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Efficacy of *Cuscuta reflexa* extract and its synergistic activity with Temephos against mosquito larvae

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Abstract
The present study was carried out to evaluate the larvicidal efficacy of Temephos and the Petroleum ether, Hexane and Methanol extracts of *Cuscuta reflexa* alone and their combinations against the anopheline and culicine larvae. It describes the compatibility of synthetic insecticide, Temephos and phytoextract, *C. reflexa.* Different combination ratios of Temephos and petroleum ether extract of *C. reflexa* viz. 1:1, 1:2 and 1:4 were prepared and evaluated for their larvicidal potentiality against larvae of *Anopheles stephensi* and *Culex quinquefasciatus*. Among these combination ratios 1:1 were found to be more effective than other ratios with LC_{50} 0.0013, 0.0010 and 0.0009 mg/L and LC_{50} of 0.0016, 0.0014 and 0.0013 mg/L against anopheline and culicine larvae after 24, 48 and 72 hours of treatment, respectively. The co-toxicity coefficient for the 1:1 mixture were 178.57, 191.67 and 181.82 and synergistic factor 1.78, 1.92 and 1.82 at LC_{50} after 24, 48 and 72 hrs against anopheline larvae. Against culicine larvae, the co-toxicity coefficient and synergistic factor for the 1:1 mixture were 375, 357.14 and 307.6 and 3.75, 3.57 and 3.08 respectively, at LC_{50} after 24, 48 and 72 hours of exposure. The combination of Temephos and *C. reflexa* is an ideal eco-friendly and cost effective approach to combat mosquito vectors.

Keywords: *Anopheles stephensi, Culex quinquefasciatus, Cuscuta reflexa,* Temephos.

1. Introduction

Mosquitoes are the tiny assassins, being vectors of malaria, filariasis, dengue, yellow fever and encephalitis, which have a significant social and economic impact, in addition to causing millions of deaths, especially in tropical and subtropical countries. The malaria in Asia is an important cause of death and illness in children and adults in tropical and subtropical countries, transmitted by *Anopheles stephensi* (Liston). Half of the world's population is at risk from malaria. According to WHO, 250 million cases causing 860000 deaths were reported each year (WHO 2010) [39]. 99 countries and territories were found with ongoing malaria transmission and 5 countries in the prevention of reintroduction phase, making a total of 104 countries and territories in 2012. (WHO 2012) [40].

Culex quinquefasciatus (Say), 1823 is generally known as the vector of the *Wuchereria bancrofti* responsible for bancroftian filariasis in tropical and humid areas of the world. Lymphatic filariasis is next to malaria as the most important vector-borne disease in India. About 31 million people are estimated to be the carriers of filaria and over 23 million suffer from filarial disease manifestations in India (WHO 2005a) [37]. It has been reported as hazardous to public health and put at risk more than a billion people in more than 80 countries (Arulpriya *et al*. 2013) [3].

The synthetic insecticides are albeit fast acting against mosquito larvae, but their indiscriminate applications have created several environmental issues, development of resistance, human health hazards and undesirable effects on non-target organisms (Curtis *et al*. 1998, Hansch and Verma 2009) [9, 14]. In order to reduce the mosquito menace and their negative effects on human health led researchers to a resurgence in interest for botanical insecticides because of their minimal costs, safe, eco-friendly and fewer ecological side effects. Recently, more than 2000 plants have been reported to produce chemical factors and metabolites of value in pest control programs (Ahmed *et al*. 1984) [2], and among these plants, the products of some 344 species have been reported to have a variety of activities against mosquitoes (Sukumar *et al*. 1991) [33].

Cuscuta reflexa (Family: Convolvulaceae) commonly known as "Amar bel", is an angiosperm parasite. It is a leafless and a rootless plant which usually grows in a prolific manner over host plants with intertwined stems, giving it a common name of Devils Hair. In Ayurvedic medicine, used in the treatment of fits, headache, jaundice, diseases of the spleen, eye and heart (Chopra *et al*. 1958, Kumar *et al*. 2012) [7, 19]. Synthetic pesticide, Temephos is an organophosphate (OP) insecticide used to control mosquito larvae and has been reported against mosquito larvae (Lek-Uthai *et al*. 2011; Mohan *et al*. 2008) [20, 26].

A synergistic combination of biological and chemical insecticides yields a promising alternative for insect pest management were supported by Morales-Rodriguez and peck (2009), Koppenhofer and Fuzy (2003) [27, 17]. If the combination of compounds is synergistic and they will provide similar control at reduced concentrations of the two compounds relative to individually applied compounds, then cost and toxic to mammalian and non-target organisms may be substantially reduced. This strategy is based on the probability that if resistance to one of the two insecticides is a rare and independent event, then the probability that resistance will occur simultaneously to both insecticides of the mixture is extremely low (Curtis 1985) [8].

In this context, the present investigation was carried out with the aim to determine the larvicidal effect of Temephos and phytoextracts, *C. reflexa* alone and synergistic properties of their combinations against third instar larvae, *An. stephensi* and *Cx. quinquefasciatus.*

2. Materials and Methods

2.1 Mosquito colony

The colony of mosquito vectors, *An. stephensi* and *Cx. quinquefasciatus* were reared in the laboratory, maintained continuously at 27±2 ºC and 70–80% relative humidity under a photoperiod of 14:10 hrs (light/dark) without exposure to pathogens or insecticides. Adults were supplied with freshly soaked deseeded raisins. Periodic blood meals were provided to female mosquitoes for egg maturation by keeping restrained albino rats in the cages. The eggs were collected in a bowl lined with Whatman filter paper and were allowed to hatch in trays filled with dechlorinated water. Larvae were fed upon a mixture of yeast powder and ground dog biscuits. The pupae formed were collected and transferred to the cloth cages for adult emergence. Freshly molted larvae were continuously available for the mosquito larvicidal experiments.

2.2 Bioassay of Temephos

Temephos were diluted to obtain stock solutions of 10 mL/L in dechlorinated tap water. Different working test concentrations were prepared by diluting the stock solution for the exposure to mosquito larvae. Larval bioassays were performed on third instance, according to the standard procedure of WHO (2005b) [38]. A batch of 20 larvae was exposed to each working concentration independently. A minimum of three replicates was kept for each concentration along with the control. The moribund and dead larvae in replicates were combined and expressed as percentage mortality at each concentration. The larvae were considered as dead or moribund, if they were not responsive to a gentle prodding with a fine needle. All bioassays were carried out at room temperature of 27±2 ºC and 70–80% relative humidity for 24 and 48 hrs of duration. Data obtained were corrected by Abbot's formula (Abbott 1925) and LC_{50} and LC_{90} values along with other statistical values were analyzed by employing probit analysis (Finney 1971) [12].

2.3 Phytoextract preparation and Bioassay

The stems of *C. reflexa* were collected from different localities of Sikandra and Dayalbagh areas of Agra, India (Fig. 1). The stems were then washed in running tap water and dried in the shade. The shade dried stems are crushed mechanically and subjected to extraction with petroleum ether, hexane and methanol subsequently in a Soxhlet apparatus for 72 hrs. Extracts were concentrated by removing the solvent by a vacuum rotatory evaporator. The crude extracts obtained are finally weighed and kept in refrigerator below 5 ºC until further use. (Bhan *et al*. 2013a) [4].

For bioassay 10 g pure residues were dissolved in ethanol to get stock solutions of 50,000 mg/L. A range of working test concentrations was prepared for each extract by further diluting these stocks and the bioassay were conducted with the same procedure as depicted as above.

2.4 Combinatorial preparation and Bioassay

For combinatorial studies, 10 mg/L stock solution of temephos and the petroleum ether extract (PEE) were prepared individually. Keeping temephos as the standard, its stock was mixed with the stock of PEE in ratios of 1:1, 1:2 and 1:4. A range of desired test concentrations for each mixed formulation ratio were prepared by further diluting the combination with water. The mortality data were recorded after 24, 48 and 72 hours of exposure and the larvicidal efficacy of each formulation was observed as above said.

2.5 Statistical Analysis

The mortality data were subjected to Probit Analysis (Finney 1971) to obtain LC_{50} and LC_{90}, standard error, regression equation and fiducial limits at 95% confidence limits. The co-toxicity coefficient (Sarup *et al*. 1980) [29] and synergistic factor (Kalayanasundaram and Das 1985) [16] for the mixed formulation were calculated.

$$\text{Coefficient} = \frac{\text{Toxicity of Co-toxicity insecticide (alone)}}{\text{Toxicity of insecticide with plant extract}} \times 100$$

$$\text{Factor (SF)} = \frac{\text{Toxicity of Synergistic insecticide (alone)}}{\text{Toxicity of insecticide with plant extract}} \times 100$$

SF value > 1, indicates synergism and SF value < 1, indicates antagonism.

3. Results

3.1 Bioefficacy of Temephos

Table 1 and 2 reveal the larvicidal potentiality of Temephos against both larvae. The larvicidal potentiality of temephos against *An. stephensi* was with the LC_{50} values 0.0025, 0.0023 and 0.0020 mL/L after 24, 48 and 72 hrs. The LC_{90} values were 0.0052, 0.0040 and 0.004 mL/L after 24, 48 and 72 hrs. The LC_{50} values were 0.006, 0.005 and 0.004 mL/L after 24, 48 and 72 hrs. The LC_{90} values were 0.018, 0.016 and 0.011 mL/L after 24, 48 and 72 hrs of treatment against culicine larvae respectively.

3.2 Bioefficacy of phytoextract

The larvicidal potentiality of crude extracts of *C. reflexa* against *An. stephensi* and *Cx. quinquefasciatus* were mentioned in table 1 and 2. The mortality data revealed that the PEE was the most effective followed by hexane and methanol extract. PEE was the most effective extract with

LC_{50} 39.251, 33.180 and 20.032 mg/L against anopheline larvae and 48.625, 31.869 and 21.667 mg/L against culicine larvae after 24, 48 and 72 hours of treatment. LC_{90} values were 292.771, 229.935 and 134.976 mg/L against anopheline larvae and 266.272, 175.041 and 156.014 mg/L against culicine larvae after 24, 48 and 72 hrs of treatment, respectively.

3.3 Bioefficacy of Combination

The bioefficacy of combinatorial studies of different ratios of synthetic insecticide, Temephos and petroleum ether extract of *C. reflexa* against anopheline larvae were depicted in Table 3 and Fig. 2. The results revealed that the combinatorial ratio 1:1 was the most effective than 1:2 and 1:4. The LC_{50} values for 1:1 were 0.0013, 0.0010 and 0.0009 mg/L after 24, 48 and 72 hrs. The LC_{90} values were 0.0103, 0.0067 and 0.0042 mg/L after 24, 48 and 72 hours of exposure, accordingly. The co-toxicity coefficient for the 1:1 were 178.571, 1.917 and 1.818 with synergistic factor 1.786, 1.917 and 1.818 at 24, 48 and 72 hrs respectively at the LC_{50} showing synergistic action. For the LC_{90} co-toxicity coefficient was 50.485, 56.338 and 80 with synergistic factor 0.504, 0.563 and 0.8 at 24, 48 and 72 hrs respectively with antagonistic action.

Table 4 and Fig. 3 illustrates the combinatorial bioassay of different combinatorial ratios of temephos and crude petroleum ether extract of *C. reflexa* against culicine larvae. The data shows that the combinatorial ratio 1:1 was the most effective than 1:2 and 1:4. The ratio 1:1 has the LC_{50} value 0.0016, 0.0014 and 0.0013 mg/L after 24, 48 and 72 hrs. The LC_{90} values were 0.0082, 0.0085 and 0.006 mg/L after 24, 48 and 72 hours of exposure, accordingly. The co-toxicity coefficient for the 1:1 were 375, 357.14 and 307.6 with synergistic factor 3.75, 3.571 and 3.076 at 24, 48 and 72 hrs respectively, for the LC_{50} and with the LC_{90} co-toxicity coefficient was 219.512, 188.235 and 183.333 with synergistic factor 2.195, 1.883 and 1.833 at 24, 48 and 72 hrs respectively with the synergistic action in each case.

4. Discussion

The pesticides of plant origin are efficient, biodegradable as well as a suitable alternative for mosquito control. Shaalan *et al.* (2005a) [31] has reviewed on different mosquito larvicidal plant species with growth retarding, reproduction inhibiting, ovicides, synergistic, additive and antagonistic activities of botanical mixture. Various other workers have reported botanicals as effective mosquito larvicides (Raveen *et al*. 2014; Ghosh *et al*. 2012; Kovendan and Murugan 2011) [28, 13, 18]. In the present study, among different solvent extracts of *C. reflexa* Petroleum ether extract was more effective against both larvae followed by hexane and methanol extracts. Our work is in agreement with Shaalan *et al*. (2005b) [32] who reported that Petroleum ether (Polarity index= 0.1) appears to have been one of the best solvents of choice for extracting non-polar bioactive phytoproducts. The present solvent extraction results are supported by findings of Mohan *et al.* (2007) that PE extract of *Solanum xanthocarpum* against *An. stephensi* exhibited maximum larvicidal activity with LC_{50} 1.41 and 0.93 ppm and LC_{90} 16.94 and 8.48 ppm.

The synergistic effect of various control agents have proved very advantageous in the control of various pests (Caraballo 2000 and Seyoum *et al.* 2002) [6, 30]. The work regarding synergism was supported by Thangam and Kathiresan 1991b) [35], who studied the synergistic properties of *Rhizophora apiculata*, *Caulerpa scalpelliformis* and *Dictyota dichotoma* individually and with DDT. The joint action using a combination of botanical extracts and different synthetic insecticide against several vectors have been supported by several previous studies (Kalayanasundaram and Das 1985, Mohan *et al*. 2006 and 2007, Shaalan *et al*. 2005a, 2005b, Thangam and Kathiresan 1990) [16, 24, 25, 31, 32, 34]. Kalayanasundaram and Das (1985) [16] studied the larvicidal activity of some plant extracts in combination with phenthoate and fenthion against *An. stephensi* and synergism were observed with fenthion and *Vinca rosea*, *Leucas aspera*, *Pedalium murex*, *Clerodendron inerme*, *Turnera ulmifolia* and *Parthenium hysterophorus* extract with SF of 1.40, 1.31, 1.61, 1.48, 1.38 and 2.23 respectively. Mohan *et al.* (2007) reported synergism of *S. xanthocarpum* with a synthetic pyrethroid, Cypermethrin against *An. stephensi* at 1:1 with SF 6.83.

In our study, combined application of Temephos and the PEE extract of *C. reflexa* revealed the synergistic action of the extract against anopheline and culicine larvae at 1:1 ratio. The binary mixture proved to be more effective than all the both insecticide and phytoextracts when applied individually. Among the different ratios studied, the 1:1 combination was the most effective followed by 1:2 and 1:4 against both the larvae. This is due to the increasing amount of plant extract, the synergistic action decreased signifying the synergistic activity of the combination decreases with the increasing concentrations of plant extract. The synergistic activity may be due to the plant extract inhibiting some factors which can act against synthetic chemicals, as reported in *Aedes aegypti* (Thangam and Kathiresan 1991a) [34]. Such strategies will minimize the problem of induction of resistance in the pest population and will apparently continue to render the extracts 'effective' for many years as pest control agents. Thus far, studies on synergistic/toxic effects of binary mixtures involving phytochemicals and synthetic insecticides have been conducted on agricultural pests (Mesbah *et al.* 2006, Mahmoud 2007, Mesbah *et al*. 2007) [22, 21, 23].

In the present study, the apparent synergistic effect of Temephos and the PEE extract of *C. reflexa* has been evident at ratio 1:1 on the basis of CTC with 178.57 and 375 and SF 1.786 and 3.75 against anopheline and culicine larvae. The CTC of the mixture was the measurement of the combined toxicity of two toxic chemicals in the mixture. Thus, the type of action involved in the mixture, has been categorized on the basis of values >100 and >1 in the case of CTC and SF, respectively. Mixtures of phytochemicals and insecticides were found to be more effective than insecticides or phytochemicals alone and could be a good ecofriendly approach to reduce the dose of chemicals to be applied in vector control programs. Besides, such mixtures could reduce the costs, prolong the lifetime of available insecticides, and regulate insecticide resistance as part of integrated vector management. The present study is in agreement with the previous studies on botanicals and mixed formulations on the mosquitocidal activity (El-Gougary 1998, Harvae and Kamath 2004, Vanmathi and Rajakumari 2004). Bhan *et al.* (2013b) [10, 15, 36, 6] observed the synergistic efficacy of Temephos and *Aspergillus flavus* against larvae, *An. stephensi* at 1:1 with SF 1.155 and 2.105. Synergism between synthetic insecticides and phytochemicals appears to be quite effective. For instance, *S. xanthocarpum* extract induced synergism with cypermethrin against larvae, *Cx. quinquefasciatus* (Mohan *et al*. 2006) [25]. Synergistic efficacy of botanical blends with and without synthetic insecticides was found against *Aedes aegypti* and *Cx. annulirostris* mosquito larvae (Shaalan *et al.* 2005a) [31]. Synergistic efficacy of piperonyl butoxide with deltamethrin as pyrethroid insecticide on *Cx. tritaeniorhynchus* and other mosquito species was reported by Fakoorziba *et al.* (2008) [11].

Bhan *et al*. (2013a) [4] studied the combinatorial potentiality of *Aspergillus flavus* and *C. reflexa* against mosquito larvae, *An. stephensi* and *Cx. quinquefasciatus.* The present study attempts to suggest a better alternative or an effective substitute in the form of a synergistic mixture of temephos and *C. reflexa* extracts, which has been widely acknowledged and currently available as a prominent biopesticide.

4.1 Tables and Figures

Fig 1: *Cuscuta reflexa* on Host plant

ST: Stem; HP: Host Plant

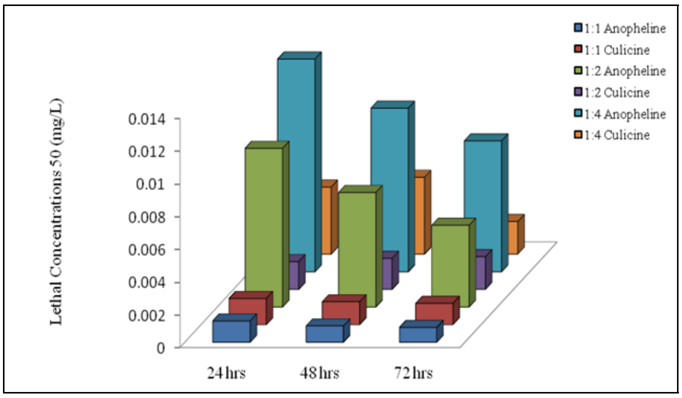

Fig 2: Comparative lethal concentrations 50 of different combination ratios of Temephos with PEE of *C. reflexa* against anopheline and culicine larvae

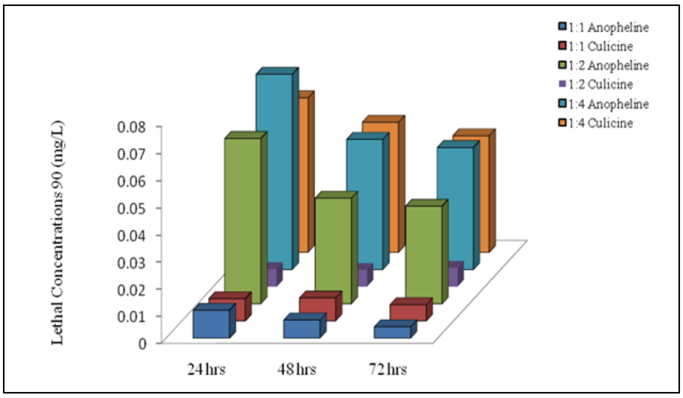

Fig 3: Comparative lethal concentrations 90 of different combination ratios of Temephos with PEE of *C. reflexa* against anopheline and culicine larvae

Table 1: Larvicidal potentiality of Temephos and phytoextracts of *C. reflexa* against, *An. Stephensi*

Substance Tested	Exposure period (Hours)	Chi-square	Regression equation	LC_{50}± SE (Fiducial limits) (mg/L)	LC_{90}± SE (Fiducial limits) (mg/L)
Temephos	24	1.351	2.735x+8.331	0.0060±0.0012 (0.0083-0.0038)	0.018±0.0085 (0.034-0.0011)
	48	2.084	2.721x+8.407	0.0055±0.0010 (0.0076-0.0035)	0.016±0.0076 (0.031-0.0016)
	72	0.564	2.990x+9.103	0.0042±0.0006 (0.0054-0.0030)	0.011±0.0038 (0.019-0.0037)
Petroleum ether	24	3.655	1.735x+0.337	48.625±12.680 (73.478-23.771)	266.272±111.698 (485.201-47.343)
	48	2.487	1.732x+0.663	31.869±9.126 (49.755-13.982)	175.041±64.716 (301.885-48.196)
	72	1.1580	1.495x+1.508	21.667±7.486 (36.341-6.994)	156.014±64.051 (281.555-30.474)
Hexane	24	1.730	2.432x-2.399	110.298±26.014 (161.285-59.311)	371.201±97.053 (561.424-180.977)
	48	1.235	2.474x-2.242	84.527±23.830 (131.235-37.819)	278.597±64.308 (404.642-152.553)
	72	1.294	2.671x-2.534	66.103±21.338 (107.927-24.279)	199.502±41.834 (281.497-117.506)
Methanol	24	2.324	3.161x-6.081	320.126 ±42.170 (402.778-237.47)	814.202±224.876 (1254.96-373.443)
	48	2.064	3.160x-5.920	285.519 ±38.428 (360.839-210.10)	726.448±185.134 (1089.31-363.486)
	72	2.202	3.231x-5.930	241.274±33.241 (306.426-176.122)	601.349±132.687 (861.416-341.281)

Table 2: Larvicidal potentiality of Temephos and phytoextracts of *C. reflexa* against *Cx. quinquefasciatus*

Substance Tested	Exposure period (Hours)	Chi-square	Regression equation	LC_{50}± SE (Fiducial limits) (mg/L)	LC_{90}± SE (Fiducial limits) (mg/L)
Temephos	24	1.351	2.735x+8.331	0.0060±0.0012 (0.0083-0.0038)	0.018±0.0085 (0.034-0.0011)
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	72	2.202	3.231x-5.930	241.274±33.241 (306.426-176.122)	601.349±132.687 (861.416-341.281)

Table 3: Larvicidal toxicity of different combination ratios of Temephos with PEE of *C. reflexa* against *An. Stephensi*

Ratio	**Exposure period (Hours)**	**Chi-square**	**Regression equation**	**LC$_{50}$ ±SE (Fiducial limits) (mg/L)**	**SF**	**CTC**	**Type of action**	**LC$_{90}$ ±SE (Fiducial limits) (mg/L)**	**SF**	**CTC**	**Type of action**
1:1	24	0.288	1.46x+7.73	0.0013±0.0004 (0.0022-.0004)	1.786	178.571	S	0.0075±0.0030 (0.013-0.0015)	0.504	50.485	A
	48	0.085	1.58x+8.13	0.0010±0.0004 (0.0018-.0003)	1.917	191.667	S	0.0067±0.0027 (0.012-0.0013)	0.563	56.338	A
	72	0.520	2.03x+9.08	0.0009±0.0003 (0.0016-0.0004)	1.818	181.818	S	0.0042±0.0013 (0.0068-.0016)	0.8	80	A
1:2	24	1.218	1.59x+6.62	0.0097±0.003 (0.0157-0.0037)	0.862	86.207	A	0.061±0.030 (0.121-0.0016)	0.391	39.097	A
	48	0.428	1.70x+6.98	0.007-0.0021 (0.011-0.003)	2.091	209.09	S	0.039±0.018 (0.074-0.004)	2	200	S
	72	0.498	1.53x+6.96	0.005±0.002 (0.009-0.002)	0.4	40	A	0.036±0.018 (0.071-0.0009)	0.111	11.111	A
1:4	24	2.040	1.74x+6.53	0.013±0.004 (0.021-0.006)	0.438	43.860	A	0.072±0.034 (0.138-0.006)	0.121	12.094	A
	48	0.767	1.83x+6.86	0.010±0.003 (0.0152-0.004)	1.917	191.67	S	0.048±0.021 (0.090-0.007)	2	200	S
	72	0.827	1.66x+6.85	0.008±0.002 (0.012-0.003)	0.25	25	A	0.045±0.021 (0.087-0.003)	0.08	8.889	A

CTC, Co-toxicity coefficient; SF, Synergistic Factor

Table 4: Larvicidal toxicity of different combination ratios of Temephos with PEE of *C. reflexa* against *Cx. quinquefasciatus*

Ratio	Exposure period (Hours)	Chi-square	Regression equation	LC_{50} ±SE (UL-LL) (mg/L)	SF	CTC	Type of action	LC_{90} ±SE (UL-LL) (mg/L)	SF	CTC	Type of action
1:1	24	0.39	1.79x+8.22	0.0016±0.0003 (0.002-0.0011)	3.75	375	S	0.0082±0.002 (0.013-0.003)	2.19	219.51	S
	48	0.59	1.66x+8.06	0.0014±0.0003 (0.0019-0.0009)	3.57	357.14	S	0.0085±0.003 (0.013-0.0031)	1.88	188.23	S
	72	1.19	1.90x+ 8.59	0.0013±0.0002 (0.002-0.0008)	3.08	307.6	S	0.006±0.002 (0.009-0.003)	1.83	183.33	S
1:2	24	1.83	2.23x+8.93	0.0017±0.0003 (0.0023-0.0011)	3.53	352.94	S	0.0065±0.0018 (0.0099-0.0030)	2.74	273.85	S
	48	2.40	2.52x+9.32	0.0019-0.0027 (0.0024-0.0014)	2.95	294.74	S	0.0062±0.0015 (0.0091-0.0033)	2.66	266.12	S
	72	2.67	2.10x+8.75	0.002±0.0003 (0.002-0.001)	2	200	S	0.007±0.002 (0.010-0.003)	1.57	157.14	S
1:4	24	0.92	1.12x+6.55	0.0041±0.0012 (0.0064-0.0018)	1.28	127.66	S	0.057±0.029 (0.114-0.0003)	0.31	31.23	A
	48	1.56	1.26x+6.67	0.0047±0.0011 (0.0070-0.0025)	1.19	119.15	S	0.049±0.021 (0.090-0.0066)	0.34	33.95	A
	72	2.01	1.00x+6.65	0.002±0.001 (0.004-0.0001)	2	200	S	0.043±0.020 (0.083-0.003)	0.26	25.58	A

CTC, Co-toxicity coefficient; SF, Synergistic Factor

5. Conclusion

It has been concluded from the results that the combined use of Temephos and *C. reflexa* against both the larvae may improve mosquito control effectively than the individual pesticides of the mixture. This reduces the indiscriminate application of harmful chemical pesticides. Therefore, synergism is a supplementary and complementary eco-friendly measure to combat mosquito vectors.

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