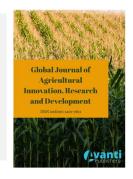


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Field Response of Tephritid Fruit Flies (Diptera) to Fruit Juice of Some Botanicals and Implications for Bio-Rationale Pest Management in Sudan

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ABSTRACT

Fruit flies belonging to the order Tephritidae are the most notorious pests that attack various fruit and vegetable species, causing severe economic losses. Guava orchards at Elfaki Hashim; North of Khartoum, (Khartoum State) and Elsawagi Elgenobia (Kassala State) in the middle and east of Sudan respectively were selected to assess the response of some Tephritids to water extracts (WE) of mango, guava, apple, cucumber, and ready-made juice of mango (Crystal®; Crystal Industrial Co. Ltd, Araak Group; crystal@araak.com) against Torula yeast (a standard fruit fly attractant) and water (control) baited in locally made traps. The trial was performed as Randomized Complete Block Design (RCBD) replicated 3 times for 5 consecutive weeks at each site. Highly significant differences were recorded between attractants and the interaction between attractants and times (weeks) on trapping different species of fruit flies. Five species of fruit flies; Ceratitis capitata, Bactrocera dorsalis, Zeugodacus cucurbitae, Ceratitis cosyra, and Ceratitis quinaria, were found to respond positively to all tested materials at both sites. The greatest number of C. capitata flies was caught by traps equipped with ready-made Crystal® mango juice at the Elfaki Hashim site. The mean number of *B. dorsalis* attracted to the WEs of mango and guava at Elsawagi Elgenobia site was statistically identical to that of the same species lured to Torula yeast, 83.7, 70.3, and 111.5 flies /trap/week, respectively. Attractants extracted from botanicals are cost-free and easily prepared by farmers for mass trapping of fruit flies. More studies regarding active ingredients, doses, and the number of traps utilized per area should be considered.

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1. Introduction

Fruit flies have been recognized as major insect pests of horticultural crops worldwide causing severe economic losses of fruits and vegetables and hindering their exportation according to the phytosanitary embargo [1]. Their significance in Africa increased after the introduction of the most seriously invasive species, *Bactrocera dorsalis* in 2003 [2, 3].

Different management tools were adopted against fruit flies around the world (field sanitation, chemical pesticide treatments, releases of natural enemies, biopesticide application, pheromone-based options (mass trapping, mating disruption) and sterile insect technique [4]. The excessive use of chemical pesticides in fruit orchards during the fruiting season pollutes the produce, causes severe health hazards, affects non-target beneficial organisms and may reduce international demand on most fruits that are tropically produced. Adoption of simple biorational alternatives to insecticides as effective eco-friendly to reduce reliance on insecticides are highly encouraged.

Fruit flies are attracted to many fruit and vegetable species; several plant extracts were examined worldwide to describe volatile compounds responsible for attraction [5-7].

In Europe, Mauritius and the United States, Nulure and Torula yeast and protein hydrolastes derived from *Zea* mays are the most widely used food bait for trapping fruit flies [8-14] reported that *Citrus aurantium* contains volatile compounds that could be used as attractants for fruit flies, while [15] found that high concentrations of grapefruit were attractive in laboratory wind-tunnel bioassay in the 1950s, protein hydrolysate baits mixed with parathion were first used in Hawaii [16]. Malathion was later used with protein hydrolaste baits in successful campaigns against the Medfly (*Ceratitis capitata*) in Florida [17]. In addition, brewery waste yeast efficacy as a successful protein source for trapping fruit flies was studied by Lloyd and Drew [18].

This study is an attempt to assess the field response of Tephritid fruit flies to water extracts (WEs) of some fruits of botanicals in order to identify plant materials for use in low-cost techniques for monitoring, mass trapping and suppressing economically important fruit fly species in Sudan.

2. Materials and Methods

The study was carried out at Elfaki Hashim, 30 km north of Khartoum, and Elsawagi Elgenobia, 2 km from Kassala city in Sudan as part of the Ph. D study of the first author. At Elfaki Hashim, the study was carried out from April to June in a 1 ha area. At Elsawagi Elgenobia, the study was conducted in 0.6 ha of an orchard between November and December 2008. In both experiments, the distance between guava trees was approximately 8 meters.

2.1. Preparation of Locally Made Trap

A plastic, orange-coloured jug (16 cm height X 12 cm diameter) was modified for use as a trap. A heated metal rod was used to perforate four equidistant openings (2 cm diameter for each) at 5 cm below the top of the jug.

2.2. Preparation of Attractants of Fruit Flies

One hundred grams each of apple, guava, mango, and cucumber were weighed individually, placed in a plastic bag, and squeezed by hand to extract the fruits' juice. A 300-ml measure of a ready-made mango juice (Crystal[®]), consisting of natural mango pulp 20%, sucrose, citric acid (E330), ascorbic acid (E300), sodium benzoate, (E211), sodium citrate (E331), gum arabic (E414), mango flavor, and sunset yellow coloring (E110) was used without dilution.

Torula yeast (5 g/100 ml water) was used for comparison between treatments, and water was used as a control. Three hundred ml of water was added to all treatments except Crystal[®], of which 300 ml was used

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undiluted as stated above. For each treatment except Torula yeast, 0.5 ml of Cypermethrin[®] EC (Eldoma Trade Company, Sudan) was dropped in traps to act as a killer of the trapped fruit flies, and 8 g of Borax (sodium borate) was added to preserve the killed insects. The content of each trap was stirred gently by a piece of wood.

Traps were suspended on guava trees approximately 2 m above the ground at the northeast side of each tree using a 50-cm length of plastic rope.

Experiments were conducted in a Randomized Complete Block Design (RCBD) with 3 replicates per treatment Trapping was continued for 5 consecutive weeks in each site.

The content of each trap was examined weekly using a fine-mesh sieve and a camel-hair brush. Trapped fruit flies were preserved in a 60-ml plastic container with 70% Alcohol, then counted, sorted, and sexed using a pictorial key prepared by Billah and Ekesi [19]. Traps were washed with powdered soap and water, and baits were renewed with the same attractants for the coming week and moved to a new position within the experimental site, following the prepared experimental design. Fruit fly counts were then expressed as numbers of insects per trap per week, and transformed using ($\sqrt{x+0.5}$) to normalize values prior to conducting the analysis of variance and.... Duncan's multiple range test and combined analysis for data of each site using the Statistical Analysis System (SAS 9) computer program.

To follow the presence of the trapped species in the experimental sites, dropped fruits were kept in ventilated rectangular containers covered with muslin mesh and left for 15 days to observe the emergence of adult flies. The emerged species and their percentages out of the total were then determined.

3. Results

3.1. Response of Different Species of Fruit Flies to the Test Attractants

Generally, at both sites, the tested extracts of botanicals were found to attract both sexes of all species of fruit flies that dominated the areas of the study (Table **1** and Table **2**).

Torula yeast caught the highest number of females (228.5) and males (167.3) per trap per week for all trapped species

A. Elfaki Hashim Site (Khartoum State)

At Elfaki Hashim Site, the study revealed 4 species of fruit flies as the most dominant species in guava ecosystem in the study area. *C. capitata* was found the most abundant species where it responded in highest numbers to all test attractants than *B. dorsalis, C. cosyra* and *C. quinaria.*

The four fruit fly species fluctuated in their response to the attractants, generally, Torula yeast significantly attracted the highest percentage of total fruit flies (42.5%) followed by Crystal (22.8%) while WE of apple, guava, mango and cucumber were found significantly same on their attraction to a total number of fruit flies with 10.6, 9.9, 8.9, 5.3% respectively. Numerically all attractants attracted higher numbers of females than males and the response of females to the test attractants was found as same as to the response of total fruit flies. The study confirmed that no fruit fly was responding to the control (water). As indicated in Table.1 No any natural enemy parasitoid or predator was found responding to all test attractants (Table **1** and Fig. **1**).

<u>B. dorsalis</u>

High significant difference was observed among the attractants and their interaction with time (weeks) on luring *B. dorsalis.* The highest number of flies of this species and the highest number of its females were attracted to Torula yeast followed by WE of guava, Crystal, apple with 61.5, 30.3, 22.9, 18.0 flies/trap/week (F/T/W) respectively while the same response was recorded to mango 13.2 F/T/W and cucumber 12.9 F/T/W (Fig. **2** and Table **1**).

Attractant	B. dorsalis				C. capitata	1		C. cosyra		Total			
	F	М	Т	F	М	т	F	М	т	F	М	т	
WE mango	2.7C	2.2B	3.4C	5.1C	5.2C	7.0CD	1.4B	0.1B	1.5B	6.0C	6.0C	8.2C	
	(7.8)	(5.4)	(13.2)	(37.0)	(31.7)	(68.7)	(1.7)	(0.3)	(2.3)	(37.1)	(38.8)	(83.3)	
WE guava	4.0B	2.7B	4.8B	5.3C	4.7CD	7.1CD	1.1BC	0.7B	1.0BC	6.7C	5.6C	8.7C	
	(21.4)	(8.9)	(30.3)	(37.0)	(27.5)	(60.2)	(0.7)	(0.0)	(0.7)	(54.8)	(37.1)	(91.9)	
WE cucumber	3.1BC	2.1C	3.3C	3.8C	3.1D	4.8D	1.3B	1.0B	1.4B	5.1C	3.9D	6.4C	
	(12.4)	(5.1)	(12.9)	(17.0)	(11.0)	(28.0)	(1.7)	(0.9)	(2.6)	(31.1)	(17.9)	(48.9)	
Crystal®	3.5BC	2.6B	4.3BC	8.2B	8.2B	11.7B	1.2BC	1.0B	1.4B	9.1B	8.8B	12.7B	
	(13.9)	(9.0)	(22.9)	(97.5)	(90.1)	(187.5)	(1.1)	(0.8)	(1.9)	(112.5)	(100.1)	(212.6)	
WE apple	3.4BC	2.2B	4.0BC	5.7C	5.5C	8.1C	1.1BC	0.9B	1.2BC	6.7C	6.1C	9.2C	
	(12.9)	(5.1)	(18.0)	(41.1)	(37.7)	(78.8)	(0.9)	(0.3)	(1.3)	(54.9)	(43.8)	(98.7)	
Torula yeast	5.6A	4.7A	7.3A	12.8A	10.8A	16.9A	2.7A	1.8A	3.2A	14.3A	12.2A	18.9A	
	(35.7)	(25.7)	(61.5)	(183.7)	(132.5)	(316.3)	(9.0)	(3.9)	(12.9)	(228.5)	(167.3)	(395.7)	
Control	0.7C	0.7C	0.7C	0.7D	0.7E	0.7E	0.7C	0.7	0.7C	0.7D	0.7E	0.7D	
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	
S.E	1.9	1.2	2.9	9.4	6.6	15.1	0.5	0.2	0.7	11.0	7.7	18.0	
C.V	40.0	42.5	39.3	47.2	48.0	43.8	46.1	48.4	51.0	43.1	44.0	40.8	
Sig	***	***	***	***	***	***	***	***	**	***	***	***	
W*T	**	**	**	*	N.S	*	**	N.S	**	*	N.S	*	

 Table 1: Mean Number of fruit flies attracted to water extract of some botanicals baited in locally-made traps at guava orchard at Elfaki Hashim. (20/4/2008-25/5/2008).

WE = water extract N.S = Not Significant, * = significant at 0.05, ** = significant at 0.001, *** = significant at 0.0001 F = Female, M = Male, T = Total. Data transformed = $\sqrt{X+0.5}$ Actual data between parenthesis.

Table 2:	Mean Number of fruit flies attracted to water extract of some botanicals baited in locally-made traps at
	guava orchard at Elsawagi Elgenobia. (2/11/2008- 6/12/2008)

Attractant	B. dorsalis			Z. cucurbitae			C. capitata			C. quinaria			Total		
	F	м	т	F	М	т	F	м	Т	F	М	Т	F	М	TFF
WE mango	6.1A	5.4B	8.2AB	0.8B	0.1	0.8B	0.8B	0.7B	0.8B	0.8B	0.7	0.8	5.5B	6.1AB	8.2A
	(45.9)	(37.1)	(83.0)	(0.3)	(0.7)	(0.3)	(0.3)	(0.0)	(0.3)	(0.2)	(0.0)	(0.2)	(37.2)	(46.5)	83.7
WE guava	5.3A	5.5B	7.7B	0.7B	0.7	0.9B	0.8B	0.8B	0.9B	0.7	0.7	0.7	5.6B	5.3B	7.8A
	(31.9)	(37.9)	(69.1)	(0.5)	(0.0)	(0.5)	(0.3)	(0.3)	(0.5)	(0.0)	(0.0)	(0.0)	(38.1)	(32.2)	(70.3)
WE	3.1B	3.3CD	4.4C	0.7B	0.7	0.8B	0.2B	0.7B	0.8B	0.7	0.7	0.7	3.3CD	3.1C	4.5B
cucumber	(12.0)	(15.8)	(55.4)	(0.0)	(0.0)	(0.3)	(0.7)	(0.0)	(0.3)	(0.0)	(0.0)	(0.0)	(15.9)	(12.3)	(28.1)
Crystal®	4.0B	4.3BC	5.8C	0.7B	0.8	0.1B	0.2B	0.7B	0.1B	0.1	0.7	0.7	4.3BC	4.0D	5.1B
	(26.9)	(28.5)	(55.4)	(0.0)	(0.2)	(0.7)	(0.7)	(0.0)	(0.7)	(0.7)	(0.0)	(0.1)	(28.7)	(27.0)	(55.8)
WE apple	3.1B	3.0CD	4.3C	0.7B	0.7	0.1B	0.7B	0.7B	0.1B	0.7	0.9	0.7	3.0D	3.2C	4.1B
	(11.6)	10.7	(22.3)	(0.0)	(0.0)	(0.7)	(0.2)	(0.0)	(0.7)	(0.0)	(0.3)	(0.0)	10.7	(11.7)	(22.3)
Torula yeast	6.5A	7.3A	9.8A	0.9A	0.7	1.5A	1.4A	0.9A	1.5A	1.1	0.7	1.2	7.3A	6.7A	9.5A
	(47.9)	(58.7)	(106.6)	(0.3)	(0.0)	(3.3)	(2.4)	(0.3)	(3.3)	(0.9)	(0.0)	(1.2)	(60.1)	(51.5)	(111.5)
Control	0.7C	0.7E	0.7D	0.7B	0.7	0.7B	0.7B	0.7B	0.7B	0.7	0.7	0.7	0.7E	0.7D	0.7C
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
Sig	***	***	***	**	N.S	*	***	***	***	***	***	***	***	***	***
Week*Treat	***	***	***	N.S	N.S	N.S	***	***	***	N.S	***	N.S	***	***	*
S.E ±	3.4	3.6	6.7	0	0	0	0.2	0.1	0.3	0.1	0.0	0.1	3.6	3.5	6.8
C.V %	41.8	39.6	38.0	17.9	18.1	24.4	32.9	19.0	35.4	26.2	13.6	28.2	38.9	41.6	49.8

WE = water extract N.S = Not Significant, * = significant at 0.05, ** = significant at 0.001, *** = significant at 0.0001, F = Female, M = Male, T = Total. Data transformed = $\sqrt{X+0.5}$ Actual data between parenthesis.

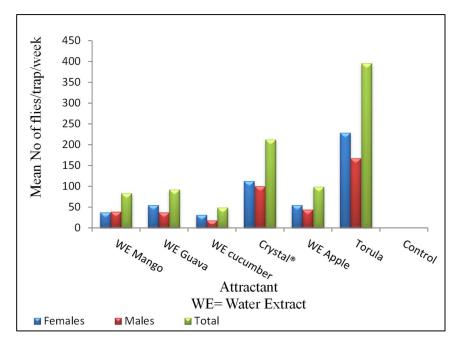


Figure 1: Means of the No of females, males and total fruit flies/ trap/week attracted to water extract of some botanicals, Guava orchard, Elfaki Hashim. (20/4/2008-25/5/2008).

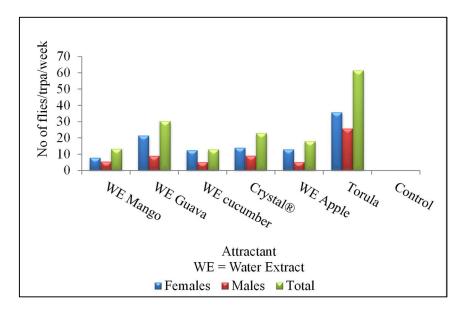


Figure 2: Means of the No of females, males and total *Bactrocera dorsalis*/trap/week attracted to water extract of some botanicals, Guava orchard, Elfaki Hashim. (20/4/2008-25/5/2008).

C. capitata

This species exhibited a significant difference in responding to different test attractants, Fig. (**3**) showed that, the highest number of flies were attracted to Torula yeast followed by Crystal and WE of apple with 316.3, 187.5 and 78.8 F/T/W respectively while it responded in the same number to WE of mango 68.7 F/T/W and guava 60.2 F/T/W and the least number was recorded for the WE of cucumber. Numerically, females of *C. capitata* were caught to different attractants in high numbers than males.

<u>C. cosyra</u>

Very few numbers of flies belonging to this species were responded to the test attractants (0.7- 12.9 F/T/W) when compared to other fruit fly species that dominated the same area. Also, attractants were found significantly

different in their attractiveness to *C. cosyra* where Torula obtained the highest number of flies/trap/week, followed same attraction to Crystal, WE of cucumber, mango and least number was found lured to WE of apple and guava (Fig. **4**).

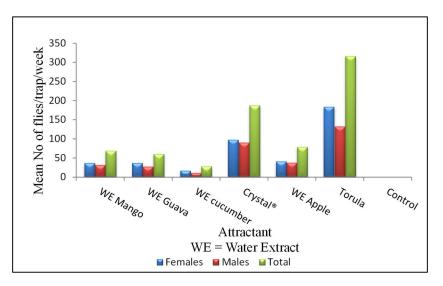


Figure 3: Means of the No of females, males and total *Ceratitis capitata*/trap/week attracted to water extract of some botanicals, Guava orchardElfaki Hashim. (20/4/2008-25/5/2008).

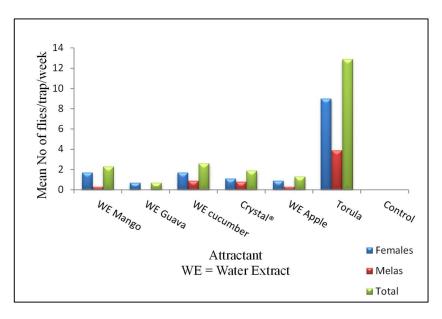


Figure 4: Means of the No of females, males and total *Ceratitis cosyra* /trap/week attracted to water extract of some botanicals, Guava orchard Elfaki Hashim. (20/4/2008-25/5/2008).

In this site, the emerged fruit flies reared out from fallen guava fruits at Elfaki Hashim confirmed the presence of the fruit flies that were trapped in different traps. These emerged fruit flies were *B. dorsalis*, *C. cosyra*, *C. capitata* and *C. quinaria* with percentages of 27%, 10%, 55%, and 8%, respectively.

B. Elsawagi Elgenobia Site (Kassala State)

In guava ecosystem in Kassala state, 4 species of fruit flies; *B. dorsalis*, *C. capitata*, *C. quinaria* and *Z. cucurbitae*, were responded positively to different attractants and *B. dorslais* was the most numerous and dominant species in the study site.

A significant difference was observed between the test attractants and their interaction with time (weeks) on their luring performances to fruit flies dominated the area. In this study same attraction results were noticed to WE of Torula yeast mango and guava 111.5, 83.7and 70.3 F/T/W followed by Crystal, WE of cucumber and WE of apple presented a statistically equal number of caught flies with 55.8, 28.1 and 22.3 F/T/W respectively (Table.2 and Fig. **5**).

<u>B. dorsalis</u>

Significantly, the number of flies of *B. dorsalis* attracted to Torula yeast was the highest (106.6) F/T/W among other attractants followed by flies attracted to WE of mango F/T/W, WE of guava, WE of cucumber, Crystal and WE of apple (83.0, 69.1, 55.4, 55.4 and 22.3) F/T/W respectively (Fig. **6**).

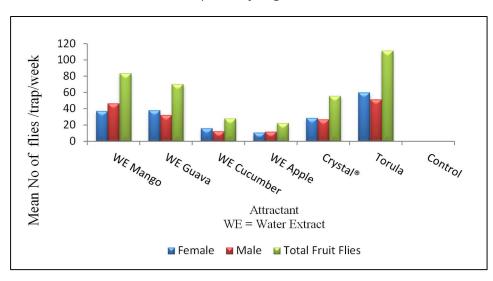


Figure 5: Mean No of females, males and total fruit flies/ trap/week attracted to water extract of some botanicals, Guava orchard, Elsawagi Elgenobia. (2/11/2008- 6/12/2008).

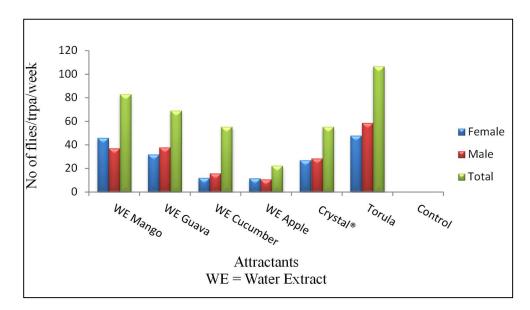


Figure 6: Means No of the females, males and total *Bactrocera dorsalis*/trap/week attracted to water extract of some botanicals, Guava orchard, Elsawagi Elgenobia (2/11/2008- 6/12/2008).

<u>C. capitata</u>

The number of flies of *C. capitata* caught to different attractants was very few (0.3-3.3) F/T/W compared to the number of *B. dorsalis* at this site. The population of this fruit fly responded significantly different to the attractants.

Torula was the best attractant. No significant difference among treatments was observed for the caught flies of *C. quinaria* and *Z. cucurbitae* (Table **2**).

At Elsawagi Elgenobia, *B. dorsalis* and *C. quinaria* were reared out from fallen guava fruits at 99.9% and 0.1%, respectively.

4. Discussion

Generally, attractant/insecticide mixtures (lure and kill technique) have been used successfully in fruit fly control since the turn of the 20th century [12]. However, the understanding of how such mixtures work, in particular the components and mechanisms involved in the attraction of fruit flies to bait, is still not fully clear. Baits consisting of carbohydrates and the juices of fresh fruit were recommended across the literature for trapping fruit flies since the early 19th century [16].

The results of trapping fruit flies obtained in this study by using extracts of botanical attractants are in agreement with the fact that fruit juices, extra-floral glandular secretions, nectar from flowers, pollen grains, honeydew, bird faeces, and bacteria are the main food sources of adult fruit flies [20-25]. Also, the obtained results in this study confirmed many studies recommend the use of botanical extracts for controlling fruit flies, especially cucumber when mixed with water or vinegar and yeast, which can attract both sexes of *Z. cucurbitae*. [24]. Also, [6] stated that extracts of mango (*Mangifera indica*), guava (*Psidium guajava*), and Sidir (*Zizyphus spina-christi*) attracted *C. cosyra*. [5] reported that the volatile compounds extracted from ripe and unripe Galia melon (*Cucumis melo* var.) were found attractive to both sexes of *Dacus ciliates*, with a preference for ripe fruit. Pineapple and brown sugar were found attractive to the papaya fruit fly [7].

In Malaysia [26] found that males of fruit flies are attracted to the flowers of Rutaceae (*Murraya panieulata*) in addition to methyl eugenol and culture, [27, 28] stated that McPhail or Multi-lure traps baited with hydrolyzed protein are used widely in the monitoring and detection of adult fruit flies.

The rearing out of fruit flies from fallen guava fruits at both study sites confirmed that the trapped flies except *Z. cucurbitae* are the most important fruit flies attack guava fruits in both areas and also it confirms the abundance of *C. capitata* at Elfaki Hashim sits and *B. dorslais* in Elsawagi Elgenobia Site. Adoption of baiting technique using locally made attractant in addition to field sanitation by burying fallen infested fruits will be of advantage in reducing the population of fruit flies.

The results of this study increase chances for environmental and biorational control tactics to reduce the ravage damages caused by fruit flies in Sudan where insecticides and mass trapping using methyl eugenol are the main control options used for management. On other hand, the availability of botanicals and simplicity of their application will decrease considerably the cost of production and open doors widely to export healthy produce free of fruit flies to meet the international highly demanded certificates especially Good Agricultural Practices (GAP) and Hazard Analysis and Critical Control Points (HACCP).

5. Conclusion

For its simplicity, availability and low cost, locally produced baiting techniques depending on extracts of botanicals and locally-modified traps are highly required for use by poor farmers for mass trapping, monitoring and suppression of fruit flies. Studies regarding the determination of active ingredients, doses, and the number of traps per area should be continued.

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