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

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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 LC_{50} value 0.010 and 0.007 ppm; LC_{90} 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 LC_{50} 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 phytopesticides. 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 phytopesticides. It is, therefore, advisable to apply a combination of synthetic and phytopesticide 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 larvicidal effects on *Anopheles stephensi* (*An. stephensi*) and to study its impact on the most common group of aquatic non-target organisms in anopheline larval habitats.

2. Materials and methods

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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 1 000 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 100 000 ppm. The stock solutions were then further diluted in ethanol to obtain concentrations of 10 000 ppm (petroleum ether), 50 000 ppm (carbon tetra chloride extract) and 100 000 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.

CTC = [toxicity of insecticide (alone) / toxicity of insecticide with plant extract] $\times$ 100

SF = toxicity of insecticide (alone) / toxicity of insecticide with plant extract

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 sluggishness 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 $\pm$ 0.002) ppm with 0.022 and 0.012 ppm upper and lower fiducial limits and (0.009 $\pm$ 0.0015) ppm with 0.011 and 0.006 ppm upper and lower fiducial limits after 24 and 48 hours of treatment. LC_{90} values were (0.063 $\pm$ 0.017) ppm with 0.097 and 0.028 ppm

Table 1
Toxicity of imidacloprid against anopheline larvae.

Exposure period (h)	*Chi*-square	Regression equation	LC_{50}±SE (Fiducial limits) (ppm)	Relative toxicity irrespective of time period	LC_{90}±SE (Fiducial limits) ppm	Relative toxicity irrespective of time period
24	2.89	2.33X+6.74	0.018 ± 0.002 (0.022 – 0.012)	2.00	0.063 ± 0.017 (0.097 – 0.028)	2.10
48	3.93	2.46X+7.56	0.009 ± 0.0015 (0.011 – 0.006)	1.00	0.030 ± 0.006 (0.042 – 0.017)	1.00

Table 2
Toxicity of different leave extracts of *O. basilicum* against anopheline larvae.

Solvent extract	Exposure period (h)	*Chi*-square	Regression equation	LC_{50} ± SE (Fiducial limits) (ppm)	Relative toxicity irrespective of time period	LC_{90}±SE (Fiducial limits) ppm	Relative toxicity irrespective of time period
Carbontetra-chloride	24	3.77	1.89X−1.47	268.61 ± 40.28 (347.56 – 189.66)	58.78	1282.45 ± 501.34 (2265.07 – 299.83)	27.14
	48	2.12	2.34X−2.39	143.85 ± 26.54 (195.87 – 91.84)	31.48	507.80 ± 95.17 (694.33 – 321.27)	10.75
Methanol	24	26.34	4.06X−9.82	446.61 ± 31.76 (508.86 – 384.36)	97.73	923.60 ± 140.33 (1198.64 – 648.56)	19.55
	48	19.69	3.53X−7.67	384.84 ± 30.70 (445.01 – 324.66)	84.21	887.00 ± 139.01 (1159.46 – 614.54)	18.77
Petroleum ether	24	3.70	1.25X+2.60	8.29 ± 1.92 (12.05 – 4.52)	1.81	87.68 ± 34.35 (154.99 – 20.36)	1.86
	48	6.02	1.26X+2.90	4.57 ± 1.24 (6.99 – 2.15)	1.00	47.25 ± 16.01 (78.61 – 15.88)	1.00

Table 3
Toxicity of different fractions of petroleum ether leave extracts of *O. basilicum* against anopheline larvae.

Fractions	Exposure period (h)	*Chi*-square	Regression equation	LC_{50}±SE (Fiducial limits) (ppm)	Relative toxicity irrespective of time period	LC_{90}±SE (Fiducial limits) ppm	Relative toxicity irrespective of time period
Fraction 1	24	8.01	1.54X+2.11	7.39 ± 1.59 (10.51 – 4.26)	5.68	50.10 ± 14.22 (77.99 – 22.22)	7.44
	48	6.91	1.48X+2.69	3.53 ± 1.13 (5.74 – 1.31)	2.72	25.62 ± 6.73 (38.82 – 12.41)	3.81
Fraction 2	24	5.64	2.8X+0.65	8.10 ± 1.35 (10.75 – 5.45)	6.23	29.55 ± 5.96 (41.22 – 17.87)	4.39
	48	3.34	2.18X+1.22	5.38 ± 1.21 (7.75 – 3.02)	4.14	20.79 ± 3.82 (28.28 – 13.31)	3.09
Fraction 3	24	5.39	1.57X+2.46	4.02 ± 0.84 (5.68 – 2.35)	3.09	26.10 ± 9.11 (43.96 – 8.24)	3.88
	48	2.68	1.86X+2.43	2.37 ± 0.53 (3.43 – 1.32)	1.82	11.58 ± 2.54 (16.56 – 6.60)	1.72
Fraction 4	24	0.47	1.57X+3.02	1.85 ± 0.45 (2.73 – 0.97)	1.42	12.15 ± 3.46 (18.92 – 5.37)	1.81
	48	0.93	1.80X+2.99	1.30 ± 0.32 (1.94 – 0.67)	1.00	6.73 ± 1.59 (9.85 – 3.61)	1.00
Fraction 5	24	2.53	1.38X+1.98	15.12 ± 3.72 (22.42 – 7.82)	11.63	127.57 ± 62.44 (249.95 – 55.19)	18.96
	48	3.32	1.43X+2.17	9.58 ± 3.17 (15.79 – 3.36)	7.37	75.38 ± 27.16 (128.61 – 22.16)	11.20

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 LC_{50} 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	LC_{50} ± SE (Fiducial limits) (ppm)	Co-toxicity coefficient	Combined factor	Nature of action	LC_{90} ± SE (Fiducial limits) ppm	Co-toxicity coefficient	Combined factor	Nature of action
1:1	24	1.162	1.504X+6.401	0.011 ± 0.002 (0.016 – 0.006)	163.63	1.636	S	0.033 ± 0.027 (0.137 – 0.028)	190.90	1.900	S
	48	0.424	1.667X+6.838	0.007 ± 0.0019 (0.011 – 0.004)	128.57	1.285	S	0.019 ± 0.012 (0.071 – 0.021)	157.89	1.578	S
1:2	24	2.497	1.451X+6.324	0.012 ± 0.0028 (0.017 – 0.006)	150.00	1.500	S	0.040 ± 0.0343 (0.160 – 0.025)	157.50	1.575	S
	48	0.764	1.520X+6.605	0.008 ± 0.002 (0.013 – 0.004)	112.50	1.125	S	0.021 ± 0.0194 (0.099 – 0.023)	150.0	1.500	S
1:4	24	2.251	2.047X+6.422	0.201 ± 0.003 (0.026 – 0.014)	90.00	0.900	A	0.0653 ± 0.022 (0.129 – 0.041)	96.92	0.960	A
	48	3.574	2.113X+6.618	0.017 ± 0.002 (0.022 – 0.012)	52.94	0.529	A	0.059 ± 0.016 (0.102 – 0.036)	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	LC_{50} ± SE (Fiducial limits) (ppm)	Co-toxicity coefficient	Combined factor	Nature of action	LC_{90} ± SE (Fiducial limits) ppm	Co-toxicity coefficient	Combined factor	Nature of action
1:1	24	0.628	1.39X+6.38	0.010 ± 0.0025 (0.0151 – 0.004)	180.00	1.80	S	0.033 ± 0.011 (0.113 – 0.033)	190.90	1.90	S
	48	0.671	1.15X+6.71	0.007 ± 0.002 (0.011 – 0.003)	128.57	1.28	S	0.023 ± 0.009 (0.043 – 0.007)	130.43	1.30	S
1:2	24	3.36	1.54X+6.18	0.019 ± 0.004 (0.025 – 0.008)	94.73	0.94	A	0.066 ± 0.047 (0.188 – 0.004)	95.45	0.95	A
	48	1.55	0.75X+6.35	0.016 ± 0.003 (0.04 – 0.009)	56.25	0.56	A	0.047 ± 0.032 (0.131 – 0.003)	63.82	0.63	A
1:4	24	2.416	2.015X+6.331	0.021 ± 0.002 (0.027 – 0.016)	85.71	0.85	A	0.0724 ± 0.026 (0.145 – 0.042)	87.50	0.87	A
	48	3.103	1.928X+6.571	0.015 ± 0.002 (0.019 – 0.0109)	60.00	0.60	A	0.051 ± 0.019 (0.107 – 0.033)	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	LC_{50}±SE (Fiducial limits) (ppm)	Relative toxicity irrespective of time period	LC_{90}±SE (Fiducial limits) ppm	Relative toxicity irrespective of time period
Anisop	24	1.499	4.054X-3.481	12.351 ± 0.819 13.957 – 10.743	3.732	25.572 ± 3.965 33.345 – 17.799	1.85
	48	1.020	4.236X-3.563	10.500 ± 0.805 12.078 – 8.922	3.173	21.072 ± 2.523 26.0183 – 16.126	1.52
Cyclop	24	2.168	1.768X-1.951	5.290 ± 0.836 6.930 – 3.649	1.598	18.058 ± 10.352 48.349 – 7.767	1.30
	48	3.839	2.063X-1.863	3.309 ± 0.610 4.506 – 2.111	1.00	13.827 ± 2.959 19.628 – 8.026	1.00

upper and lower fiducial limits after 24 and 48 h of exposure, accordingly. The PEE followed, CEE with LC_{50} 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 LC_{50} 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 LC_{90} values also show the same trend as shown by LC_{50} values. In case of PEE LC_{90} 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 LC_{90} values were (1 282.45 ± 501.34) ppm with 2 265.07 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 LC_{90} values were (923.60 ± 140.33) ppm with 1 198.64 and 648.56 ppm upper and lower fiducial limits and (887.00 ± 139.01) with 1 159.46 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 anopheline larval (Table 3). The LC_{50} and LC_{90} 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 LC_{50} and LC_{90} values 8.10 and 29.55 ppm after 24 hours and 5.38 and 20.79 ppm after 48 hours. The LC_{50} of fraction III (FPE3) was 4.02 and 2.37 ppm and LC_{90} was 26.10 and 11.58 ppm after 24 hours and 48 hours of exposure period, respectively. Fraction IV (FPE4) was observed with LC_{50} value 1.85 and 1.30 ppm

and LC_{90} 12.15 and 6.73 ppm after 24 hours and 48 hours of treatment period, respectively. Fraction V (FPE5) had the LC_{50} and LC_{90} 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 LC_{50} and LC_{90} 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 LC_{50} and LC_{90} values 0.012 and 0.040 ppm after 24 hours and 0.008 and 0.021 ppm after 48 hours of treatment. The LC_{50} and LC_{90} of ratio 1:4 were 0.0201 and 0.0653 ppm after 24 hours and LC_{50} 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 LC_{50} values, and with LC_{90} 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 LC_{50} 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 LC_{90} 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 LC_{50} were 90.00 and 52.94 and combined factor values were 0.9 and 0.52, the co-toxicity coefficient values of LC_{90} 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 LC_{50} and LC_{90} 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 LC_{50} and LC_{90} values 0.019 and 0.066 ppm after 24 hours and 0.016 and 0.047 after 48 hours of treatment. The LC_{50} and LC_{90} of ratio 1:4 were 0.021 and 0.072 ppm after 24 hours and LC_{50} 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 LC_{50} values and co-toxicity coefficient of LC_{90} 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 LC_{50} was 94.73 and 56.25 and combined factor was 0.94 and 0.56 and the co-toxicity coefficient of LC_{90} 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 LC_{50} were 85.71 and 60.00 and combined factor values were 0.85 and 0.60 and the co-toxicity coefficient values of LC_{90} 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 LC_{50} and LC_{90} 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 LC_{50} (10.500±0.805) ppm with 12.078 and 8.922 ppm upper and lower fiducial limits and LC_{90} (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 LC_{50} (5.290±0.836) ppm with 6.930 and 3.649 ppm being upper and lower fiducial limits and LC_{90} (28.058±10.352) ppm with 48.349 and 7.767 ppm upper and lower fiducial limits after 24 hours and LC_{50} was (3.309±0.610) ppm and LC_{90} 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 LC_{50} 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 LC_{50} 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 *Abrus precatorius* (*A. precatorius*), *Croton bonplandianum* (*C. bonplandianum*), *Cynodon dactylon*, *Musa paradisiaca* (*M. paradisiaca*) and *Syzygium aromaticum* (*S. aromaticum*) were tested against fourth instar larvae of *Anopheles vagus* (*An. 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 *An. vagus* with LC_{50} values of 19.31, 39.96, 35.18, 79.90 and 85.90 μg/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 μg/mL, respectively[9].

Mulla and Su[10] 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 gigantica* 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 larval 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 offinale* (*Z. offinale*), *Curcuma longa* (*C. longa*)] and vegetable waste (*Solanum 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. offinale*, *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 caspius* (Insecta) > *Gambusia affinis* (Poeciliidae) > 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 reticulate* (*P. reticulata*) (predatory fishes), *Diplonychus indicus* (*D. indicus*) (predatory water-bug), 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 mauritiana* (*S. mauritiana*) on non-target aquatic invertebrates and vertebrates, damselfly and dragonfly nymph, macro dytiscids, micro dytiscids, 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. mauritiana*, respectively, after 24 h. Promsiri *et al*[27] reported that out of 14 plant extracts found to be larvicidel, 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*, *Stemona tuberosa* (*S. tuberosa*), *Strychnos nux-vomica*, and *Kaempferia galanga* (*K. galanga*) extracts were toxic to guppy fish at concentrations of 100, 100, 50, 50, 50, 100, 100, and 50 μg/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, Pelvicachromis pulcher, Anacardium occidentale, Musa siamensis, Apium graveolens,* 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 μg/mL, respectively.

It is concluded that the present combination is one of the best larvicidal combination in the management of anopheline 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.

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