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Efficacy of Seed Extracts of *Cuscuta chinensis* and *Asparagus setaceus* as Assumed Synergist against Larval Immatures of *Culex quinquefasciatus*

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Abstract As one kind of cost-effective, efficient and environment-friendly materials for direct control of mosquitoes, botanicals have been widely concerned. The present study has evaluated the synergistic effect of different blends of seed methanolic extract of *Cuscuta chinensis* (*C. chinensis*) with its assumed synergist *Asparagus setaceus* (*A. setaceus*) against larval instars of *Culex quinquefasciatus* (*Cx. quinquefasciatus*). Different larval instars were exposed to 100 ppm to 300 ppm concentrations separately and in blends at different ratios of prepared extracts of tested plants as per WHO protocol. Major seed secondary metabolites of interest were analysed. This study recorded the highest mortality at 300 ppm for both the plants against all instars in 72 h. In combinatorial bioassay, *A. setaceus* and *C. chinensis* at the ratio of 1:2 was more effective as a larvicide and it encountered (98.68±0.00)%, (97.32±0.33)%, (85.32±0.33)% and (94.68±0.33)% death of 1st, 2nd, 3rd and 4th instars larvae at 300 ppm concentration respectively after 72 h of exposure. In addition, the SF (Synergistic Factor) and CTC (Co-toxicity Coefficient) values indicated synergism. These results have suggested that the botanical blend of tested plants possesses a suitable property which may be exploited as a source of the larvicidal product to replace harmful chemical pesticides.

Keywords *Cuscuta chinensis*; *Asparagus setaceus*; Botanical derivatives; Mosquito control; Synergism

Background

Mosquitoes create an essential group of dipterans as far as human health is concerned. Among all the vector insects, mosquito alone can transmit diseases to more than seven hundred million people annually (Singha et al., 2011). Lymphatic filariasis occupies the second highest position after malaria in severity amongst the vector-borne diseases. *Wuchereria bancrofti*, transmitted by *Culex quinquefasciatus* is principally responsible for filarial burden in tropical and humid areas of the world. About 31 million people are carriers of filarial parasites and over 23 million people suffer from filarial disease manifestations in India (WHO, 2005a). Though synthetic insecticides are fast acting against mosquito larvae, their indiscriminate application has created several environmental issues like development of resistance, human health hazards and undesirable effects on non-target organisms (Hansch and Verma, 2009). Insecticides of botanical origin are more preferable for its minimal cost and eco-friendly nature. The scientific report suggests that about 344 species of plants have a variety of activities against mosquitoes (Sukumar et al., 1991) and phytochemicals have received more attention as potential mosquito larvicide (Ghosh et al., 2012; Bhattacharya and Chandra, 2013; Bhattacharya and Chandra, 2014). However, synergists are effective for overcoming metabolic resistance and are more potent than the individual component of any mixture. Moreover, scientists have been conducted studies on the synergistic and toxic effect of mixtures of botanical principles against agricultural pests rather than vectors of diseases (Essam et al., 2005; Hari and Mathew, 2018) and have reported these compounds as very advantageous (Caraballo, 2000). In this context, it is necessary to study the efficacy of the combination of active principles of botanical origin to diminish disease vectors. In the present investigation, we conducted experiments to measure the individual and combined effects of seed extracts of *Cuscuta chinensis* and *Asparagus setaceus* on larval instars of *Cx. quinquefasciatus* mosquito.

1 Materials and Methods

1.1 Phytoextract preparation

Mature seeds of *Cuscuta chinensis* and *Asparagus setaceus* were collected manually from different localities of Kulti and Polba, West Bengal. Professor Dr. Ambarish Mukherjee, Department of Botany, The University of Burdwan authenticated the plants. The voucher specimen (Voucher number- GCSC05, GCSC06) submitted as herbarium in Mosquito, Microbiology, and Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan. Tap-water washed seeds were soaked in a paper towel and dried in good air draft in shaded condition. Shed dried seeds were crushed into a fine powder. Methanol extracts of plants were obtained by taking 300 g of seed powder in a glass container previously filled with 1,500 mL of methanol (Merck) and extracted for 72 h by cold maceration (Okoye and Osadede, 2009). The extraction was processed by stirring it thrice per day (morning, noon, and afternoon) in the Mosquito, Microbiology and Nanotechnology Research Units, Parasitology laboratory Department of Zoology, The University of Burdwan. The maceration process was then repeated twice for maximal extraction. After that, a solvent with active principles was filtered with Whatman No-1 filter paper (size 24 cm, England) and the filtrate was concentrated almost to dryness at 40°C in rotary vacuum evaporator RE300 ROTAFLO (Fisher Scientific UK Ltd., UK). The filtrate was stored at 4°C in an air-tight container for future laboratory assay (Dong et al., 2005).

1.2 Test insect

Different larval instars of *Cx. quinquefasciatus* were collected by scooping and dipping process (Robert et al., 2002) from neighbour drains of Burdwan University campus (23°16'N, 87°54'E) to establish the colony. The collected assorted larval colony were maintained in a shallow plastic tray filled with tap water at (27±2)°C and 75%~85% relative humidity under a photoperiod of 13:11 hrs (light/dark) without exposure to pathogens or insecticides in Mosquito, Microbiology and Nanotechnology Research Units, Department of Zoology, The University of Burdwan. Larvae served at regular intervals with a combination of brewer yeast, dog biscuits and algae in 3:1:1 ratio (Kamaraj et al., 2011). We transferred pupae from the trays to insectary (45×45×40 cm) for adult emergence. The freshly hatched mosquitoes were identified according to the guidelines of Christophers (1933), Barraud (1934) and Chandra (2000). Nurtured adults provided with 10% sucrose solution with cotton balls within a Petri dish. Blood meals were provided to female mosquitoes in a periodic fashion for egg maturation by keeping restrained albino rats in the cages. The eggs were collected in a glass Petri dish lined with Whatman filter paper and were allowed to hatch in trays filled with dechlorinated tap water. Freshly moulted 1st generation larvae were used for the larvicidal experiments.

1.3 Larvicidal bioassay

Bioassay experiments were set up according to the standard WHO larval susceptibility test methods (WHO, 1981; 2005) with minor modification under similar conditions used for rearing. Stock solutions (1,000 ppm) for both seed extracts were prepared by dissolving 250 mg of methanolic extract in 5 mL of methanol and making the volume up to 250 mL by adding distilled water in a standard flask. All the test concentrations 100 ppm, 200 ppm, and 300 ppm were prepared by diluting the known volume of stock solution of the extract with water (Karmgam et al., 1997). Then 25 larvae of each instar (1st, 2nd, 3rd and 4th) of the tested mosquito were selected and were released into separate disposable test cup containing 100 mL of test concentrations. The larvae were considered and counted as dead when they remained non-motile after being pricked by a sharp needle in the siphon or cervical region or they were incompetent to arrive at the water surface (Macêdo et al., 1997). Larval mortality was recorded after 24 h, 48 h, and 72 h respectively. For the combinatorial experiment, *C. chinensis* (CC) and *A. setaceus* (AS) extract weighed according to required amounts and mixed according to the ratios like AS:CC (1:1); AS:CC (2:1) and AS:CC (1:2). For all the test concentrations stock solutions of *A. setaceus* and *C. chinensis* were prepared separately. For the toxicity test of mixed formulations, the same procedure we followed with similar larval instars of the tested mosquito. A control set up for independent and combined experiment was also maintained with 25 larvae of each instar by adding 2 mL of methanol to 98 mL of distilled water. A mixture of yeast powder and biscuit powder (1:3) sprinkled on the surface of the water on a daily basis for a larval meal. For each of the concentrations, three replicates were maintained against all the instars. The larval mortality was

counted at the end of 24, 48 and 72 h. Dead larvae were periodically removed to avoid decomposition. The median lethal concentration (LC_{50}) was determined. The synergistic factor calculated using the formula: $SF = LC_{50}$ value of individual plant extract / LC_{50} value of plant with assumed synergist (George and Vincent, 2005). If the value of $SF > 1$, so indicates synergism or $SF < 1$, signifies antagonism. The intended SF value was tabulated.

1.4 Phytochemical analysis

To search out an assumption on active constituent responsible for larval mortality, phytochemical analysis of extracts of both seeds were carried out accordingly. The presence of tannins, flavonoids, saponins, and alkaloids was examined.

1.5 Test for tannins

In a test tube about 0.5 g of the extract was boiled with 10ml of water and then filtered. Few drops of ferric chloride (1%) added and observed for blue-black or brownish-green colouration (Trease and Evans, 2002).

1.6 Test for flavonoids

In a test tube, about 0.5 g of extract was boiled with distilled water and then filtered. Few drops of ferric chloride solution (10%) were then added with 2 mL of the filtrate. Green-blue or violet colouration indicated the presence of a phenolic hydroxyl group (Trease and Evans, 2002).

1.7 Test for saponin

In a test tube with 0.5 g of the extract 5 mL of distilled water was added. The solution was vigorously shaken and observed for a stable persistent froth (Sofowora, 1993). The presence or absence of saponins was noted and recorded.

1.8 Test for alkaloids

About 1 g of extract was stirred with 5 mL aqueous HCl (1%) on water bath and then filtered. Few drops of Dragendorff's reagent were added to the 1 ml filtrate in a test tube; the occurrence of orange-red precipitate was taken as positive (Sofowora, 1993).

1.9 Effects on non-target organisms

Non-target organisms share the same habitat with mosquito larvae. The efficacy of both the plant extracts was tested against aquatic forms of non-target insect e.g. *Chironomus circumdatus* and *Diplonychus annulatum* (predatory water-bug). The individual and combined concentration that is similar to its LC_{50} value at 24 h against *Cx. quinquefasciatus* was applied to the non-target organisms with the proper experimental set-up. The mortality and other abnormalities were observed up to 72 h after exposure.

1.10 Statistical analysis

The mortality data were subjected to probit analysis (Finney, 1971) to obtain LC_{50} , standard error (SE), regression equation and fiducial limits at 95% confidence limits. The synergistic factor (SF) (Kalayanasundaram and Das, 1985) and co-toxicity coefficient (CTC) (Sarup et al., 1980) for the mixed formulations also performed using the computer software "MS EXCEL 2003" after calculating the LC_{50} for each combination.

2 Results

Mean values on larval mortality of three experiments on different instars of *Cx. quinquefasciatus* immature, when exposed separately to *A. setaceus* and *C. chinensis* seed extracts are depicted in Table 1. Mean larval mortalities (three replicates) of different instars of *Cx. quinquefasciatus* exposed to 1:1, 2:1 and 1:2 combination ratios of *A. setaceus* and *C. chinensis* seed extract are presented in Table 2. The output of Log-probit analysis (at 95% confidence level) indicated that LC_{50} values gradually decreased with the exposure period. The lowest value was 50.424 ppm for the first instar larvae at 72 h of exposure followed by second, third and fourth instar larvae in *C. chinensis* seed extract. The mortality rate (Y) was also positively correlated with concentration (X) for both of the plant extracts as evident from regression equations (Table 3). The result of log-probit analysis in combinatorial toxicity experiment (three replicates) with the different combinations of *A. setaceus* and *C. chinensis* extract revealed that the lowest LC_{50} value was 27.453 ppm for the first instar larva after 72 h of exposure in 1:2

combinations followed by second, third and fourth instars of *Cx. quinquefasciatus* (Table 4). Both the resultant SF values and CTC values were greater than 1 and 100 respectively and strongly indicated synergism against all the instars at 24, 48 and 72 h (Table 4). Presence of some secondary metabolites such as tannin, flavonoid, saponin, and alkaloid in *A. setaceus* was recorded through preliminary phytochemical analysis. In *C. chinensis*, amongst the tested secondary metabolites, saponin was absent. There was no change in swimming behaviour and survival rate among nontarget creatures recorded within 72 h of post-exposure to component extracts as well as combined extracts.

Table 1 Larvicidal toxicity of seed methanolic extracts of *Asparagus setaceus* and *Cuscuta chinensis* on *Cx. quinquefasciatus*

Plant	Concentration (ppm)	Instars	Mean Mortality % $\pm$ SE		
			24 h	48 h	72 h
<i>A.setaceus</i>	100	First	49.32 $\pm$ 0.33	52.00 $\pm$ 0.00	56.00 $\pm$ 0.57
		Second	49.32 $\pm$ 0.33	52.00 $\pm$ 0.57	53.32 $\pm$ 0.33
		Third	42.68 $\pm$ 0.66	46.68 $\pm$ 0.33	50.68 $\pm$ 0.33
		Fourth	42.68 $\pm$ 0.33	44.00 $\pm$ 0.57	45.32 $\pm$ 0.33
	control		00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00
	200	First	50.68 $\pm$ 0.33	54.68 $\pm$ 0.33	62.68 $\pm$ 0.33
		Second	49.32 $\pm$ 0.33	52.00 $\pm$ 0.00	54.68 $\pm$ 0.33
		Third	46.68 $\pm$ 0.33	46.68 $\pm$ 0.33	52.00 $\pm$ 0.33
		Fourth	45.32 $\pm$ 0.33	48.00 $\pm$ 0.00	49.32 $\pm$ 0.33
	control		00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00
	300	First	58.64 $\pm$ 0.33	64.00 $\pm$ 0.57	69.32 $\pm$ 0.33
		Second	54.68 $\pm$ 0.33	60.00 $\pm$ 0.57	65.32 $\pm$ 0.66
		Third	50.68 $\pm$ 0.66	54.68 $\pm$ 0.33	56.00 $\pm$ 0.57
		Fourth	50.68 $\pm$ 0.33	53.32 $\pm$ 0.33	54.68 $\pm$ 0.66
	control		00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00
<i>C.chinensis</i>	100	First	54.68 $\pm$ 0.33	57.32 $\pm$ 0.33	66.68 $\pm$ 0.66
		Second	54.68 $\pm$ 0.33	54.68 $\pm$ 0.33	57.32 $\pm$ 0.33
		Third	50.68 $\pm$ 0.33	52.00 $\pm$ 0.57	57.32 $\pm$ 0.66
		Fourth	45.32 $\pm$ 0.33	49.32 $\pm$ 0.66	52.00 $\pm$ 0.57
	control		00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00
	200	First	65.32 $\pm$ 0.33	68.00 $\pm$ 0.57	70.68 $\pm$ 0.33
		Second	61.32 $\pm$ 0.33	64.00 $\pm$ 0.00	64.00 $\pm$ 0.00
		Third	57.32 $\pm$ 0.33	60.00 $\pm$ 0.00	62.68 $\pm$ 0.33
		Fourth	50.68 $\pm$ 0.33	54.68 $\pm$ 0.33	61.32 $\pm$ 0.33
	control		00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00
	300	First	70.68 $\pm$ 0.33	76.00 $\pm$ 0.57	86.68 $\pm$ 0.33
		Second	69.32 $\pm$ 0.33	69.32 $\pm$ 0.33	82.68 $\pm$ 0.33
		Third	60.00 $\pm$ 0.57	62.68 $\pm$ 0.88	68.00 $\pm$ 1.00
		Fourth	61.32 $\pm$ 0.33	65.32 $\pm$ 0.88	66.68 $\pm$ 1.20
	control		00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00

Table 2 Combinatorial toxicity of different combination ratios of seed methanolic extracts of *Asparagus setaceus* and *Cuscuta chinensis* at different ratios against different larval instars of *Cx. quinquefasciatus*

Conc.(ppm)	First instars (Mean Mortality % $\pm$ SE)			Second instars (Mean Mortality % $\pm$ SE)			Third instars (Mean Mortality % $\pm$ SE)			Fourth instars (Mean Mortality % $\pm$ SE)		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
100												
AS:CC (1:1)	68.00 $\pm$ 0.57	76.00 $\pm$ 1.15	77.32 $\pm$ 1.20	65.32 $\pm$ 0.33	73.32 $\pm$ 0.33	76.00 $\pm$ 0.00	62.68 $\pm$ 0.33	66.68 $\pm$ 0.88	70.68 $\pm$ 0.33	53.32 $\pm$ 0.66	56.00 $\pm$ 0.57	62.68 $\pm$ 0.33
AS:CC (2:1)	70.68 $\pm$ 0.33	73.32 $\pm$ 0.33	77.32 $\pm$ 0.66	66.68 $\pm$ 0.33	70.68 $\pm$ 0.33	73.32 $\pm$ 0.88	64.00 $\pm$ 1.00	65.32 $\pm$ 1.33	69.32 $\pm$ 0.88	57.32 $\pm$ 0.33	58.68 $\pm$ 0.33	62.68 $\pm$ 0.33
AS:CC (1:2)	77.32 $\pm$ 0.66	82.68 $\pm$ 0.33	86.68 $\pm$ 0.33	73.32 $\pm$ 1.20	78.68 $\pm$ 0.88	81.32 $\pm$ 1.20	66.68 $\pm$ 1.20	74.68 $\pm$ 1.33	77.32 $\pm$ 1.66	58.68 $\pm$ 0.33	62.68 $\pm$ 0.33	65.32 $\pm$ 0.88
control	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00
200												
AS:CC (1:1)	82.68 $\pm$ 0.33	85.32 $\pm$ 0.33	85.32 $\pm$ 0.33	78.68 $\pm$ 0.88	81.32 $\pm$ 1.20	85.32 $\pm$ 1.20	72.00 $\pm$ 0.57	78.68 $\pm$ 0.33	81.32 $\pm$ 0.33	58.68 $\pm$ 0.33	61.32 $\pm$ 0.33	66.68 $\pm$ 0.33
AS:CC (2:1)	82.68 $\pm$ 0.33	85.32 $\pm$ 0.33	86.68 $\pm$ 0.33	77.32 $\pm$ 0.33	81.32 $\pm$ 0.33	82.68 $\pm$ 0.66	77.32 $\pm$ 0.66	77.32 $\pm$ 0.33	81.32 $\pm$ 0.33	61.32 $\pm$ 0.33	62.68 $\pm$ 0.33	66.68 $\pm$ 0.66
AS:CC (1:2)	85.32 $\pm$ 0.33	89.32 $\pm$ 0.66	93.32 $\pm$ 0.33	81.32 $\pm$ 0.33	85.32 $\pm$ 0.33	89.32 $\pm$ 0.33	82.68 $\pm$ 0.88	84.00 $\pm$ 0.57	86.68 $\pm$ 0.33	65.32 $\pm$ 0.33	69.32 $\pm$ 0.33	73.32 $\pm$ 0.88
Control	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00
300												
AS:CC (1:1)	89.32 $\pm$ 0.33	90.68 $\pm$ 0.66	94.68 $\pm$ 0.33	86.68 $\pm$ 0.33	88.00 $\pm$ 0.00	90.68 $\pm$ 0.33	81.32 $\pm$ 0.33	82.68 $\pm$ 0.33	85.32 $\pm$ 0.33	70.68 $\pm$ 0.66	73.32 $\pm$ 0.33	77.32 $\pm$ 0.33
AS:CC (2:1)	89.32 $\pm$ 0.33	89.32 $\pm$ 0.33	92.00 $\pm$ 0.00	85.32 $\pm$ 0.66	88.00 $\pm$ 0.57	88.00 $\pm$ 0.57	81.32 $\pm$ 0.66	82.68 $\pm$ 0.33	85.32 $\pm$ 0.88	73.32 $\pm$ 0.88	78.68 $\pm$ 0.33	82.68 $\pm$ 0.33
AS:CC (1:2)	94.68 $\pm$ 0.33	97.32 $\pm$ 0.33	98.68 $\pm$ 0.00	94.68 $\pm$ 0.33	96.00 $\pm$ 0.00	97.32 $\pm$ 0.33	89.32 $\pm$ 0.57	93.32 $\pm$ 0.33	94.68 $\pm$ 0.33	81.32 $\pm$ 0.33	85.32 $\pm$ 0.33	85.32 $\pm$ 0.33
Control	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00

Table 3 Log-probit analysis and regression analyses of seed methanolic extracts of *Asparagus setaceus* and *Cuscuta chinensis* exposed separately on different larval instars of *Cx. quinquefasciatus*

plant	Instar	Exposure	LC ₅₀ ± SE (ppm)	Fiducial limit (ppm)	Regression equation	R ²
<i>A. setaceus</i>	First	24 h	125.370±72.621	369.126-42.581	Y=0.01x+11.111	0.68
		48 h	92.582±56.581	29.800-287.631	Y=0.015x+11.222	0.77
		72 h	64.145±46.555	17.129-240.210	Y=0.0167x+12.333	0.83
	Second	24 h	140.584±141.005	794.288-24.882	Y=0.0067x+11.444	0.50
		48 h	88.801±97.815	13.759-573.108	Y=0.01x+11.667	0.50
		72 h	83.745±58.318	23.438-299.220	Y=0.015x+11.444	0.67
	Third	24 h	298.120±282.210	57.109-1556.241	Y=0.01x+9.667	0.50
		48 h	216.838±129.904	71.221-660.176	Y=0.01x+10.333	0.60
		72 h	111.909±165.019	11.064-1131.868	Y=0.0067x+11.889	0.50
	Fourth	24 h	313.518±257.628	72.149-1362.361	Y=0.01x+9.5556	0.72
		48 h	233.422±115.014	92.060-591.854	Y=0.0117x+9.7778	0.75
		72 h	186.736±78.082	84.125-414.503	Y=0.0117x+10.111	0.67
<i>C. chinensis</i>	First	24 h	70.683±40.198	204.416-24.440	Y=0.0183x+12.111	0.86
		48 h	68.687±98.935	114.223-1.204	Y=0.0233x+12.111	0.87
		72 h	50.424±62.902	90.371-1.497	Y=0.025x+13.667	0.85
	Second	24 h	83.316±38.763	201.238-34.494	Y=0.0217x+11.111	0.82
		48 h	77.525±269.463	128.142-0.592	Y=0.0217x+11.556	0.91
		72 h	65.098±76.518	108.482-2.151	Y=0.025x+12.222	0.86
	Third	24 h	90.757±68.836	357.809-23.020	Y=0.0117x+11.667	0.68
		48 h	79.727±58.978	305.229-20.825	Y=0.0133x+11.889	0.60
		72 h	67.344±52.832	276.453-16.405	Y=0.015x+12.222	0.69
	Fourth	24 h	152.137±39.703	252.309-91.736	Y=0.02x+9.111	0.89
		48 h	114.041±39.534	222.097-58.557	Y=0.02x+10.111	0.73
		72 h	86.703±50.578	257.059-29.244	Y=0.0183x+11.333	0.63

Note: LC₅₀ = lethal concentration that kills 50% of tested organism

3 Discussion

The importance of the appropriate selection of plants as a synergist in mixed formulations with other phytochemical is being increasingly recognized in mosquito control strategy (Caraballo, 2000; Singha et al., 2011). Insecticides obtained from botanical resources are efficient, biodegradable as well as potent alternative for effective mosquito control. Several of these secondary metabolites are produced by some plants for their own defense from their enemies, and have been found to have good larvicidal activity such as steroids, essential oils, triterpenes etc. (Chowdhury et al., 2008; Benelli, 2015b; Benelli et al., 2017a; Benelli et al., 2017b; Benelli et al., 2017c). Saponin extracted from the fruits of *Balanites aegyptica* showed 100% larvicidal activity against *Ae. aegypti* mosquito larvae (Wiesman and Chapagain, 2006). Synergistic effects of various control agents have proved very advantageous in the control of various pests (Seyoum et al., 2002). Different mosquito larvicidal plant species with growth retarding, reproduction inhibiting and ovicidal activity were studied. In addition, synergistic, additive and antagonistic actions of botanical mixtures were also reviewed (Shaalan et al., 2005). Blends of *Pongamia glabra* and *Annona squamosa* extracts exhibited a synergistic effect against larvae of mosquitoes (George and Vincent, 2005). From the results of the present study, we obtained that the methanolic seed extracts of *Asparagus setaceus* and *Cuscuta chinensis* divulged larvicidal performance individually and in combinations indicating the mosquitocidal potentials of the plants. The results of this study show that mortalities increased with concentration ($p<0.05$) and there was a positive correlation between concentration and percentage of larval mortality. The lower LC₅₀ values indicated that the combinations of the extracts were much more effective than the individual extracts. At 300 ppm concentrations, the combination AS: CC (1:2) was the most effective against first instar larvae and showed 98.68% mortality in 72 h with an LC₅₀ value of 27.453 ppm. SF and CTC indicate that were also determined and it was observed that all the combinations showed good synergistic response on mortality of *Cx. quinquefasciatus* larvae. Secondary metabolites present in tested plants may be the key factor for

larval mortality (Singha et al., 2012; Rawani et al., 2012). No mortality was recorded in the non target organism that indicated both of the plant extracts are ecologically safe.

Table 4 Log-probit analysis, regression analysis, estimation of synergistic factor and co-toxicity coefficient of *Asparagus setaceus* and *Cuscuta chinensis* seed extracts in different combination ratios exposed separately on different larval instars of *Cx. quinquefasciatus*

Plant ratio	Instar	Exposure	Regression equation	LC ₅₀ ± SE (ppm)	Fiducial limits(ppm)	R ²	SF	CTC	Type of action
AS:CC (1:1)	First	24 h	Y=0.0267x+14.667	51.586±31.488	84.049-8.731	0.8889	1.3701	137.019	S
		48 h	Y=0.0183x+17.333	27.873±104.875	64.623-0.022	0.6302	2.4642	246.428	S
		72 h	Y=0.0217x+17.111	35.897±37.752	68.227-1.87	0.7369	1.4046	140.468	S
	Second	24 h	Y=0.0267x+13.889	54.749±37.111	89.725-7.462	0.8610	1.3603	136.031	S
		48 h	Y=0.0183x+16.556	28.334±337.922	68.466-0.001	0.6823	2.7311	273.113	S
		72 h	Y=0.0183x+17.333	27.873±104.874	64.622-0.022	0.6722	2.9510	295.102	S
	Third	24 h	Y=0.0233x+13.333	54.219±84.323	96.871-0.795	0.9074	1.6738	167.389	S
		48 h	Y=0.02x+15	39.591±162.511	82.147-0.021	0.7500	2.0137	201.376	S
		72 h	Y=0.0183x+16.111	30.943±379.719	72.443-0.0002	0.8561	1.6033	160.336	S
	Fourth	24 h	Y=0.0217x+10.889	88.454±979.589	142.756-0.001	0.7922	1.7199	171.995	S
		48 h	Y=0.0217x+11.556	75.054±792.431	127.095-0.001	0.8564	1.5194	151.945	S
		72 h	Y=0.0183x+13.556	44.622±38.828	208.471-9.551	0.8561	1.9430	194.305	S
AS:CC (2:1)	First	24 h	Y=0.0233x+15.556	42.452±42.611	77.451-2.421	0.9188	1.6650	166.501	S
		48 h	Y=0.02x+16.667	33.502±63.631	69.797-0.291	0.8571	2.0502	205.023	S
		72 h	Y=0.0183x+17.667	27.911±77.976	63.301-0.066	0.8403	1.8066	180.660	S
	Second	24 h	Y=0.0283x+14.444	46.661±60.087	85.619-1.286	0.8855	1.5961	159.610	S
		48 h	Y=0.0217x+15.667	38.593±61.829	76.107-0.566	0.8802	2.0051	200.513	S
		72 h	Y=0.0183x+16.667	28.722±222.456	68.185-0.001	0.6722	2.8637	286.379	S
	Third	24 h	Y=0.0217x+14.222	47.476±91.086	89.438-0.361	0.6671	1.9116	191.164	S
		48 h	Y=0.0217x+14.222	45.257±91.258	86.781-0.294	0.6778	1.7616	176.165	S
		72 h	Y=0.02x+15.667	36.291±109.022	76.384-0.06	0.6667	1.3670	136.708	S
	Fourth	24 h	Y=0.02x+12	67.193±39.397	200.217-22.551	0.7500	2.2641	226.417	S
		48 h	Y=0.025x+11.667	70.834±93.831	116.289-1.599	0.8523	1.6099	160.997	S
		72 h	Y=0.025x+12.667	58.871±79.212	101.755-1.333	0.8152	1.4752	147.526	S
AS:CC (1:2)	First	24 h	Y=0.0217+17.111	35.897±37.753	68.227-1.871	0.8741	1.9690	196.905	S
		48 h	Y=0.0183x+18.778	30.247±38.927	61.1071-0.9198	0.8326	2.2708	227.086	S
		72 h	Y=0.015x+20.222	27.453±41.874	57.2324-0.5011	0.8679	1.8367	183.673	S
	Second	24 h	Y=0.0267x+15.444	47.518±28.675	78.209-8.314	0.7967	1.5673	156.732	S
		48 h	Y=0.0217x+17.333	35.824±35.407	67.376-2.198	0.8284	2.1601	216.011	S
		72 h	Y=0.02x+18.333	33.569±34.192	64.0328-1.9217	0.7059	1.9392	193.922	S
	Third	24 h	Y=0.0283x+14.222	55.905±28.248	87.161-12.973	0.7423	1.6234	162.341	S
		48 h	Y=0.0283x+16.333	40.199±35.503	72.714-3.206	0.7101	1.9833	198.330	S
		72 h	Y=0.0217x+17.222	36.567±35.303	68.2633-2.3752	0.6094	1.8416	184.166	S
	Fourth	24 h	Y=0.0283x+11.444	74.133±48.078	113.931-10.427	0.9107	2.0522	205.221	S
		48 h	Y=0.0283x+12.444	63.707±42.641	101.217-8.654	0.9107	1.7900	179.008	S
		72 h	Y=0.025x+13.667	52.288±55.597	91.431-2.43	0.7813	1.6581	165.818	S

Note: LC₅₀ = lethal concentration that kills 50% of tested organism, SF, synergistic factor, CTC, co-toxicity coefficient

4 Conclusions

From the present study, it can be concluded that the formulation of the blend was novel, targeted mosquitocide and that synergism enabled a reduced dose to be applied for vector control potentially leading to improved resistance management, cost effectivity, and environmental concerns. Additional studies are recommended to determine the active principles against mosquitoes for further development of new and safe insecticides.

Authors' contributions

SC selected the plants, carried out the laboratory experiments, performed the statistical analysis, and drafted this manuscript. GC designed the work, supervised the laboratory experiments and reviewed the drafted manuscript. All authors read and approved the final manuscript.

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