Larvicidal efficacy of *Ocimum basilicum* extracts and its synergistic effect with neonicotinoid in the management of *Anopheles stephensi*

Prejwltta Maurya, Preeti Sharma, Lalit Mohan, Manish Mohan Verma, Chand Narayan Srivastava*  

Applied Entomology & Vector Control Laboratory, Department of Zoology, Faculty of Science, Dayalbagh Educational Institute (Deemed University), Dayalbagh, Agra – 282 110, India

**ARTICLE INFO**

**Article history:**
Received 15 October 2011  
Received in revised form 27 December 2011  
Accepted 28 February 2012  
Available online 28 April 2012

**Keywords:**  
*Ocimum basilicum*  
Neonicotinoid  
Mosquito larvicide  
Imidacloprid  
Synergism

**ABSTRACT**

**Objective:** To evaluate the larvicidal activities of different combinations of synthetic nicotinoid insecticide, imidacloprid with an insecticidal plant, *Ocimum basilicum* (*O. basilicum*) against malaria vector, *Anopheles stephensi*, with reference to the impact of most potent combination on some non–targets, *Anisops bouvieri* (*A. bouvieri*) and cyclop.  

**Methods:** The larvicidal activity was determined against mosquito larvae in various concentrations, 1:1, 1:2 and 1:4 under laboratory conditions. These experiments were conducted according to WHO standard procedure.  

**Results:** In bioassays, binary mixtures of different combinations of synthetic insecticide (imidacloprid) with crude petroleum ether leave extract of *O. basilicum* and then with most potent 4th fraction of it, produced promising results. Therefore, ratio 1:1 of all the binary mixtures was most effective as compared to 1:2 and 1:4 against mosquito larvae and showed synergism in all cases. The combinatorial ratio 1:1 of imidacloprid and most potent 4th fraction of petroleum ether extract of *O. basilicum* with LC50 value 0.010 and 0.007 ppm; LC90 value 0.033 and 0.023 ppm for anopheline larvae after 24 and 48 h of treatment, respectively, showed high toxicity. This effective ratio was found safe to aquatic mosquito predator, *A. bouvieri* and other aquatic non–target cyclops with the respective LC50 values 12.351 and 5.290 ppm after 24 h of exposure.  

**Conclusions:** It is, therefore, concluded that the tested combination is more effective than its individual constituents. Further, this formulation is cost–effective and ecofriendly to the aquatic fauna.

1. Introduction

Due to the public concern over the health and environmental hazards of conventional synthetic pesticides, exploitation and utilization of naturally occurring products in order to combat harmful agricultural and public health pests, the researchers and environmentalists have paid their attention towards the development of biodegradable phytocides. Repeated use of a single synthetic pesticidal ingredient can result in resistance amongst the target populations. On the contrary the pests rarely develop resistance against pesticides of plant origin. However, a lot of plant material is required to get a small amount of phytocides. It is, therefore, advisable to apply a combination of synthetic and phytocide instead of their individual application in insect pest management.

The introduction of synergists in pest control method could be great benefit both economically and ecologically, thereby, reducing the cost and increasing toxicity of a given treatment[1]. The few studies on the mosquitocidal activity of binary mixtures have investigated the combined effects of phytochemicals with insecticides or microbial control agents. Synergism between synthetic insecticides and phytochemicals appears to be more common than among different phytochemicals, with some phytochemicals producing varied results depending on which synthetic insecticides they are mixed with. The present investigation, thus, aimed to identify alternative active botanical metabolites that could be combined with existing synthetic insecticide to produce synergistic or additive larvicial effects on *Anopheles stephensi* (*A. stephensi*) and to study its impact on the most common group of aquatic non–target organisms in anopheline larval habitats.

2. Materials and methods

*Corresponding author: Prof. CN Srivastava, Applied Entomology & Vector Control Laboratory, Department of Zoology, Faculty of Science, Dayalbagh Educational Institute (Deemed University), Dayalbagh, Agra – 282 110, India.  
Tel: 91-09319103817  
Fax: 91-562-2801226  
E-mail: chandnarayan_dei@rediffmail.com  
Foundation Project: Supported by University Grant Commission under Dr. DS Kothari Post Doctoral Scheme [No. F. 4–2/2006(BSR)/13–277/2006(BSR)].
2.1. Bioassay of synthetic insecticide

The imidacloprid (97.6% SL) provided by District Malaria Office, Circuit House, Agra (India), were used for bioassay test against An. stephensi larvae. The stock solutions of 50 ppm for the imidacloprid were prepared by dissolving 0.05 mL of imidacloprid in 1000 mL distilled water independently. The prepared stock solutions were further diluted to get working concentrations. Twenty third instar larvae of An. stephensi were exposed to different concentrations, independently. All experiments were conducted in triplicate along with control, following the standard procedure[2]. The larval mortality in both the treatment and the control were recorded after 24 and 48 h of treatment. All data were then subjected to probit analysis[3] to calculate the LC\(_{50}\) and LC\(_{90}\) values.

2.2. Bioassay of phytoextracts

Leaves of the selected plant, Ocimum basilicum (O. basilicum) were collected from Dayalbagh Educational Institute (Deemed University) campus. After washing the leaves were dried in the shade and powdered manually. The powdered leaves were subjected separately to different solvents (petroleum ether, carbon tetrachloride and methanol) in a Soxhlet apparatus (Borosil, Mumbai, India) for up to 72 h, in each solvent for complete extraction[4]. Each extract was subjecting to rotary vacuum evaporator to remove solvent and get concentrated crude. The crude (10 g) obtained from each solvent was dissolved in 100 mL of ethanol independently to obtain stock solutions of 10000 ppm. The stock solutions were then further diluted in ethanold to obtain concentrations of 10000 ppm (petroleum ether), 50000 ppm (carbon tetra chloride extract) and 100000 ppm (methanol extract). These stocks were further diluted to get desired working concentrations and follow the same procedure for bioassay as abovesaid. Experiments were set in triplicates along with control.

2.3. Chromatographic fractionation, isolation and bioefficacy of bioactive chemical group present in the most potent extract

The potent crude extract (24 g) was subjected to column chromatographic separation [Length: 55 cm, diameter: 2.5 cm, stationary phase: silica gel (50 g)] and yielded five different compounds, FPE1 (fraction petroleum ether) (yellow), FPE2 (light yellow), FPE3 (light brown), FPE4 (yellowish brown) and FPE5 (dark yellow oily) by increasing polarity of eluents hexane and ethyl acetate in ratio 98:2, 95:5, 90:10, 80:20 and 50:50, respectively. All fractions were monitored by thin layer chromatography (precoated plate, 0.02 mm thick, E. merck, Germany 60 F254) until a single spot was obtained by using solvent system hexane: ethyl acetate (80:20). The pure fractions were carefully evaporated to dryness and subsequently characterized on the basis of their Rf values and the bioactive group was identified by conducting standard qualitative analytical tests. All the recovered compounds FPE1, FPE2, FPE3, FPE4 and FPE5 were screened for their larvicidal activity against anopheline mosquitoes by adopting bioassay procedure as above.

2.4. Combined efficacy of imidacloprid and phytoextract

For combinatorial studies, 50 ppm stock of imidacloprid and the most efficient phytoextract (crude and fraction, independently) was prepared. Keeping imidacloprid as the standard, its stock was mixed with the stock of phytoextract in ratios of 1:1, 1:2 and 1:4. Test concentrations 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 concentrations LC\(_{50}\) as well as LC\(_{90}\) were determined. A co–toxicity coefficient (CTC)[5] and a synergistic factor (SF)[6] for mixed formulation experiments were calculated after calculating LC\(_{50}\) and LC\(_{90}\) for each combination.

\[
\text{CTC} = \frac{[\text{toxicity of insecticide (alone) / toxicity of insecticide with plant extract}]}{100}
\]

\[
\text{SF} = \frac{\text{toxicity of insecticide (alone) / toxicity of insecticide with plant extract}}{100}
\]

A value of SF > 1 indicates synergism and SF < 1 indicates antagonism.

2.5. Effect on non–target organisms

The effect of the most potent combination was tested against non–target mosquito predator, Anisops bouvieri (An. bouvieri) (Notonecta sp.) along with other aquatic organism cyclops. They were obtained from the field from where mosquito larvae were collected. Twenty of each non target was exposed to test concentrations ranging from 1 to 50 mg/L separately. Three replicates were performed for each test concentration along with controls. The organisms were observed for mortality and other abnormalities such as sluggisnness and reduced swimming activity after 24 and 48 h of exposure. LC\(_{50}\) and LC\(_{90}\) values were obtained by probit analysis[3].

3. Results

3.1. Bioefficacy of neonicotinoid insecticide (imidacloprid)

The larvicidal potential of imidacloprid against An. stephensi is depicted in Table 1. The LC\(_{50}\) values for imidacloprid against An. stephensi were (0.018 ± 0.002) ppm with 0.022 and 0.012 ppm upper and lower fiducial limits and (0.009 ± 0.0015) ppm with 0.011 and 0.006 ppm upper and lower fiducial limits after 24 and 48 hours of treatment. LC\(_{50}\) values were (0.063 ± 0.017) ppm with 0.097 and 0.028 ppm...
upper and lower fiducial limits and (0.030 ± 0.006) ppm with 0.042 and 0.017 ppm upper and lower fiducial limits after 24 and 48 hours of treatment, respectively. The results reveal that the target species of mosquito was susceptible against imidacloprid.

3.2. Bioefficacy of crude petroleum ether extracts of *O. basilicum*

The data mentioned in Table 2 reveal that the crude petroleum ether extracts (PEE) of *O. basilicum* were the most effective against the anopheline larvae as compared to their carbon tetrachloride (CEE) and methanol extracts (MEE). The PEE was observed more effective with LC₅₀ values of (8.29 ± 1.92) ppm with 12.05 and 4.52 ppm upper and lower fiducial limits, (4.57 ± 1.24) ppm with 6.99 and 2.15 ppm
Table 4

Toxicity of different combinations of imidacloprid with petroleum ether crude extract of *O. basilicum* against anopheline larvae.

| Combinations | Exposure period (h) | Chi-square | Regression equation | LC50 ± SE (Fiducial limits) (ppm) | Co-toxicity coefficient | Combined factor | Nature of action | LC90 ± SE (Fiducial limits) (ppm) | Co-toxicity coefficient | Combined factor | Nature of action |
|---------------|---------------------|------------|---------------------|-----------------------------------|-------------------------|-----------------|-----------------|-----------------------------------|-------------------------|-----------------|-----------------|
| 1:1           | 24                  | 1.612      | 1.10X+6.401         | 0.011 ± 0.003                     | 103.63                  | 1.860           | S               | 0.035 ± 0.027                                    | 190.90                  | 1.900           | S               |
| 1:2           | 24                  | 0.424      | 1.69X+6.878         | 0.007 ± 0.003                     | 128.57                  | 1.285           | S               | 0.019 ± 0.012                                    | 157.89                  | 1.578           | S               |
| 1:4           | 24                  | 2.497      | 1.40X+6.254         | 0.012 ± 0.002                     | 150.00                  | 1.900           | S               | 0.040 ± 0.034                                    | 157.50                  | 1.575           | S               |
| 1:1           | 24                  | 0.704      | 1.52X+6.005         | 0.017 ± 0.003                     | 112.50                  | 1.125           | S               | 0.021 ± 0.0194                                   | 190.1                   | 1.500           | S               |
| 1:2           | 24                  | 2.251      | 2.04X+6.212         | 0.011 ± 0.002                     | 90.00                   | 0.900           | A               | 0.055 ± 0.032                                    | 96.92                   | 0.960           | A               |
| 1:4           | 24                  | 1.574      | 2.12X+6.618         | 0.017 ± 0.02                      | 52.94                   | 0.529           | A               | 0.109 ± 0.046                                    | 60.00                   | 0.600           | A               |

Table 5

Toxicity of different combinations of imidacloprid with the most potent 4th fraction of petroleum ether extract of *O. basilicum* against anopheline larvae.

| Combinations | Exposure period (h) | Chi-square | Regression equation | LC50 ± SE (Fiducial limits) (ppm) | Co-toxicity coefficient | Combined factor | Nature of action | LC90 ± SE (Fiducial limits) (ppm) | Co-toxicity coefficient | Combined factor | Nature of action |
|---------------|---------------------|------------|---------------------|-----------------------------------|-------------------------|-----------------|-----------------|-----------------------------------|-------------------------|-----------------|-----------------|
| 1:1           | 24                  | 0.628      | 1.30X+6.38          | 0.010 ± 0.002                     | 100.00                  | 1.80            | S               | 0.031 ± 0.011                                    | 190.90                  | 1.00            | S               |
| 1:2           | 24                  | 0.671      | 1.55X+6.71          | 0.007 ± 0.002                     | 128.57                  | 1.28            | S               | 0.023 ± 0.009                                    | 150.43                  | 1.30            | S               |
| 1:4           | 24                  | 3.36       | 1.58X+6.18          | 0.019 ± 0.004                     | 94.73                   | 0.94            | A               | 0.046 ± 0.047                                    | 95.45                   | 0.95            | A               |
| 1:1           | 24                  | 1.55       | 0.75X+6.55          | 0.016 ± 0.003                     | 56.25                   | 0.86            | A               | 0.047 ± 0.012                                    | 63.64                   | 0.63            | A               |
| 1:2           | 24                  | 2.416      | 2.01X+6.531         | 0.021 ± 0.002                     | 65.71                   | 0.85            | A               | 0.074 ± 0.026                                    | 67.90                   | 0.87            | A               |
| 1:4           | 24                  | 3.103      | 1.02X+6.571         | 0.015 ± 0.002                     | 60.00                   | 0.80            | A               | 0.051 ± 0.019                                    | 60.00                   | 0.60            | A               |

Table 6

Effect of most potent combination (imidacloprid and most potent 4th fraction of petroleum ether leaves extract of *O. basilicum* in ratio 1:1) on non-target organisms.

| Non target species | Exposure period (h) | Chi-square | Regression equation | LC50/SE (Fiducial limits) (ppm) | Relative toxicity irrespective of time period | LC50/SE (Fiducial limits) (ppm) | Relative toxicity irrespective of time period |
|--------------------|---------------------|------------|---------------------|---------------------------------|---------------------------------------------|---------------------------------|---------------------------------------------|
| Anisop             | 24                  | 1.499      | 4.05X-3.481         | 12.351 ± 8.19                   | 3.732                                      | 25.572 ± 9.65                   | 1.85                                        |
|                    | 48                  | 1.020      | 4.23X-3.563         | 10.500 ± 8.05                   | 3.173                                      | 21.072 ± 2.53                   | 1.52                                        |
|                    | 48                  | 2.168      | 1.76X-1.951         | 5.290 ± 0.836                   | 1.598                                      | 18.058 ± 10.352                 | 1.30                                        |
|                    | 48                  | 3.839      | 2.06X-1.863         | 3.309 ± 0.610                   | 1.00                                       | 13.827 ± 2.959                  | 1.00                                        |
|                    |                     |            |                     | 4.506 – 2.111                   |                                            | 19.628 – 8.026                  |                                            |

upper and lower fiducial limits after 24 and 48 h of exposure, accordingly. The PEE followed, CEE with LC50 value of (268.61 ± 40.28) ppm with 347.56 and 189.66 ppm upper and lower fiducial limits, (143.85 ± 26.54) ppm with 195.87 and 91.84 ppm upper and lower fiducial limits after 24 and 48 hours of exposure, respectively. The MEE possess least potency with LC50 value of (446.61 ± 31.76) ppm with 508.86 and 384.36 ppm upper and lower fiducial limits and (63.48 ± 20.78) ppm with 104.21 and 22.76 ppm upper and lower fiducial limits after 24 and 48 hours of exposure, accordingly[7].

The LC50 values also show the same trend as shown by LC90 values. In case of PEE LC50 values were (87.68 ± 34.35) ppm with 154.99 and 20.36 ppm after 24 hours and (47.25 ± 16.01) ppm with 78.61 and 15.88 ppm upper and lower fiducial limits after 48 hours of exposure against anopeline. The CEE have LC50 values were (128.45 ± 501.34) ppm with 2265.07 and 299.83 ppm upper and lower fiducial limits and (507.80 ± 95.17) ppm with 694.33 and 321.27 ppm upper and lower fiducial limits after 24 and 48 hours of exposure, accordingly. The MEE hold the LC50 values were (923.60 ± 140.33) ppm with 1198.64 and 648.56 ppm upper and lower fiducial limits and (887.00 ± 139.01) with 1159.46 and 614.54 ppm upper and lower fiducial limits after 24 and 48 hours of exposure, respectively[7].

3.3. Fractional bioassay

The bioassay of each fraction was conducted against anopeline larval (Table 3). The LC50 and LC90 of fraction I (FPE1) were 7.39 and 50.10 ppm after 24 hours and 3.53 and 25.62 ppm after 48 hours. Fraction II (FPE2) had the LC50 and LC90 values 8.10 and 29.55 ppm after 24 hours and 5.38 and 25.62 ppm after 48 hours. Fraction II (FPE2) had the LC50 and LC90 values 8.10 and 29.55 ppm after 24 hours and 5.38 and 25.62 ppm after 48 hours. Fraction III (FPE3) was observed with LC50 value 1.85 and 1.30 ppm.
and LC₉₀ 12.15 and 6.73 ppm after 24 hours and 48 hours of treatment period, respectively. Fraction V (FPE) had the LC₅₀ and LC₉₀ values 15.12 and 127.57 ppm after 24 hours and for 48 hours 9.58 and 75.38 ppm. The bioassay of all the five fractions highlighted the fraction FPE4 as most effective component with RF value 0.3 and found saponin in nature after chemically tested.

3.4. Combinatorial bioassay

3.4.1. Crude plant extract and synthetic insecticides

The bioefficacy of different combinations of synthetic insecticide, imidacloprid and crude petroleum ether leave extract of O. basilicum against anopheline larvae is represented in Table 4. The combinatorial ratio 1:1 had the LC₅₀ and LC₉₀ value 0.011 and 0.033 after 24 hours and 0.007 and 0.019 ppm after 48 hours of exposure, accordingly. Ratio 1:2 had the LC₅₀ and LC₉₀ values 0.012 and 0.040 ppm after 24 hours and 0.008 and 0.021 ppm after 48 hours of treatment. The LC₅₀ and LC₉₀ of ratio 1:4 were 0.201 and 0.063 ppm after 24 hours and LC₉₀ 0.017 ppm and 0.059 ppm after 48 hours of exposure, accordingly.

The co-toxicity coefficient and combined factor of these combinations were depicted in Table 4. The co-toxicity coefficient was 163.63 and 128.57 and combined factor was 1.63 and 1.28 with LC₅₀ values, and with LC₉₀ values co-toxicity coefficient was 190.90 and 157.89 and combined factor was 1.90 and 1.57 after 24 and 48 hours of treatment, respectively, with synergistic action in both cases. For 1:2, the co-toxicity coefficient of LC₅₀ was 150.0 and 112.5 and combined factor was 1.50 and 1.12 with synergistic activity after both 24 and 48 hours of exposure, respectively. The co-toxicity coefficient of LC₉₀ was 157.5 and 150.00 and combined factor was 1.57 and 1.50 and shows synergistic action after 24 hours and 48 hours of treatment, accordingly. For ratio 1:4, the co-toxicity coefficient values of LC₅₀ were 90.00 and 52.94 and combined factor values were 0.9 and 0.52, the co-toxicity coefficient values of LC₉₀ were 96.92 and 60.00 and combined factor values were 0.96 and 0.60 after 24 and 48 hours, respectively and show antagonistic activity in both the cases.

3.4.2. Plant fraction and synthetic insecticide

The combinatorial bioassay of imidacloprid and most potent 4th fraction of petroleum ether extract of O. basilicum in different ratios against anopheline larvae are depicted in Table 5. The combinatorial ratio 1:1 has the LC₅₀ and LC₉₀ value 0.010 and 0.033 ppm after 24 hours and 0.007 and 0.023 ppm after 48 hours of exposure. Ratio 1:2 has the LC₅₀ and LC₉₀ values 0.019 and 0.066 ppm after 24 hours and 0.016 and 0.047 ppm after 48 hours of treatment. The LC₅₀ and LC₉₀ of ratio 1:4 were 0.021 and 0.072 ppm after 24 hours and LC₉₀ 0.015 and 0.051 after 48 hours of exposure.

The co-toxicity coefficient and combined factor of different combinations against anopheline larvae were depicted in Table 5. When the combinatorial ratio was 1:1, the co-toxicity coefficient was 180.00 and 128.57 and combined factor was 1.80 and 1.28 with LC₅₀ values and co-toxicity coefficient of LC₉₀ values, 190.90 and 130.43 and combined factor was 1.90 and 1.30 after 24 and 48 hours of treatment, respectively, the nature of action was synergistic. When the combinatorial ratio was 1:2, the co-toxicity coefficient of LC₅₀ was 94.73 and 56.25 and combined factor was 0.94 and 0.56 and the co-toxicity coefficient of LC₉₀ was 95.45 and 63.82 and combined factor was 0.95 and 0.63 after 24 hours and 48 hours of treatment accordingly, the nature of action was antagonistic. When the combinatorial ratio was 1:4, the co-toxicity coefficient values of LC₅₀ were 85.71 and 60.00 and combined factor values were 0.85 and 0.60 and the co-toxicity coefficient values of LC₉₀ were 87.50 and 60.00 and combined factor values were 0.87 and 0.60 after 24 and 48 hours, respectively, with antagonism.

3.5. Effects on non–target organisms

The effect of the most potent combination was tested against non–target mosquito predator, A. bouvieri (Notonecta sp.) along with aquatic organism cyclops and the results is presented in Table 6. The LC₅₀ and LC₉₀ against A. bouvieri were (12.351±0.819) ppm and (25.572±3.965) ppm with 13.957–10.743 ppm being upper and lower fiducial limits for former and 33.345–17.799 ppm for later after 24 hours and LC₅₀ (10.500±0.805) ppm with 12.078 and 8.922 ppm upper and lower fiducial limits and LC₉₀ (21.072±2.523) ppm with 26.0183 and 16.126 ppm upper and lower fiducial limits after 48 hours of exposure, accordingly. Cyclop have LC₅₀ (5.290±0.836) ppm with 6.930 and 3.649 ppm being upper and lower fiducial limits and LC₉₀ (28.058±10.352) ppm with 48.349 and 7.767 ppm upper and lower fiducial limits after 24 hours and LC₅₀ was (3.309±0.610) ppm and LC₉₀ was (13.827±2.959) along with 4.506 and 2.111 ppm upper and lower fiducial limits for former and 19.628 and 8.026 ppm for later after 48 hours of exposure period, respectively. Therefore, A. bouvieri were the least susceptible as compared to cyclops.

4. Discussion

The combination of imidacloprid and most potent, 4th fraction of petroleum ether leaves extract of O. basilicum in ratio 1:1 was the most toxic to anopheline larvae with LC₅₀ 0.010 ppm, as compared to combinations in ratios 1:2 and 1:4. Larvicidal potential of the extracts from different parts viz. green and red fruits, seeds, fruit without seeds, leaves and roots of Withania somnifera in different solvents was evaluated against larvae of An. stephensi, Aedes aegypti (Ae. aegypti) and Culex quinquefasciatus (Cx. quinquefasciatus). The 24 h LC₅₀ values as observed for whole green fruits in water, methanol and petroleum ether were 350.9, 372.4, 576.9; 115.0, 197.1, 554.6; 154.9, 312.0, 1085.0 while corresponding values for red fruits were 473.5, 406.4, 445.2; 94.7, 94.5, 1013.0; 241.8, 535.0, 893.3 mg/L for An. stephensi, Ae. aegypti and Cx. quinquefasciatus, respectively, showing that methanol extracts were more effective against anophelines as compared to culicines when whole fruits were taken[8]. To evaluate the mosquito larvicidal activity of plant extracts,
hexane, chloroform, ethyl acetate, acetone, and methanol leaf, flower and seed extracts of *Abras precatorius* (*A. precatorius*), *Croton bonplandianum* (*C. bonplandianum*), *Cynodon dactylon*, *Musa paradisiaca* (*M. paradisiaca*) and *Syzyzygium aromaticum* (*S. aromaticum*) were tested against fourth instar larvae of *Anopheles vagus* (*A. vagus*), and *Culex vishnui* (*Cx. vishnui*). The highest larval mortality was found in seed ethyl acetate extracts of *A. precatorius* and leaf extracts of *C. bonplandianum*, flower chloroform and methanol extracts of *M. paradisiaca*, and flower bud hexane extract of *S. aromaticum* against *A. vagus* with LC$_{50}$ values of 19.31, 39.96, 35.18, 79.90 and 85.90 $\mu$g/mL; and seed methanol of *A. precatorius*, flower methanol extract of *M. paradisiaca*, flower bud hexane extract of *S. aromaticum* against *Cx. vishnui* with LC$_{50}$ values of 136.84, 103.36 and 149.56 $\mu$g/mL, respectively.[9]

Mulla and Su[i] showed that neem seed kernel extract has synergistic effects when combined with the juvenile hormone analog methoprene against *Ae. aegypti*, *Ae. togoi* and *An. stephensi*. Moawed[11] studied the joint action of binary mixtures of some plant extracts, *Ruta graveolens* ethanol and petroleum ether extract, aqueous *Haplophyllum tuberculatum* (*H. tuberculatum*) extract and *H. tuberculatum*, *Euphorbia peplus* ethanol extracts with each other and with the synthetic pyrethroid, cypermethrin at doses 175, 225, 38, 12.8, 380 mg/L showing synergistic and additive effects, respectively against *Cx. pipiens* larvae. For instance, non–lethal concentrations of the volatile oil thymol and an unsaponifiable portion isolated from *Thymus capitatus* synergized the toxicity of malathion but induced additive and antagonistic effects when mixed with permethrin or pirimiphos–methyl insecticide against *Cx. pipiens* larvae.[12]

The mechanism of synergism is not well studied; however, Thangam and Kathiresan[9] stated that synergism might be due to phytochemicals inhibiting the ability of mosquito larvae to employ detoxifying enzymes against synthetic chemicals.

Ethanolic, acetone and petroleum ether extracts of leaves from the Egyptian plant *Cupressus sempervirens* (*Cupressaceae*) were tested against 3rd instar larvae of the mosquito *Cx. pipiens* L. The results indicated that petroleum ether extracts were more efficient than ethanolic and acetone extracts with LC$_{50}$ 37.8 ppm.[13] Larvicidal activity of crude chloroform, dichloromethane and methanol extracts of the leaves and roots of six Indian plants, *Aegle marmelos* L., *Balanites aegyptica* (*B. aegyptica*) L., *Calotropis gigantea* L., *Murraya koenigii* L., *Nyctanthes arbor–tristis* L. and *Plumbago zeylanica* (*P. zeylanica*) L., were tested against the early fourth instar larvae of *Ae. aegypti* L. and *An. stephensi*. However, the highest larvae mortality was found in methanol extracts of *P. zeylanica* roots and *B. aegyptica* roots against *Ae. aegypti* (LC$_{50}$ 169.61 mg/L, 289.59 mg/L) and *An. stephensi* (LC$_{50}$ 222.34 mg/L, 102.29 mg/L), respectively.[14] The larvicidal activities of crude and chloroform: methanol (1:1 v/v) extracts of some common spices [*Cuminum cyminum* (*C. cyminum*), *Allium sativum* (*A. sativum*), *Zingiber officinale* (*Z. officinale*), *Curcuma longa* (*C. longa*)] and vegetable waste (*Solunum tuberosum* germinated tuber) were examined against *An. stephensi* and *Cx. quinquefasciatus* mosquito larvae, mortality in mosquito species were recorded in the following sequences: *C. cyminum* > *A. sativum* > *Z. officinale*, *C. longa* > *S. tuberosum* germinated tuber[15].

Results of the effect on non–target organisms have revealed that combinations of imidacloprid and most potent 4th fraction of petroleum ether leaves extract of *O. basilicum* in ratio 1:1 are safe to certain non–targets tested. It shows that the present combination is ecofriendly to aquatic ecosystem. el–Shazly and el–Sharnoubi[16-24] tested the neem based insecticides on certain aquatic non targets. The order of tolerance of the organisms to different concentrations of the insecticide was: larvae of *Bufo regularis* (*Amphibia*) > *Aedes caspis* (*Insecta*) > *Gambusia affinis* (*Poeclialiidae*) > *Cyclops sp.* > *Daphnia magna* (*Crustacea*). At a concentration of 20 ppm, all the tadpoles died within 9 days, while all other individuals died within 5 to 8 days after exposure to a concentration of 10 ppm of Neem Azal insecticide. Sivagnaname and Kalyanasundaram[25] studied the effect of methanolic extract of *Atlantia monophylla* against non–target organisms *Toxorhynchites splendens* (*Tx. splendens*) (mosquito predator), *Gambusia affinis* (*G. affinis*), *Poecilia reticulata* (*P. reticulata*) (predatory fishes), *Diploynychus indicus* (*D. indicus*) (predatory water–bugs), and *A. bouvieri* (*Notonecta sp.*), *Tx. splendens* larvae. *G. affinis* and *P. reticulata*, were the least susceptible, with LC$_{50}$ values of 23.4 mg/L and 21.3 mg/L, respectively. The extract was found to be highly lethal to *A. bouvieri*, with a LC$_{50}$ of 0.15 mg/L. *D. indicus* was less susceptible to the plant extract than *Tx. splendens*. Ohaga et al.[26] assessed the impact of powders of *Piper guineense* (*P. guineense*) and *Spilanthes mauritian* (*S. mauritian*) on non–target aquatic invertebrates and vertebrates, damselly and dragonfly nymph, macro dystiscids, micro dystiscids, notonectids, fresh water shrimp, tadpoles and tipapia fish with LD$_{50}$ varied from 12.2 to 39.2 g/L and 13.6 to 41.83 g/L for *P. guineense* and *S. mauritian*, respectively, after 24 h. Promsiri et al.[27] reported that out of 14 plant extracts found to be larvicidal, eight plant extracts were toxic to guppy fish and six extracts were found to be not so toxic. *Abutilon indicum*, *Samanea saman*, *Costus speciosus*, *Acorus calamus*, *Knema globularia*, *Stemonia tuberosa* (*S. tuberosa*), *Strychnos nux–vomica*, and *Kaempferia galanga* (*K. galanga*) extracts were toxic to guppy fish at concentrations of 100, 100, 50, 50, 100, 100, and 50 $\mu$g/mL, respectively. They all produced 100% mortality for guppy fish except for *S. tuberosa* and *K. galanga*, which resulted in 80% mortality. Six plant species, *Cinnamomum porrectum*, *Pelvisfuckomis pulcher*, *Anacardium occidentale*, *Musa siamensis*, *Aegle marmelos*, and *Annona muricata* were not toxic to guppy fish at concentrations of 50.0, 12.5, 6.3, 3.2, 12.5 and 50.0 $\mu$g/mL, respectively. It is concluded that the present combination is one of the best larvicidal combination in the management of anopheles mosquito. Thus, the present synergistic approach can be used as an eco–safe popular combination in mosquito management with lesser toxicity to aquatic non–targets.
Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Authors are thankful to University Grant Commission, New Delhi for providing financial assistance under Dr. DS Kothari Post Doctoral Scheme, F.4-2/2006(BSR)/13-277/2008(BSR)

References

[1] Metcalf RL. The synergistic activity of various selected chemicals on the insecticides carbofuran and malathion against brine shrimp larvae [B.Sc. (Hons.) Theses]. UK: Department of Agricultural and Environmental Sciences, University of Newcastle upon Tyne; 1992. p. 53.

[2] WHO. Instructions for determining the susceptibility of resistance mosquito larvae to insecticides. Geneva: WHO; 1975.

[3] Finney DJ. Probit analysis. 3rd ed. Cambridge: Cambridge University Press. 1971.

[4] Maurya P, Sharma P, Mohan L, Batabyal L, Srivastava CN. Evaluation of larvicidal nature of fleshy fruit wall of Momordica charantia Linn. (family: cucurbitaceae) in the management of mosquitoes. Parasitol Res 2009; 105(6): 1653–1659.

[5] Sanup P, Dingra S, Agarwal KN. Newer dimensions for evaluating the synergistic effect of non-toxic chemicals in the mixed formulations against the adults of Cylas formicarius Fabricius. J Entomol Res 1980; 4: 1–14.

[6] Kalyansundaram M, Das PK. Larvicidal and synergistic activity of plant extracts for mosquito control. Indian J Med Res 1985; 82: 19–21.

[7] Maurya P, Sharma P, Mohan L, Batabyal L, Srivastava CN. Evaluation of larvicidal potential of different plant parts of Withania somnifera against Anopheles stephensi and Culex quinquefasciatus. J Asia–Pac Entomol 2009; 12(2): 113–115.

[8] Bansal SK, Singh KY, Sharma S, Sherwani MR. Comparative larvicidal potential of different plant parts of Withania somnifera against vector mosquitoes in the semi-arid region of Rajasthan. J Environ Biol 2011; 32(1): 71–75.

[9] Bagavan A, Rahuman AA. Evaluation of larvicidal activity of medicinal plant extracts against three mosquito vectors. Asian Pac J Trop Med 2011; 4(1): 29–34.

[10] Mulla MS, Su T. Activity and biological effects of neem products against arthropods of medical and veterinary importance. J Am Mosq Control Assoc 1999; 15: 133–152.

[11] Moawed HAM. Joint action of some plant extracts against the mosquito larvae of Culex pipiens and their physiological impact [M.S. thesis]. Egypt: Faculty of Science–Dmietta, Mansoura University; 1998.

[12] Mansour SA, Messeha SS, Gengaihi SE. Botanical biocides, 4. Mosquitocidal activity of certain Thymus capitatus constituents. J Nat Toxins 2000; 9: 49–62.

[13] Sheikh TMY, Hassan MI, Moselhy WA, Amer MS, Shehata AZ. Evaluation of the biological activity of some Cupressus sempereirenus (Cupressaceae) extracts against the mosquito vector Culex pipiens L. (Diptera: Culicidae). Egypt Acad J biol Sci 2011; 4(1): 33–48.

[14] Patil SV, Patil CD, Salunkhe RB, Salunke BK. Larvicidal activities of six plants extracts against two mosquito species, Aedes aegypti and Anopheles stephensi. Trop Biomed 2010; 27(3): 360–365.

[15] Singha S, Chandra G. Mosquito larvicidal activity of some common spices and vegetable waste on Culex quinquefasciatus and Anopheles stephensi. Asian Pac J Trop Med 2011; 4(4): 288–293.

[16] el-Shazly MM, el-Sharmoubi ED. Toxicity of a neem (Azadirachta indica) insecticide to certain aquatic organisms. J Egypt Soc Parasitol 2003; 30(1): 221–231.

[17] Warikoo R, Ray A, Sandhu JK, Samal R, Wahab N, Kumar S. Larvicidal and irritant activities of hexane leaf extracts of Citrus sinensis against dengue vector Aedes aegypti L. Asian Pac J Trop Biomed 2012; 2: 152–155.

[18] Prabhu K, Murugan K, Narasubramanian N, Bragadeeswaran S. Larvicidal and repellent potential of Morinda oleifera against malarial vector, Anopheles stephensi Liston (Insecta: Diptera: Culicidae). Asian Pac J Trop Biomed 2011; 1: 124–129.

[19] Singha S, Adhikari U, Chandra G. Smoke repellency and mosquito larvicidal potentiality of Mesua ferrea L. leaf extract against filarial vector Culex quinquefasciatus Say. Asian Pac J Trop Biomed 2011; 1: S119–S123.

[20] Beula JM, Ravikumar S, Ali MS. Mosquito larvicidal efficacy of seaweed extracts against dengue vector of Aedes aegypti. Asian Pac J Trop Biomed 2011; 1: S143–S146.

[21] Koyel Mallick Haldar, Papiya Ghosh, Goutam Chandra. Evaluation of target specific larvicidal activity of the leaf extract of Typhonium trilobatum against Culex quinquefasciatus Say. Asian Pac J Trop Biomed 2011; 1: S199–S203.

[22] Das D, Chandra G. Mosquito larvicidal activity of Rauwolfia serpentina L. seeds against Culex quinquefasciatus Say. Asian Pac J Trop Med 2012; 5: 42–45.

[23] Govindarajan M, Sivakumar R, Rajeswari M. Larvicidal efficacy of Cassia fistula L. leaf extract against Culex tritaeniorynchus Giles and Anopheles subpictus Grassi (Diptera:Culicidae). Asian Pac J Trop Med 2012; 4: 295–298.

[24] Kamaraj C, Rahuman AA, Bagavan A, Elango G, Zahir AA, Santhoshkumar T. Larvicidal and repellent activity of medicinal plant extracts from Eastern Ghats of South India against malaria and filariasis vectors. Asian Pac J Trop Med 2012; 4: 698–705.

[25] Sivagnaname N, Kalyansundaram M. Laboratory evaluation of methanolic extract of Atlanticia monophylla (family: Rutaceae) against immature stages of mosquitoes and non-target organisms. Mem Inst Oswaldo Cruz 2004; 99(1): 115–118.

[26] Ohaga SO, Ndzie Og, Kubasu SS, Beier JC, Mhogo CM. Susceptibility of non target aquatic macro-invertebrates and vertebrates to Piper guineense (Piperaceae) and Spilanthes mauritiana (Asteraceae) powder in Kilifi district, Kenya. Int J Zoological Res 2007; 3(2): 86–93.

[27] Promsiri S, Naksathit A, Kruatrachue M, Thavara U. Evaluations of larvicidal activity of medicinal plant extracts to Aedes aegypti (Diptera: Culicidae) and other effects on a non target fish. Insect Sci 2006; 13: 179–188.