

BIOCONTROL OF TAGETES ERECTA, CHOMOLAENA ODORATA AND LANTANA CAMARA LEAF EXTRACTS ON BACTROCERA CORRECTA (BEZZI)

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Abstract

The leaf extracts of *Tagetes erecta* (L.) (TLE), *Chromolaena odorata* (L.) (CLE) and *Lantana camara* (L.) (LLE) were investigated for the biocontrol against guava fruit flies, *Bactrocera correcta*, on their adult repellent, insecticidal activities, egg hatching, and larval development. The ethanol extracts were more likely potent than the water extracts. CLE was most effective, followed by TLE for the adult control and LLE for the egg and the larval controls. CLE/e repellent efficacy against the adult flies with EC₅₀ of 35.42 mg/ml, 15 min, followed by TLE/e with EC₅₀ of 36.58 mg/ml. CLE/e expressed highest insecticidal activity with LC₅₀ of 67.32 mg/ml at 24 h. The insecticidal efficacy of the extracts on the adult flies were ranged as CLE/e > CLE/w > TLE/e > LLE/e > LLE/w > TLE/w. The insecticidal activity of the pair-wise combination was depending on the higher ratio, 3-fold CLE/e enhanced the activities of TLE/e and LLE/e and vice versa. CLE/e exhibited most effective against egg hatching with EC₅₀ of 44.54 mg/ml at 24 h. CLE/e caused highest mortality to the 2nd instar larvae by feeding assay with LC₅₀ of 55.56 mg/ml and by dipping assay with LC₅₀ of 52.99 mg/ml. CLE/e inhibited molting of the 3rd instar larvae with LC₅₀ of 69.55 mg/ml. The larvicidal activities of the extracts was ranged as CLE/e > LLE/e > CLE/w > TLE/e > LLE/w > TLE/w. This study demonstrates that *T. erecta*, *C. odorata* and *L. camara* leaf extracts are likely to be one of good alternatives of plant-based insecticides on *B. correcta* biocontrol.

Keywords: Biocontrol, *Tagetes erecta*, *Chromolaena odorata*, *Lantana camara*, *Bactrocera correcta*

Introduction

Fruit fly, “*Bactrocera* spp.” (Diptera: Tephritidae) is one of the most economically destructive crop pests. There are a few methods to control this fruit fly have been conducted (Sarango, 2009; Vargas *et al.*, 2015), such as insecticide sprays, bait sprays, male annihilation, parasitoids, and cultural controls. Some fungal pathogenic strains against the fruit fly had also been investigated (Jiji *et al.*, 2006). Guava

fruit fly, *Bactrocera correcta* (Bezzi) (White and Elson-Harris, 1994), is also belongs to Tephritidae family, it is mostly limited to the tropics, including Thailand (Smith, 2001; Puanmanee, *et al.*, 2010; Weems and Fasulo, 2014). *B. correcta* is polyphagous fruit pest which is able to economically damage a wide variety of vegetables and orchard fruits such as guava, mango, banana,

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sweet star fruit and rose apple. *B. correcta* infests fruits from the early fruiting stage until the harvest stage. This makes an effective investigation on the fly at the early infestation difficult and its population largely grows during fruit production period. This can allow it to damage fruit production up to 100% (Sarango, 2009). *B. correcta* lays eggs underneath fruit skin. Within 2-3 days, the eggs hatch and the larvae feed on fruit tissue (Kumar *et al.*, 2011). The routine *B. correcta* management involves in the effective applications of chemically synthetic insecticides, which most of them are left and persistent in plants and animals. They are not only toxic to fruit flies but also to human, other lives, and environment. The accumulation of toxic chemicals in the environment as well as the insect resistance to insecticides can lead to long term human health problems and fruit fly outbreaks instead.

Intervention strategies for controlling large population of fruit flies and interruption of their proliferation were described (CTA, 2007). Gamma ray from ^{137}Cs source had been attempted to make sterility to *B. correcta* pupae (Puanmaneel *et al.*, 2010) and *B. zonata* (Mahmoud and Barta, 2011). The gamma radiation could reduce the infestation of the flies (Kabbashi *et al.*, 2012). Wrapping guava fruits with transparent polypropylene bags and partial papers cover inside was efficiently decreased *B. correcta* infestation (Mondal *et al.*, 2015). Essential oil extracted from basil (*Ocimum* spp.) and nanogel of methyl eugenol were able to be used as pheromone to attract fruit flies in bait trapping (Bhagat *et al.*, 2013; Kardinan, 2014; Dharmadasa *et al.*, 2015). This study attempted to investigate the effects of *Tagegetes erecta*, *Chromolaena odorata* and *Lantana camara* leaf extracts to control *B. correcta*. The repellent, insecticidal, and antibiosis properties (inhibition of reproduction) of the extracts were investigated.

Materials and Methods

Plant Collection and Extract Preparation

The fresh leaves of *C. odorata* and *L. camara* were collected from Suranaree University of Technology (SUT) campus. *T. erecta* leaves were collected from a local farm near SUT campus. All leaves were cleaned, sun dried and ground to powder. The powder was extracted in water and 70% ethanol in Soxhlet extraction apparatus (Buchi model B 811, Germany). The extracts were evaporated, dried and stored at -20°C until use. The dried extracts were dissolved in their original solvents for use in all experiments. The water and 70% ethanol were also used as controls.

Insect Rearing

B. correcta pupae were obtained from the Thailand Institute of Nuclear Technology (Public Organization), Ministry of Science and Technology, Thailand. The flies were reared in the laboratory of Suranaree University of Technology, Nakhon Ratchasima, Thailand. Adult flies emerged in 7 days and were cultured in wire-net cages under laboratory conditions at $28\pm 2^\circ\text{C}$, 65-70% relative humidity, and 12 h light: 12 h dark. The flies were fed with artificial food (Walker *et al.*, 1997) and allowed to mate. The female flies laid eggs in the egg dome containing guava juice. After hatching, the larvae were fed on artificial food and allowed to develop to pupa stage in wood chip trays.

Repellent Tests

Individual females or males of *B. correcta* were introduced through the hole at the middle of the olfactometer which was made of a 4-cm diameter and a 75-cm long plastic tube with a 29-mm diameter pore in the middle and covered with gauze (Boeke *et al.*, 2004). At one end of the tube, a 10-ml beaker containing 1 ml of 20, 40, 60, 80, and 100 mg/ml extracts and the other end containing 1 ml distilled water or 1 ml 70% ethanol were placed. After introducing, the movement of the flies to either end was observed and counted for 15, 30 min and 1, 3, and 6 h. The tests were repeated for 30 flies (15 females and 15 males). The percentage of repellency was calculated as the following formula (Tawatsin *et al.*, 2001) and the median effective concentration, EC_{50} , was determined.

$$\% \text{ Repellent} = \frac{\text{Number collected from control} - \text{Number collected from treated}}{\text{Number collected from control}} \times 100$$

Insecticidal Activity on Adult Flies

Thirty *B. correcta* adults were placed into a plastic box (100×100×60 mm) with a punched-hole lid and covered with gauze. Five milliliters of 20, 40, 60, 80, and 100 mg/ml and combinations of the extracts (1:1, 3:1, and 1:3) were sprayed into the box. The dead flies were counted after 1, 6, 12, and 24 h treatments. The tests were performed in triplicate and repeated twice. The mortality was calculated as the following formula (Abbott, 1925) and analyzed for LC_{50} .

$$\% \text{ Mortality} = \frac{\% \text{ Test mortality} - \% \text{ Control mortality}}{100 - \% \text{ Control mortality}} \times 100$$

Egg Hatching

Thirty eggs were gently moved and placed on Whatman disc filter papers #3 (42.5 mm in diameter), damped with 10 ml of 20, 40, 60, 80, and 100 mg/ml extracts. The papers were then placed on

the artificial food. The eggs were allowed to hatch into larvae and counted within 24 h. The experiments were performed in triplicate. The results were calculated using the same formula as for repellent tests and expressed as percentage of inhibition. The EC₅₀ was also calculated.

Feeding Deterrence

Thirty 2nd instar larvae were cultured in 30 g artificial food mixed in 30 ml of 20, 40, 60, 80, and 100 mg/ml extracts. Mortality of larvae was counted after 6, 12, and 24 h. The experiments were performed in triplicate. The LC₅₀ was calculated.

Dipping Assay

Thirty 2nd instar larvae were dipped into 10 ml of 20, 40, 60, 80, and 100 mg/ml extracts for 3 sec and then put in a 100-ml bottle, covered with a small hole lid, and counted after 6, 12, and 24 h. The experiments were performed in triplicate. The mortality was calculated and analyzed for the LC₅₀.

Pupa Molting

Thirty 3rd instar larvae were cultured in 30 g artificial food mixed in 30 ml of 20, 40, 60, 80, and 100 mg/ml extracts. The larvae were allowed to develop into pupae, approximately in 7 days. The molted flies were counted. The experiments were

performed in triplicate. The molting inhibition and the EC₅₀ were calculated.

Data Analysis

All data were analyzed using analysis of variance (ANOVA) and in completely randomized design (CRD) using Statistical Package for the Social Sciences (SPSS) program for Windows v.17. The mean was separated by the Duncan's Multiple Range Test (DMRT) when ANOVA was significant ($P < 0.05$). The LC₅₀ and EC₅₀ values were determined by Probit analysis (Finny, 1971). The percentages of the repellence, mortality and inhibition rate were corrected by Abbott's formula (1925).

Results

Repellent Effect

The repellent effects of *T. Erecta* (TLE), *C. odorata* (CLE) and *L. camara* (LLE) extracts on *B. correcta* adults were concentration- and time dependent, the effects were decline along with treatment time. It was most active during 15-30 min (Table 1). The repellent activities of the ethanol extracts expressed slightly higher than those of the water extracts. Both *C. odorata* extracts had the highest repellent efficacy, at 100 mg/ml, 15 min.

Table 1. Repellent activities of *T. erecta* (TLE), *C. odorata* (CLE) and *L. camara* (LLE) leaf water (/w) and 70% ethanol (/e) extracts on *B. correcta* adults at various concentrations and designated times

Extract	Conc. mg/ml	% Repellent (Mean±SE)					EC ₅₀ mg/ml, 15 m
		15 m	30 m	1 h	3 h	6 h	
TLE/w	20	11.79±9.06 ^a	29.22±6.87 ^b	9.13±9.78 ^a	0.80±5.16 ^a	1.04±2.60 ^a	43.23
	40	26.28±4.59 ^b	34.80±8.39 ^b	25.04±7.72 ^{ab}	13.27±7.26 ^a	9.74±5.37 ^{ab}	
	60	54.77±1.44 ^c	63.18±3.49 ^c	49.71±7.72 ^c	35.02±5.03 ^b	21.45±1.90 ^{bc}	
	80	68.88±1.88 ^d	75.60±0.98 ^{cd}	73.06±2.55 ^d	47.46±5.59 ^b	29.76±6.77 ^{cd}	
	100	74.98±4.18 ^d	78.64±1.33 ^d	74.40±1.25 ^d	69.49±4.46 ^c	41.09±8.20 ^d	
CLE/w	20	18.68±5.91 ^b	21.67±3.80 ^b	20.76±1.62 ^b	11.85±4.05 ^a	9.35±2.69 ^{ab}	37.66
	40	69.59±2.25 ^c	43.65±5.20 ^c	48.08±8.88 ^c	17.07±6.35 ^a	14.58±2.33 ^b	
	60	40.63±1.73 ^d	62.09±2.59 ^d	63.76±1.01 ^d	37.30±11.43 ^b	42.71±6.70 ^c	
	80	78.37±4.87 ^d	73.87±3.60 ^e	68.99±3.56 ^d	52.53±7.54 ^{bc}	48.10±2.95 ^c	
	100	85.43±3.90 ^e	79.61±1.34 ^e	73.45±1.74 ^d	64.14±2.53 ^c	67.85±5.15 ^d	
LLE/w	20	16.28±7.53 ^b	38.87±5.63 ^b	27.93±7.00 ^b	11.85±4.05 ^a	6.17±6.17 ^a	39.17
	40	31.65±3.95 ^c	45.84±6.27 ^b	29.15±8.58 ^b	16.43±2.23 ^b	18.72±3.12 ^{ab}	
	60	66.82±3.12 ^d	64.58±2.46 ^c	48.23±3.98 ^c	32.24±6.14 ^c	26.70±1.26 ^{bc}	
	80	68.35±3.95 ^d	75.97±1.67 ^d	64.13±1.94 ^d	53.52±0.84 ^d	33.88±4.49 ^{cd}	
	100	81.72±2.97 ^e	83.08±2.46 ^d	71.73±3.88 ^d	63.06±3.89 ^d	39.90±7.89 ^d	
TLE/e	20	27.80±9.14 ^b	29.46±7.61 ^b	20.55±4.44 ^b	3.61±6.03 ^a	10.46±3.38 ^b	36.58
	40	34.22±1.70 ^b	42.98±2.86 ^c	30.51±7.48 ^b	18.93±7.40 ^b	17.03±3.24 ^b	
	60	66.97±2.65 ^c	68.19±4.14 ^d	61.80±6.91 ^c	38.72±1.82 ^c	28.92±2.49 ^c	
	80	72.87±7.19 ^{cd}	75.05±3.78 ^{de}	73.70±2.77 ^{cd}	52.90±1.46 ^d	32.92±1.58 ^c	
	100	86.60±2.54 ^d	80.63±0.32 ^e	80.45±3.86 ^d	74.78±3.05 ^c	42.21±3.97 ^d	
CLE/e	20	24.68±5.87 ^b	27.26±6.46 ^b	26.01±3.17 ^b	21.43±6.27 ^b	17.08±2.55 ^b	35.42
	40	38.99±3.45 ^c	48.03±5.26 ^c	53.44±7.61 ^c	36.19±2.10 ^{bc}	22.31±3.26 ^b	
	60	75.33±2.94 ^d	73.20±3.53 ^d	62.93±4.55 ^c	42.60±7.75 ^c	48.77±5.64 ^c	
	80	81.20±3.75 ^{de}	77.57±3.63 ^d	74.12±1.61 ^d	62.90±7.78 ^d	50.05±3.02 ^c	
	100	88.46±1.24 ^e	85.29±2.95 ^d	79.35±1.06 ^d	73.15±4.11 ^d	67.13±2.47 ^d	
LLE/e	20	0.00±0.00 ^a	48.31±5.52 ^b	33.44±5.36 ^b	17.05±2.43 ^b	10.46±5.23 ^a	38.16
	40	27.46±6.64 ^b	53.54±6.27 ^b	35.84±7.89 ^b	25.36±0.36 ^b	25.52±4.14 ^b	
	60	41.54±3.42 ^c	73.54±2.15 ^c	55.85±4.81 ^c	37.86±6.91 ^c	26.80±3.42 ^b	
	80	70.86±2.12 ^d	81.55±1.40 ^{cd}	70.27±1.46 ^d	59.91±1.77 ^d	39.58±3.84 ^c	
	100	75.03±2.19 ^d	86.95±2.38 ^d	79.00±1.46 ^d	67.10±3.82 ^d	60.22±5.17 ^d	
H ₂ O		0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	
70% EtOH		0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	

Note: Each value is the mean ± standard error, n = 9. Numbers with different letters within the same column are significant differences ($P \leq 0.05$).

CLE/w repelled the flies 85.43±3.90% with EC₅₀ 37.66 mg/ml and CLE/e repelled the flies 88.46±1.24% with EC₅₀ 35.42 mg/ml.

Insecticidal Effect

Individual extracts demonstrated similar pattern of the insecticidal effect on *B. correcta* adults to the repellent effects, i.e., the ethanol extracts had

higher activities than the water extracts and were concentration- and time dependent (Table 2). CLE/e expressed highest insecticidal activity with LC₅₀ 67.32 mg/ml at 24 h. It was the only extract that could kill the flies more than 50% at 80 mg/ml within one hour and up to 80% at 100 mg/ml within 24 h. TLE/w had least activity with LC₅₀ 93.67 mg/ml, while the rest had similar activity with LC₅₀

Table 2. Insecticidal effects of *T. erecta* (TLE), *C. odorata* (CLE) and *L. camara* (LLE) leaf water (/w) and 70% ethanol (/e) extracts on *B. correcta* adults by direct spray assay at various concentration and designated times

Extract	Conc. mg/ml	% Mortality (Mean ± SE)				LC ₅₀ mg/ml, 24 h
		1 h	6 h	12 h	24 h	
TLE/w	20	1.11±1.11 ^a	11.11±2.94 ^b	13.33±1.92 ^b	16.67±1.92 ^b	93.67
	40	4.44±1.11 ^a	14.44±2.22 ^b	16.67±1.92 ^b	20.00±1.92 ^b	
	60	15.56±2.22 ^b	21.11±1.11 ^c	23.33±1.92 ^c	28.89±1.11 ^c	
	80	15.56±2.22 ^b	23.33±1.92 ^c	27.78±1.11 ^c	37.78±2.94 ^d	
	100	33.33±3.33 ^c	42.22±2.94 ^d	47.78±2.22 ^d	52.22±1.11 ^e	
CLE/w	20	2.22±1.11 ^{ab}	4.44±1.11 ^{ab}	7.78±1.11 ^a	10.00±0.00 ^a	80.92
	40	7.78±1.11 ^b	14.44±1.11 ^b	20.00±1.92 ^b	23.33±1.92 ^b	
	60	16.67±1.92 ^c	27.78±6.19 ^c	34.44±6.19 ^c	41.11±6.19 ^c	
	80	23.33±1.92 ^d	34.44±6.19 ^{de}	41.11±4.84 ^c	50.00±5.09 ^{cd}	
	100	34.44±4.01 ^e	41.11±2.94 ^e	53.33±1.92 ^d	57.78±4.01 ^d	
LLE/w	20	1.11±1.11 ^a	2.22±1.11 ^a	3.33±0.00 ^a	5.56±1.11 ^b	89.88
	40	4.44±1.11 ^a	8.89±1.11 ^b	10.00±1.92 ^b	11.11±1.11 ^c	
	60	14.44±2.22 ^c	22.22±2.22 ^c	25.56±2.94 ^c	31.11±1.11 ^d	
	80	20.00±1.92 ^c	31.11±2.94 ^d	32.22±2.22 ^d	38.89±2.22 ^e	
	100	31.11±4.84 ^d	47.78±2.22 ^e	50.00±0.00 ^e	55.56±2.94 ^d	
TLE/e	20	3.33±0.00 ^{ab}	4.44±1.11 ^a	5.56±1.11 ^b	11.11±2.94 ^b	87.90
	40	8.89±1.11 ^b	11.11±2.94 ^b	15.56±1.11 ^c	20.00±1.92 ^c	
	60	20.00±1.92 ^c	22.22±1.11 ^c	24.44±1.11 ^d	27.78±1.11 ^d	
	80	22.22±2.94 ^c	25.56±1.11 ^c	27.78±1.11 ^e	40.00±5.09 ^e	
	100	37.78±4.84 ^d	43.33±1.92 ^d	48.89±1.11 ^f	58.89±2.22 ^f	
CLE/e	20	3.33±1.92 ^{ab}	3.33±1.92 ^a	3.33±1.92 ^a	5.56±2.22 ^a	67.32
	40	10.00±1.92 ^b	12.22±1.11 ^b	12.22±1.11 ^b	16.67±1.92 ^b	
	60	42.22±2.94 ^c	46.67±1.92 ^c	46.67±1.92 ^c	53.33±1.92 ^c	
	80	51.11±2.94 ^d	55.56±2.94 ^d	55.56±2.94 ^d	66.67±3.33 ^d	
	100	63.33±3.33 ^e	72.22±1.11 ^e	72.22±1.11 ^e	80.00±1.92 ^e	
LLE/e	20	1.11±1.11 ^a	3.33±1.92 ^a	7.78±1.11 ^{ab}	8.89±1.11 ^b	88.20
	40	7.78±1.11 ^b	10.00±0.00 ^b	14.44±1.11 ^b	17.78±2.94 ^c	
	60	16.67±1.92 ^c	18.89±1.11 ^c	22.22±2.94 ^c	26.67±1.92 ^d	
	80	23.33±1.92 ^d	26.67±1.92 ^d	33.33±5.09 ^d	38.89±1.11 ^e	
	100	32.22±2.94 ^d	34.44±2.94 ^e	55.56±2.94 ^e	60.00±1.92 ^f	
H ₂ O		0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	
70% EtOH		0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	

Note: Each value is the mean ± standard error, n = 9. Numbers with different letters within the same column are significant differences (P≤0.05).

Table 3. Insecticidal effects of the pair-wise combinations of *T. erecta* (TLE), *C. odorata* (CLE) and *L. camara* (LLE) leaf water and ethanol extracts on *B. correcta* adults by direct spray assay at different times

Combined extract	Ratio mg/ml	% Mortality (Mean ± SE)			
		1 h	6 h	12 h	24 h
CLE/w + TLE/w	1:3	16.67±1.92 ^b	15.11±3.62 ^b	41.11±2.94 ^b	53.33±1.92 ^b
	1:1	23.33±1.92 ^b	27.78±6.19 ^b	34.44±6.19 ^b	50.00±5.09 ^{bc}
	3:1	34.44±4.01 ^c	34.44±6.19 ^b	41.11±4.84 ^c	57.78±4.01 ^c
CLE/w + LLE/w	1:3	4.44±1.11 ^a	8.89±1.11 ^a	10.00±1.92 ^a	58.89±2.22 ^d
	1:1	31.11±4.84 ^b	47.78±2.22 ^b	50.00±4.01 ^b	55.56±2.94 ^c
	3:1	20.00±1.92 ^c	31.11±2.94 ^c	32.22±2.22 ^c	38.89±2.22 ^d
LLE/w + TLE/w	1:3	3.33±4.01 ^a	4.44±1.11 ^b	5.56±1.11 ^b	34.44±4.01 ^c
	1:1	22.22±2.94 ^b	25.56±1.11 ^c	27.78±1.11 ^c	40.00±5.09 ^c
	3:1	37.78±4.84 ^c	43.33±1.92 ^d	48.89±1.11 ^d	58.89±2.94 ^d
CLE/e + TLE/e	1:3	6.22±1.18 ^b	6.89±0.11 ^b	12.33±1.45 ^b	15.89±0.48 ^b
	1:1	12.22±1.11 ^c	12.22±1.11 ^c	16.67±1.92 ^b	55.56±2.94 ^c
	3:1	46.67±1.92 ^d	46.67±1.92 ^d	53.33±1.92 ^c	72.22±1.11 ^d
CLE/e + LLE/e	1:3	7.78±1.11 ^b	13.33±1.92 ^b	21.11±1.11 ^b	17.78±2.94 ^b
	1:1	16.67±1.92 ^c	18.89±1.11 ^c	22.22±2.94 ^b	27.78±1.11 ^c
	3:1	23.33±1.92 ^d	26.67±1.92 ^d	33.33±5.09 ^c	60.00±1.92 ^d
LLE/e + TLE/e	1:3	3.33±0.21 ^b	4.44±1.11 ^a	11.11±2.94 ^b	27.78±1.18 ^c
	1:1	8.89±1.11 ^c	11.11±2.94 ^b	15.56±1.11 ^c	45.56±1.11 ^c
	3:1	20.00±1.92 ^d	22.22±1.11 ^c	26.67±0.51 ^d	54.44±2.22 ^d
H ₂ O		0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
70% EtOH		0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

Note: Each value is the mean ± standard error, n = 9. Numbers with different letters within the same column are significant differences (P≤0.05).

between 80.92 to 89.88 mg/ml. The insecticidal efficacy of the extracts on the flies were ranged as CLE/e > CLE/w > TLE/e > LLE/e > LLE/w > TLE/w.

The pair- wise combination of the water extracts at ratio 1:1, 3:1, and 1:3 at 24 h treatment showed that TLE/w slightly decreased (antagonistic effect) the insecticidal activities of CLE/w and LLE/w. LLE/w somewhat enhanced (synergistic effect) the activities of CLE/w and TLE/w (Table 3). For the combination of the ethanol extracts, it was found that the insecticidal activity was depending on the higher proportion of the individual extracts as compared to the 1:1 ratio. Three fold of CLE/e in the combination enhanced the activities of TLE/e and LLE/e, while 3 fold of TLE/e and LLE/e reduced the activities of CLE/e.

Egg Hatching

The hatching of guava fruit fly eggs was inhibited by these three plant extracts in concentration dependent fashion. Even though the effects of ethanol extracts were higher than those of water extracts. At 100 mg/ml, CLE/e was most active with the egg hatching inhibition of $82.22 \pm 6.19\%$, which was approximately 15 fold of the control (Table 4). The egg hatching efficacy of the extracts was CLE/e > LLE/e > TLE/e > CLE/w > LLE/w > TLE/w with EC_{50} 44.54, 53.42, 55.92, 65.66, 72.03, and 75.41 mg/ml at 24 h, respectively.

Feeding Deterrence

Giving artificial food mixed with the extracts to the 2nd instar larvae of *B. correcta* caused dead to the larvae depending on concentration and time.

Table 4. Egg hatching inhibition of *T. erecta* (TLE), *C. odorata* (CLE) and *L. camara* (LLE) leaf water (/w) and 70% ethanol (/e) extracts on *B. correcta* eggs at various concentrations

Conc. mg/ml	% Inhibition (Mean $\pm$ SE)					
	TLE/w	CLE/w	LLE/w	TLE/e	CLE/e	LLE/e
H ₂ O	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a
70% EtOH	5.56 $\pm$ 1.11 ^b	5.56 $\pm$ 1.11 ^a	5.56 $\pm$ 1.11 ^b	5.56 $\pm$ 1.11 ^a	5.56 $\pm$ 1.11 ^a	5.56 $\pm$ 1.11 ^a
20	12.22 $\pm$ 1.11 ^c	14.44 $\pm$ 2.22 ^b	14.44 $\pm$ 2.94 ^c	44.44 $\pm$ 2.94 ^b	51.11 $\pm$ 5.56 ^b	44.44 $\pm$ 2.94 ^b
40	27.78 $\pm$ 1.11 ^d	35.56 $\pm$ 4.84 ^c	31.11 $\pm$ 1.11 ^d	46.67 $\pm$ 0.00 ^b	63.33 $\pm$ 3.33 ^{bc}	46.67 $\pm$ 0.00 ^b
60	44.44 $\pm$ 1.11 ^e	48.89 $\pm$ 2.94 ^d	45.56 $\pm$ 2.22 ^e	57.78 $\pm$ 1.11 ^c	70.00 $\pm$ 6.94 ^{bc}	57.78 $\pm$ 1.11 ^c
80	55.56 $\pm$ 1.11 ^f	60.00 $\pm$ 1.92 ^e	56.67 $\pm$ 0.00 ^f	67.78 $\pm$ 4.84 ^d	78.89 $\pm$ 6.76 ^c	67.78 $\pm$ 4.84 ^d
100	62.22 $\pm$ 1.11 ^g	76.67 $\pm$ 1.92 ^f	66.67 $\pm$ 1.92 ^g	72.22 $\pm$ 1.11 ^d	82.22 $\pm$ 6.19 ^c	80.00 $\pm$ 1.92 ^e
EC ₅₀ (mg/ml)	75.41	65.66	72.03	55.92	44.54	53.42

Note: Each value is the mean $\pm$ standard error, n = 9. Numbers with different letters within the same column are significant differences ($P \leq 0.05$).

Table 5. Feeding deterrent effects of *T. erecta* (TLE), *C. odorata* (CLE) and *L. camara* (LLE) leaf water (/w) and 70% ethanol (/e) extracts on *B. correcta* 2nd instar larvae at various concentration and designated times

Extract	Conc. mg/ml	% Mortality (Mean $\pm$ SE)			LC ₅₀ mg/ml, 24 h
		6 h	12 h	24 h	
TLE/w	20	7.78 $\pm$ 1.11 ^b	11.11 $\pm$ 2.22 ^b	15.56 $\pm$ 1.11 ^b	80.14
	40	8.89 $\pm$ 1.11 ^b	16.67 $\pm$ 1.92 ^c	23.33 $\pm$ 1.92 ^c	
	60	17.78 $\pm$ 1.11 ^c	31.11 $\pm$ 2.94 ^d	37.78 $\pm$ 2.94 ^d	
	80	31.11 $\pm$ 2.94 ^d	42.22 $\pm$ 1.11 ^e	47.78 $\pm$ 1.11 ^e	
	100	43.33 $\pm$ 1.92 ^e	55.56 $\pm$ 2.22 ^f	62.22 $\pm$ 2.22 ^f	
CLE/w	20	11.11 $\pm$ 2.22 ^b	13.33 $\pm$ 1.93 ^b	20.00 $\pm$ 1.92 ^b	67.23
	40	16.67 $\pm$ 1.93 ^c	20.00 $\pm$ 1.92 ^c	30.00 $\pm$ 1.11 ^c	
	60	28.89 $\pm$ 1.11 ^d	38.89 $\pm$ 1.11 ^d	47.78 $\pm$ 1.11 ^d	
	80	42.22 $\pm$ 1.11 ^e	54.44 $\pm$ 2.94 ^e	62.22 $\pm$ 2.22 ^e	
	100	56.67 $\pm$ 1.93 ^f	66.67 $\pm$ 1.93 ^f	73.33 $\pm$ 5.09 ^f	
LLE/w	20	10.00 $\pm$ 1.92 ^b	12.22 $\pm$ 1.11 ^b	15.56 $\pm$ 1.11 ^b	74.67
	40	12.22 $\pm$ 1.11 ^b	17.78 $\pm$ 1.11 ^c	24.44 $\pm$ 2.22 ^c	
	60	18.89 $\pm$ 2.94 ^c	37.78 $\pm$ 2.94 ^d	44.44 $\pm$ 1.11 ^d	
	80	35.56 $\pm$ 2.94 ^d	46.67 $\pm$ 1.92 ^e	54.44 $\pm$ 2.94 ^e	
	100	47.78 $\pm$ 1.11 ^e	60.00 $\pm$ 1.92 ^f	65.56 $\pm$ 1.11 ^f	
TLE/e	20	11.11 $\pm$ 1.11 ^b	15.56 $\pm$ 1.11 ^b	21.11 $\pm$ 1.11 ^b	72.33
	40	15.56 $\pm$ 2.22 ^b	20.00 $\pm$ 1.92 ^b	25.56 $\pm$ 1.11 ^b	
	60	24.44 $\pm$ 2.22 ^c	34.44 $\pm$ 2.94 ^c	41.11 $\pm$ 2.94 ^c	
	80	37.78 $\pm$ 2.94 ^d	51.11 $\pm$ 2.22 ^d	57.78 $\pm$ 2.22 ^d	
	100	52.22 $\pm$ 1.11 ^e	61.11 $\pm$ 2.94 ^e	68.89 $\pm$ 1.11 ^e	
CLE/e	20	14.44 $\pm$ 1.11 ^b	21.11 $\pm$ 1.11 ^b	28.89 $\pm$ 1.11 ^b	55.56
	40	23.33 $\pm$ 1.92 ^c	31.11 $\pm$ 1.11 ^c	40.00 $\pm$ 1.92 ^c	
	60	38.89 $\pm$ 1.11 ^d	48.89 $\pm$ 1.11 ^d	60.00 $\pm$ 1.92 ^d	
	80	48.89 $\pm$ 1.11 ^e	63.33 $\pm$ 1.92 ^e	73.33 $\pm$ 1.92 ^e	
	100	61.11 $\pm$ 1.11 ^f	74.44 $\pm$ 4.01 ^f	83.33 $\pm$ 1.92 ^f	
LLE/e	20	14.44 $\pm$ 1.11 ^b	11.11 $\pm$ 1.11 ^b	21.11 $\pm$ 1.11 ^b	65.95
	40	17.78 $\pm$ 1.11 ^b	20.00 $\pm$ 1.92 ^c	31.11 $\pm$ 1.11 ^c	
	60	27.78 $\pm$ 2.22 ^c	40.00 $\pm$ 1.92 ^d	48.89 $\pm$ 1.11 ^d	
	80	41.11 $\pm$ 2.94 ^d	55.56 $\pm$ 2.94 ^e	63.33 $\pm$ 1.92 ^e	
	100	54.44 $\pm$ 1.11 ^e	64.44 $\pm$ 1.11 ^f	74.44 $\pm$ 4.01 ^f	
H ₂ O		0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	
70% EtOH		0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	

Note: Each value is the mean $\pm$ standard error, n = 9. Numbers with different letters within the same column are significant differences, $P \leq 0.05$.

The higher doses and the longer treatments demonstrated the most efficacy on larval mortality. It was likely that the ethanol extracts were more potent than the water extracts. At 100 mg/ml extract and 24 h feeding, CLE/e expressed the highest mortality of $83.33 \pm 1.92\%$ with LC_{50} 55.56 mg/ml and TLE/w produced the lowest one with $62.22 \pm 2.22\%$, LC_{50} 80.14 mg/ml (Table 5). The potent of the extracts was ranged as CLE/e > LLE/e = CLE/w > TLE/e > LLE/w > TLE/w and their efficacy, LC_{50} at 24 h, was 55.56, 65.95, 72.33, 74.67, and 80.14 mg/ml respectively.

Larvicidal Effect

The larvicidal effects of the extracts on the 2nd instar larvae of *B. correcta* by dipping assay showed

similar pattern to feeding assay but very slightly lower. The LC_{50} values of CLE/e, LLE/e, CLE/w, TLE/e, LLE/w, and TLE/w were 52.99, 63.43, 64.08, 70.29, 71.77, and 77.52 mg/ml respectively (Table 6).

Molting Effect

All extracts inhibited the molting of the 3rd instar larvae of *B. correcta* into adults in concentration dependent manner. The molting inhibition was over 50% at high concentrations of 80-100 mg/ml (Table 7). CLE was most effective. The efficacy of the extracts at 24 h was ranged as CLE/e, TLE/e, CLE/w, LLE/e, TLE/w, and LLE/w with EC_{50} values of 69.55, 72.49, 72.91, 77.23, 78.38, and 83.44 mg/ml, respectively.

Table 6. Larvicidal effects of *T. erecta* (TLE), *C. odorata* (CLE) and *L. camara* (LLE) leaf water (/w) and 70% ethanol (/e) extracts on the 2nd instar larvae of *B. correcta* by dipping assay at different concentrations and times

Extract	Conc. mg/ml	% Mortality (Mean $\pm$ SE)			LC_{50} mg/ml, 24 h
		6 h	12 h	24 h	
TLE/w	20	7.78 $\pm$ 1.11 ^b	11.11 $\pm$ 1.11 ^b	15.56 $\pm$ 1.11 ^b	77.52
	40	10.00 $\pm$ 1.92 ^b	17.78 $\pm$ 1.11 ^c	24.44 $\pm$ 2.22 ^c	
	60	20.00 $\pm$ 0.00 ^c	33.33 $\pm$ 1.93 ^d	40.00 $\pm$ 0.00 ^d	
	80	31.11 $\pm$ 1.11 ^d	44.44 $\pm$ 1.11 ^e	48.89 $\pm$ 1.11 ^e	
	100	45.56 $\pm$ 2.22 ^e	57.78 $\pm$ 2.22 ^f	65.56 $\pm$ 1.11 ^f	
CLE/w	20	11.11 $\pm$ 1.11 ^b	15.56 $\pm$ 1.11 ^b	22.22 $\pm$ 1.11 ^b	64.08
	40	17.78 $\pm$ 1.11 ^c	22.22 $\pm$ 2.22 ^c	32.22 $\pm$ 1.11 ^c	
	60	33.33 $\pm$ 1.93 ^d	42.22 $\pm$ 1.11 ^d	51.11 $\pm$ 1.11 ^d	
	80	44.44 $\pm$ 1.11 ^e	57.78 $\pm$ 2.22 ^e	64.44 $\pm$ 1.11 ^e	
	100	57.78 $\pm$ 2.22 ^f	67.78 $\pm$ 1.11 ^f	76.67 $\pm$ 1.92 ^f	
LLE/w	20	11.11 $\pm$ 1.11 ^b	13.33 $\pm$ 0.00 ^b	16.67 $\pm$ 0.00 ^b	71.77
	40	13.33 $\pm$ 0.00 ^b	18.89 $\pm$ 1.11 ^c	26.67 $\pm$ 0.00 ^c	
	60	21.11 $\pm$ 2.22 ^c	40.00 $\pm$ 1.92 ^d	46.67 $\pm$ 0.00 ^d	
	80	38.89 $\pm$ 4.44 ^d	46.67 $\pm$ 1.92 ^e	57.78 $\pm$ 2.22 ^e	
	100	48.89 $\pm$ 1.11 ^e	63.33 $\pm$ 1.92 ^f	67.78 $\pm$ 1.11 ^f	
TLE/e	20	12.22 $\pm$ 1.11 ^b	15.56 $\pm$ 1.11 ^b	22.22 $\pm$ 1.11 ^b	70.29
	40	16.67 $\pm$ 1.93 ^c	21.11 $\pm$ 1.11 ^c	26.67 $\pm$ 1.93 ^c	
	60	25.56 $\pm$ 1.11 ^d	35.56 $\pm$ 2.22 ^d	43.33 $\pm$ 1.92 ^d	
	80	38.89 $\pm$ 2.22 ^e	52.22 $\pm$ 1.11 ^e	58.89 $\pm$ 1.11 ^e	
	100	53.33 $\pm$ 0.00 ^f	62.22 $\pm$ 2.22 ^f	71.11 $\pm$ 1.11 ^f	
CLE/e	20	16.67 $\pm$ 0.00 ^b	22.22 $\pm$ 1.11 ^b	30.00 $\pm$ 0.00 ^b	52.99
	40	24.45 $\pm$ 2.22 ^c	32.22 $\pm$ 1.11 ^c	42.22 $\pm$ 1.11 ^c	
	60	38.89 $\pm$ 1.11 ^d	50.00 $\pm$ 0.00 ^d	61.11 $\pm$ 2.22 ^d	
	80	51.11 $\pm$ 1.11 ^e	64.44 $\pm$ 1.11 ^e	75.56 $\pm$ 1.11 ^e	
	100	62.22 $\pm$ 1.11 ^f	76.67 $\pm$ 1.92 ^f	87.78 $\pm$ 1.11 ^f	
LLE/e	20	15.56 $\pm$ 1.11 ^b	12.22 $\pm$ 1.11 ^b	22.22 $\pm$ 1.11 ^b	63.43
	40	18.89 $\pm$ 1.11 ^b	21.11 $\pm$ 2.22 ^c	32.22 $\pm$ 1.11 ^c	
	60	28.89 $\pm$ 1.11 ^c	42.22 $\pm$ 1.11 ^d	51.11 $\pm$ 1.11 ^d	
	80	42.22 $\pm$ 2.22 ^d	57.78 $\pm$ 1.11 ^e	65.56 $\pm$ 1.11 ^e	
	100	56.67 $\pm$ 1.92 ^e	65.56 $\pm$ 1.11 ^f	77.78 $\pm$ 1.11 ^f	
H ₂ O		0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	
70% EtOH		0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	

Note: Each value is the mean $\pm$ standard error, n = 9. Numbers with different letters within the same column are significant differences, $P \leq 0.05$.

Table 7. Molting inhibition of *T. erecta* (TLE), *C. odorata* (CLE) and *L. camara* (LLE) leaf water (/w) and 70% ethanol (/e) extracts on the 3rd instar larvae of *B. correcta* at various concentrations and 24 h

Conc. mg/ml	% Inhibition (Mean $\pm$ SE)					
	TLE/w	CLE/w	LLE/w	TLE/e	CLE/e	LLE/e
H ₂ O	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a
70% EtOH	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a
20	12.22 $\pm$ 1.11 ^b	18.89 $\pm$ 1.11 ^b	13.33 $\pm$ 1.92 ^b	15.56 $\pm$ 2.94 ^b	23.33 $\pm$ 1.92 ^b	21.11 $\pm$ 2.22 ^b
40	27.78 $\pm$ 1.11 ^c	34.45 $\pm$ 1.11 ^c	25.56 $\pm$ 2.94 ^c	30.00 $\pm$ 1.92 ^c	37.78 $\pm$ 2.94 ^c	28.89 $\pm$ 1.11 ^c
60	41.11 $\pm$ 1.11 ^d	45.56 $\pm$ 1.11 ^d	36.67 $\pm$ 1.92 ^d	44.44 $\pm$ 1.11 ^d	46.67 $\pm$ 3.85 ^d	43.33 $\pm$ 1.86 ^d
80	53.33 $\pm$ 1.92 ^e	51.11 $\pm$ 1.11 ^e	47.78 $\pm$ 2.22 ^e	55.56 $\pm$ 1.11 ^e	54.44 $\pm$ 2.22 ^e	51.11 $\pm$ 1.11 ^e
100	58.89 $\pm$ 1.11 ^f	66.67 $\pm$ 1.92 ^f	55.56 $\pm$ 2.94 ^f	67.78 $\pm$ 2.22 ^f	68.89 $\pm$ 2.94 ^f	60.00 $\pm$ 1.92 ^f
EC_{50} (mg/ml)	78.38	72.91	83.44	72.49	69.55	77.23

Note: Each value is the mean $\pm$ standard error, n = 9. Numbers with different letters within the same column are significant differences ($P \leq 0.05$).

Discussion

It is widely known that natural products, especially plant extracts, have been proved as effectively alternative methods in controlling fruit flies. The present study demonstrated that the leaf extracts of three aromatic plants *T. erecta*, *C. Odorata*, and *L. camara* possess properties as control agents against *B. correcta* adults, eggs, larvae and pupae. Like some other aromatic plants containing chemicals can strongly repel insect pests (Olonisakin *et al.*, 2006; Mwangangi and Mutisya, 2013) by disrupting respiratory activity and resulting in asphyxiation and subsequent death (Tawatsin *et al.*, 2001; Kumar, *et al.*, 2011). The aromatic *Alpinia galingale* tuber fermented extract substantially contained fragrant oil was traditionally and effectively used in a large orchard to protect fruits from *B. dorsalis* and *B. correcta*. Its foul odor highly repelled and prevented various fruits from flies laying eggs. It also killed the flies (Food and Fertilizer Technology Center, 2005). As well as the *Azadirachta indica* leaf methanol extract inhibited oviposition of Mediterranean fruit flies on grave fruits (Silva *et al.*, 2012).

The insecticidal activities of *T. erecta*, *C. odorata* and *L. camara* extracts in this study showed high potential against *B. correcta* larvae and adults. It is evidenced that *T. erecta* cultured callus produced insecticidal pyrethrins (Sarin, 2004). *T. erecta* leaf extract possibly contained pyrethrins as well, which could cause deadly toxic to *B. correcta* larvae and adults. Moreover, *T. erecta* extract affected against beetles, the stored product insect pests, such as *Tribolium castaneum* (Islam and Talukder, 2005; Nikkon *et al.*, 2009), *Sitophilus oryzae* (Broussalis *et al.*, 1999) and *Sitophilus zeamais* (Parugrug and Roxas, 2008) by repellence, adult mortality, antioviposition and growth inhibition. There are some pieces of evidence support our study that *L. camara* and *C. odorata* had insecticidal activities against adult *S. zeamais* (Bouda *et al.*, 2001). In addition, the leaf extracts of *L. camara* had strongly insecticidal activities against adult and larvae of mosquitoes *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles culicifacies*, *An. fluviatilis* and *An. stephensi* (Tanprasit, 2005; Kumar and Maneemegalai, 2008; Dua *et al.*, 2010). The effects of these three plant extracts on *B. correcta* were also in agreement with Casana-Giner *et al.* (1999) who tested insect growth regulators (IGRs) as chemosterilants for *Ceratitis capitata* (Diptera: Tephritidae) by mixing them with the fly food. The IGRs inhibited the fly egg hatching and induced the male sterility. It was also in agreement with Arif *et al.* (2014) investigation of IGRs

on repellent, oviposition-deterrent, development, metamorphosis, reproduction, and insecticidal properties against *B. correcta*. Our extracts may possibly express their actions on the esterase activity in resisting to toxic plant allelochemicals and many commercial insecticides on *B. zonata* (Halawa *et al.*, 2013) and *B. cucurbitae* (Kaur *et al.*, 2014) and on the AChE activity in interfering the passage of impulses in the insect nervous system by *L. camara* (Rajashekar *et al.*, 2014).

Conclusions

In conclusion, *T. erecta*, *C. odorata* and *L. camara* leaf extracts exhibited high potential as repellent and insecticidal agents against guava fruit fly *B. correcta*. The ethanol extracts showed slightly more potent than the water extracts in controlling of all studied aspects on *B. correcta*. CLE/e contained highest repellent and insecticidal activities against the adult flies. The pair-combination tests showed that the insecticidal activity of the extracts depended on the higher proportion of each individual extract in the combination. Three fold of CLE/e in the combination enhanced the activities of TLE/e and LLE/e and *vice versa*. CLE/e showed highest repellency followed by TLE/e. CLE/e also expressed the most effectiveness against egg hatching, larval growth of the 2nd and 3rd instar larvae of *B. correcta*, followed by the others. Therefore, *T. erecta*, *C. odorata* and *L. camara* leaf extracts can potentially be one of the alternative choices for biological control agents against *B. correcta*, dipterans and other insect pests.

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