Research Article CODEN: IJPRNK ISSN: 2277-8713
C. N. Srivastava, IJPRBS, 2015; Volume 4(3): 69-82 IJPRBS

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

SYNERGISTIC ACTIVITY OF TEMEPHOS AND *PSEUDOCALYMMA ALLIACEUM* LEAVES AGAINST *ANOPHELES STEPHENSI* AND *CULEX QUINQUEFASCIATUS* LARVAE

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Accepted Date: 04/05/2015; Published Date: 27/06/2015

Abstract: The larvicidal activity of different combinations of synthetic insecticide, Temephos with leaves extract of *Pseudocalymma alliaceum* against *Anopheles stephensi* and *Culex quinquefasciatus.* The experiments were conducted according WHO standard procedure. Combination 1:2 was found to be the most potent as compared to 1:1 and 1:4 ratio and shows synergism against both the target species and shows LC_{50} values 0.0008 and 0.0004 ppm and LC_{90} values were 0.0047 and 0.0013 ppm after 24 and 48 hours of treatment, respectively. In case of *Cx. quinquefasciatus* the ratio 1:2 exhibits LC_{50} values 0.0016 and 0.0003 ppm and LC_{90} values were 0.0139 and 0.0022 ppm after 24 and 48 hours of treatment, respectively. The present study concluded that the combination of tested extract possesses more effective as compared to its individual components. So, this formulation is cost-effective and eco- friendly and also used to control the mosquito larval population.

Keywords: *Pseudocalymma alliaceum,* Temephos, Mosquito larvae, Synergism, Arbovirus, Synthetic insecticide.

PAPER-QR CODE

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Access Online On:

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How to Cite This Article:

C. N. Srivastava, IJPRBS, 2015; Volume 4(3): 69-82

INTRODUCTION

Mosquito, a flying insect of family Culicidae, serves as a crucial vector for a number of arboviruses and parasites that are maintained in nature through biological transmission between susceptible vertebrate hosts by blood sucking arthropods. They are responsible for encephalitis, dengue, malaria, rift valley fever, yellow fever and filariasis. Mosquitoes are not only the vector for the transmission of these diseases, they also act as an irritating agent to man by causing allergic responses that include local skin reactions as well as systemic reactions, such as angioderma and urticaria(Peng et al. 1999)[1].

Malaria is one of the most serious diseases of the tropical regions and accounts for 310-515 million clinical cases annually with 1.5-3.0 million deaths per year (Snow et al. 2005)[2]. In India, 2-3 million malaria cases and about 1,000 deaths are reported every year (Lal et al. 2010)[3]. There were 216 million cases of malaria and an estimated 6,55,000 deaths in 2010 (WHO, 2011)[4]. Lymphatic filariasis transmitted by *Cx. quinquefasciatus*, infects 120 million people worldwide with 44 million people demonstrating chronic manifestations of enlarged limbs and/or genital regions (Bernhard et al. 2003[5]; Rahuman and Venkatesan, 2008[6]). Since from several years, synthetic insecticides were used. They are fast acting, highly active and cost effective use in integrated pest management programmes is made difficult by their wide spectrum of activity and, therefore, toxic to natural enemies. Therefore, new tools of insect pest management are needed, and botanical insecticides as well as plant derived semio-chemicals are now actively sought as leads towards more eco-friendly alternatives (Mohan et al. 2013)[7]. Several plant extracts have been evaluated for their activity against mosquito species, but they are time consuming and the excessive amount of plant material needed to get the required amount of residue. Therefore, to enhance their slower action and to reduce their requirement, an integrated approach is required for efficient, eco-friendly and cost effective management of vectors covering the combined application of different aspects of vector control.

Insecticide synergists have been recommended as a powerful research tools for diagnosing resistance mechanisms, reduce the usage of plant materials, determining the confirmation of target sites and elucidating metabolic pathways (Bernard and Philogene, 1993)[8]. Therefore, the present study demonstrates the synergistic action of an insecticidal plant, *Pseudocalymma alliaceum* and Temephos against *Anopheles stephensi* and *Culex quinquefasciatus*.

MATERIALS & METHODS

Maintenance of mosquito colony

The mosquito species i.e. *An. stephensi* and *Cx. quinquefasciatus* were reared in our laboratory under control conditions of 27±1 ºC, 85 % relative humidity (RH) and a normal photoperiod from eggs initially collected from cyclic colony at the National Institute of Malaria Research, New Delhi. The eggs were immersed in dechlorinated tap water in enamel basins of 30 cm diameter. The hatched larvae were fed brewer's yeast. The transformed pupae were separated manually with a glass dropper into a 500 ml beaker with water and introduced into adult cages of 12″ x 12″ x 12″ for adult emergence. Adult mosquitoes were fed a glucose meal (cotton soaked in 10 % glucose solution). Albino rabbits were used to provide a blood meal for adult female mosquitoes after the second day of emergence, and every third day thereafter. Moist filter paper was kept in a beaker in the cages for mosquitoes to lay eggs on. Eggs laid on the filter paper were immersed in larval basins containing water for the maintenance of the colony.

Bioassay of synthetic insecticide

The temephos (50 % EC) was obtained from District Malaria Office, Agra (India), were used for bioassay test against *Anopheles* and *Culex* larvae. Temephos was diluted to obtain stock solutions of 10 ppm by dissolving 0.01 ml of temephos in 1000 ml of dechlorinated tap water. Different working test concentrations of 250 ml ranging from 0.0005 ppm to 0.16 ppm were prepared in 500 ml capacity of Borosil glass beakers by diluting the stock solution for the exposure to mosquito larvae. Twenty, third instar larvae of *Anopheles* and *Culex* were exposed to different concentration independently. The experiments were conducted in three replicates with a control parallel. Mortality observations were recorded 24 and 48 hrs post-exposure separately. All the experiments were devised according to WHO standard procedure (2005)[9]. The mortality data were then subjected to probit analysis (Finney, 1971)[10] to calculate LC_{50} and LC_{90} values with other statistical analysis. Experiments with >20% mortality in the controls were discarded and repeated. Mortality values ranging from 5-20% in the controls were corrected by using Abbott's formula (Abbot, 1925)[11] so as to remove the factors working other than the larvicidal combinations:

Corrected % mortality = [T-C]/[100-C] × 100

Where, T is the per cent mortality in the test concentrations and C is the per cent mortality in the control.

Bioassay of Phytoextracts

Leaves of *Pseudocalymma alliaceum* (*P. alliaceum*) were collected from the botanical garden of Dayalbagh Educational Institute (Deemed University), campus (Figure 1). After washing the leaves were dried in the shade and crushed manually. The crushed leaves were subjected separately to different solvents (petroleum ether, hexane and methanol successively) in a Soxhlet apparatus (Borosil, Mumbai, India) for up to 72 h in each solvent for complete extraction. Each extract was subjected to vacuum rotary evaporator to remove solvent and get concentrated crude extract. The crude extract was dissolved in different amount in ethanol to get a stock solution. The residue was stored in a glass beaker and covered it with aluminium foil and maintain at 3ºC in a refrigerator until further use. The dry leaves of *P. alliaceum* yields petroleum ether, hexane and methanol extracts were 60.80, 2.50 and 216.40 g/kg, respectively. Stock solution of the petroleum ether extract of *P. alliaceum* was prepared by dissolving 1.6 g crude extract in 100 ml ethanol to obtain a final concentration of 16,000 ppm. For the hexane extract, 0.16 g extract was dissolved in 100 ml ethanol to obtain a final concentration of 1,600 ppm as a stock solution and for the methanol extract, 2 g extract was dissolved in 100 ml of ethanol to obtain a final concentration of 20,000 ppm as stock solution. These stocks were further diluted to get desired working concentration and follow the same procedure for bioassay as above said. Experiments were set in triplicates along with control.

Figure 1: *Pseudocalymma alliaceum*

Combined efficacy of Temephos and Phytoextract

For combinatorial studies, 10 ppm stock of temephos and the most efficient phytoextract was prepared. Keeping temephos as a standard, its stock was mixed with the stock of phytoextract in ratios of 1:1, 1:2 and 1:4. Test concentration for each of the mixed formulation ratios were prepared by further diluting the combined mixture in water. Larval efficacy for each formulation was observed as above and lethal concentration LC_{50} as well as LC_{90} were determined. Co-toxicity coefficient (CTC) (Sarup et al. 1980)[12] and a synergistic factor (SF) (Kalyansundaram and Das, 1985)[13] for mixed formulation were calculated after calculating LC_{50} and LC_{90} for each combination.

$$\text{Co-toxicity coefficient} = \frac{\text{[Toxicity of insecticide alone]}}{\text{[Toxicity of insecticide alone with plant extract]}} \times 100$$

$$\text{Synergistic factor} = \frac{\text{[Toxicity of insecticide alone]}}{\text{[Toxicity of insecticide with plant extract]}}$$

SF value > 1; indicates synergism and

SF value < 1; indicates antagonism.

RESULTS

Bioefficacy of synthetic insecticide (Temephos)

The larvicidal potential of temephos against *Anopheles* and *Culex* is depicted in Table 1 & 2. The LC_{50} values for temephos against *An. stephensi* were 0.0024 and 0.00085 ppm after 24 and 48 hr of exposure respectively. The LC_{90} values were 0.0071 and 0.0019 ppm after 24 and 48 hr of treatment respectively (Table 1, Fig. 2).The LC_{50} values for temephos against *Cx. quinquefasciatus* were 0.0023 and 0.0014 ppm after 24 and 48 hr of exposure respectively. The LC_{90} values were 0.0128 and 0.0054 ppm after 24 and 48 hr of treatment respectively (Table 2, Fig. 3). The result shows that the target species *Cx. quinquefasciatus* larvae was the most susceptible against Temephos as compared to *An. stephensi*.

Bioefficacy of crude hexane extract of *P. alliaceum*

The LC_{50} values of the most effective hexane extract of *P. alliaceum* against *An. stephensi* were 8.65 and 7.49 ppm after 24 and 48 hr of exposure respectively. The LC_{90} values were 31.00 and 22.11 ppm after 24 and 48 hr of treatment respectively (Shrankhla et al. 2012)[14].The LC_{50} values of the most effective hexane extract of *P. alliaceum* against *Cx. quinquefasciatus* were 2.49 and 1.16 ppm after 24 and 48 hr of exposure respectively. The LC_{90} values were 15.06 and 8.45 ppm after 24 and 48 hr of treatment respectively (Shrankhla et al. 2011)[15].This data reveals that the crude hexane extract of *P. alliaceum* were the most effective against *Cx. quinquefasciatus* larvae as compared to other tested extracts.

Combinatorial bioassay (Synthetic Insecticide + Phytoextract)

The results of the bioassay of different ratios of Temephos and hexane extract of *P. alliaceum* leaves against *An. stephensi* larvae are both the species shown in same table (Table 2). The ratio 1:1 shows LC_{50} and LC_{90} values were 0.0135 and 0.0014 ppm and 0.1007 and 0.0317 ppm after 24 and 48 hr of exposure, respectively. The LC_{50} and LC_{90} values for ratio 1:2 were 0.0008 and 0.0004 ppm and 0.0047 and 0.0013 ppm after 24 and 48 hr of exposure, respectively. The LC_{50} and LC_{90} values for ratio 1:4 were 0.0014 and 0.0007 ppm and 0.0085 and 0.0036 ppm after 24 and 48 hr of treatment, respectively.

For the LC_{50} value, the combined factor of the ratio 1:1 was 0.178 and 0.607 after 24 and 48 hr of treatment, respectively, antagonism was seen at booth time points. In case of LC_{90} value, the combined factor was 0.071 and 0.059 after 24 and 48 hr of treatment, respectively, antagonism was seen at both time points. For the ratio 1:2, the combined factor was 2.927 and 2.125 for LC_{50} after 24 and 48 hr of treatment, respectively, synergistic activity was seen against the *An. stephensi* larvae at both time points. In case of LC_{90} value, the combined factor was 1.511 and 1.462 after 24 and 48 hr of treatment, respectively: synergism was seen at both time points. The ratio 1:4 had a combined factor was 1.714 and 1.133 for LC_{50} after 24 and 48 hr of treatment, respectively, synergistic activity was seen against the *An. stephensi* larvae at both the points. In case of LC_{90} value, the combined factor was 0.835 and 0.531 after 24 and 48 hr of treatment, respectively, antagonism was seen in both time points which indicates that the efficacy of the combined extract reduces its efficiency when the time increases.

The results of the bioassay of different ratios of Temephos and hexane extract of *P. alliaceum* leaves against *Cx. quinquefasciatus* larvae are shown in Table 2. The ratio 1:1 had LC_{50} and LC_{90} values were 0.0153 and 0.0028 ppm and 0.1070 and 0.0299 ppm after 24 and 48 hr of exposure, respectively. The LC_{50} and LC_{90} values for the ratio of 1:2 of Temephos and plant extract were 0.0016 and 0.0003 ppm and 0.0139 and 0.0022 ppm after 24 and 48 hr of

exposure, respectively. The LC_{50} and LC_{90} values for the ratio of 1:4 were 0.0014 and 0.0008 ppm and 0.0085 and 0.0046 ppm after 24 and 48 hr of treatment, respectively.

For the LC_{50} value, the combined factor of the ratio 1:1 was 0.150 and 0.5 after 24 and 48 hr of treatment, respectively, antagonism was seen at booth time points. In case of LC_{90} value, the combined factor was 0.119 and 0.181 after 24 and 48 hr of treatment, respectively, antagonism was seen at both time points. For the ratio 1:2, the combined factor was 1.437 and 5.185 for LC_{50} after 24 and 48 hr of treatment, respectively, synergistic activity was seen against the *Cx. quinquefasciatus* larvae at both time points indicates that the efficacy of the combination increases 5 times as compared to the 24 hr. In case of LC_{90} value, the combined factor was 0.921 and 2.7 after 24 and 48 hr of treatment, respectively, antagonism was seen in 24 hr and synergism was seen at 48 hr indicates that the efficacy of the combination of synthetic insecticide and plant extract is directly proportional to the time. The ratio 1:4 had a combined factor was 1.642 and 1.867 for LC_{50}after 24 and 48 hr of treatment, respectively, synergistic activity was seen against the *Cx. quinquefasciatus* larvae at both the points. In case of LC_{90} value, the combined factor was 1.506 and 1.174 after 24 and 48 hr of treatment, respectively; synergism was seen in both time points.

DISCUSSION

The present investigation reveals that the combination of temephos and the hexane extract of *Pseudocalymma alliaceum* show the optimum efficacy in ratio 1:2 against *An. stephensi* and *Cx. quinquefasciatus*. The efficacy of hexane extract of *Pseudocalymma alliaceum* was found to be the more effective against *Anopheles stephensi* larvae with LC_{50} value 8.65 ppm and *Culex quinquefasciatus* larvae with LC_{50} value 2.49 ppm as compared to the other tested extracts of *Pseudocalymma alliaceum.* The crude extract of *Murraya koenigii* (L.) hexane extract was found effective against *Anopheles stephensi* with its LC_{50} value 418.7 ppm (Arivoli et al. 2011)[16]. The larvicidal effect of *Moringa oleifera* against *An. stephensi* with LC_{50} value 72.45 ppm was studied by Prabhu et al. (2011)[17]. The ethanol extract of *Cassia occidentalis* was effective against *An. stephensi* with LC_{50} value 70.56 ppm (Dhandapani et al. 2011)[18]. The LC_{50} value of ethyl acetate extract of *Abrus precatorius* was 19.31 µg/ml against *An. Vagus* (Bagavan and Rahuman, 2011)[19].

The susceptibility of fenthion in Hyderabad city with an LC_{50} value 0.1 ppm against the *Cx. quinquefasciatus* larvae and reported the 99 to 100% mortality (Rao et al. 2004)[20]. The larvicidal susceptibility of Melathion in Kuala Lumpur city of Malaysia with an LC_{50} value 0.0078 mg/L against *Cx. quinquefasciatus* was observed by Nazni et al. (2005)[21]. The mortality rates of *Cx. quinquefasciatus* to different insecticides, in which Cyfluthr in shows the highest mortality rate i.e. 100% and 99% in the lab strains and field strains, respectively (Vatandoost et al. 2004)[22].

The synergistic effects observed in bioassays using a combination of botanical extracts and different synthetic insecticides have been observed in several previous studies (Kalyansundaram and Babu, 1982)[23]. The larvicidal activity of some plant extracts in combination with phenthoate and fenthion against *An. stephensi* (Kalyansundaram and Das, 1985)[13]. The three different insecticides with the plant, *Bougainvillea glabra* with LC_{50} value of DDT were 0.69 and 0.74 µg/l, BHC value were 10.96 and 11.22 µg/l and melathion were 14.45 and 18.20 µg/l after 24 and 48 hr of treatment, respectively against the *Cx. sitiens* (Thangam and Kathiresan, 1990)[24]. The joint action of binary mixtures of some plant extracts with each other and with the synthetic pyrethroid insecticide cypermethrin against *Cx. pipiens* larvae (Moawed, 1998)[25]. Neem kernel extract has synergistic effects when combined with juvenile hormone along methoprene against *Ae. aegypti*, *Ae. togoi* and *An. stephensi* (Mulla and Su, 1999)[26]. Bioassay of the combination of cypermethrin and petroleum ether root extract of *Solanum xanthocarpum* with LC_{50} value of ratio 1:1 were 0.0055 and 0.0050 after 24 and 48 hr of exposure. At ratio 1:2, the LC_{50} values were 0.0058 and 0.0054 ppm at 24 and 48 hr of exposure, respectively against *Cx. quinquefasciatus* (Mohan et al. 2006)[27]. The cypermethrin and *Solanum xanthocarpum* ratio 1:2 gave an LC_{50} value 0.0057 and 0.0047 ppm against *An. stephensi* after 24 and 48 h of exposure, respectively (Mohan et al. 2007)[28]. The larvicidal activity on the combination of malathion and the two plants *Ficus benghalensis* and *Calotrophis procera* (Ali and El-Rabaa, 2012)[29].The combinations of imidacloprid with petroleum ether crude extract of *Occimum basilicum* with LC_{50} value 0.011 and 0.007 ppm of ratio 1:1 after 24 and 48 h of exposure against *An. stephensi* (Maurya et al. 2012)[30]. The hexane extract of *P. alliaceum* is an effective mosquito larvicides and its efficacy enhanced by using the synthetic insecticide temephos due to synergism. This approach helps in minimizing the usage of both the materials (plant and synthetic) and make the application more effective, economical and comparatively less hazardous to the environment. This investigation helps in the proper management to control the mosquito larvae.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

ACKNOWLEDGEMENT

The authors are thankful to Prof. Sant Prakash, Head of Zoology Department for providing laboratory facilities and Dr. V. P. Bhatnagar, Professor Emeritus, Department of Botany, Dayalbagh Educational Institute for his generous support in the authentification of plant material. The authors are also grateful to the UGC for providing the financial assistance for our research.

Table 1. Joint action of binary mixtures of temephos and hexane leaf extract of *Pseudocalymma alliaceum* against *Anopheles stephensi.*

	Ratio	Exposure period	Regression equation	Chi-square	LC_{50}±SE (Fiducial limits) ppm	SF	Type of action	LC_{90}±SE (Fiducial limits) ppm	SF	Type of action
Temephos	-	24	Y= 2.73X+9.42	1.58	0.0024±0.0005 (0.0033-0.0015)	-	-	0.0071±0.0019 (0.0109-0.0032)	-	-
		48	Y= 3.49X+12.3	0.65	0.00085±0.0002 (0.0011-0.0006)	-	-	0.0019±0.0004 (0.0027-0.0012)	-	-
**P. alliaceum* (Hexane)		24	Y= 2.31X+0.52	1.53	8.65±1.36 (11.32-5.99)	-	-	31.00±7.05 (44.81-17.19)	-	-
		48	Y= 2.73X-0.11	4.49	7.49±1.08 (9.60-5.38)	-	-	22.11±3.93 (29.82-14.39)	-	-
Temephos+ *P. alliaceum*										
	1:1	24	Y= 1.46X+6.27	2.52	0.0135±0.0036 (0.0205-0.0064)	0.178	A	0.1007±0.0451 (0.189-0.0124)	0.071	A
		48	Y= 0.94X+6.75	1.83	0.0014±0.0005 (0.0024-0.0004)	0.607	A	0.0317±0.0193 (0.0695-0.006)	0.059	A
	1:2	24	Y= 1.69X+8.54	1.74	0.0008±0.0002 (0.0012-0.0004)	2.927	S	0.0047±0.0016 (0.0077-0.0016)	1.511	S
		48	Y= 2.45X+10.9	0.95	0.0004±0.00008 (0.0006-0.0002)	2.125	S	0.0013±0.0005 (0.0023-0.0004)	1.462	S
	1:4	24	Y= 1.67X+8.07	1.69	0.0014±0.0004 (0.0022-0.0007)	1.714	S	0.0085±0.0030 (0.0144-0.0025)	0.835	A
		48	Y= 1.89X+9.02	1.79	0.0007±0.0002 (0.0011-0.0004)	1.133	S	0.0036±0.0013 (0.0061-0.0011)	0.531	A

SF = Synergistic factor, A = Antagonism, S = Synergism

***Shrankhla *et al*, (2012)**

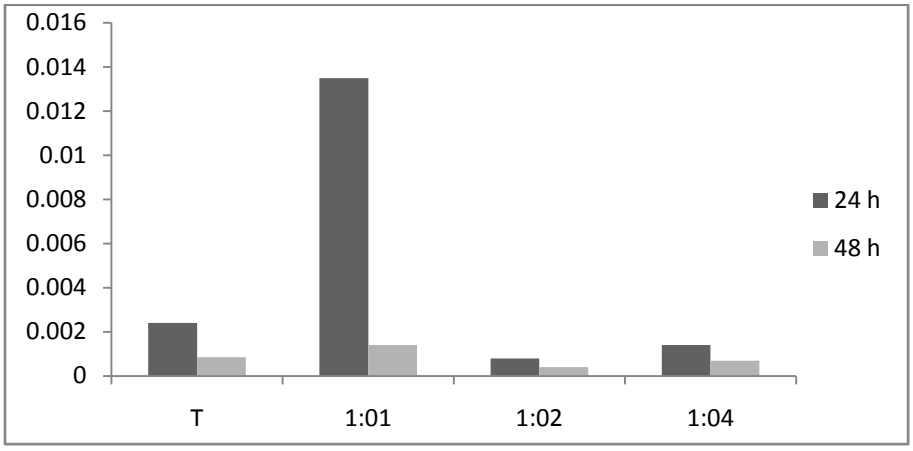

Figure 2: Comparative efficacy of Temephos and a combination of Temephos and hexane extract of *Pseudocalymma alliaceum* against *Anopheles stephensi* larvae.

Table 2. Joint action of binary mixtures of temephos and hexane leaf extract of *Pseudocalymma alliaceum* against *Culex quinquefasciatus.*

	Ratio	Exposure period	Regression equation	Chi-square	LC_{50}±SE (Fiducial limits) (ppm)	SF	Type of action	LC_{90}±SE (Fiducial limits) (ppm)	SF	Type of action
Temephos	-	24	Y= 1.75X+7.84	1.25	0.0023±0.0004 (0.0031-0.0016)	-	-	0.0128±0.0039 (0.0205-0.0051)	-	-
		48	Y= 2.17X+9.03	3.23	0.0014±0.0019 (0.0018-0.001)	-	-	0.0054±0.0012 (0.0078-0.0031)	-	-
P. alliaceum (Hexane)	-	24	Y= 1.64X+2.71	15.19	2.49±0.42 (3.32-1.67)	-	-	15.06±3.88 (22.66-7.46)	-	-
		48	Y= 1.49X+3.42	14.79	1.16±0.27 (1.68-0.63)	-	-	8.45±2.12 (12.59-4.31)	-	-
Temephos+ *P. alliaceum*										
	1:1	24	Y= 1.51X+6.23	3.54	0.0153±0.0039 (0.0231-0.0075)	0.150	A	0.1070±0.0427 (0.192-0.024)	0.119	A
		48	Y= 1.24X+6.94	1.68	0.0028±0.0011 (0.0048-0.0007)	0.5	A	0.0299±0.0142 (0.0578-0.0019)	0.181	A
	1:2	24	Y= 1.36X+7.45	0.87	0.0016±0.0007 (0.0029-0.0003)	1.437	S	0.0139±0.0057 (0.0253-0.0026)	0.921	A
		48	Y= 1.40X+8.59	1.012	0.0003±0.00009 (0.0005-0.00008)	5.185	S	0.0022±0.0009 (0.0042-0.0003)	2.7	S
	1:4	24	Y= 1.65X+8.05	1.586	0.0014±0.0005 (0.0024-0.0004)	1.642	S	0.0085±0.0031 (0.0145-0.0025)	1.506	S
		48	Y= 1.62X+8.45	1.133	0.0008±0.0003 (0.0014-0.00008)	1.867	S	0.0046±0.0017 (0.008-0.0012)	1.174	S

SF= Synergistic factor, A= Antagonism, S= Synergism

***Shrankhla *et al*, (2011)**

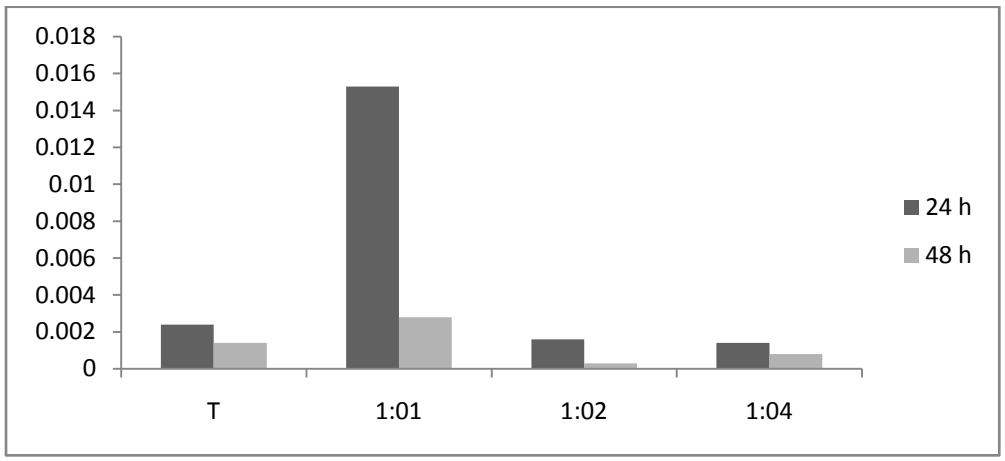

Figure 3: Comparative efficacy of Temephos and a combination of Temephos and hexane extract of *Pseudocalymma alliaceum* against *Culex quinquefasciatus* larvae.

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