International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 04-05-2026, Accepted: 04-06-2026, Published: 19-06-2026
Volume 11, Issue 2, 2026, Page No. 498-504

Variation of synergistic larvicidal activity of petroleum ether plant extracts against *Culex quinquefasciatus* and *Aedes albopictus*

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DOI: https://doi.org/10.66856/ijer.2026.11.2.11209

Abstract
This paper will examine the larvicidal properties of petroleum ether extracts of *Jacquinia ruscifolia* flower and *Thuja orientalis* strobili which have been combined to produce greater synergistic effects against *Culex quinquefasciatus* and *Aedes albopictus* mosquitoes. They were carried out in three conditions of concentration (50 ppm, 100 ppm, and 150 ppm plus in different proportions, 1:1, 1:2 and 2:1) and at different times (24, 48 and 72 hrs) of the extracts in various conditions (as extracts alone and in different mixtures). Findings were that there was high mortality of larvae with 100 percent death in *Cx. quinquefasciatus* with the 2: 1 mixture after 72 hours, and in *Ae. Albopictus* with the 1:2 mixture. The calculated synergy factors (SF) were always more than 1 in all treatments, which indicates a synergistic effect of the extracts, especially in *Cx. quinquefasciatus* in comparison with *Ae. Albopictus*. This study also brings out the possibility of using plant extracts as powerful larvicides in the control of the mosquitoes.

Keywords: *Jacqunia ruscifolia, Thuja orientalis, Culex quinquefasciatus, Aedes albopictus*, synergism, synergistic factor

Introduction

Culex quinquefasciatus and *Aedes albopictus* are the main disease-carrying mosquitoes responsible for lymphatic filariasis and dengue in rural areas of India. As socio-economic issues increase, people around the world are facing increased risk of these diseases (Ghosh *et al*., 2012) [1]. *Culex* mosquitoes can breed rapidly in dirty water sources such as clogged drains (Chandra, 2001) [2] poorly maintained pits, septic tanks and other similar places. They complete their life cycle in about 7 to 10 days (Chandra & Hati, 1993) [3]. These mosquitoes bite mainly in the third part of the night and rest in the darkest corners of houses during the day (Chandra, 1995) [4]. *Aedes albopictus* mosquitoes, on the other hand, are more active during the day and bite outdoors (Chamberlain & Sudia, 1961) [5]. *Aedes* species are known to breed in small containers such as coconut shells, flower pots, buckets, empty cans, earthen pots, open water tanks, old tyres, unused shoes and even ice cream cups (Getachew *et al*., 2015) [6].

We believe that it is better to prevent diseases than to cure them. Controlling the population of these disease-carrying mosquitoes can help reduce the spread of infection. There has been a long-running debate about the effectiveness and safety of chemical pesticides. Unlike traditional insecticides, which target only a part of the insect's biology, plant-based botanical mixtures can affect both the behaviour and physiological processes of the mosquito. Research has shown that these natural insecticides are less likely to develop resistance in mosquitoes. To find new and effective solutions, we chose two plants, *Jacquinia ruscifolia* and *Thuja orientalis*, for chemical extraction and tested their combined effects on the larvae of *Culex quinquefasciatus* and *Aedes albopictus*.

Jacquinia ruscifolia belongs to Theophrastaceae family and is well known as garden plants in India (CSIR, 1959) [7]. This evergreen shrub grows 2.4 to 3.0 m tall (Bose, 1965) [8], has dark brown, almost black branchlets, and has dense, lance-shaped leaves that are spiny at the ends (Ganguly *et al*., 1969) [9]. During summer it produces small red or orange flowers. Earlier studies on its seeds isolated compounds such as β-sitosterol acetate, its glucosides and four triterpenes along with other bioactive substances from its stem bark and leaves (Jain & Sharma, 2017) [10].

Thuja orientalis plant belongs to the Cupressaceae family. Various parts of this plant have been used for various medicinal purposes, including promoting hair growth, treating viral infections, reducing allergies and managing inflammation, bacterial infections, fungal infections, etc. It has also shown effectiveness against pests such as insects, worms and snails.

This study sought to ascertain the synergistic effectiveness of petroleum extracts from *J. ruscifolia* flowers and *T. orientalis* strobili when applied in various combinations against *Cx. quinquefasciatus* and *Ae. albopictus* larvae. We will contrast these outcomes with those of well-known, widely used medications like BTI and temephos. By maintaining a low mosquito population, the proper combination may help regulate the growth of mosquito larvae and so stop the spread of dengue and filariasis.

Materials and Methods

Composition of Plant Materials

Fresh brilliant red-coloured flowers of *J. ruscifolia* and mature green spherical *T. orientalis* strobili were collected randomly from the campus during April to May in the year 2021. After proper identification, a voucher sample was submitted to the Mosquito, Microbiology and Nanotechnology Research Units, Department of Zoology, University of Burdwan.

Breeding of Mosquito Larvae

In Burdwan city, mosquito larvae, *Cx. quinquefasciatus* and *Ae. Albopictus*, were collected from drains. Once correctly identified, the larvae were stored in plastic trays. Additionally, a carefully maintained mosquito colony was established in the Mosquito, Microbiology and Nanotechnology research units of the Department of Zoology at the University of Burdwan. All contaminants including pesticides, disinfectants, pathogens and repellents were kept out of the colony. The setup was kept at a temperature of 27 ± 1.00°C and relative humidity of 80 ± 1.00% in a laboratory setting. During the light and dark cycle there were thirteen hours of light and eleven hours of darkness. The larvae were reared in plastic containers measuring approximately 35 × 25 × 10 cm and were provided with a diet composed of dry brewer's yeast powder, wheat flour, glucose and algae mixed in a ratio of 3:1:1:1.

Preparation of Solvent Extracts

To prepare solvent extracts, *J. ruscifolia* fresh bright red flowers of *J. ruscifolia* and mature green spherical *T. orientalis* strobili were spread and dried for a few days. Once dried, 100 g of dried flowers and strobili were chopped and then thoroughly ground into a fine powder. This powder was placed in a BOD bottle in a ratio of 1:10 with the solvent, which was petroleum ether. Each sample was processed separately. The resulting liquid from the extraction was first filtered using Whatman No. 41 filter paper, followed by a second filter paper.

Dose-Dependent Larvicidal Bioassay

Twenty-five third instar larvae of *Cx. quinquefasciatus* according to WHO standard protocol (WHO, 2005). *Cx. Quinquefasciatus* and *Ae. Albopictus* was placed in a 150 ml glass beaker containing 100 ml tap water. For larvicidal bioassay, *J. ruscifolia* petroleum ether extracts of *T. orientalis* flowers and *T. orientalis* strobili were prepared at concentrations of 50, 100, 150, 200, 250 ppm, respectively. To check coordination, *J. ruscifolia* extracts of *J. ruscifolia* red flower (F_P) and *T. orientalis* strobili (S_P) were used in combination in various ratios of 1:1, 1:2 and 2:1. Each combination was mixed well and tested in triplicate for each concentration. A control group experiment was also conducted. A larva was considered dead if no movement was seen when touched gently with a fine-tipped needle, without causing any damage to the test specimen.

Statistical Analysis

Statistical analysis involves using Abbott's formula (Abbott, 1925) [12] to determine the percentage of larval mortality (%M) when looking at the larvicidal effect of plant extracts individually and in combination. Mean percentage of mortality, standard errors and LC_{50} values were calculated using MS Excel 2016 and Stat Plus 2009.

Calculating Synergistic Effects

A true synergistic effect occurs when two or more chemicals work together to produce an effect that is stronger than the combined effect of each chemical used alone (Yuana & Chenb, 2019) [13] The SF is determined by dividing the LC_{50} value of the larvicide by the LC_{50} value of the larvicide in combination with a potential synergist. This can also be done by dividing the LC_{50} value of a single plant extract by the LC_{50} value of a mixture of plant extracts or synergists (Cortina-Borja *et al*., 2009) [14]. If the SF value is greater than 1, it indicates that there is synergy. To evaluate how well the drug combination works, the combination index analysis method was used, as described by Chou & Talalay (Chou, 2010) [15]. This method was performed using CompuSyn software version 1 developed by Ting-Chao Chou and Nick Martin.

Semi-Field Trials

Semi-field trials were conducted following WHO guidelines (WHO, 2005; Srivastava *et al*., 2020[11, 16]; Rej *et al*., 2022; Mandal & Chandra, 2024) [18]. Artificial containers, which were 5-litre jars with a base diameter of 30 cm or 20 cm, were used. These containers were filled with debris-free drain water to test against *Cx. quinquefasciatus* and rainwater stored in earthen pots for *Ae. Albopictus*. A group of 100 laboratory-reared third-instar larvae of each species were tested, with three test replicates for each. Larval food was added to containers used in the semi-field study. After allowing the larvae to adapt for 2 to 3 h, the containers were treated with five different doses (50,100, 150, 200 and 250 ppm, respectively). Nylon mesh was placed over the containers to prevent other mosquitoes and insects from depositing eggs and to prevent debris from entering. Three replicates of each dose and three separate control groups were also tested. Containers were inspected after 24-, 48-, and 72-hour treatment.

Result

A bioassay test using petroleum ether extracts from red flowers of *J. ruscifolia* (F_P) and strobili of *T. orientalis* (S_P) showed better results when the extracts were used together rather than separately. The highest number of larvae died after 72 hours of exposure at a concentration of 250 ppm, and this was observed in both *Cx. quinquefasciatus* (Table 1) and *Ae. Albopictus* (Table 4). In a semi-field trial, it was found that as concentrations increased, more larvae died over time for *Cx. quinquefasciatus* (Table 3) and *Ae. Albopictus* (Table 6). LC_{50} values, regression equations and R^2 values for larval mortality at different exposure times are listed in Table 2 (*Cx. quinquefasciatus*) and Table 5 (*Ae. albopictus*). The synergistic effects of F_P and S_P in the ratio of 1:1, 1:2 and 2:1 was also studied and are given in Table 7 (*Cx. quinquefasciatus*) and Table 9 (*Ae. albopictus*). The most effective combinations were shown by dose-effect curve and combination index (CI) plot (FA-CI plot) in Table 8 (*Cx. quinquefasciatus*) and Table 10 (*Ae. albopictus*). These plots show how the partial percent mortality (FA) changes with different concentrations, calculated using CompuSyn software from FA = 0.10 to 0.99. A CI value less than 1 means the effect is antagonistic, equal to 1 means it is additive, and greater than 1 means it is synergistic. Table 11 shows the effect of *J. ruscifolia*. The larvicidal effects of petroleum ether extracts from *J. ruscifolia* (F_P) and *T. orientalis* (S_P) have been compared *Cx. quinquefasciatus* and *Ae. Albopictus*.

Table 1: Bioassay experiment to assess the percentage of larval mortality, expressed as the average with standard error, in third-instar *Culex quinquefasciatus* larvae. The study utilized extracts from the red flowers of *Jacquinia ruscifolia* (Fp), the cones of *Thuja orientalis* (Sp), and their mixtures in the ratios 1:1, 1:2, and 2:1, all prepared using petroleum ether. The experiment involved the application of five distinct concentrations at regular intervals

Conc. (ppm)	Time (h)	F_p	S_p	1:1	1:2	2:1
50	24	23.67±2.67	18.33±3.53	23.67±3.53	46.00±1.33	54.33±1.33
	48	54.33±3.53	45.00±0.23	65.33±1.33	66.67±2.67	69.33±2.67
	72	62.33±3.53	75.67±3.53	81.00±0.33	77.00±2.31	86.33±1.33
100	24	34.67±1.33	34.33±3.53	32.00±1.33	57.33±3.53	61.33±2.67
	48	58.33±3.53	58.67±3.53	85.00±2.31	68.00±2.31	73.00±2.31
	72	65.33±1.33	82.33±1.33	94.67±1.33	85.00±0.33	87.67±1.33
150	24	43.67±3.53	42.00±2.31	54.33±3.53	74.00±0.33	73.33±1.33
	48	72.67±3.53	64.00±2.31	90.67±3.53	81.00±2.31	84.00±2.31
	72	81.00±2.31	90.67±3.53	96.00±0.33	96.00±2.31	97.00±0.33
200	24	62.33±1.33	57.00±2.31	75.67±1.33	74.67±3.53	78.33±1.33
	48	79.67±2.67	85.33±3.53	96.00±2.31	89.00±2.31	88.33±1.33
	72	90.67±3.53	98.67±1.33	98.00±0.33	93.33±1.33	97.33±1.33
250	24	68.33±1.33	79.67±1.33	93.00±0.33	87.67±3.53	90.33 ± 1.33
	48	89.67±3.53	89.67±1.33	95.00±1.33	98.67±1.33	97.33±1.33
	72	98.67±1.33	98.67±3.53	100.00±0	100.00±0	100.00±0

Table 2: Bioassays employing petroleum ether extracts of Fp, Sp, and their combinations (Fp:Sp ratios of 1:1, 1:2, and 2:1) against third-instar *Cx. quinquefasciatus* larvae

Plant extract	Time (h)	Regression equation	R^2 value	LD_{50} value
Flower extract (F_p)	24	y = 0.25x + 11.13	0.98	154.01
	48	y = 0.18x + 40.33	0.97	56.98
	72	y = 0.20x + 39.19	0.97	45.80
Strobilus extract (S_p)	24	y = 0.30x + 1.56	0.96	151.76
	48	y = 0.28x + 32.73	0.99	68.38
	72	y = 0.13x + 69.66	0.96	29.95
F_p:S_p (1:1)	24	y = 0.38x - 1.06	0.98	117.91
	48	y = 0.17x + 64.40	0.77	38.93
	72	y = 0.08x + 82.33	0.68	28.11
F_p:S_p (1:2)	24	y = 0.21x + 35.14	0.97	66.70
	48	y = 0.19x + 52.00	0.89	42.05
	72	y = 0.12x + 70.66	0.87	24.14
F_p:S_p (2:1)	24	y = 0.18x + 42.53	0.98	51.71
	48	y = 0.15x + 61.39	0.97	29.59
	72	y = 0.081x + 80.89	0.90	14.88

Table 3: Bioassay experiment to determine the percentage of larvicidal mortality (mean ± standard error) on third instar *Culex quinquefasciatus* larvae using petroleum ether extracts from *Jacquinia ruscifolia* red flowers (Fp), *Thuja orientalis* strobili (S_p), and their combinations (F_p:S_p = 1:1, 1:2, and 2:1) at five consecutive concentrations applied at regular intervals under semi-field conditions

Cons. (ppm)	Time (h)	Flower extract (F_p)	Strobilus extract (S_p)	F_p:S_p (1:1)	F_p:S_p (1:2)	F_p:S_p (2:1)
50	24	21.67±1.20	17.33±0.88	36.67±1.20	44.00±1.20	53.33±0.88
	48	53.33±0.88	44.00±1.15	62.33±0.88	66.67±1.20	70.33±0.88
	72	62.33±0.88	74.67±1.20	79.00±1.15	76.33±1.20	82.33±1.20
100	24	34.67±1.33	32.33±0.88	41.00±1.15	57.33±0.88	61.33±0.88
	48	55.33±0.88	58.33±0.88	72.00±0.58	66.00±0.58	74.00±1.15
	72	65.33±0.88	80.00±0.58	81.67±1.20	84.00±0.58	86.67±1.20
150	24	42.67±1.20	40.00±1.15	53.33±0.88	72.00±0	73.33±1.33
	48	70.67±1.20	64.00±1.15	74.67±1.20	80.00±0.58	84.00±1.15
	72	71.00±0.58	90.67±1.20	87.00±0.58	89.00±1.15	96.00±0.58
200	24	61.33±0.88	58.00±0.58	74.67±1.33	74.67±1.20	77.33±0.88
	48	73.67±1.20	84.33±0.88	84.00±1.15	86.00±0.58	89.33±0.88
	72	80.67±1.20	92.67±1.20	90.00±1.15	93.33±0.88	96.67±1.20
250	24	69.33±0.88	78.33±0.88	83.00±1.15	86.67±1.20	89.67±1.20
	48	89.67±1.20	85.67±1.20	89.00±1.15	92.67±1.20	97.33±0.88
	72	88.67±1.20	95.67±1.20	93.00±0.58	96.00±1.15	100.00±0.58

Table 4: Bioassay experiment to determine the mean percent larvicidal mortality on third instar larvae of *Aedes albopictus* using crude extracts from *Jacquinia ruscifolia* red flowers (F_c), *Thuja orientalis* strobili (S_c), and a mixture of F_c:S_c in a 1:1 ratio. The experiment involved five consecutive concentrations applied at regular intervals under laboratory conditions

Cons. (ppm)	Time (h)	Flower extract (F_p)	Strobilus extract (S_p)	F_p:S_p (1:1)	F_p:S_p (1:2)	F_p:S_p (2:1)
50	24	26.67 ± 1.33	21.33 ± 1.33	25.33 ± 1.33	22.67 ± 1.33	24.00 ± 0
	48	33.33 ± 1.33	34.67 ± 1.33	33.33 ± 1.33	34.67 ± 1.33	30.67 ± 1.33
	72	41.33 ± 1.33	38.67 ± 1.33	41.33 ± 1.33	38.67 ± 1.33	41.33 ± 1.33
100	24	45.33 ± 2.67	42.67 ± 2.67	38.67 ± 1.33	38.67 ± 1.33	38.67 ± 1.33
	48	50.67 ± 1.33	45.33 ± 1.33	49.33 ± 2.67	52.00 ± 0	48.00 ± 2.31

	72	57.33 ± 1.33	65.33 ± 1.33	66.67 ± 1.33	66.67 ± 1.33	62.67 ± 1.33
150	24	53.33 ± 1.33	54.67 ± 1.33	50.67 ± 3.53	49.33 ± 2.67	46.67 ± 2.67
	48	62.67 ± 1.33	60.00 ± 0	61.33 ± 1.53	60.67 ± 1.76	59.33 ± 0.67
	72	69.33 ± 1.33	70.67 ± 2.67	73.33 ± 2.67	70.67 ± 2.67	66.67 ± 1.33
200	24	70.67 ± 1.33	68.00 ± 2.31	69.33 ± 2.67	68.00 ± 2.31	65.33 ± 1.33
	48	73.33 ± 1.33	69.33 ± 1.33	70.67 ± 1.33	72.00 ± 2.31	71.00 ± 3.00
	72	78.67 ± 2.67	78.67 ± 2.67	81.33 ± 2.67	82.67 ± 1.33	80.00 ± 2.31
250	24	82.67 ± 1.33	80.00 ± 2.31	81.33 ± 2.67	81.33 ± 1.33	80.00 ± 2.31
	48	89.33 ± 1.33	86.67 ± 3.53	92.00 ± 2.31	94.67 ± 1.33	93.33 ± 1.33
	72	98.67 ± 1.33	93.33 ± 1.33	98.67 ± 1.33	100.00 ± 0	97.33 ± 1.33

Table 5: Bioassays employing petroleum ether extracts from Sp, Fp, and their combinations (Fp: Sp ratios of 1:1, 1:2, and 2:1) against third instar larvae of *Aedes albopictus* at varying exposure times (24, 48, and 72 hours)

Plant extract	Time (h)	Regression equation	R^2 value	LD_{50} value
Flower extract (F_p)	24	y = 0.2747x + 14.532	0.9889	111.87
	48	y = 0.2693x + 21.468	0.9931	91.554
	72	y = 0.272x + 28.26	0.9864	73.6
Strobilus extract (S_p)	24	y = 0.2853x + 10.533	0.9853	120.95
	48	y = 0.256x + 20.8	0.9918	97.35
	72	y = 0.2453x + 32.536	0.9281	71.37
F_p:S_p (1:1)	24	y = 0.2853x + 10.268	0.9949	121.53
	48	y = 0.2774x + 19.728	0.9833	93.12
	72	y = 0.2587x + 33.465	0.9458	71.25
F_p:S_p (1:2)	24	y = 0.2933x + 8.005	0.9951	125.8
	48	y = 0.28x + 20.802	0.9738	89.39
	72	y = 0.2753x + 30.238	0.9393	67.07
F_p:S_p (2:1)	24	y = 0.2773x + 9.336	0.988	129.03
	48	y = 0.2966x + 15.97	0.9841	97.44
	72	y = 0.2587x + 30.801	0.9652	71.28

Table 6: Results of a bioassay experiment that demonstrates the mean percent larvicidal mortality against third instar *Aedes albopictus* larvae. The study utilized three different treatments: crude extract from *Jacquinia ruscifolia* red flowers (F_c), crude extract from *Thuja orientalis* strobili (S_c), and a 1:1 mixture of F_c and S_c. The experiment was conducted using five consecutive concentrations, with equal intervals between each concentration, under semi-field conditions

Cons. (ppm)	Time (h)	Flower extract (F_p)	Strobilus extract (S_p)	F_p:S_p (1:1)	F_p:S_p (1:2)	F_p:S_p (2:1)
50	24	24.67 ± 1.33	27.33 ± 0.88	25.67 ± 1.20	22.67 ± 1.33	24.00 ± 0
	48	31.33 ± 1.20	31.67 ± 1.20	33.67 ± 1.20	34.67 ± 1.33	30.67 ± 1.33
	72	42.33 ± 1.33	38.67 ± 1.33	43.33 ± 1.53	44.67 ± 1.33	41.33 ± 1.33
100	24	46.33 ± 2.67	41.67 ± 1.20	39.67 ± 1.33	38.67 ± 1.33	38.67 ± 1.33
	48	49.67 ± 1.33	49.33 ± 0.88	45.33 ± 2.67	52.00 ± 0	48.00 ± 2.31
	72	57.67 ± 1.20	62.33 ± 0.88	61.67 ± 1.33	66.67 ± 1.33	62.67 ± 1.33
150	24	51.33 ± 1.33	56.67 ± 1.20	50.33 ± 1.53	49.33 ± 2.67	46.67 ± 2.67
	48	60.67 ± 1.33	60.33 ± 0.88	59.33 ± 1.53	60.67 ± 1.76	59.33 ± 0.67
	72	67.33 ± 1.33	69.67 ± 1.67	67.33 ± 2.67	70.67 ± 2.67	66.67 ± 1.33
200	24	70.33 ± 1.33	65.00 ± 1.15	69.33 ± 1.53	68.00 ± 2.31	65.33 ± 1.33
	48	73.67 ± 1.20	70.33 ± 0.88	70.67 ± 1.33	72.00 ± 2.31	71.00 ± 2.31
	72	78.33 ± 2.67	76.67 ± 1.20	81.33 ± 1.67	82.67 ± 1.33	80.00 ± 2.31
250	24	80.67 ± 1.33	78.00 ± 1.15	79.33 ± 0.88	81.33 ± 1.33	80.00 ± 2.31
	48	85.33 ± 1.20	81.67 ± 1.53	88.00 ± 1.15	94.67 ± 1.33	93.33 ± 1.33
	72	91.67 ± 1.15	93.33 ± 0.88	91.67 ± 1.33	100.00 ± 0	97.33 ± 1.33

Table 7: Estimation of the synthetic fungicidal (SF) activity of the petroleum ether extracts from the flowers of *Jacquinia ruscifolia* (F_p) and the strobili of *Thuja orientalis* (S_p) when used in combination in the ratios of 1:1, 1:2, and 2:1 against the third instar larvae of *Culex quinquefasciatus*

Time (h)	Combination 1:1		Combination 1:2		Combination 2:1	
	F_p	S_p	F_p	S_p	F_p	S_p
24	1.33	1.33	2.26	2.27	2.96	2.97
48	1.48	1.78	1.36	1.65	1.95	2.35
72	1.54	0.99	1.09	1.26	3.01	1.94

Table 8: Dose-effect curve and combination index plot for the different ratios of *Jacquinia ruscifolia* flower (Fp) and *Thuja orientalis* strobili (Sp) petroleum ether extracts, specifically the 1:1, 1:2, and 2:1 combination, against third instar *Culex quinquefasciatus* larvae. The results show that the combination treatment yielded the highest level of mortality

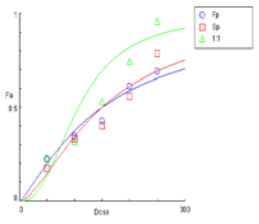
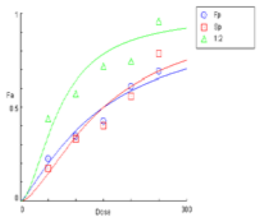
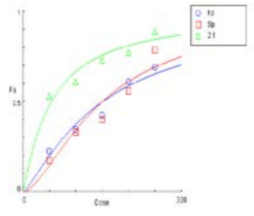
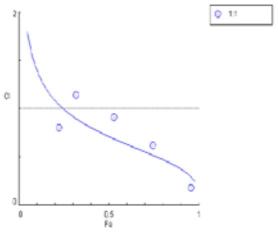
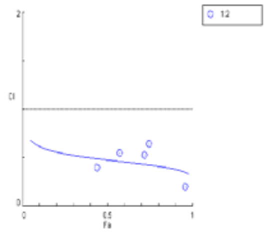
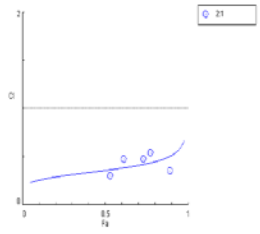
Curve type	24 h plot		
	1:1	1:2	2:1
The dose-effect curve			
Index composite plot			

Table 9: Estimation of the SF (Synthetic Formula) for the petroleum ether extract of *Jacquinia ruscifolia* flowers (F_p) and *Thuja orientalis* strobili (S_p), including their combinations in the ratios of 1:1, 1:2, and 2:1, against *Aedes albopictus*

Time (h)	1:1		1:2		2:1	
	F_p	S_p	F_p	S_p	F_p	S_p
24	0.921	0.995	0.889	0.961	0.867	0.937
48	0.938	1.045	1.024	1.046	0.939	0.999
72	1.097	1.064	1.033	1.063	1.033	1.001

Table 10: Dose-effect curve and combination index plot for various ratio 1:1, 1:2, and 2:1 of the petroleum ether extracts from *Jacquinia ruscifolia* flowers (F_p) and *Thuja orientalis* strobili (S_p), against third instar *Aedes albopictus* larvae, showing that the combined treatment yielded the highest mortality rate

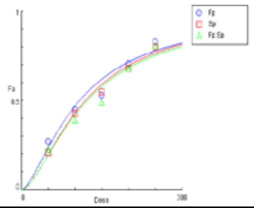
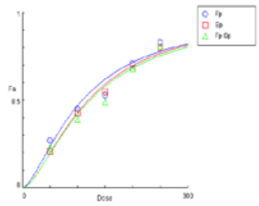
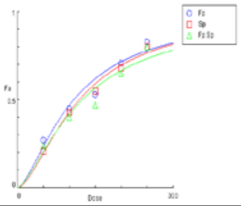
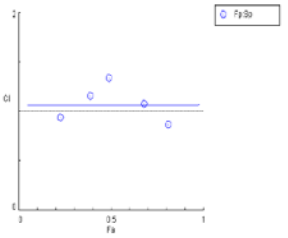
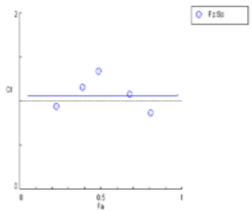
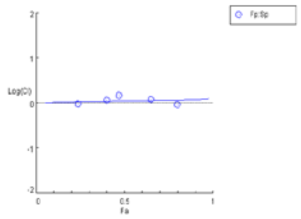
Curve type	24 h plot		
	1:1	1:2	2:1
The dose-effect curve			
Index composite plot			

Table 11: Bioassay experiment, showing the percentage of mortality (mean ± standard error) observed in third instar larvae of the mosquitoes *Cx. quinquefasciatus* and *Ae. Albopictus* when exposed to various treatments. The treatments included extracts from F_p and S_p, used in different combinations (F_p: Sp = 1:1, 1:2, and 2:1), as well as the commercial products Temephos, BTI (ABIL), and BTI (164), all applied at a concentration of 250 ppm

Test Organism	Time	F_p:S_p(1:1)	F_p:S_p(1:2)	F_p:S_p(2:1)	Temephos	BTI(1)	BTI(2)
Cx. quinquefasciatus	24	96.00 ± 0	86.67±3.53	89.33±1.33	89.33 ± 1.65	44.00±0.33	49.33±1.33
	48	100.00 ± 0	98.67±1.33	97.33±1.33	94.33 ± 1.25	61.33±1.63	57.33±1.33
	72	100.00 ± 0	100.00 ± 0	100.00 ± 0	100.00±0.33	79.00±0.33	72.00 ± 0
Ae. albopictus	24	79.33±0.88	81.33 ±1.33	80.00 ±2.31	87.33 ± 1.33	49.33±1.33	36.33±1.33
	48	88.00 ±1.15	94.67 ±1.33	93.33 ±1.33	96.33 ± 1.33	61.33±1.33	58.00±0.88
	72	91.67 ±1.33	100.00 ± 0	97.33 ±1.33	100.00±1.20	69.67±0.88	75.33±0.33

Discussion

When we emphasize on the compatibility and acceptability of phytochemicals over any chemical larvicide, various viewpoints come to our mind. There is no doubt that we cannot ignore the wide acceptance of efficiency, biodegradability of phyto-larvicides which aptly act as alternative mosquito control strategy.

Bioassay experiment with petroleum ether extract from *J. ruscifolia* red flower (Fp) and *T. orientalis* strobili (S). The highest mortality was observed at exposure for *Cx. quinquefasciatus* at 250 ppm for 72 hours (Table 1) and *Ae. Albopictus* (Table 4). Semi-field results indicated the highest mortality was observed in Fp: sp = 2:1 in *Cx. quinquefasciatus* (Table 3) and Fp: Sp = 1:2 in the case of *Ae. Albopictus* (Table 6). Lowest LC50 value found is 14.88 ppm (Table 2) against *Cx. quinquefasciatus* and 67.07 ppm (Table 5) against Ae. Albopictus. Again, synergistic factor of Fp and Sp against their combined form Fp: Sp = 1:1, 1:2 and 2:1 was estimated in Table 7 (*Cx. quinquefasciatus*) and Table 9 (*Ae. albopictus*). The most effective synergistic dose response was as indicated by the dose effect curve and the combination index plot (fa-CI plot) in Table 8 *Cx. quinquefasciatus* and Table 10 *Ae. Albopictus*. In the fa-CI plot, the values are plotted as a function of the fractional percent response to (fa) by computer stimulus (CompuSyn) from fa= 0.10 to 0.99, were CI < 1, =1, > 1 representing antagonism, additive and synergism.

Previously, plant extracts have been studied for larvicidal and synergistic activity (Singha *et al*., 2011) [19] and for synergistic activity with synthetic larvicides such as phenthoate (Kalyanasundaram & Das, 1985) [20], phenanthion (Kalyanasundaram & Das, 1985) [20] and pyrethrum (Subramonia & Kathiresan, 1997) [21]. Determination of SF of red flowers of F_P and S_P in combination (F_P: SP = 1:1, 1:2 and 2:1) was done at three consecutive time points (24, 48 and 72 h) using the LC_{50} value of individual plant extracts and the LC_{50} value of the combination plant extracts. SF value >1 indicates the presence of synergy. When combinations showed high synergy in mortality against *Cx. quinquefasciatus* (Table 7) but less synergistic, more additive effect against *Ae. Albopictus* (Table 9).

Dose effect curves (in Tables 8 and 10) showed larvicidal mortality response in the form of F_P (blue line), S_P (red line) and the combination (green line). From the dose effect curve, it is observed that the combination was found to work more effectively than either one used alone. Similarly, the conjunction index plot (FA-CI plot) of the blue lines pointed above the gray horizontal line (representing the additive effect line) shows a trend toward opposition. Lower CI values along with higher FA values indicate better drug-drug compatibility and higher synergy between the tested combinations.

It is clear that Fp: Sp = 1:2 worked best among the three combinations (Fp: Sp = 1:1, 1:2 and 2:1) showing synergy against *Cx. quinquefasciatus.* The effect of *Cx. quinquefasciatus* against *Ae. Albopictus* was a simple additive effect or negligible synergy.

Furthermore, Table 11 shows the comparative analysis of larvicidal mortality between third instar larvae (*Cx. quinquefasciatus* and *Ae. albopictus*) with F_P, S_P petroleum ether extract in their combinations (1:1, 1:2 and 2:1), temephos, BTI (ABIL) and BTI (164) using 250 ppm concentration. This suggested combinations (Fp: Sp = 1:1, 1:2 and 2:1) as effective as temephos and more capable of showing rapid activity rather than BTI.

Acknowledgement

The authors thank Professor Ambarish Mukherjee, Department of Botany, Burdwan University, for his contribution to the identification of the species used in this paper work.

Funding

No funding was provided for this study.

Availability of data and materials

All data presented in this article were generated and analysed during the course of the study. The raw data are available at any time upon request of the corresponding Author. The abovementioned databases have been used for the analysis of the FT-IR, GC-MS and FE-SEM and may be provided at any time if requested.

Authors' contribution

SM was responsible for collecting the raw material, setting up the experiment, and generating data based on observations.

GC analysis was performed on the data, and conclusions were drawn from the results.

Ethical approval and consent to participate

No plants or animals were harmed during the course of this experiment.

Consent to publication

Not applicable.

Declaration of competing interest

The author declares that there are no known competing financial or personal interests that could have influenced the work reported in this paper.

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