GC-MS of methanolic leaf extract of *Euphorbia heterophylla*

Sr No	Retention Time	Area %	IUPAC name	Molecular Formula	Structure	Nature of compound	Biological activity
1	20.735	9.28	Lup-20(29)-en-3-ol, acetate 3(beta)	$C_{32}H_{52}O_2$		Triterpene	Anti-diabetic, stimulates melanogenesis (Kumari, 2023)
2	20.735	9.28	Lupeol	$C_{30}H_{50}O$		Triterpene	Anti-cancer, anti-tumor, immunostimulant, pesticide (Kumari, 2023)
3	20.368	5.78	Lup-20(29)-en-3-ol, acetate 3(beta)	$C_{32}H_{52}O_2$		Triterpene	Anti-diabetic, stimulates melanogenesis (Kumari, 2023)
4	20.042	2.19	24- Norusa 3,12-diene	$C_{29}H_{46}$		Non-triterpene	Not reported
5	19.880	2.74	24- Norusa 3,12-diene	$C_{29}H_{46}$		Non-triterpene	Not reported
6	19.391	10.51	gamma-sitosterol	$C_{29}H_{50}O$		Phytosterol	Anti-diabetic (Kumari, 2023)
7	17.366	1.82	1-Pentacosanol	$C_{25}H_{52}O$		Fatty alcohols	Not reported
8	17.311	0.14	Tetracosane	$C_{24}H_{50}$		Alkane hydrocarbon	Not reported
9	17.203	0.77	Cholesta-4-6-dien-3-ol (3, beta)	$C_{27}H_{44}O$		Cholesterol	Not reported
10	16.806	0.44	Heptacosanal	$C_{27}H_{54}O$		Fatty alcohol	Not reported
11	15.506	1.92	1-Pentacosanol	$C_{25}H_{52}O$		Fatty alcohols	Not reported
12	13.842	7.95	Octadecanoic acid 2,3-dihydroxyl propyl ester	$C_{21}H_{42}O_4$		Stearic acid	Toxic (Kumari, 2023)
13	11.890	10.17	Hexadecanoic acid 2-hydroxy 1-(hydroxymethyl)ethyl-ester	$C_{19}H_{38}O_4$		Monoacylglycerols	Toxic, nematicide, insecticide, anti-microbial (Kumari, 2023)

Discussion

The ovicidal (egg hatching) property of plant extracts was first reported by Ouda *et al*., (1998). Seed extract of *Atriplex canescen*s exhibited complete ovicidal effect at 1000 ppm on eggs of *Culex quinquefasciatus*.

Maximum egg mortality rate (EMR) at lower concentration is an indication of potential ovicidal activity. Ovicidal activity is more influenced by concentration, freshly laid egg rafts and mosquito species. Freshly laid eggs are white, soft but later undergo sclerotization during embryogenesis and appears black and dark in colour. Ovicidal compounds are able to interrupt embryo development, impair the survival of larva inside the egg or block hatching of the eggs (Raveen *et al*., 2017).

Gokulakrishnan *et al*., (2012) [11] also tested for larvicidal and ovicidal efficacies of different solvent leaf extracts of *Aristolochia indica* against *Anopheles stephensi* Liston. Ethyl acetate and benzene extracts were tested for larvicidal, ovicidal and repellent property of *Ervatamia coronaria* and *Caesalpinia pulcherrima* against all three mosquito species *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus.* Highest larvicidal and ovicidal activity was observed in benzene leaf extract of *E. coronaria* against *Anopheles* than *A. aegypti* and *C. quinquefasciatus*. 100% mortality (zero hatchability) was observed at 300, 250 and 200 ppm for *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* respectively (Govindrajan *et al*., 2011) [14]. They also have stated that freshly laid eggs showed poor hatchability in higher concentrations whereas the older rafts showed higher hatchability at lower concentrations. The percent hatchability was inversely proportional to the concentration of extract and directly proportional to eggs. Amongst different solvent leaf extracts (benzene, ethyl acetate, methanol and chloroform) of *Eclipta alba*, the methanol extract showed zero hatchability at 300 ppm (Govindrajan and Karuppannam, 2011). Similar study of efficacy of methanolic extracts of *Erythrina indica* on was tested against all three major vectors *A. stephensi*, *A. aegypti* and *C. quinquefasciatus.* Zero hatchability (100% mortality) was reported at concentrations 150, 200 and 250 ppm for *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* respectively (Govindrajan and Sivakumar, 2013).

Ethanolic extracts of plants used for study exhibited 50-70% ovicidal activity whereas, butanolic extract of *A.sessilis*

caused highest egg mortality of 86% as per research conducted by Raveen *et al*., (2017). The activity may be due to high amounts of polyphenols extracted by ethanol. Kuppusamy and Murugan (2008) [18] reported leaf extracts of *Andrographis paniculata* with potential ovicidal activity after mortality of hatched larvae of *Anopheles subpictus* within one or two hours. Similar observations as damage of eggs and egg shells due to endosmosis of plant extracts led to desiccation and shrinkage of eggs followed by low adult emergence (Subramaniam *et al*., 2011) [36]. Silva (2017) [33] could isolate 15 major compounds from leaves and 5 from roots of *C. sulphureus* that are sesquitepenes lactones, costunolide, reynosin and santamarin along with stigmasterol, phenylpropanoids by GC-MS analyses. Coreopsin, a glycoside of butein was isolated from ray florets of *Cosmos* as reported by Geissman (1942) [7] but, later "sulphurein" a new crystalline 6-glycoside of sulphuretin (3,'4, 6-trihydroxybenzalcourmaranone) was detected from ray florets of *C. sulphureus* by Masami *et al*., (1952) [21]. According to Lim (2014) [19] *Cosmos* leaves contain quercetin and stigmasterol-3-O-β-D-glucopyranosid. Similarly, β-sitosterol- 3-O-β-D-glucoside a phytosterol that possess larvicidal property was isolated from *Acanthus montanus* (Amin *et al*., 2012) [1]. The results of aqueous extracts of *Cosmos sulphureus* showed no effect on larvicidal and ovicidal activity of *Aedes aegypti* are corroborating with results of *C. bipinnatus* on larvae and pupae of *Culex quinquefasciatus* mosquitoes (Modise *et al*., 2015).

Vani *et al*. (2017) [40] has reported presence of 23 major compounds from mixture of hexane and chloroform stem fractions of *E. heterophylla* such as as cyclohexasiloxane, dodecamethyl., octadecamethyl, hexadecenoic acid, methyl ester., octadecanoic acid, methyl ester and 13-Docoenamide, (Z). Whereas, Nwankudu *et al*., (2016) [24] has isolated Furan-2-ylmethanol, 2-ethyl-2 hexen-1-al, 2,5-dimethyl nona-2, 5-8- trien-1-ylium, acifluofen, isobutyl ester from leaf ethanolic extract of *Euphorbia*. β-sitosterol, stigmasterol, esters of lupeol, germanicol, taraxasterol, psuedotarasterol, α-amyrin and β-amyrin were isolated from root methanolic extracts of wild poinsettia by Silva *et al*., (2018) [39]. As per the literature β-sitosterol, a ubiquitous phytosterol present in all plants, animals and fungi belonging to group of 4-desmethyl sterols possess many biological activities as anti- microbial, anti-inflammatory, cytotoxic and insecticidal/ toxic larvicidal properties (Gonzalez, 2013; Soodabeh *et al*., 2014; Sogan *et al*., 2020) [35].

The chemical composition of botanicals depends on several attributes like plant phenological stage, plant part, age of the plant, geographical location, seasonality and method of solvent extraction that consequently affects the insecticidal activity (Shaalan *et al*., 2005; Maira *et al*., 2020) [32]. Steroids and alkaloids are extracted with mid-polar solvents (Tehri, 2014) [38]. Alkaloids along with tannins, steroids, glycosides, triterpenoids and saponins present in plants possess insecticidal properties (Pedro *et al*., 2014: Subramaniam *et al*., 2016) [26, 37]. Alkaloids are the nitrogenous compounds that show insecticidal properties at a very low concentrations which affects the larvae by constricting blood vessels and affects the autonomous nervous system. Liu *et al*., (2012) considered alkaloids to be toxic to larvae of *Aedes aegypti, Culex* and *Anopheles* mosquito species.

Conclusion

There is an urgent need for an alternative source as botanical pesticides in the form of essential oils, extracts or isolated pure compounds. Many plant species produce these secondary metabolites which possess repellent/ attractant, antifeedant, toxic and larvicidal property. Medicinal plants like *Azadirachta indica*, *Ocimun santum*, *Cinnamomum zeylanicum* (Cinnamon oil) are known to exhibit larvicidal/ ovicidal and deterrent properties but study on invasive weeds is very scanty which needs to be explored. Formulations derived from weeds will lead to eradication of these invasive weeds which are found gregariously growing near agricultural crop fields and hampering yield of the crops. Bio-pesticides of plant origin will be economically available at an affordable price, environmentally safe and reduce the cost of synthetic chemicals.

Many studies have been conducted on immature larval stages of mosquito vectors but, further isolation of bio-active compounds and assessment of the larvicidal activity of these compounds needs to be done in order to understand the action of biopesticide on large scale production. A new pharmaceutical technique of silver-based nanoparticles can be synthesized and studied for their further biological activity in future. Further studies are necessary in order to understand the target sites/ mechanisms of botanical pesticides.

Acknowledgement

The authors are thankful to the Principal, B G College, Ross Life Science, Dr Rahul Marathe and Dr Megha Biware for compilation and interpretation of FTIR and GC-MS results.

Conflict of interest: The authors declare no conflict of interest

References

1. Amin E, Radwan MM, El Hawary SS, Fathy MM, Mohammad R, Becnel J J. Potent insecticidal secondary metabolites from the medicinal plant *Acanthus montanus*, Records of Natural products,2012:6:01-305.
2. Bansod V, Gharpure P, Ghayal NA. Bioefficacies of different solvent extracts of *Cosmos sulphureus* on 3rd instar larvae of *Aedes aegypti*. International Journal of Mosquito Research,2024:11(1):99-104.
3. Bowers WS. Biorational approaches for insect control, Korean Journal of Applied Entomology, 1992:31:289–303.
4. Dhole J A, Bodke, S S, Dhole, NA. Allelopathic effect of aqueous extract of five selected weeds species on seed mycoflora, seed germination and seedling growth of *Sorghum vulgare* Pers. (Jawar), Research Journal of Pharmaceutical, Biological and Chemical Sciences,2011:2(3):142.
5. Dua VK, Pandey AC, Kamaraju R, Gupta A, Sharma T, Dash AP. Larvicidal activity of neem oil (*Azadirichta indica*) formulations against mosquitoes., Malaria Journal., doi:10.1186/1475-2875-8124, Volume 8, 2009, Pages 124
6. Falodun A, Ali S, Quadir IM, Iqbal M, Choudhary I. Phytochemical and biological investigation of chloroform and ethyl acetate fractions of *Euphorbia heterophylla* leaf (Euphorbiaceae). Journal of Medicinal Plants Research,2008:2(12):365-369.

7. Geissman TA. Anthochlor Pigments. 111. The Pigments of *Cosmos sulphureus*., Contribution from The Department of Chemistry, University of California, Los Angeles,1942.
8. Gharpure P, Bansod V, Ghayal N. Phytotoxic effects of aqueous leaf extracts of *Cosmos sulphureus* on *Sorghum bicolor* L, *Vigna aconitifolia* L, Triticum *aestivum* L. Journal of Tropical agriculture,2023:61(2):319-327.
9. Gharpure P, Ghayal N, Bansod V. Comparative studies of allelopathic effects of composite plant extracts of *Alternanthera tenella* Colla and *Euphorbia heterophylla* L on *Triticum aestivum* L. South Asian Journal of Experimental Biology,2023:5(13):366-372.
10. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides., Indian Journal of Medical Research, 2012:135:581-598.
11. Gokulakrishnan J, Selvakumar B, Elumalai K, Krishnappa K. Mosquito larvicidal and ovicidal efficacy of *Aristolochia indica* L. (Aristolochiaceae) leaf extracts against malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae)., International Journal of Current Life Sciences,2012:2:48-52.
12. Gonzalex Coloma A, Reina M, Diaz CM, Fraga BM, Santa Meridas O. Natural Product based Biopesticides for Insect Control., Reference Module in Chemistry, Molecular Science and Chemical Engineering., http://dx.doi.org/10.1016/B978-0-12-409547-2.02770-0, 2013, Pages 1-55
13. Govindarajan MT, Mathivanan K, Elumalai K, Krishnappa A, Anandan. (2011) Mosquito larvicidal, ovicidal, and repellent properties of botanical extracts against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* (Diptera: Culicidae)., Parasitology Research., doi 10.1007/s00436-011-2263-1., 109:353–367
14. Govindrajan M, Karuppanam P. Mosquito larvicidal and ovicidal properties of *Eclipta alba* (L) Hassk (Ateraceae) against chikungunya vector, *Aedes aegypti* L (Diptera: Culicidae)., Asian Pacifiic Journal of Tropical Medicine,2011:4:24-28.
15. Govindrajan M, Sivakumar R. Larvicidal, ovicidal and adulticidal efficacy of *Erythrina indica* (Lam) (Family: Fabaceae) against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitology Research,2014:113:777-791.
16. Kumar V, Shririam V, Bhagat R, Khare T, Kapse S, Kadoo N. Phytochemical profile, anti-oxidant, anti-inflammatory and anti-proliferative acitivites of Pogostemon decacanensis essential oils. Biotech (online),2019:9(1):31.
17. Kumari AA, Patnaik V, Oraron L, Sharan. GC-MS profiling of phytochemicals present in ethanolic extracts of *Bauhinia variegata* L leaves. South Asian Journal of Experimental Biology,2023:13(3):173-180. [DOI: 10.38150/sajeb.13(3):173-180]
18. Kuppusamy C, Murugan K. Oviposition deterrent, ovicidal and gravid mortality effects of ethanolic aextract of *Andrographis paniculata* Nees against the malarial vector *Anopheles stephensi* L (Diptera: Culicidae)., Entomological Research, 2008:38:119-125.
19. Lim TK. *Cosmos sulphureus.* Edible Medicinal and Non-Medicinal Plants. **7**, Flowers, doi: 10.1007/978-94-7395-0_19, 2014
20. Maria Silverio Rosato Silveiral, Laila Salmen Epsindola, Norberto Peporine Lopes, Paulo Cezar Vieria. Plant natural products for the control of *Aedes aegypti*: The main vector of important Arboviruses, Molecules,2020:25:3484. doi 10.3390/molecules/25152484
21. Masami S, S Hattori. Anthochlor Pigments of *Cosmos sulphureus*, *Coreopsis lanceolata* and *C. Saxicola*. Contribution from The Department of Chemistry, University of California, Los Angeles,1952.
22. Miguel M G. Betalains in some species of the Amaranthaceae family: A review, Volume *7*, Issue 4, 2018, Pages, 53, https://doi.org/10.3390/antiox7040053
23. Modise SA, Ashafa AOT. Larvicidal, pupicidal and insecticidal activites of *Cosmos bipinatus, Foeniculum vulgare* and *Tagetes minuta* against *Culex quinquefasciatus* mosquitoes. Tropical Journal of Pharmaceutical Research,2016:15(5):965-972.
24. Nwankudu ON, Ijioma SN, Igwe KK, Madubuike AJ, Achi NK. Preliminary studies of phytochemicals in *Euphorbia heterophylla* ethanol extract using GC-MS analysis, Journal of Science and Technology Advances,2016:1(2):85-92.
25. Ouda NAA, Al chalabi BBM, Al mchurchaftchi FFMR, Mobsen ZZH. Extract of *Atriplex canescens* against *Culex quiquefasciatus*. Pharma Biology,1998:366(1):69-71.
26. Pedro MGJr, Antepuesto AN, Eugenio BAL, Santos MFL. Larvicidal activity of selected plant extracts against the Dengue vector *Aedes aegypti* mosquito. International Research Journal of Biological Sciences,2014:3(4):23-3.
27. Ramani K, Poonguzhali TV. Quantitiative estimation of flavonoid content of the leaf and root of *Alternanthera tenella* Colla (Amaranthaceae). International Journal of Current Science, 15S, E 27-30.
28. Rathinam K, Bhavan PS, Rajkumar G, Nathiya V, Satgurunathan T, Manjula T. Phytochemical characterisation of *Alternanthera sessilis* and assessment of its growth promoting potential on the fresh water Prawn *Macrobrachium rosenbergii*. International Journal of Research Studies in Zoology,2017:3(4):25-38.
29. Rodrigues Brandão I, Kneinowski AM, Einhardt AM, Lima MC, Amarante MCLL, Peters JA *et al*. Salicylic acid on antioxidant activity and betacyanins in production from leaves of *Alternanthera tenella*. Ciênc Rural,2014:44:1893-1898.
30. Salvador M, Ferreira E, Mertens Talcott S, De Castro W, Butterweck V, Derendorf H *et al*. Isolation and HPLC Quantitative Analysis of Antioxidant Flavonoids from *Alternanthera tenella* Colla. Zeitschrift für Naturforschung C,2006:61(1-2):19-25. https://doi.org/10.1515/znc-2006-1-204
31. Salvador MJ, Eliane O Ferreirab, Susanne U, Mertens Talcottb, Whocely Victor De Castrob, Veronika Butterweckb *et al*. Isolation and HPLC Quantitative Analysis of Antioxidant Flavonoids from *Alternanthera tenella* Colla. Zeitschrift für Naturforschung C. Volume 61c, 2006, Pages 1-25, https://doi.org/10.1515/znc-2006-1-204
32. Shaalan EAS, Canyonb D, Younesc MWF, Abdel Wahaba H, Mansoura AH. A review of botanical

phytochemicals with mosquitocidal potential. Environment International,2005:31:1149-1166.
33. Silva Bruna Pires da. Allelopathic potential of *Cosmos sulphureus* Cav., Doctoral Thesis, 2017.
34. Sogan N, Kapoor N, Singh H, Kala S, Nayak A, Nagpal BN. Larvicidal activtity of *Ricinus communis* extract against mosquitoes., Journal of Vector Borne Disease,2018:55:282-290.
35. Soodabeh S, Azadeh Manayi, Ahmad R Gohari, Mohammad A. The story of Beta sitosterol a review., European Journal of Medicinal Plants,2014:4(5):590-609.
36. Subramaniam A, Tennyson S. Larvicidal and adult emergence inhibition activity of *Abutilon indicum* Linn (Malvaceae) leaf extracts against vector mosquitoes (Diptera: Cuculidae). Journal of Biopesticide,2011:4(1):27-35.
37. Subramaniam AS, Tennyson R, Raveen M, Jaykumar B, Senthilkumar M, Govindarajan R, *et al*. Larvicidal activity of fractions of *Sphaeranthus indicus* Linnaeus (Asteraceae) ethyl acetate whole plant extract against *Aedes aegypti* Linnaeus 1762, *Anopheles stephensi* Liston 1901 and *Culex quinquefasciatus* Say 1823 (Diptera: Cuculidae)., International Journal of Mosquito Research,2016:3(2):18-30.
38. Tehri K, Singh N. The role of botanicals as green pesticides in integrated mosquito management. International Journal of Mosquito Research,2014:2(1):18-23.
39. Ueveton Pimental da Silva, Gabriela MIlane Furlani, Antonio Jacinto Demurer, Otavio Luis Marques da Silva, Eduardo Vinicius Vieira Varejao. (2018) Allelopathic activity and chemical constituents of extracts from roots of *Euphorbia heterophylla*. Natural Product Research, 2018, 1-4. DOI: 10.1080/14786419.2018.1460829
40. Vani M, Abdul Rahaman SK, Rani AP. Phytochemical composition of *Euphorbia heterophylla* L. International Journal of Advances Life Sciences,2017:10(2):247-257.