Bioactivity of some Essential Oils against the Mediterranean fruit fly (C*eratitis capitata*) under Laboratory Condition

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Abstract

Mediterranean fruit fly is one of the most important fruit damaging pests. It is a major pest in Ethiopian orchards causing annual loss of about 1,500 tone of orange and mandarin in Upper Awash Agro-Industry Enterprise. Different pest management strategies are currently employed against the pest and the use of bio-derived pesticides are known to be widely adopted practical intervention as major components of integrated pest management (IPM) to tackle the med-fly problems in fruit production. Hence, the bioactivities of essential oils from local plants were tested against the adult and immature stages of the fruit fly (*C. species*). Essential oil bearing local plant materials was collected from Wondo Genet and Addis Ababa areas. Essential oils were produced using hydro-distillation method using Clavenger Apparatus. The repellent action of essential oil of *Chenopodium ambrosioides, Laggera tomentosa, Schinus molle,* and *Ocimum suavae* were tested against the Mediterranean fruit flies by standard bioassay procedure. The result indicated that more than 90% of the adult flies were repelled due to application of essential oils on guava fruits in a choice bioassay. Essential oils extracted from the leaves and succulent parts of *O. suavae*, showed no significant difference (P> 0.05) in repellency. To determine the prophylactic effects of each essential oil against immature stages (maggots), six levels concentrations (100,150,200,250,300,500 ppm) were studied. Each test preparations were sprayed on pre-infested guava fruits at 12, 24, 36 and 48 hours between infestations with three replicates for each period. Number of adults emerged from each cage after 21- 30 days were counted and comparison was made with untreated cages to know the efficacy of the oils. The essential oil extracts caused significant reduction in number of progeny (> 74% mortality) except for extract from *O. suave*. Thus, products of essential oils both from *C. ambrosiodes* and *L. tomentosa* are found to be promising against the Mediterranean fruit fly *Ceratitis* species. Hence, the current findings deserve to be considered as sound inputs for future promotion of detailed practical experiments both under laboratory and field circumstances to enhance the ongoing Integrated Pest Management Strategy of the country.

Introduction

Mediterranean fruit fly is one of the most important fruit pests worldwide with its origin believed to be sub-Saharan tropical country (White and Elson-Harris, 1992). Currently, it is found in most tropical and Sub-tropical areas of the world and is a major pest in Ethiopian orchards causing annual loss of about 1,5,00 tones of orange and mandarin in Upper Awash Agro-Industry Enterprise (UAAIE) alone (Eshetu *et al.*, 2006).

Fruit production is constrained by lack of adequate pest control, poor soil fertility management practices, lack of attention to product quality and prevention of physical damage, as well as the lack of appropriate storage and packaging facilities (Fekadu and Dandena, 2006). Annual reports from Upper Awash Agro-Industry Enterprise (UAAIE), the largest producer of variety of horticultural fruits and vegetables for local and export markets confirmed that insect pest damages are among the most critical factors threatening citrus production in the enterprise in particular and the country in general. Of the records made so far, fruit fly (*Ceratitis capitata*) and false codling moth damages are the key pests the enterprise (UAAIE, 2008).

Currently, monthly bait spray and physical control measures are the only methods being used to control these pests. The application of synthetic insecticides can cause undesirable effects on the environment and on non-target organisms of the surrounding and the use of physical control measures may be less applicable under big farms like UAAIE, where fruits are cultivated in thousands of hectares. Thus, it is more demanding and pertinent to develop either single sound or integrated pest management (IPM) strategies to enhance fruit production and marketing in the country.

Hence, the aim of this study was to evaluate repellency and bioactivity of essential oils of some local plants against immature stages of the Mediterranean fruit fly, and to identify the preliminary mode of action of the selected natural products against the target pest.

Materials and Method

The study area

All the investigations were carried out in the Insect Science laboratory of Addis Ababa University and in the previous Essential Oil Research Center of Institute of Agricultural Research (EIAR) laboratory at Kality/Addis Ababa.

Rearing of Insect (*Ceratitis capitata*)

The flies were reared using Guava, *Psidium guajava* L. (Myrtacae). Fruits infested with fruit flies were collected from UAAIE and brought to the Insect Science laboratory .The infested fruits were placed in plastic cages of 30 X 30 X 30 cm with insect proof ventilation net at the center of the corner. The cage was half filled with sterilized soil at 60^{o}c for 240 minutes for pupation. The soil was disinfected using an oven in order to remove soil born pathogen. Five infested guava fruits were introduced into each cage and kept until flies were emerged. The rearing room was maintained at 60-70% Rh and 24-26^{o}c.

Plant Collection and Essential Oils Extraction

Plant materials, *C. ambrosiodes, L. tomentosa S. molle,* and *O. suavae,* were collected from Wondo Genet and around Addis Ababa areas. Samples were dried under ventilated room condition and their moisture contents before extraction were determined by using standard oven-dry method. The volatile oils were extracted from the plants by the process of hydro-distillation using a Clavenger apparatus (Gunter, 1946). For each of the plant samples, 200gm leaves were taken for the hydro-distillation and the extraction took 3 hrs. The distillates were collected in separating funnels in which the aqueous portions were separated from the volatile oil. The water layer was slowly drawn off until the oil layer remained. The resulted oils were used for the bioassays.

Bio-assay test: Samples of citrus fruits (n=2) were placed in two bottles separately. Samples in one of the glass bottle were treated with essential oil products (100, 150,200,250,300 and 500 ppm) and while the other was not treated (control). Then, air was pumped at a rate of 1.5 liters per minute with regulated air pump into the gas washing bottles containing activated charcoal for filtration through Teflon tube. The filtered air then passed into the gas washing bottles having different treatments (Essential oil and guava as compared with air and guava). After the set up was prepared, the method of Jembere *et al.* (1995) was followed where 25 adults of Mediterranean fruit flies of mixed sex and age were released into the "Y" olfactometer glass. After 30 minutes, the numbers of insects which moved into the untreated (Nc) and treated bottle (Nt) were counted. After each test the "Y" glass tube and the glass jars were washed with water, rinsed with acetone and dried at 80^{0}C for 1h. Each treatment was replicated three times and percentage repellency (PR) was computed using the methods of Jilani *et al.* (1988) as:

$$PR\,(\%) = \left(\frac{N_c - N_t}{N}\right) x100$$

Where Nc is the individuals in the control bottle (untreated Guavas). Nt is the individuals on the treated bottle, N is the total number of insects before the test. The assay for each dose of test material replicated three times for each Oil. A negative PR value was treated as zero and the data was analyzed after transforming them into arcsine values when necessary. All repellency assays carried out in the laboratory at 27 ± 2^0$_C$ and 65-70 r.h. The experiment was done twice to confirm its experimental validity. Finally, the insects were counted and the attraction was determined statistically by using independent t- test.

Bioassay for Prophylactic Effects of the Essential Oils

Samples of citrus fruits (n=2) per each cage were introduced in to a cage containing fruit flies and subjected to artificial infestation with adult Mediterranean fruit flies for overnight in rearing cages. The infested fruits were taken out and introduced in to a new cage prepared in the same way for stock population. Each new cage was contained two infested fruits. Essential oils were prepared at different concentrations (100,150,200,250,300,500 ppm). Each test preparations were sprayed on pre-infested citrus fruits after 12, 24, 36 and 48 hours of infestation with three replicates for each period. Number of adults emerged from each cage after 21- 30 days were counted and comparison was made with untreated cages to examine the efficacy of the oil. Percent progeny reduction was calculated following the method of Tapondjou *et al.* (2002), Method as follows:

$$IR\,(\%) = \left(\frac{C_n - T_n}{C_n}\right) X100$$

Where Cn is the number of newly emerged insects in the untreated (control) cage and Tn is the number of insects in the treated cage. In cases where the number of adults emerged from treated cages are greater than of control, 0 was taken as mortality record. Each treatment was replicated three times and percent mortality was computed using two ways ANOVA. The bioassay was conducted twice to check verification of the method and repeatability.

Statistical Analysis

Data entry and analysis were done using Microsoft Excel and SPSS Version 15.0. The median effective dosage (ED_{50}) and ED_{95} of the oils were determined using computer software for probit analysis (Finney, 1971) by SPSS 15.0 for windows. Effectiveness of the tested oils was determined by comparing the 95% confidence intervals of the ED_{50} values. Significant differences between the means were separated using Tukey's honestly significant difference (HSD) test (Gomez and Gomez, 1984).

Results

Repellency Effects of Essential Oils against Fruit flies

The repellency activity of *Chenopodium ambrosioides* against fruit fly increased in concentration-dependent manners. As shown in Fig 1, almost 50 % of the fruit fly populations were repelled with 500ppm where 1000ppm repelled more than 90% of the studied fruit flies.

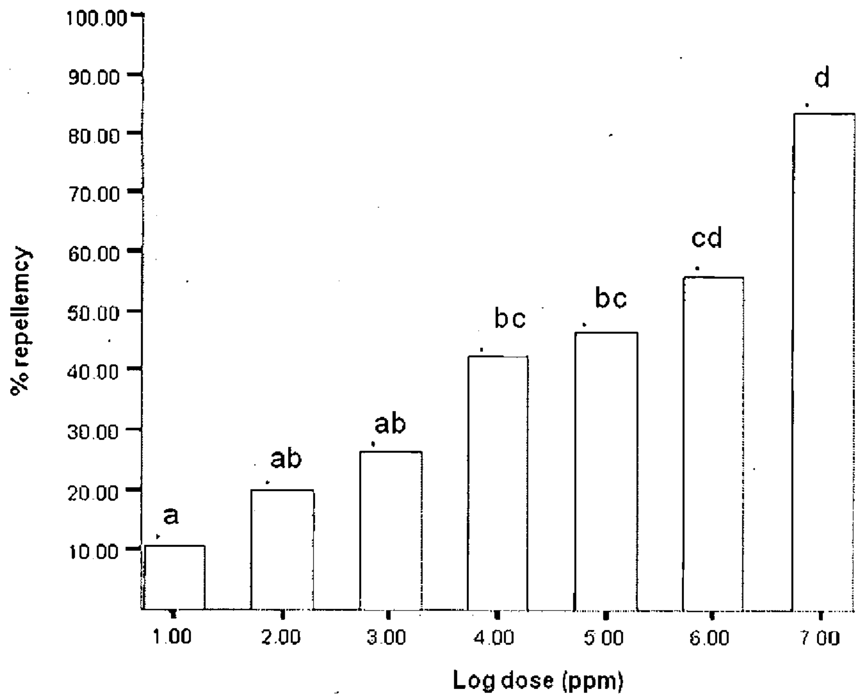

Figure. Percent repellency of *C. ambrosioides* against *C. capitata* in laboratory trials (bars with similar letter are not statistically different at 95% CI, * significant differences between treated and untreated test materials, concentration in part per million, ppm,).

Isolated tested materials from the *L. tomentosa* was found to be the second most repelling plant extract when compared with the other essential oil due to its repelling effects accounting about 50% of the test fruit fly population at the rate of 500ppm and more than 85% of the fruit fly population when the application rate was 1000ppm (Fig 2).

The independent analysis of two most repellent doses 500ppm and 1000ppm for extracts both from *L. tomentosa* and *C. ambrosioides* showed no significant difference between them, which may indicates both products consisted of more number of active principles at higher concentration to be considered as good source of natural insecticidal products for further practical investigations.

The essential oils of test plant species showed high level of repellency with ED_{50} values ranging from .927 (95% CI) for *C. ambrosiodes* to 4.910 (95% CI) for *S. mollae* and ED_{95} values ranging from 6.472 (95% CI) for *C. ambrosiodes*, to 1558.511 (95% CI) for *S. mollae*. *L. tomentosa* stands as the second essential oil possessing high level of repellency after *C. ambrosiodes* while the lowest ED_{50} value was that of *S. mollae*, with ED_{50} and ED_{95} values of 4.910 (95% CI = 3.095-229.009) and 1558.511 (95% CI = 8.485-1558.511), respectively (Table 1).

Interestingly, the rate of larval mortality due to the different essential oils and concentrations was significantly different to the time of exposure (Table 2). The longer exposure periods allowed the surface treated fruits; the higher percentage, about 70% due to application of *C. ambrosioides* to 74% due to that from *L. tomentosa* extracts, of larval mortality inside those tested fruits was recorded for the different natural products.

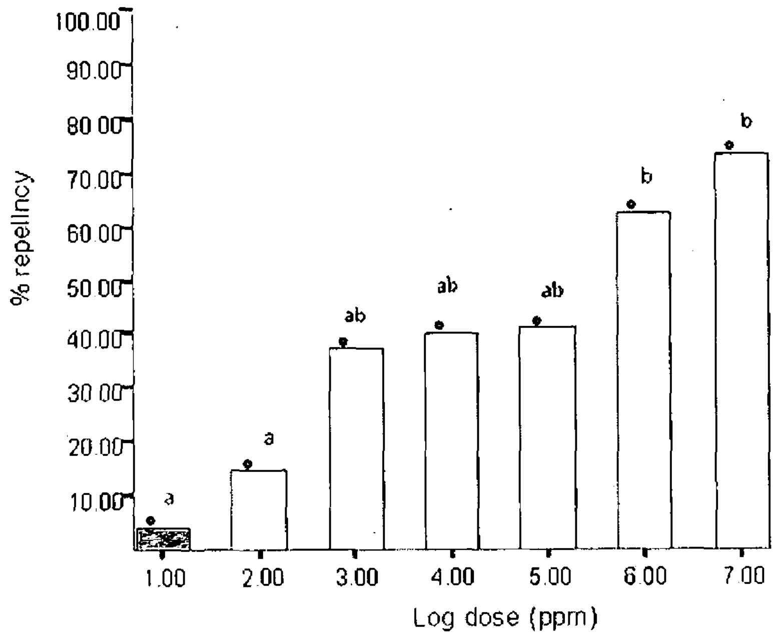

Figure 2. Percent repellency of *L. tomentosa* against *C. capitata* in laboratory trials (bars with similar letter are not statistically different at 95% CI, * significant differences between treated and untreated test materials, concentration in part per million, ppm,).

Table 1. The ED_{50} and ED_{95} values of essential oils from *Chenopodium*, *Laggara* and *Shinus* plants against fruit flies (*Ceratitis* species).

Plant species	LD50(95% CI)	LD95 (95% CI)
C. ambrisoides	0 .927 (0.000)	6.472(0.000)
L. tomentosa	2.291(0.000)	6.465(0.000)
S. mollae	4.910 (3.095-229.009)	1558.511(8.485-1558.511)

Table 2: Average (%) mortality (±SE) of *Ceratitis spp.* due to application of the different rates of essential oils and after different periods of infestation. Means followed by different letter (s) within a column (lower case letter) and rows (uppercase letter) are significantly different at 5% using Tukey studentized test (HSD).

Treatment	Dose	Mean % larval mortality, after treatment application			
	(ppm)	12hrs	24hrs	36hrs	48hrs
C. ambrisoides	100	22.67+3.53.51bB	37.33+2.67bcBC	46.67+4.06cC	43.00+8.54bcBC
	150	25.00 + 5.77abB	34.90 +6.92bB	22.77+6.31abB	26.33 +5.27bB
	200	33.33 + 8.33bB	33.37 +4.77bB	39.13 +2.51bB	50.67+3.53bB
	250	26.40+1.40abB	50.00+8.65bB	48.80+3.60bB	43.83+ 8.77bB
	300	18.33+3.33aA	51.80+4.8bB	66.70+ 7.67bB	59.67+3.53bB
	500	26.37+3.67bB	58.67+7.06cC	52.97+8.98cBC	**70.20+3.50**cC
L.tomentosa	100	21.73 + 2.51bB	36.53+4.20bcBC	52.0 + 4.62cd	58.70+ 4.18b
	150	19.30 + 4.65aA	50.00+ 5.25bB	57.33+ 7.42bB	53.97+ 6.89bB
	200	42.67 + 2.67bB	46.07+ 3.17bB	58.13+ 6.31bB	55.00+13.23bB
	250	17.30 + 1.92aA	21.57+10.38aA	55.00+ 10.0bB	71.03 + 3.85bB
	300	33.33 + 4.77a	47.03+14.82b	54.67+ 7.42b	66.67 + 3.93b
	500	24.07 + 1.85aA	55.00 + 5.77bB	**75.00+ 5.77**bB	**74.03+10.31**bB
	100	26.67+ 2.67bB	27.00+ 8.41bB	33.33+1.47bB	47.63+ 4.77bB
	150	7.33 + 9.94aA	41.2 + 3.17bcBC	53.83+5.87cC	47.60+12.59cC
S. mollae	200	18.33+ 3.33aA	33.33+3.93bcBC	49.97+4.53cC	54.53+11.44cC
	250	14.03+ 1.77aA	49.97+ 8.47bB	60.00+2.31bB	56.53+ 2.51bB
	300	44.00+8.33bcBC	27.43+ 1.97bB	62.30+2.90cC	49.13 + 1.73cC
	500	26.33 + 8.03bB	45.00+5.77bcBC	58.93+5.13cC	**63.47+ 4.20**cC
O.suavae	100	7.93 + 4.20aA	00 + 00aA	12.51+8.68aA	13.33 + 7.26aA
	150	21.70+ 5.02abB	3.50 +3.50aA	**28.00+6.11**bB	21.23+6.07abAB
	200	18.33 + 3.33aA	00 + 00aA	8.83 + 8.83aA	16.67 + 9.21aA
	250	18.33 + 1.67aA	3.50 + 3.50aA	11.53+6.67aA	15.27 + 7.73aA
	300	7.93 + 5.70aA	3.70 + 1.85aA	4.17 + 4.17aA	6.67 + 4.41aA
	500	5.00 + 5.00aA	6.33 + 3.17aA	17.33+4.57aA	18.17 + 4.57aA
Control		00 + 00aA	00 + 00aA	00 + 00aA	00 + 00Aa

Discussion

The base line study carried out (at Upper Awash showed the presence of two species of the fruit fly (Bezawork 2007, personal communication). The majority of the population (94%) was found to be *C. capitata* and the remaining was *C. rosa*. The population variation of the two species has also been reported in studies carried out elsewhere confirming that; *C. capitata* is more tolerant to wider range of climatic conditions than *C. rosa* (Meyer et al., 2008).

Practically, the use of bio-pesticides is the most recommended control measure against these flies and remains most important aspect as it is economically sound and environmentally friendly. More over it increase both the quality and quantity of fruit production. To this end, no effective alternatives have been developed so far in Ethiopia as far as fruit flies are concerned. Plant derived compounds are being used as oviposition deterrent, repellent, larvicidal, and malfunctioning of adult physiology to control the pest population elsewhere in the world. With respect to this there are base line studies to develop biopesticides against fruit flies in Upper Awash Agro industry Enterprise (UAAIE). In a work done in 2007 by UAAIE in collaboration with ex-Essential Oils Research center, 13 botanicals were selected and essential oils and solvent extracts were prepared and tested against the larvae of fruit flies by filter paper bioassay. Among the selected plants three plant extracts (Jatropha, Eucalyptus and Black cumin) were found to be most promising in their larvicidal activity causing more than 85% mortality with in 30 minute exposure time using at the rate of 0.1ml of the active ingredient (Mekuria, 2007, personal communication). Thus, the current study further evaluated the repellency effect of essential oil products and prophylactic effects of the same products against the fruit fly larvae. The findings confirmed that the tested essential oils from *C. ambrosiodes*, *L. tomentosa*, *S. mollae* and *O.suavae* each with different concentrations displayed their naturally repellent behaviors. With regard to this ends, the essential oils from *C. ambrosioides* and *L. tomentosa* had embedded with significant repellent activity. Such efforts to search and research activities, experimenting with essential oils from the above plants revealed in for natural active insecticidal products potent against the Mediterranean fruit flies.

On the other side the prophylactic effect of same essential oils were tested for their systemic action on the immature stages of Mediterranean fruit fly. The results were found to be evoking higher mortality of the larvae, inhibiting considerable number of progenies. Exceptionally *O. suavae* has not caused significant number of adult mortality. Number of adults emerged were not significantly different from the number of adults emerged from control cages. The highest mortality (28%) was found using 150ppm applied after 36 hours of infestation. All the rest doses have induced lower percent of mortality and it was the least in its systemic effect compared to the other oils tested.

The bio-insecticidal behaviors detected from essential oils of *C. ambrosioides* by causing more than 70% of adult fruit flies mortality using at the rate of 500ppm when it was applied after 48 hours of infestation seems the product consisted of bio-active substances with fast knock-down effects. Similarly, the works Mouhssen (2004), Araya (2007), and Dembitsky *et al.* (2008), in their previous studies on essential oils from *C. ambrosioides* confirmed with its strong knock-down insecticidal activity against storage pests. The insecticidal property of *L. tomentosa* was found to be another most promising bio-activity, which is the first time reported in Ethiopia in this study, though some comparative bio-medicinal effects by reducing adult malarial mosquitoes *A. arabiensis*, in which, more than 98% repellency for about 1 hour using 20% concentration in confirming its strong repellent activity (Solomon 2007). Likewise, in this study, essential oil from *S. mollae* inhibited more than 60% Of progeny emergency using 500ppm applied after 48 hours of infestation. The result was well in agreement with the insecticidal, bactericidal and fungicidal activity of the plant (Laurent *et al.*, 1997; Cariac *et al.*, 2003; Richard and Ronald, 2006; DiAz *et al.*, 2008; Belhamel *et al.*, 2009)

Results of this study suggested that essential oils from *Chenopodium ambrosioides* and *Laggera tomentossa* have shown strong repellent activity against Mediterranean fruit flies, *C. capitata*. Essential oils from *S. molle* were less toxic to larvae of fruit flies where as *O. suavae* was not effective in causing mortality. Tests of each essential oils for their prophylactic effect against different immature stages of *Ceratitis spp.* has revealed that both *L. tomentosa* and *C. ambrosioides* can be considered as the future plant species with promising sources of bioactive insecticidal substances because both of them evoked significant high mortality in either egg, larvae or pupa of the insect when applied on host fruits. Hence, the current findings deserve to be considered as sound inputs for future promotion of detailed practical experiments both under laboratory and field circumstances to enhance the ongoing Integrated Pest Management Strategy of the country.

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