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Heterocyclic thiophene extraction and their Antilarvacidal activity against potential pathogens

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Abstract
Natural thiophene and its compounds are a class of heterocyclic compounds having therapeutic potential. Thiophene is a natural substance that is synthesized to satisfy specific needs. It's become a critical pillar in the production of combinatorial tools for medicinal chemists. It is reported to offer a wide range of medicinal qualities, as well as several medical applications. In terms of physiological and biological qualities, thiophene is a highly effective chemical. Antihypertensive, analgesic, antibacterial, antifungal, anticancer, biocidal, and anti-inflammatory activities, In present investigation naturally extracted four thiophene, 5-(3-buten-1-ynyl)-2,2-bithienyl (BBT), 5-(4-hydroxy-1-butynyl)-2,2- bithienyl (BBTOH), 5-(4-acetoxy-1-butynyl)-2,2-bithienyl (BBTOAc), and α-T (2,20:50,20- terthienyl) from flowered plants viz Tagetes erecta, Calendula officinalis, Eclipta prostrate, and Echinops echinatus Roxb. evaluated for free radical scavenging activities. Finding suggested significant scavenging response.

Keywords: *Heterocyclic compound, natural and synthetic thiophene, antilarval activity.*

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I. Introduction

Thiophene is a five-membered heteroaromatic molecule with a single sulphur element. It is a structural alert with the formula C4H4S and the chemical name thiacyclopentadiene [1-3]. Thiophene was detected in benzene as a contaminant [4,5]. Most organic solvents, such as alcohol and ether, are soluble in it, but it is insoluble in water. Thiophene derivatives are important heterocycles in medicinal chemistry, with several uses in various fields. Thiophene derivatives have antibacterial, analgesic, and anti-inflammatory [6,7], hypertension [8,9], and anticancer [9] properties in medicine, and they are also utilised as metal corrosion inhibitors [10]. Compounds containing the thiophene nucleus have a wide range of biological functions. Antimicrobial activity of thiophene derivatives against a variety of microbial illnesses has been demonstrated [11,12]. Antioxidant activities created a new family of substituted 2-(2-cyanoacetamido)thiophenes by cyanoacetylating substituted 2-aminothiophene with a powerful cyanoacetylating agent, 1-cyanoacetyl- 3,5-dimethylpyrazole, as shown in scheme [14]. At a concentration of 100 M, all of the produced compounds were tested for in vitro antioxidant activity by scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) and nitric oxide free radicals. In both free radical scavenging models, the chemical 2-(2-cyanoacetamido)-4,5-dimethylthiophene- 3-carboxamide was found to have the best anti-oxidant activity. However, in a nitric oxide free radical scavenging assay, 2-(2-cyanoacetamido)-4,5-dimethylthiophene-3-carboxamide (56.9%) and 2-(2-cyanoacetamido)-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxamide (56.9%) showed the best activity. The polar character of the carboxamide or nitrile group at the 3rd position on the thiophene ring was linked to the higher activity of these compounds [15].

Thiophene was also discovered as a natural substance derived from many plants. Four thiophenes were isolated from the Tagetes patula plant in this study. Tagetes patula belongs to the Asteraceae family's marigold marigold division. Its essential oil content has been studied in a variety of ways [16-21]. The predominant chemical constituents of the essential oil isolated from Tagetespatula capitula and leaves were terpenes, particularly (Z) and (E) ocimenone, as well as limonene, caryophyllene, piperitone, and piperitenone [22]. The biosynthesis of T. patula essential oil has recently gotten a lot of attention [23].

II. Material and Method

PLANTS COLLECTION AND PROCESSING

Fully flowered plants viz *Tagetes erecta, Calendula officinalis, Eclipta prostrate,* and *Echinops echinatus Roxb.* were collected from Jawaharlal Nehru Agricultural University, Jabalpur. The whole plants were brought to the laboratory as soon as possible in a sterile polythene bag. Plant material was washed appropriately

under running tap water to remove soil, dust and other debris then rinsed using distilled water. After washing the plants were soaked onto a newspaper to get rid of extra water.

Whole plant was dried as such under shade for one week or longer till a constant weight was achieved. The air dried whole plant material was pulverized to a coarse powder in a mechanical grinder, passed through 100 µM mesh sieve (Sonar, India). The remaining coarse powder was again subjected to grinding and sieving and the process was continued till no further material passed through test sieve (100 µM) and was stored in an air tight container for further use.

EXTRACTION OF THIOPENE FROM WHOLE PLANT

Under decreased ambient light, thiophene extraction was carried out using a modified approach [24,25]. Dried plant powders were separated using a Soxhlet extractor at room temperature (JSGW, India). 250 mL of methanol:water (3:1 v/v) was added to the extractor reservoir (Qualigens, India). A heating mantle kept the reservoir at a consistent 45°C temperature. The vapours of methanol are condensed in the water-cooled condenser and drip into the extraction device, which contains a thimble loaded with plant material. The thiophene extracts come from the thimble, which is formed of filter paper. Once the extracting arm is full, a looped leveller arm from the extraction unit transports the methanol: water mixture containing the extracted plant material to the solvent reservoir.

The thiophene-rich extract was dissolved in a little amount of hexane, filtered, and dried. The extracting process was repeated to recover a considerable quantity of extracts, which were then stored at 4°C in the refrigerator for later use. The extract's percentage yield (w/w) was calculated [26]. By comparing their retention durations and absorption spectra with those of legitimate standards, four thiophene compounds in the sample, namely BBT, BBTOH, BBTOAc, and -T, were identified as having larvicidal activity.

III. Results And Discussion

LARVICIDAL ACTIVITY

Using larval mortality bioassays at 26-28 °C and 78 percent–80 percent relative humidity with extract concentrations of 0.2, 0.3, and 0.4 percent, the toxic impact of thiophene isolated from several tested plants against *Aedes aegypti* and *Culex quinquefasciatus* was determined. *Aedes aegypti* and *Culex quinquefasciatus* were studied for 24 hours after being exposed to thiophene extract at 12-hour intervals, and the results revealed that each plant had a different level of larvicidal activity that was dose and time dependent. Each experiment was carried out three times and the results were provided as a mean SD.

Larvicidal activity of *T. erecta*

Effect of *T. erecta* thiophene extract on mosquito larvae after 12 and 24 hr of exposure was presented Fig. 1. Results revealed that after 12 hr of exposure to different concentration of thiophene extract larvae mortality ranged from 33.35 % - 63.35% for *Aedes aegypti* and 45% - 61.65% for *Culex quinquefasciatus.* After 24 h of exposure, it was observed that 0.3% and 0.4 % extract was lethal for *Aedes aegypti* and *Culex quinquefasciatus* larvae, respectively with 100 % mortality. In control, 100% larvae survived.

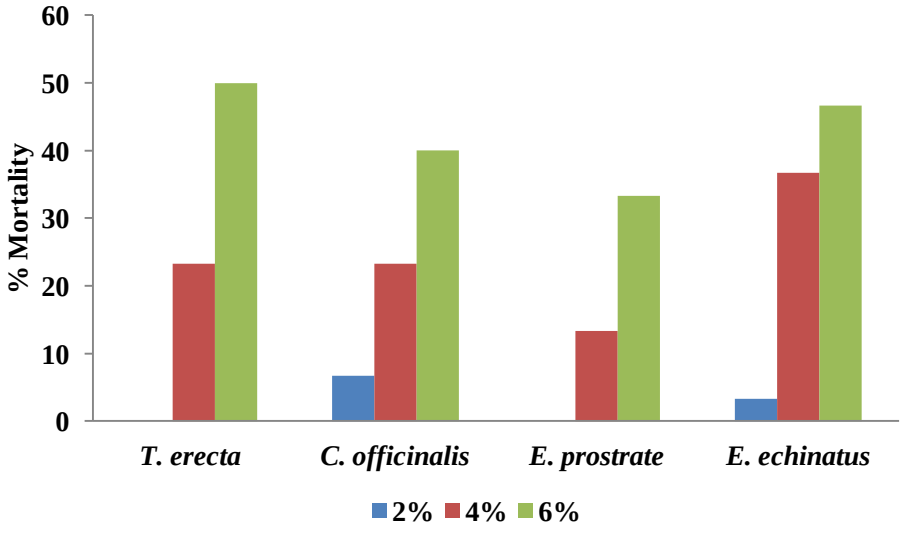


Fig. 1 Comparative analysis of % mortality of *Sitophilus oryzae* after 4 hr of thiophene extracts exposure.

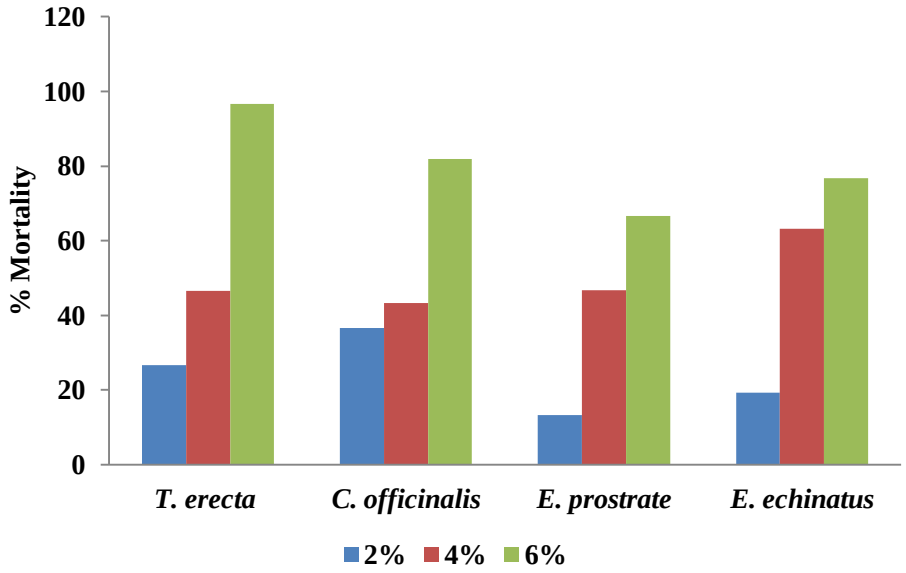


Fig. 2 Comparative analysis of % mortality of *Sitophilus oryzae* after 8 hr of thiophene extracts exposure.

Larvicidal activity of *C. officinalis and C. officinal*

Thiophene on mosquito larvae after 12 and 24 hr of exposure was presented in Fig 2. Results revealed that after 12 hr of exposure to different concentration of thiophene extract larvae mortality ranged from 23.33 %-51.67% for *Aedes aegypti* and 28%-66.67% for *Culex quinquefasciatus.* After 24 h of exposure, it was observed that at 0.2% concentration 80 % larvae of *Culex quinquefasciatus* dies while 61.67% of *Aedes aegypti*. 0.4 % extract was lethal for both *Aedes aegypti* and *Culex quinquefasciatus* larvae, with 100 % mortality. 0% mortality was observed in control. Results revealed maximum mortality at highest concentration with maximum exposure time.

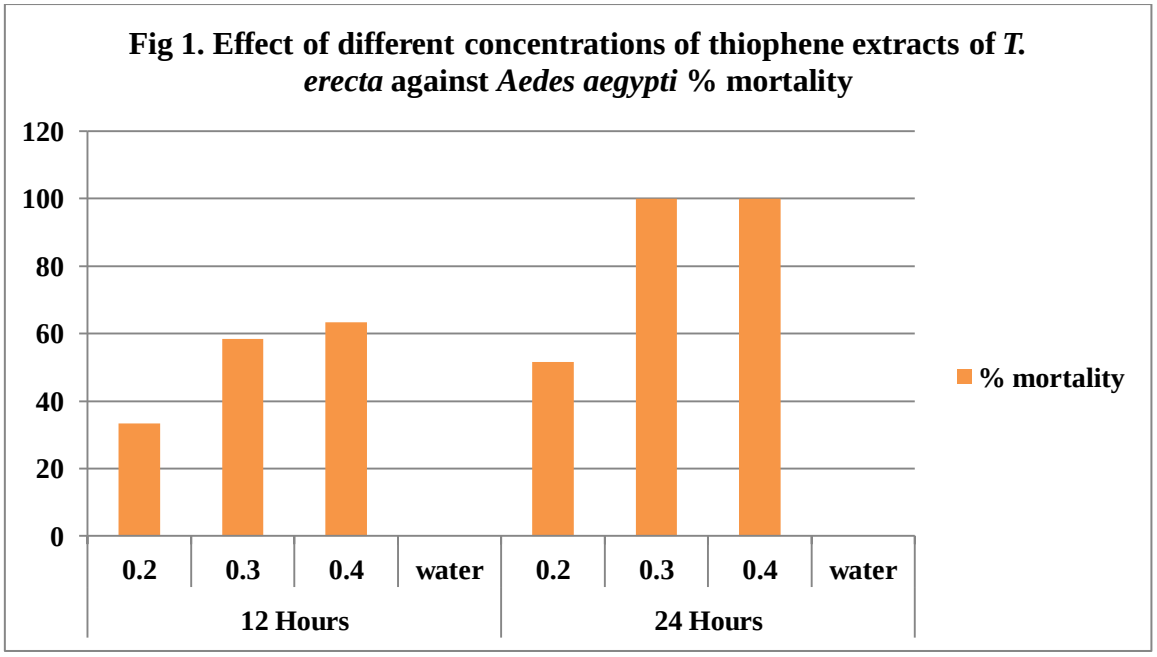
Larvicidal activity of *E. prostrate* and *E. echinatus*

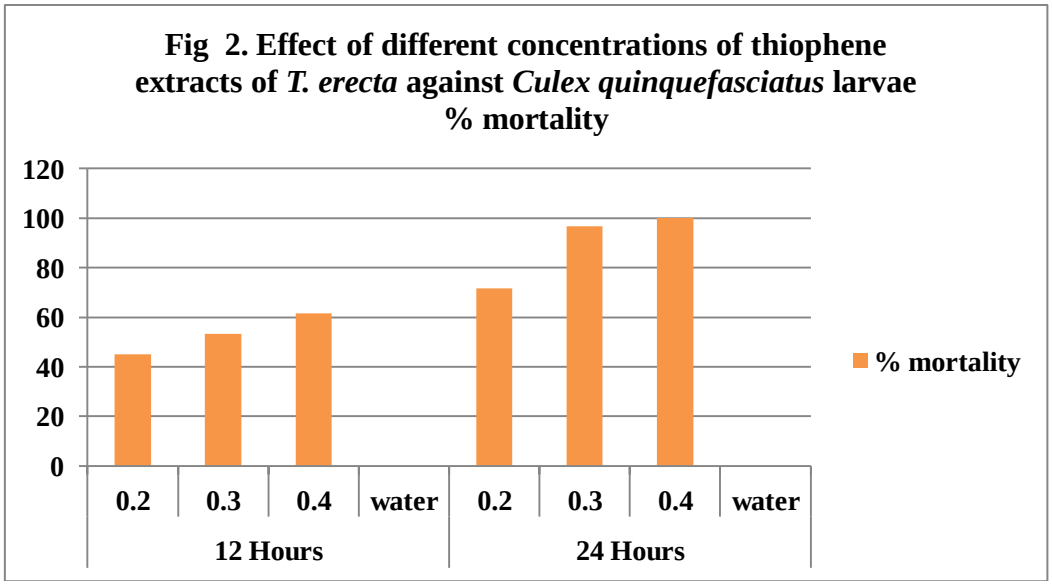
Effect of different concentrations of thiophene extract of *E. prostrate* against *Aedes aegypti* and *Culex quinquefasciatus* larvae after 12 and 24 hr of exposure was presented in Fig 3. Results revealed maximum mortality at highest concentration with maximum exposure time. In control, no mortality was observed. After 12 hr of exposure to different concentration of thiophene extract larvae mortality ranged from 0 % - 31.67% for *Aedes aegypti* and 8.33% - 38.33% for *Culex quinquefasciatus* and was lowest among all tested plant. After 24 h of exposure, mortality ranged from 25 % - 100% for *Aedes aegypti* and 16.7% - 100% for *Culex quinquefasciatus*. Mortality of *Aedes aegypti* and *Culex quinquefasciatus* larvae was more than 70% with 0.3 % extract. Effect of *E. echinatus* thiophene against *Aedes aegypti* and *Culex quinquefasciatus* larvae after 12 and 24 hr of exposure was presented in Fig 4. Results revealed that after 12 hr of exposure mortality ranged from 3.33 % - 36.67% for *Aedes aegypti* and 1.67% - 41.67% for *Culex quinquefasciatus.* After 24 h of exposure, it was observed that at 0.2% concentration only 38.33 % larvae of *Aedes aegypti* dies while 30% larvae of *Culex quinquefasciatus.* 0.3% and 0.4 % extract was 100 % lethal for *Aedes aegypti* and *Culex quinquefasciatus* larvae, respectively with 100 % mortality while 93.33 % larvae of *Culex quinquefasciatus* died with 0.3% *E. echinatus* thiophene. Mortality in control was observed to be 0%.

Table 1 Effect of different concentrations of thiophene extracts of *T. erecta* against *Aedes aegypti* and *Culex quinquefasciatus* larvae and control after continuous exposure for 24hrs.

Exposure time (hr)	Concentration (%)	*Aedes aegypti*		*Culex quinquefasciatus*	
		Mean number of larvae dies	% Mortality	Mean number of larvae dies	% Mortality
12	0.2	6.67±0.58	33.35	9.00±0.00	45.00
	0.3	11.67±1.15	58.35	10.67±0.58	53.35
	0.4	12.67±0.58	63.35	12.33±0.58	61.65
	Water	0	0.0	0	0.0
24	0.2	10.33±1.15	51.65	14.33±0.58	71.65
	0.3	20.00±0.00	100.0	19.33±0.58	96.65

	0.4	20.00±0.00	100.0	20.00±0.00	100.0
	Water	0	0.0	0	0.0





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Exposure time (hr)	Concentration (%)	*Aedes aegypti*		*Culex quinquefasciatus*	
		Mean number of larvae dies	% Mortality	Mean number of larvae dies	% Mortality
12	0.2	4.67±0.58	23.35	5.67±0.58	28.35
	0.3	5.67±1.15	28.35	7.67±0.58	38.35
	0.4	10.33±0.58	51.65	13.33±0.58	66.65
	Water	0	0.0	0	0.0
24	0.2	12.33±0.58	61.65	16.00±0.00	80.00
	0.3	18.33±0.58	91.65	18.00±1.00	90.00
	0.4	20.00±0.00	100.0	20.00±0.00	100.0

	Water	0	0.0	0	0.0

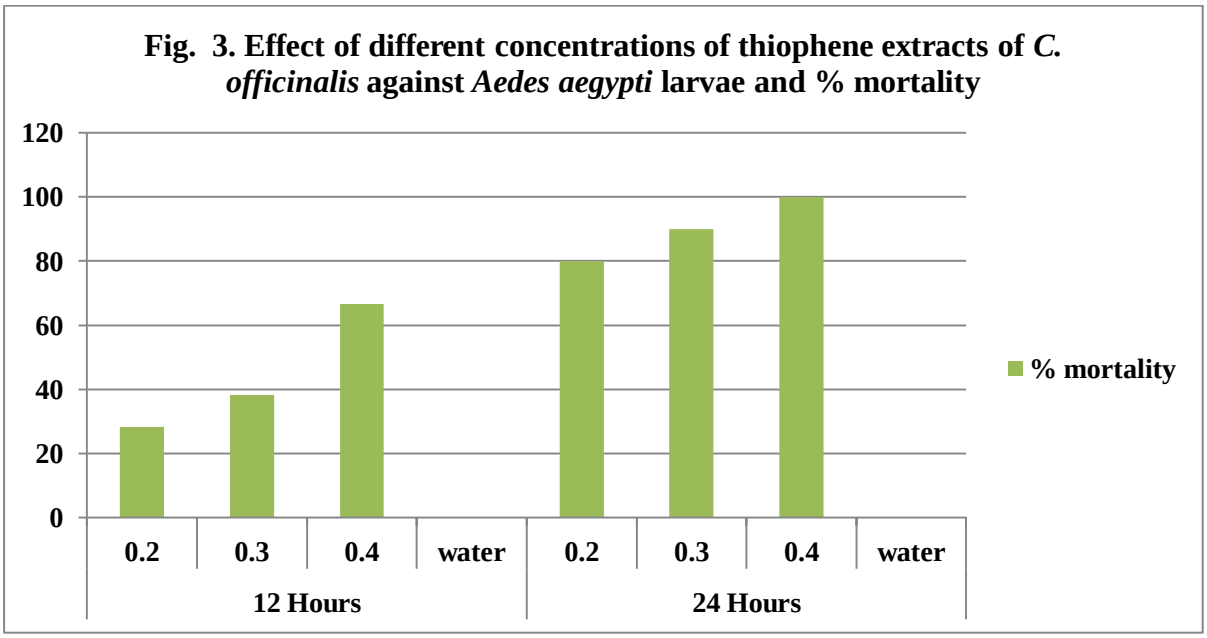


Fig. 3. Effect of different concentrations of thiophene extracts of *C. officinalis* against *Aedes aegypti* larvae and % mortality

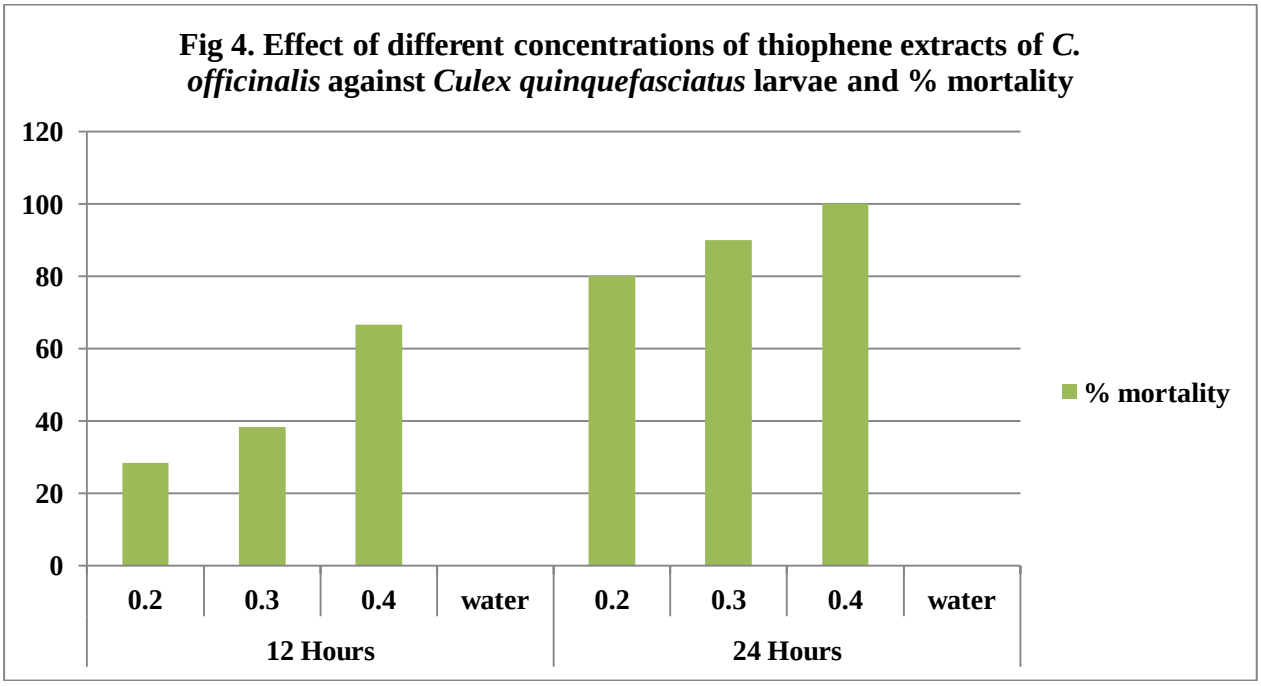
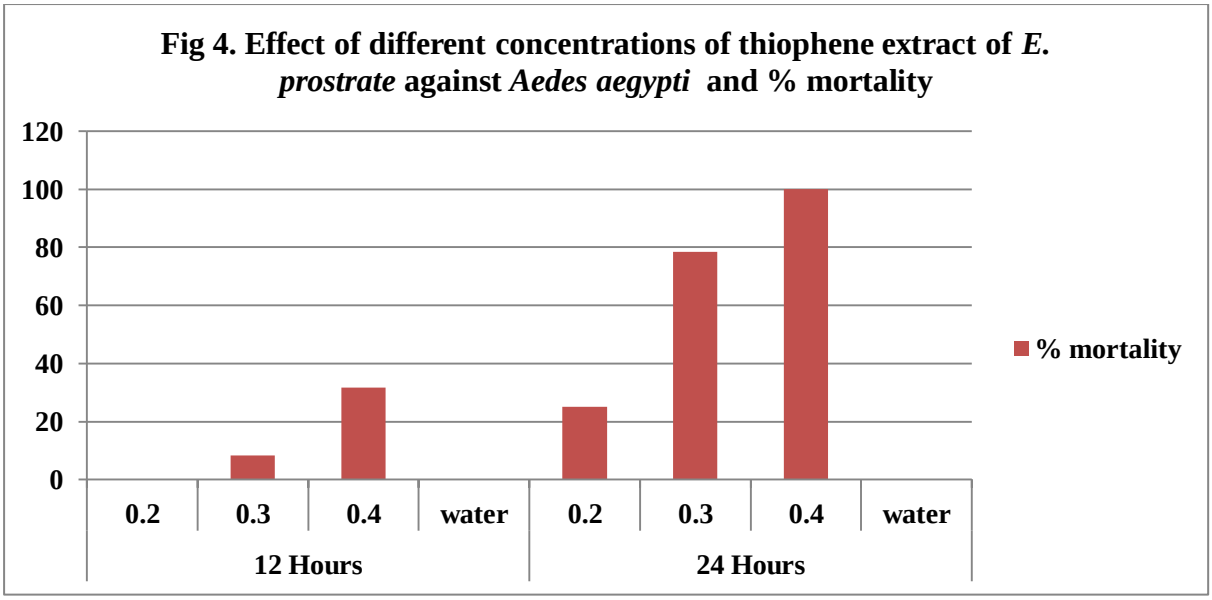


Fig 4. Effect of different concentrations of thiophene extracts of *C. officinalis* against *Culex quinquefasciatus* larvae and % mortality

Table 3. Effect of different concentrations of thiophene extract of *E. prostrate* against *Aedes aegypti* and *Culex quinquefasciatus* larvae and control after continuous exposure for 24hours.

Exposure time (hr)	Concentration (%)	*Aedes aegypti*		*Culex quinquefasciatus*	
		Mean number of larvae dies	% Mortality	Mean number of larvae dies	% Mortality
12	0.2	0.00±0.00	0.00	1.67±0.58	8.35
	0.3	1.67±0.58	8.35	5.67±1.15	28.35
	0.4	6.33±0.58	31.65	7.67±0.58	38.35
	Water	0	0.0	0	0.0
24	0.2	5.00±0.00	25.00	3.33±0.58	16.65
	0.3	15.67±0.58	78.35	14.67±0.58	73.35

	0.4	20.00±0.00	100.0	20.00±0.00	100.0
	Water	0	0.0	0	0.0



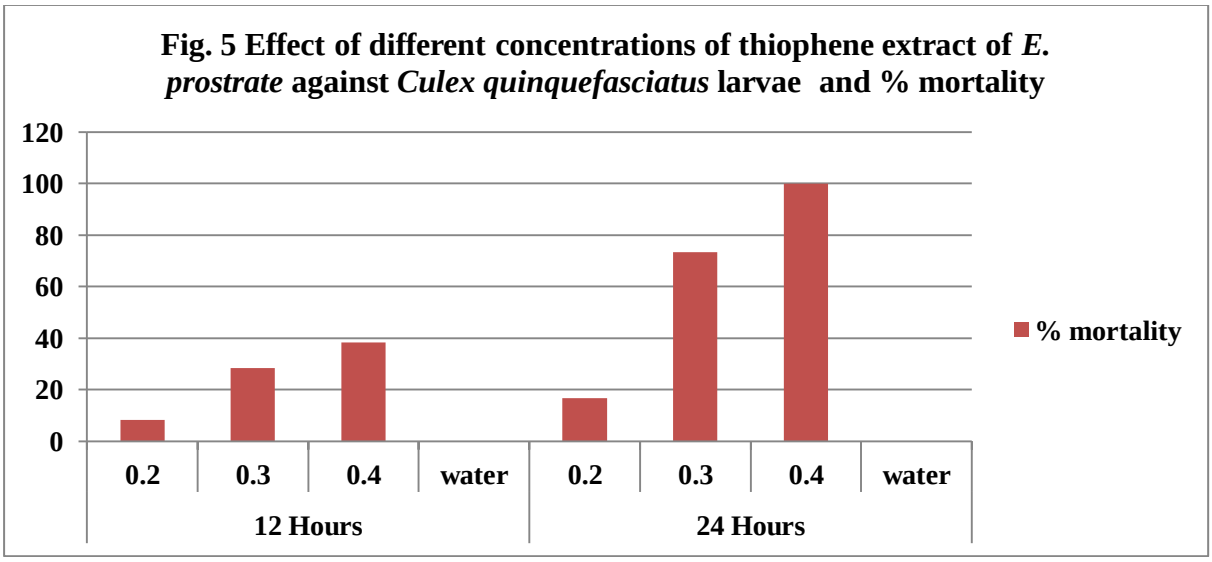


Table 4. Effect of different concentration of thiophene extracts of *E. echinatus* against *Aedes aegypti* and *Culex quinquefasciatus* larvae and control after continuous exposure for 24hours.

Exposure time (hr)	Concentration (%)	*Aedes aegypti*		*Culex quinquefasciatus*	
		Mean number of larvae dies	% Mortality	Mean number of larvae dies	% Mortality
12	0.2	0.67±0.58	3.35	0.33±0.58	1.65
	0.3	2.67±0.58	13.35	3.33±1.15	16.65
	0.4	7.33±1.15	36.65	8.33±0.58	41.65
	Water	0	0.0	0	0.0
24	0.2	7.67±0.58	38.35	6.00±0.00	30.00
	0.3	20.00±0.00	100.0	18.67±0.58	93.35
	0.4	20.00±0.00	100.0	20.00±0.00	100.0
	Water	0	0.0	0	0.0

Fig 5. Effect of different concentration of thiophene extracts of *E. echinatus* against *Aedes aegypti* and larvae % mortality

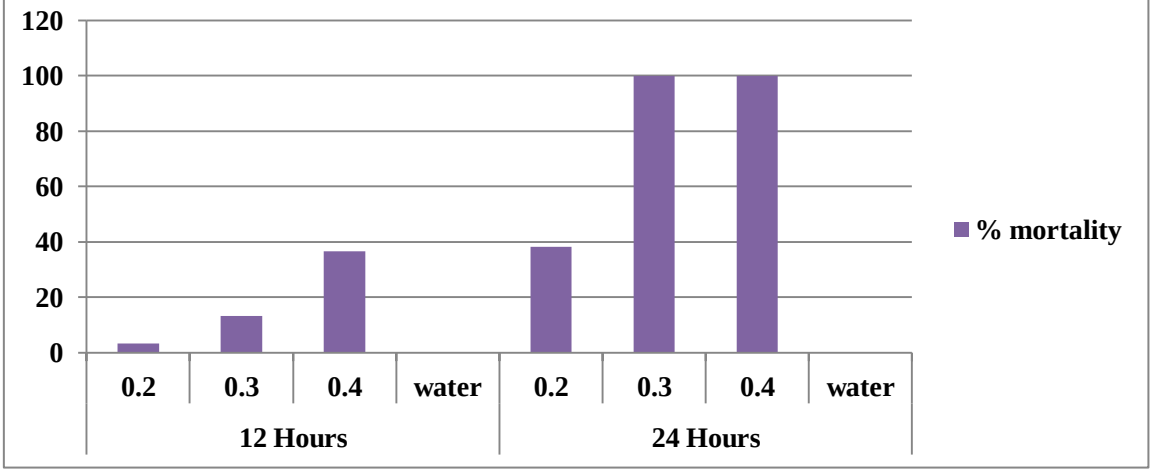
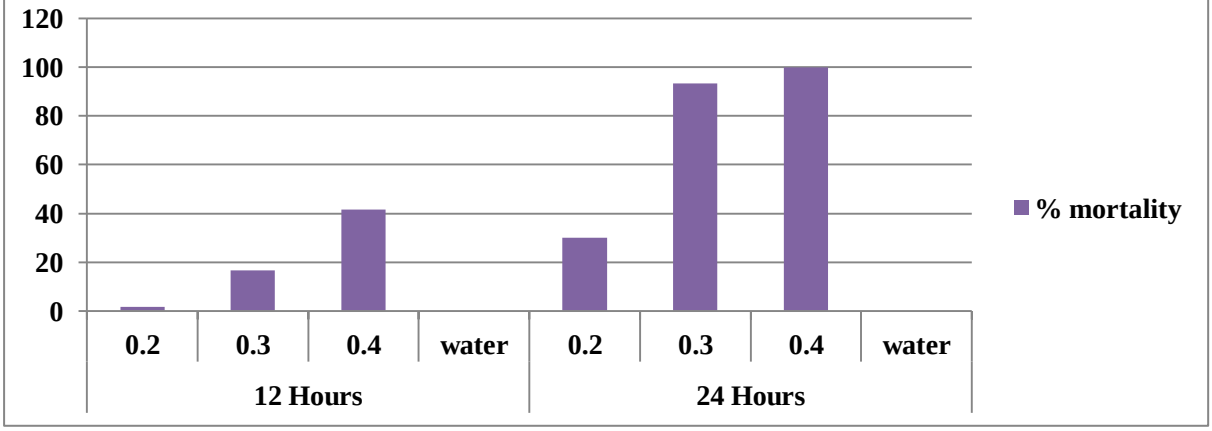


Fig. 6. Effect of different concentration of thiophene extracts of *E. echinatus* against *Culex quinquefasciatus* larvae and % mortality



IV. Conclusion

Different concentration of thiophene extracts of experimentally taken plant were shown potential larvicidal activity against the *Culex* and *Aedes aegypti quinquefasciatus* larvae species with desired % mortality. *T. erecta* plant extracted thiophene shown high mortality of insect larval stage. The extracted thiophene compound of flowering plant shown better results. Selected plant was found suitable for this potential activity. In future more plants and their different parts will allow for similar other activity. Investigated naturally extracted four thiophene, 5-(3-buten-1-ynyl)-2,2-bithienyl (BBT), 5-(4-hydroxy-1-butynyl)-2,2- bithienyl (BBTOH), 5-(4-acetoxy-1-butynyl)-2,2-bithienyl (BBTOAc), and α-T (2,20:50,20- terthienyl) will be shown more activity also.

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