AN INNOVATED OF AN AIR FLOW OLFACTOMETER FOR MEASURING RESPONSE OF THE MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA* (WEID.) TO ODORS

EL-ABBASSI,T.S. and A.A. EL-ZOUK

Plant Protection Research Institute, ARC, Dokki, Giza

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Abstract

The present work was carried out to describe a new innovation of a simple detachable olfactometer for measuring the olfactory responses of fruit flies to different odors. Such device may be useful to discover the efficiency of current attractive or repellent odors or new odors that could be used for controlling fruit flies in fields. It could save effort, time and money required to investigate any new attractive or repellent material prior usage in the field Many experiments were conducted to standardize this device using the standard attractive material " Buminal " . These trials included determination of optimum concentration of test odor, optimum dose to test, velocity of air flow inside device, optimum number of flies that could be used in every test, optimum light intensity, best exposure time to test material and relationship between response of flies to test odor and temperature.

INTRODUCTION

Fruit flies are considered to be of the most world's destructive and injurious pests that widely distributed host range (White and Elson-Harris, 1994). They are high – priority quarantine pests, therefore, intensive control applications are being applied. Partial spray using a bait consisting of Malathion and protein hydrolysate is used worldwide for controlling these pests as an element in integrated pest management techniques (IPM). The use of malathion is controversial because of human health concerns (Flessel *et al.,* 1993 and Marty *et al.,* 1994) and the harmful effects on beneficial insects activity and survival of natural enemies and non-target organisms (Hoelmer and Dahlsten, 1993) Therefore, this insecticide has recently been excluded from annex 1 of the Directive 91/414 CEE, which lists authorized active ingredients for pest control in the European Union (Mapa,2009). Olfaction is the primary sense used by insects to detect and locate various resources (Tumlinson *et al.*, 1993). Insects respond to different olfactory cues or stimulates like volatile oils released from plants (e.g., phytophagous insects), host odors (e.g., parasitoids and predators), and pheromones for mate searching and aggregation. Plant chemical cues play a crucial role in mediating host findings and oviposition of fruit flies (Fletcher and

Prokopy,1991). Studies on the interactions among insect and other organisms revealed the sources and identities of substances that insects use to locate food and other resources (Carde´ & Bell, 1995).

Olfactometer and wind tunnels are often used to monitor the responses of insects to odor cues. McIndoo (1926) invented an apparatus (Y-tube olfactometer) that revolutionized the study of insect behavior. Afterward, various kinds of Y- or T-tube olfactometers have been designed to provide methods for detecting olfactory responses of insects (Janssen *et al*., 1990 and Martin *et al.,* 1990) An olfactometer with four arms has been also evolved to offer more choices of odor. (Turlings *et al.* 2004) innovated a six-arm olfactometer. Precise of olfactometry testing method depends on the quality assurance / quality control (QA/QC) statistics associated with the laboratory’s results. Many researches have been conducted to develop an effective control method as alternative to chemical control. These methods included usage of traps baited with the female-targeted or male-targeted lures (Papadopoulos *et al.,* 1998; Broughton,S. and de Lima, 2002; Heath *et al*., 2004 and Toth *et al.,* 2004). Mass trapping has proven to be a powerful weapon in the control of *C.capitata* and olive fruit fly *Bactrocera oleae* (Broumas *et al*., 2002). Therefore, it could be used as an alternative method for controlling fruit flies.. Behavioral assays to study insect attraction to specific odors are tedious, time consuming and often require large numbers of replications (Alemany *et al*. 2004; Navarro-Lopis *et al.*, 2008, Alonso Munoz and Garcia Mari 2009). The present paper describes an innovation of a simple detachable olfactometer for the analyses of olfactory responses of med fly to different odors. Some odors may act as attractants while others may have repellency effect. The innovated olfactometer was designed to test response of some odors against fruit flies as either attractants or repellents. Data that could be obtained with this olfactometer may explain to what extend volatile compounds emitted at different concentrations are used by fruit flies to localize their food plant and /or mating sites. Further, we can evaluate how the actual feeding stimulation of fruit flies corresponds to findings from olfactometer experiments. Therefore, results expected to be obtained from screening tests may be useful for controlling these insect pests.

MATERIALS AND METHODS

This new olfactometer is designed to test the response of fruit flies to different odors either as attractants or repellents. It consists of the following parts:

i. Glassware part:

As shown in Fig. 1 each olfactometer unit consists of five glass tubes, 90 cm long and 3.5 cm in diameter, opened from both ends. Each tube composed of three detachable compartments. The opening of 1st compartment is attached to the source of air flow and allocated for test material which could be introduced through an opening of 1 cm in diameter at its middle top side . The 2nd (middle) compartment is allocated for test fly (males and/or females) which could be introduced through an opening of 1 cm in diameter at its middle top side. The 3rd and last compartment (terminal) is designated to receive flies trying to escape away from odor where the terminal opening is covered with muslin cloth to capture flies inside the tube while allowing air flow to pass out.

ii. An electric air pump through which air flow rate could be controlled.

iii. Anemometer for measuring speed of air flow.

iv. Activated charcoal.

v. Adjustable illuminating system.

Assisting devices, chemicals and tools:

1- Luxmeter.
2- Thermo hygrograph.
3 Aspirators.
4- Micro pipettes
5- Eppendorf tubes.
6- Fine forceps.
7- Fine water brushes (camel's hair).
8- Small sized cotton balls
9- Ethyl Alcohol.
10- Detergents

Test insects:

Fruit flies used in these experiments (the Mediterranean fruit fly, *Ceratitis capitata* and the peach fruit fly, *(Bactrocera zonata*) were reared in the laboratory of Horticultural Insect Research Department (HIRD) , Plant Protection Research Institute (PPRI). Adult flies were kept inside wooden cages (40 x 40 x 40 cm) covered with fine wire nettings and provided with water and food consisting of enzymatic yeast hydrolysate + sugar at a rate of 4:1. Only one side of rearing cage was covered with fine muslin cloth. Deposited eggs were collected and scattered on an artificial medium diet. Full grown larvae popped out of medium were received in fine sterilized sand to pupate. Pupae were obtained by sieving the sand and kept inside Eppendorff tube till emergence.

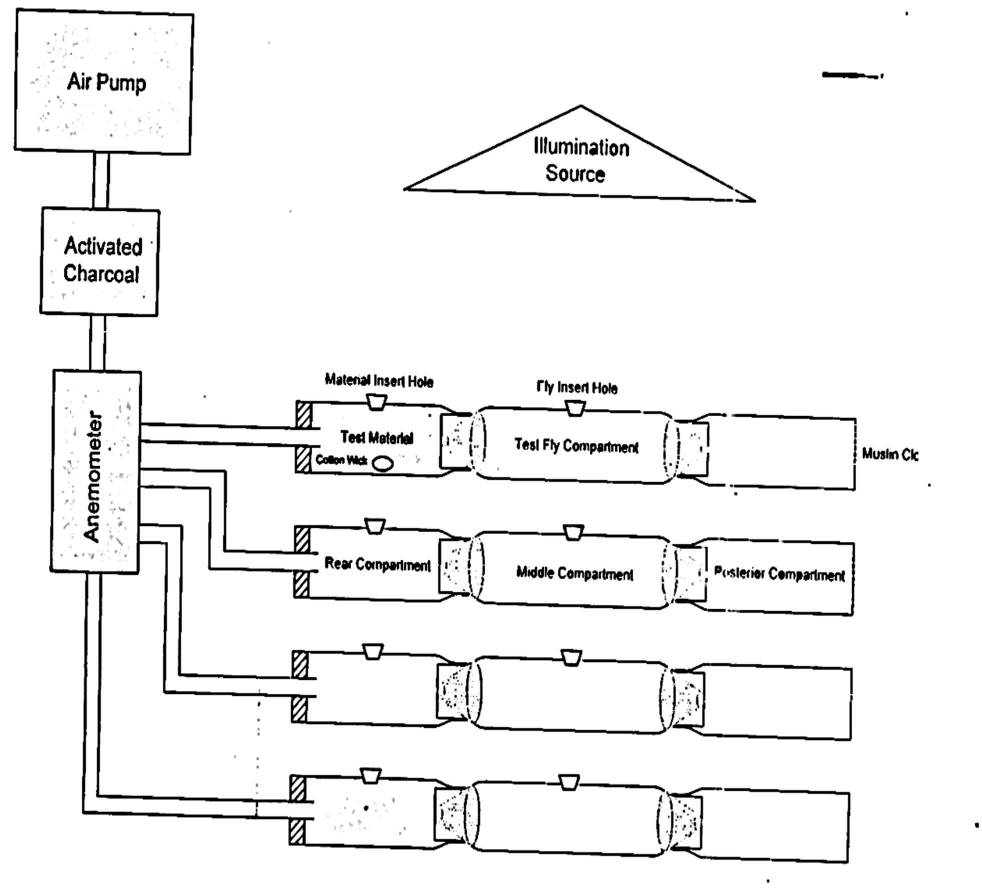

Fig.(1): The Innovated Olfactometer Model

Preparation of test flies:

Emerged flies kept inside Eppendorff tubes were separated into males and females, starved for 24 hrs. and kept in darkness for 12 hrs. prior exposure to test odors .

Optimization of Apparatus:

1- Standardization of odor concentration:

The concentration of the standard food attractant material (Buminal 36-38% protein hydrolysate) being used in open fields ranges between 5-10%. Therefore, these standard concentrations were tested. However, due to the small size of test apparatus a range of lower concentrations (1/4, 1/2, 1, 2, 3, and 4 % were tested**.** Untreated control was a small cotton piece saturated with water only.

2- Determination of test odor dose:

Small cotton pieces were loaded with different doses from the determined effective concentration of the standard material (Buminal) by using micropipettes. The tested doses were 25, 50,100,150,200 and 300 micro liters.

3- Adjusting proper speed of air flow:

Different air flow rates adjusted by an anemometer were tested to realize the optimum response of flies to odor. These air speeds were 10, 20, 30, 40, 50, 60, 70, 80, 90.and 100 meters/second.

4- Determining optimum number of test flies inside each tube unit:

The relationship between number of flies inside test tube and their response to odor was tested. Different fly numbers,4,6,8,10,12,14,16,18 and 20 flies were introduced in fly holding compartment to determine the optimum fly density. Sex ratio of tested flies was 1:1.

5- Adjustment of light intensity:

Fruit flies are photopositive insects. Therefore, light direction and/or intensity may affect their response to odors. Different light intensities ranged between 1500-5000 Lux (adjusted through controllable light system) , measured by a Luxmeter and response of flies to odors were recorded. Illumination source was positioned vertically above test insects to avoid influence of light on their movement to either right or left directions.

6- Determining proper time of exposure:

Time of exposure may play an important role in fly response to odors. Therefore, different exposure times (5, 10, 15, 20, 25, and 30 minutes) were tested.

7- Effect of temperature & relative humidity :

Temperature and relative humidity may positively or negatively affect flies activities (feeding, flying, mating, and ovipositing ….etc.). Therefore, a range of temperatures (18-26 ˚ C) and degrees of relative humidity (40-60%) were tested in relation to response of test flies to odor.

RESULTS AND DISCUSSION

Data in table (1) represent the response of med fly to the odor of the standard attractant material (Buminal) when used at different concentrations. Results indicated that the highest response was obtained at concentration 1%. Response of flies to odor decreased with the decrease or increase of concentration below or above 1%.

Table (2) show the response of flies when exposed to different doses from the effective concentration of the standard attractant material (Buminal) which was determined from experiments conducted before (1%). Data revealed that either 100 or 150 μ liter were the most effective doses in attracting test flies, where 80% flies positively responded to odor.

The relationship between velocity of airflow inside the olfactometer and response of med flies to test odor is shown in table (3). A series of airflow speeds were adjusted and ranged between 10-100 m/s. Data indicated that the best response of flies to odor (80%) is recorded when airflow speeds were 70 &80 m/s. Hao *et al*. (2012) stated also that wind speed significantly affected the capture rate of mosquitoes where the wind speed of 0.2 m/s exhibited a higher capture rate, which was significantly different from those at either 0.1 m/s or 0.4 m/s.

Data in table (4) indicate the relationship between intensity of test flies inside the olfactometer under investigation and response to odor. The optimum number of flies was ten flies. Positive response of flies to odor decreased with increase or decrease in number of flies. Moreover, 10 -30 % of flies escaped to the compartment away from odor direction (repelled) when number of flies inside tube increased from 14- 20 flies. This result disagree with Hao *et al*. (2012) who found that capture rates of mosquitoes increased as the number of testing insects in the olfactometer increased.

Med flies are photopositive insects and light intensity may play important role in response of flies to test odors. Therefore, the response of flies inside

Table 1. Effect of Buminal concentrations of on attraction of med flies

Concentration %	**Att. %**	**Neu. %**	**Rep. %**
0,25	40	60	0
0,5	70	30	0
1	90	10	0
2	70	30	0
3	70	30	0
4	40	50	10
5	40	50	10
6	30	50	20
7	30	40	30
8	40	20	40
9	30	30	40
%10	%30	%30	%40

(Att. = attractant Neu. = neutral Rep. = Repellent

Table 2. Relationship between dose of test attractive material and response of med flies

Quantity of Buminal μ L	**Att.%**	**Neu.%**	**Rep.%**
25	40	50	10
50	50	50	0
100	80	20	0
150	80	20	0
200	50	30	20
300	40	40	20

Table 3. Effect of air flow rates on response of med flies to odor

Air flow rates (m\s)	**Att. %**	**Neu. %**	**Rep. %**
0	0	100	0
10	10	80	10
20	10	80	10
30	40	60	0
40	50	50	0
50	60	40	0
60	70	30	0
70	80	20	0
80	80	20	0
90	70	20	10
100	60	20	20

Table 4. Effect of the insect density on the rate of attraction and repellent.

No. of insects	**Att. %**	**Neu. %**	**Rep. %**
4	20	80	0
6	30	70	0
8	50	50	0
10	80	20	0
12	70	30	0
14	60	30	10
16	60	30	10
18	50	30	20
20	40	30	30

The olfactometer under investigation to standard test odor was tested using various light intensities ranged between 1500- 5000 lux. Data represented in table (5) show that the best light intensity was 2500 lux where 80% of flies positively reponded.

Proper time of exposure to test odor is investigated and shown in table (6). Data clearly revealed that the best time of exposure was 20 minutes, where 90% of flies positively responded to test odor. Increasing time of exposure caused a repellency effect on test flies, where 10 and 20% of flies moved to the compartment away from of odor after exposure for 25 and 30 minutes, respectively. Hao *et al*. (2012) found that the capture rate of mosquito was highly correlated with time of exposure to three air-flow speeds. The capture rates increased linearly between 2 and 8 minutes, with correlation coefficients (r2) 0.985, 0.997, and 0.995, respectively. However, further increase in time duration to 10 minutes did not result in further increase in rate of attracted or responded insects.

Data presented in table (7) show the effect of different room temperature on response of flies to test attractant odor. Flies' response was higher when room temperatures were 22 and 25 ℃, where 80% of flies responded positively to odor. Higher or lower temperatures negatively affected flies response to odor. Also,. Hao *et al.* (2012) indicated that variation in temperature from 25 to 28º C had no apparent effect on mosquito capture under experimental condition.

Table 5. Effect of intensity of light on response of med flies to odors

Intensity of light (lux.)	**Att. %**	**Neu. %**	**Rep. %**
1500	40	50	10
2000	60	40	0
2500	80	20	0
3000	70	30	0
3500	70	30	0
4000	60	40	0
4500	50	40	10
5000	50	40	10

Table 6. Relationship between exposure time to test material and response of med flies

exposure period / min.	Att. %	Neu. %	Rep. %
5	10	90	0
10	20	80	0
15	60	40	0
20	90	10	0
25	70	20	10
30	50	30	20

Table 7. Effect of temperature on response of med flies to test material

Temperature (˚C)	Att. %	Neu. %	Rep. %
18	60	40	0
20	70	20	10
22	80	10	10
25	80	20	0
26	70	30	0
28	70	30	0
30	70	30	0

CONCLUSION

Fruit flies respond positively or negatively to certain odors either emitted from plant or chemically synthesized. Such odor could be useful and may play an important role for controlling fruit flies when used as a component in an IPM program. Therefore, many researchers devoted their efforts to find out effective materials that could be used for detecting and/or controlling these pests. Establishment of an apparatus through which screening several numbers of plant odors or chemically synthesized odors in laboratory to find out the most effective materials as attractants or repellents is highly recommended.

The present investigation is an attempt to innovate an olfactometer suitable for fruit flies to achieve this goal. The design of this olfactometer is privileged by the following:

i. It is made of glass which facilitates observing and recording movement of test insects.

ii- Composed of three detachable glass parts which facilitate cleaning and replacement of any part that could be broken or damaged.

iii- its small size which enable conducting several treatments and/or replicates at the same time.

iv- Easy to operate and maintain.

v- Its lower costs.

To ensure the efficiency of this new designed olfactometer, a series of trials were conducted on all factors that may affect the behavioral response of flies to test odors.

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ابتكار جهاز معملي جديد لقياس استجابة ذبابة فاكهة البحر المتوسط للروائح

طلال صلاح الدين العباسي و عبد الله علي عبد الحميد الزوق

معهد بحوث وقاية النباتات – مركز البحوث الزراعية

يوضح هذا البحث شرحا لابتكار جهاز معملي جديد سهل الفك والتركيب لقياس القدرة الشمية واستجابة ذباب الفاكهة للعديد من الروائح سواءا كانت جاذبة أو طاردة . و قد يسهم هذا الجهاز فى اكتشاف و تحديد كفاءة أي مادة جاذبة أو طاردة لذباب الفاكهة يمكن استخدامها في برامج مكافحة هذه الآفات وما يستتبع ذلك من توفير الوقت والجهد والتكاليف اللازمة لاختبار اي مادة قبل استخدامها تحت الظروف الحقلية. ولقد أجريت العديد من التجارب على هذا الجهاز لاختباركفاءتة ولوضع المعايير التي يجب اتباعها عند استخدامه حيث تم استخدام المادة الجاذبة الغذائية القياسية (البومينال) لتحديد أفضل تركيز وجرعة يمكن استخدامها ، كذلك تحديد سرعة تدفق الهواء المناسبة داخل الجهاز ، أفضل عدد من الحشرات التي يمكن اجراء التجريب عليها داخل الجهاز ، تحديد أفضل شدة اضاءة ، تحديد أفضل مدة تعريض للمادة بالاضافة الى تأثير درجة الحرارة على استجابة الحشرات للمادة المختبرة .