ISSN 0974 – 5211

Journal of Natural Products

Volume 6 (2013)

www.JournalofNaturalProducts.Com

Research Paper

Bactrocera Cucurbitae response to four *Cymbopogon* species essential oils.

F.T.D. Bothon[1*], D. Gnanvossou[2], J.P. Noudogbessi[1], R. Hanna[2], D. Sohounhloue[1]

[1]Laboratoire d'Etude et de Recherche en Chimie Appliquée, Ecole Polytechnique d'Abomey-Calavi, 01 BP 2009 Cotonou, Rép. du Bénin

[2]Institut International d'Agriculture Tropicale, Centre de Lutte Biologique pour l'Afrique, 08 BP 0932 Cotonou, Rép. du Bénin.

*Corresponding Author

(Received 04 July 2013; Revised 08-20 July 2013; Accepted 24 July 2013)

ABSTRACT

GC/MS analysis of essential oils extracted from four *Cymbopogon* species revealed that the majors compounds were *trans*-p-mentha-1(7),8-dien-2-ol (21.9%), *cis*-p-mentha-1(7),8-den-2-ol (19.4%), *trans*-p-mentha-2,8-dien-1-ol (9.6%), *cis*-p-mentha-2,8-dien-1-ol (7.2%), *cis*-p-menth-2-en-1-ol (7.2%), limonene (6.3%) in *C. giganteus*; piperitone (68.4%); δ-2-carene (11.5%) and α-eudesmol (4.9%) in *C. schoenanthus*, while citronellal (41.6%); geraniol (28.2%); citronellol (12.6%) and geranial (41.3%); neral (33.0%); myrcene (10.4%), geraniol (6.5%) were recorded in *C. nardus* and *C. citratus*, respectively.Tephritid fruit flies use both olfactory and visual cues to seek food and ovipositional resources. Olfactive effects for *C. citratus*, *C. nardus*, *C. giganteus* and *C. schoenanthus* essential oils on melon fly (*B. cucurbitae*) were evaluated using a four-arm olfactometer. The results showed that *C. giganteus* and *C. schoenanthus* repel mostly the fruit fly *B. cucurbitae*, compared with *C. nardus* and *C. citratus* and that female and male *B. cucurbitae* responded similarly to odours emitted from all essential oils evaluated. The number of pupae collected from zucchini treated with *C. giganteus* was significantly lower than that collected from zucchini treated with *C. nardus* when exposed to female *B. cucurbitae*, regardless of the concentrations.

Keywords: Chemical compounds; Olfactometer; *Cymbopogon.*

INTRODUCTION

The cucurbits such as cucumber, bitter gourd, sponge gourd, ridge gourd, bottle gourd are some of the major vegetables grown across in Africa. Several biotic factors limit the production and productivity of cucurbits, of which cucurbit fruit fly. The melon fly, *Bactrocera cucurbita*e (Coquillett) (*Diptera*:*Tephritidae*) is an invasive pest species in Africa and it is distributed widely in temperate, tropical, and sub-tropical regions of the world. It has been reported to damage over 125 fruit species (Sapkota, et al., 2010).The extent of losses in Tropical Africa varies between 30 to 100%, depending on the cucurbit species and the season (Gnanvossou, et al., 2008).The damage is mostly caused by the larvae of the female fly as they feed inside the fruits. After egg hatching, the larvae bore into the pulp tissue of fruit by making the feeding galleries. Young larvae usually leave the necrotic region and move to healthy tissue, where they often introduce various pathogens and hasten fruit decomposition and distortion (Dhillon, et al., 2005). Nowadays control of insect pests is primarily dependent

upon synthetic insecticides such as organophosphates, carbamates, pyrethroids and neonicotinoids (Ebadollahi, 2013). This results in environmental health risks and pest resistance may increase the tendency for vector borne disease outbreaks. An alternative to this situation could be the use of plant derived-products as low-risk botanical insecticides. The insecticidal activity of plant-products has been reported extensively against fruit flies pests (Bowers, 1993; Jang and Light, 1996). Tephritid fruit flies have evolved mechanisms which use olfactory and visual signals to find and recognize suitable host fruits in which they lay eggs (Cornelius, et al., 2000). The use of bio pesticides such as the essential oils to control tephritid fruit flies seems to be the most convenient solution - less expensive and weak environmental impact (Ebadollahi, 2013). Little is known, however, about the details of their effects on tephritid species and especially on *B. cucurbitae* behaviour. In the present study, experiments were conducted to determine (1) whether essential oils attract or repel mated and unmated female/male *B. cucurbitae*, (2) *B. cucurbitae* avoid ovipositing in cucurbit fruits treated with either essential oils and (3) whether the essentials odour blends differ, there by providing a chemical basis for the discrimination behaviour in *B. cucurbitae.*

MATERIALS AND METHODS

Plants**:** *Cymbopogoncitratus, Cymbopogongiganteus* and *Cymbopogonnardus* leaves were collected in September 2006 from Porto-Novo, Setto and Tori-Cada towns in southern of Benin respectively, while *Cymbopogon Schoenanthus* leaves were sampled from Boukoumbé town in northern of Benin in November 2006. All plants were identified at Abomey-Calavi University National Herbarium and voucher specimen numbers: AA6456/HNB, AA6457/HNB, AA6458/HNB and AA6459/HNB respectively, were deposited.

Extraction of the essential oils*:* The leaves were air-dried at room temperature (17-20°C) and were distilled for 3h using a Clevenger type apparatus. The essentials oils collected at the end of this process were dried with anhydrous sodium sulphate prior to their use in the analysis of volatile compounds.

Essential oils volatile components Analysis

GC/MS:The essential oils were analyzed on a Hewlett-Packard gas chromatograph Model 7890, coupled to a Hewlett-Packad MS model 5875, equipped with a DB5 MS column (30m x 0.25mm; 0.25µm), programming from 50°C (5 min) to 300°C at 5°C/min, 5 min hold. Helium as carrier gas (1.0ml/min); injection in split mode (1:30); injector and detector temperature, 280 and 280°C respectively. The MS working in electron impact mode at 70 eV; electron multiplier, 2500V; ion source temperature, 180°C; mass spectra data were acquired in the scan mode in *m/z* range 33-450.

GC/FID:The essential oils were analyzed on a Hewlett-Packard gas chromatograph Model 6890, equipped with a DB5 MS column (30m x 0.25mm; 0.25µm), programming from 50°C (5min) to 300°C at 5°C/min, 5min hold. Hydrogen as carrier gas (1.0ml/min); injection in split mode (1:60); injector and detector temperature, 280 and 300°C respectively. The essential oil is diluted in hexane: 1/30. The compounds assayed by GC in the different essential oils were identified by comparing their retention indices with those of reference compounds in the literature and confirmed by GC/MS by comparison of their mass spectra with those of reference substances (Adams, 1989; Rösch, et al., 1999)

Insects: The population of *B. cucurbitae* (Diptera: *Tephritidae*) was reared in the insectary (Temperature= 25±3°C; Relative humidity =80%) at the International Institute of Tropical Agriculture (IITA-Benin). Flies were allowed to oviposit on cucurbit fruits kept in wooden cages (1m x 1m x 1m) with the upper side made in glass. After 72 hours of exposure, fruits were removed from the cages and incubated in transparent plastic buckets containing sterilized sand to allow pupation of third instar larvae. First generation flies emerged 5-6 days

after pupae collection. Unmated flies were isolated from adults colony when they just emerged, while in case of mated flies, male and female flies were kept together to allow mating. All categories of flies were fed sugar, yeast hydrolysate enzymatic ultra-pure and water for 10 days to reach sexual maturity prior to their use in the olfactometer experiments**.**

Bioassay*:* The behaviour of the melon fly, *B. cucurbitae* using the odour emitted from various essential oils was evaluated in a closed four-arm olfactometer (Figure 1) at IITA-Benin. This system was identical to that described by Suazo, et al., 2003. It was set-up in a climate room (26±2°C; 70-80%RH) with a humidified charcoal-filtered provided into the olfactometer at a rate of 0.25 l/min/quadrant. Odour sources included cotton + distilled water, cotton + essential oil, air and dry cotton, each kept in a glass jar of 2L volume, connected to each arm of the olfactometer. One ml volume of each type of essential oils was used per test. Ten (10) males and 10 female *B. cucurbitae* were tested alternatively – one at a time at the center of the olfactometer and their behaviour recorded for 10 min using a hand timer. After a series of five males/females, the odour sources were connected to the opposite arm of the olfactometer to correct for any unforeseen asymmetry in the experimental set-up. Each experiment was repeated 3 times on the same day. For each fly, frequency of visit and time spent per visit in each quadrant were recorded. The total time spent in each quadrant was calculated for all the flies and all these values were there after expressed as a proportion of time spent in each quadrant.The effect of essential oils *C. giganteus* and *C. nardus* on eggs deposition by *B. cucurbitae* in zucchini treated with *C. giganteus* and zucchini treated with *C. nardus* was evaluated. The essential oils were first dissolved in a solution of ethanol 95% and tween-80 with different concentrations: 10ppm, 100ppm and 1000ppm.

Statistical analysis*:*On way ANOVA (analysis of variance) with JMP (SAS Institute 2005) was used to test for significant differences caused by essential oils for time per visit and total time spent in the vicinity of odour sources as well as mean number of pupae collected from zucchini treated with either *C. nardus* or *C. giganteus*. The means of the parameters were compared using the Student-Newman-Keuls test (SNK) only when the ANOVA F-values were significant at $P<0.05$. Data in proportions were first Arcsin-trans formed before the analysis.

RESULTS

Volatile compounds collected from the essential oils: Table 1 shows that the majority of chemical compounds in *C. citrates* were myrcene (10.4%), neral (33.0%), geranial (41.3%) while in *C. giganteus*, *trans*-mentha-1(7),8-dien-2-ol (21.86%); *cis*-mentha-1(7),8-dien-2-ol (19.4%); *trans*-mentha-2,8-dienol (9.6%) and *cis*-mentha-2,8-dienol (7.1%) were the majors compounds. The last oils were rich on citronellal (41.6%); citronellol (12.6%), geraniol (28.2%) for *C. nardus* and piperitone (68.4%), δ-2-carene (11.5%) for *C. schoenanthus.*

Fly behaviour in the four-arm olfactometer*:* Males and females were observed to have similar pattern of responses to odors from essential oils in the olfactometer experiments, so all values presented herein are means of both fly sexes.

a. Experiment 1*:* Responses of flies to odours from either *C. citratus* versus water+cotton, cotton only and clean air. When mated flies were tested, statistical analysis revealed significant differences between treatments in terms of total time spent in the vicinity of odour sources ($P=0.002$; Table 2). Mated *B. cucurbitae* significantly avoided to be in the vicinity of *C. citratus* compared with the control treatments (water+cotton, cotton only and clean air). Total time in the vicinity of *C. citratus* was significantly reduced (140.32±28.22 s), compared with that of water+cotton (214.36±33.73 s).When unmated *B.cucurbitae* flies were offered the same odour sources as in previous test, total time in the vicinity of *C. citratus* was significantly (52.95±5.24 s) compared with water+cotton (293.43±18.47s) $P<0.001$; Table 2).

b. Experiment 2*:* Responses of flies to odours from either *C. giganteus* versus water+cotton, cotton only and clean air. Mated *B. cucurbitae* discriminated odors emitted from *C. giganteus* and control treatments and strongly avoided immediate vicinity of source with oils of *C. giganteus*, in terms of total time spent and time per visit ($P<0.001$ and $P<0.0001$;Table 2). Same trend in the response was observed in unmated *B. cucurbitae* when offered the same odor sources ($P<0.001$ and $P<0.002$ for total time spent and time per visit; Table 2).

c. Experiment 3*:* Responses of flies to odours from either *C. nardus* versus water+cotton, cotton only and clean air. When odors emitted from essential oils extracted from *C.nardus* were offered versus controls, mated *B. cucurbitae* did discriminate odors from *C. nardus* and those from the controls (water + cotton, cotton alone and clean air) and total time spent was much less (109.84±13.51s) compared to time spent at the vicinity of clean air (206.00±15.62s) ($P=0.006$; Table 2). The same pattern in the response was observed for total time spent by unmated *B. cucurbitae* when offered the same odor sources ($P=0.0008$; Table 2), while in terms of time spent per visit, the analysis revealed significant difference between treatments ($P = 0.026$; Table 2).

d. Experiment 4*:* Responses of Flies to odours from either *C. schoenanthus* versus water+cotton, cotton only and clean air. When either mated *B. cucurbitae* flies were offered *C. schoenanthus* and controls (water + cotton, cotton only and clean air), they spent less time (total time=34.86±5.06s; time per visit = 14.93±1,60s) compared with the controls. Significant difference exists between treatments in terms of total time spent and time per visit ($P=0,001$ and $P=0.0002$, Table 2). Same trend in the response was observed in unmated *B. cucurbitae* when offered the same odor sources ($P<0.019$ and $P<0.009$ for total time spent and time per visit Table 2).

Table 3 gives summary responses of flies to odour in terms of proportion of total time and time per visit. Statistical analysis revealed significant differences between essential oils when compared among themselves, based on total time ($P<0.001$) andtime per visit ($P=0.022$). Globally, *C. citratus* and *C. nardus* attract, while *C. giganteus* and *C. schoenanthus* repel the melon fly. Based on the above results, we used *C. giganteus* and *C. nardus* to treat zucchini and exposed fruits to melon fly *B. Cucurbitae* to test the oviposition behaviour. The results indicated that.after fruit incubation process, the mean number of pupae collected from zucchini treated with *C. nardus* was higher than that collected from zucchini treated with *C. giganteus*, regardless of the concentrations ($P=0.016$; Table 4). *B. cucurbitae* preferred to oviposit in zucchini treated with *C. nardus* than in zucchini treated with *C. giganteus.*

DISCUSSION

When using the four-arm olfactometer, the essential oils extracted from *C. giganteus* and *C. schoenanthus* repelled the melon fly, *B. cucurbitae*, compared with essential oils extracted from *C. citratus* and *C. nardus*. Moreover, the number of pupae collected from zucchini treated with *C. giganteus* was significantly lower than that collected from zucchini treated with *C. nardus*, 24 hours after exposure to female *B. cucurbitae*, and this was regardless of the concentrations. Differences in the composition of volatile blends in *C. giganteus*, *C. schoenanthus*, *C. citratus* and *C. nardus* may explain differences in the behaviour of the melon fly, *B. cucurbitae*. GC/MS analysis of *C. giganteus* leaves essential oils showed that *trans*-p-mentha-1(7),8-dien-2-ol, *cis*-p-mentha-1(7),8-den-2-ol, *trans*-p-mentha-2,8-dien-1-ol, *cis*-p-mentha-2,8-dien-1-ol, *cis*-p-menth-2-en-1-ol and limonene were the majors components. Those major components are similar compared with data reported by Bassolé, et al., (2011) and Nonviho, et al., (2010). In our study, *C. Schoenanthus* essential oil was rich in pipéritone; this corroborates the results reported by Ketoh, et al., (2006) and by Yentema, et al., (2007).

Like in our study, previous research works by Schreck, et al., in 1991; Bowers, et al., in 1993 had also showed that volatile compounds such as p-menthane-diol, *trans*-menthane-dienol, isopiperitolin *C. giganteus*repelled some Dipterian species including mosquitoes. Peperitone extracted from *C. schoenanthus* has been proven by Ketoh, et al., 2002, to have a toxic effect on *Callosobruchusmasculatus* (Fabricius) repellent effect on mosquitoes (Pal, et al., 2011**)**. The presence of menthanes and piperitone in *C. giganteus* and *C. schoenanthus*, respectively. And which derive from each other would explain why these essential oils repelled *B. cucurbitae* in the olfactometer. This could also explain the lower number of pupae collected from zucchini treated with *C. giganteus*, compared with zucchini treated with menthanes-free *C. nardus*. The latter two volatiles *C. nardus* and *C. citratus* contain higher concentration of citral, 82.4% and 86.7% respectively. For *C. citratus* this composition was similar to essential oils from studied by Matasyoh, et al., (2011) and by Sessou, et al., (2012). Concerning the composition of our *C. nardus* essential oil, it is similar compared with data provided by Kanko, et al., (2004) and Noudogbessi, et al., (2013). While citral is usually known to repel some dipteran species like *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* Say (Trongtokit, et al., 2005), our study revealed that *C. nardus* and *C. citratus* (rich in citral) rather attracted the melon fly *B. cucurbitae*. Differences in ways leaves were sampled the synergy which may occur between chemical compounds as reported by Enan, 2001; Lee, et al., 2001 may have contributed to the differences in insect species behaviour. However our study corroborates other studies (Robacker, et al., 2007) which demonstrated that some pheromones which contain high concentration of citrals and mainly geraniol like in *C. nardus* and *C. citratus* also attract insect species. In generally, our studies showed that fruit flies can use volatiles from host fruits treated with essential oils for discrimination in oviposition, a crucial stage on ability to enhance reproductive success. To some extent, essential oils may play a role in the control of fruit flies infesting fruit trees and fruit vegetables, especially *B. cucurbitae*. As the essential oils are extremely volatile, further research should put emphasis on the appropriate formulation to ensure higher persistence of their odours in an integrated management of melon fly in particular and of various fruit fly species in general, infesting cucurbits.

Acknowledgements: The authors thank Drs A.H. Bokonon-Ganta and M.Toko for reviewing earlier version of the manuscript, Ms J. Winsou for flies rearing. Funding by IITA donors and BMZ is gratefully acknowledged.

REFERENCES

Adams, R.P., (1989): Identification of essential oils by ion mass spectroscopy, Academy Press Inc, New-York.

Bassolé, I.H., Lamien-Meda, A., Bayala, B.B., Obame, L.C., Ilboudo, A.J., Franz, C., Novak, J., Nebié, R.C., Dicko, M.H., (2011): Chemical composition and antimicrobial activity of *Cymbopogon citratus* and *Cymbopogongiganteus* essential oils alone and in combination. *Phytomedicine*, 18: 1070-1074.

Bowers, W.S., Ortego, F., Xianoqing, Y., P.H., Evans, P.H., (1993): Insect Repellents from the Chinese Prickly Ash *Zanthoxylum Bungeanum*. *J. Nat. Prod*., 56: 935-938.

Cornelius, M.L., Nergel, L., Duan, J.J., Messing, R.I., (2000): Responses of female oriental fruit flies (Diptera: Tephritidae) to protein and host fruit odors in field cage and open field tests. *Environ. Entomol*., 29:14-19.

Dhillon, M.K., Singh, R., Naresh, J.S. Sharma, H.C., (2005): The melon fruit fly, *Bactroceracucurbitae*: A review of its biology and management. *J. Insect Sci*., 5: 40

Ebadollahi, A., (2013): Essential oils isolated from *Myrtaceae* family as natural insecticides. *A.R.R.B.*,3(3): 148-175.

Enan, E.E., (2001): Insecticidal activity of essential oil octopaminergic sites of action. The ESA 2001 Annual Meeting.*An Entomological Odyssay of ESA san Diego CA. USA* DO579.

Gnanvossou, D., Hanna, R., Azandeme, G., Goergen, G., Tindo, T., Agbaka, A., (2008): Inventaire et importance des dégâts des mouches de fruits sur quelques espèces de cucurbitacées au Bénin. *Compilation de résumé, 3ème édition de l'Atelier Scientifique national de la recherche agricole*, 19-22 Décembre 2006.

Jang, E.B., Light, D.M., (1996): Olfactory semiochemicals of tephritids. Fruit fly pests: A world assessment of their biology and management (ed. by B. A. McPheron and G. J. Steck), 73-90. St Lucie Press, Hampton, FL, USA.

Kanko, C.,Sawailiho, B.E., Kone, S., Koukoua, G., N'guessan, Y.T., (2004): Etudes physico-chimiques des huiles essentielles de *Lippiamultiflora, Cymbopogoncitratus, Cymbopogonnardus, Cymbopogongiganteus. C. R. Chim.,* 7: 1039-1042.

Ketoh, C.K., Glitho, A., Huignard, J., (2002): Succeptibility of bruchide *Callosobruchusmaculatus* (Coleoptera: Bruchidae) and its parasitoid *Dinamusbasalis* (Hymenoptera: Pteromalidae) to three essential oils. *J. Econ. Entomol*., 95: 174-182.

Ketoh, G.K., Koumanglo, H.K., Glitho, I.A., (2006): Inhibition of *Callosobruchusmaculatus* (F) (Coleoptera: Bruchidae) development with essential oil extracted from *Cymbopogonschoenanthus* L. spreng (Poaceae) and the wasp *Dinarmusbasalis* (Rodani) (Hymeneptera: Pteromalidae). *J. Stored Prod. Research,* 41: 363-371.

Lee, B.H., Choi, W.S., Lee, S.E., Park, B.S., (2001): Fumigant toxicity of essentials oils and their constituent compounds towards the rice weevil, *Sitophilusoryzae* (L). *Crop Protect*., 20: 317-320.

Matasyoh, J.C., Wagara, I.N., Nakavuma, J.L., Kiburai, A.M., (2011): Chemical composition of *Cymbopogoncitratus* essential oil and its effect on Mycotoxigenic Aspergillus species. *Afr. J. Food Sci*., 5: 138-142.

Nonviho, G., Wotto, V.D., Noudogbessi, J.P., Avlessi, F., Akogbeto, M., Sohounhloué, D.C.K., (2010): Insecticidal activities of essential oils extracted from three species of Poaceae on *Anopheles Gambiae Spp.*, Major vector of malaria. *St. Cerc. St. CICBIA* 11(4): 411–420.

Noudogbessi, J.P., Alitonou, G.A, Djènontin, T., Avlessi, F., Figueredo, G.,Chalard, P., Chalchat, J.C., Sohounhloue, D.C.K., (2013): Chemical compositions and physico-chemical properties of three varieties essential oils of *Cymbopogongiganteus* growing to the spontaneous state in Benin. *Orient. J. Chem.*, 29(1): 59-67.

Pal, M., Kumar, A., Tewari, K.S., (2011): Chemical composition and mosquito repellent activity of the essential oil of *Plectranthus incanus* link. *Physics, Chemistry and Technology*,9(1):57-64.

Robacker, D.C., (2007): Attractiveness to *Anastrepha* (Diptera: *Tephritidae*) of plant essential oils and synthetic food-odour lure. *J. Appl. Entomol*., 131; 202-208.

Rösch, P., Popp, J., Kiefer, W., (1999): Raman and SERS Investigations on *Lamiaceae*. *J. Mol. Struct.,* 121: 480-481.

Sapkota, R., Dahal, K.C.,Thapa, R.B., (2010): Damage assessment and management of cucurbit fruit flies in spring-summer squash *J. Entomol. Nematol.* 2(1): 007-012.

SAS, (2005): The SAS System for Windows, Version 9.1. SAS Institute Inc.

Schreck, C.E., Leonard, B.A., (1991): Efficacy assessment of quwenlung: a mosquito repellent from China. *J. Am. Mosquito Control Assoc*., 7: 433-436.

Sessou, P., Farougou, S., Kaneho, S., Djenontin, S., Alitonou,G. A., Azokpota, P., Youssao, I., Sohounhloué, D., (2012): Bioefficacy of *Cymbopogoncitratus* essential oil against foodborne pathogens in culture medium and in traditional cheese wagashi produced in Benin., *IRJM*., 3(12): 406-415.

Suazo, A., Torto, B., Teal, P.E.A., Tumlinson, J.H., (2003): Response of the small hive beetle (*Aethinatumida*) to honey bee (*Apis melliffera*) and beehive-produced volatiles. *Apidologie,* 34: 525-533.

Trongtokit, Y., Rongsrivam, Y., Komalamisra, N., Apiwathnason, C., (2005): Comparative repellency of 38 essential oils mosquito bites. *Phytother. Res.,* 19: 303-339.

Yentema, O., Alioune, O., Abdoul-Dorosso, S., (2007): Chemical composition and physical caractéritics of the essential oil of *Cymbopogonschoenanthus* (L). Spreng of Burkina Faso. *J. Appl. Sci*., 7: 503-506.

Table-1: Chemical composition of the essential oils extracted from the four *cymbopogon* species used in the experiments.

Chemical components	**IK**	***Cc***	***Cg***	***Cn***	***Cs***
4-hydroxy-4-methyl-pentan-2-one	839	-	-	-	0.1
6-methyl-hept-5en-2-one	985	1.2	-	-	-
dehydro-1,8-cineole	990	-	-	-	0.1
Myrcene	**991**	**10.4**	0.3	-	-
δ-2-carene	**998**	-	-	-	**11.5**
α-phellandrene	1006	-	-	-	0.1
p-cymene	1025	-	0.2	-	0.1
Limonene	**1031**	t	**6.3**	2.5	2.2
(Z) β-ocimene	1036	0.2	-	-	0.1
(E) β-ocimene	1047	0.2	-	-	0.1
Bergamal	1054	-	-	0.2	-
Terpinolene	1085	-	-	0.6	-
Fenchone	1089	-	-	-	0.1
p-cymenene	1190	-	0.2	-	-
6,7- epoxymyrcene	1091	0.2	-	-	-
Pirillene	1098	0.1	-	-	-
Linalool	1100	0.5	-	0.1	-
2,2-dimethyl-octa-3,4-dienal	1106	0.1	-	-	-
***cis*-p-menth-2-en-1-ol**	**1122**	-	**7.2**	1.1	0.9
***trans*-p-mentha-2,8-dien-1-ol**	**1125**	-	**9.6**	-	-
***cis*-p-mentha-2,8-dien-1-ol**	**1140**	-	**7.2**	-	-
trans-p-menth-2-en-1-ol	1145	-	-	-	0.6
cis-verbenol	1141	0.1	-	-	-
Isopulegol	1151	-	-	0.4	-
Menthone	1147	0.1	-	0.1	-
Citronellal	**1153**	0.4	-	**41.6**	-
cis-chrysanthenol	1162	0.7	-	-	-
p-mentha-1,5-dien-8-ol	1171	-	-	-	1.3
***trans*-p-mentha-1(7),8-dien-2-ol**	**1194**	-	**21.9**	-	-
α-terpineol	1198	-	-	-	1.3
***trans*-piperitol**	**1212**	-	**6.3**	-	0.3
Verbenone	1216	-	1.5	-	-
Nerol	1221	0.3	-	-	0.1
Citronellol	**1231**	-	-	**12.6**	-
***cis*-p-mentha-1(7),8-den-2-ol**	**1237**	-	**19.4**	-	-
Neral	**1245**	**33.0**	2.3	0.6	-
Carvone	1243	-	2.8	-	-
carvotanacetone	1247	-	-	-	0.4
Geraniol	**1256**	**6.5**	2.5	**28.2**	-
Piperitone	**1265**	-	-	-	**68.4**
Geranial	**1276**	**41.3**	3.7	0.8	-
aldehyde pirillique	1279	-	0.6	-	-
neryl formate	1285	0.1	-	-	-
geranyl formate	1299	t	-	-	-
mentha-1-en-9-ol	1308	-	0.4	-	-
citronellyl acetate	1350	-	-	0.2	-
Eugenol	1354	-	-	0.8	-
nerylacetate	1365	-	-	0.1	-
geranylacetate	1378	2.4	0.1	0.1	-
β-elemene	1392	t	-	-	0.3
β-caryophyllene	1424	t	-	-	0.1
trans-α- bergamotene	1435	t	-	-	-
germacrene-D	1485	-	-	0.6	0.1

Table-1 : Continue…..

β-selinene	1493	-	-	-	0.1
Viridiflorene	1499	-	-	-	0.1
α-muurolene	1505	-	-	0.1	-
germacrene-A	1511	-	-	0.1	0.2
Cubebol	1614	-	-	0.1	-
γ-cadinene	1516	-	-	0.1	0.1
δ-cadinene	1520	-	-	0.6	0.2
Elemol	1552	0.1	-	3.5	3.9
germacrene-D-4- ol	1581	-	-	1.8	-
caryophyllene oxide	1587	0.1	0.9	-	0.4
γ-eudesmol	1636	-	-	0.2	0.3
epi-α-cadinol	1646	-	0.1	0.2	0.2
epi-α-muurolol	1648	-	-	1.6	-
α-eudesmol	**1662**	-	-	-	**4.9**
(E)-nerolidylacetate	1715	-	-	0.2	-
palmitic acide	1960	-	0.7	-	-
Total		**98.0**	**94.2**	**99.1**	**98.6**
t ≤ 0.05% ; *CC = C. citratus ; Cg = C. giganteus ; Cn = C. nardus* ; *Cs = C. schoenanthus*					

Table-2: **Total time and time per visit per quadrant† by mated and unmated *B.cucurbitae* in the vicinity of the four essentials oils**

Treatment	Mated *B. cucurbitae*		Unmated *B. cucurbitae*	
	Total time	**Time per visit**	**Total time**	**Time per visit**
	C. citratus			
Essential oil + cotton	140.3 ± 28.2b	29.6 ± 2.6a	52.9 ± 5.2c	27.5± 0.8b
Water + cotton	214.4± 33.8a	38.5 ± 5.0a	293.4 ± 18.5a	46.5 ± 1.9a
Cotton only	83.5 ± 16.9b	26.7± 2.0a	120.0 ± 7.0b	33.3± 0.3b
Clean air	126.7± 19.6b	31.5 ± 1.4a	91.4 ± 6.3bc	35.7 ±0.4ab
	C. giganteus			
Essential oil + cotton	21.7 ± 3.9c	21.7 ± 2.0b	13.7 ± 1.9c	13.2 ± 1.8b
Water + cotton	223.3 ± 20.5a	51.6 ± 5.6a	322.8 ± 12.9a	46.4± 0.7a
Cotton only	167.9 ± 10.78b	49.1 ± 3.37a	127.5 ± 20.7b	35.8 ± 1.3a
Clean air	138.3± 12.4b	52.0± 0.18a	133.0 ± 37.4b	53.6 ± 4.1a
	C. nardus			
Essential oil + cotton	109.8 ± 13.5b	51.1± 5.7a	79.1 ± 11.6c	39.7 ± 1.5b
Water + cotton	140.1± 300b	49.1 ± 9.7a	200.2 ± 14.7a	65.4 ± 2.5a
Cotton only	97.5 ± 18.0b	51.5 ±12.7a	150.1 ± 13.8b	63.7± 7.2a
Clean air	206.0 ± 15.6a	64.7 ± 1.3a	135. ± 12.7b	63.9 ± 1.9a
	C. schoenanthus			
Essential oil + cotton	34.9 ± 5.1b	14.9 ± 1.6c	46.8 ± 3.1c	17.4± 4.3c
Water + cotton	198.4± 22.9a	30.3 ± 0.7ab	226.0 ± 27.7a	28.2 ± 4.0ab
Cotton only	98.3 ± 16.4b	24.8 ± 5.9b	119.0 ± 9.8bc	22.8 ± 8.5bc
Clean air	218.8± 31.6a	35.6± 3.8a	179.9 ± 34.8ab	32.3 ± 8.7a

- †Mean±SE in the same column for each essential oil, followed by same letter are not significantly different at *P*=0.05 (Student-Newman-Keuls multiple range test).

Table-3: Proportion of total time and time per visit (mean±SE)[†] by mated and unmated flies in the vicinity of the four essential oils.

Essential oils	Total time	Time per visit
C. citratus	0,25 ± 0,04a	0,24 ± 0,03a
C. giganteus	0,04 ± 0,01c	0,13 ± 0,01c
C. nardus	0,17 ± 001b	0,20 ± 0,02ab
C. schoenanthus	0,06 ± 0,01c	0,14 ± 0,02bc

- [†]Proportions in the same column followed by same letter are not significantly different at *P*=0.05 (Student-Newman-Keuls multiple range test).

Table-4: Mean number of pupae (±SE) collected from 100g of treated C. *giganteus* and C. *nardus* zucchini, exposed to *B. cucurbitae* for 72 hours.

Treatment	Mean number (± SE) of pupae per fruit
Distilwater (control 1)	168.1 ± 19.7a
Water+Tween+Ethanol (control 2)	124.1 ± 31.2abc
C. giganteus	
10 ppm	85.0 ± 23.4bcd
100 ppm	66.7 ± 19.7cd
1000 ppm	61.9 ± 1.7d
C. nardus	
10 ppm	134.0 ± 10.7ab
100 ppm	135.7 ± 21.6ab
1000 ppm	137.6 ± 28.6ab

- Means ± SE in the second column followed by the same letter are not significantly different at *P*=0.05 (Student-Newman-Keuls multiple range test).

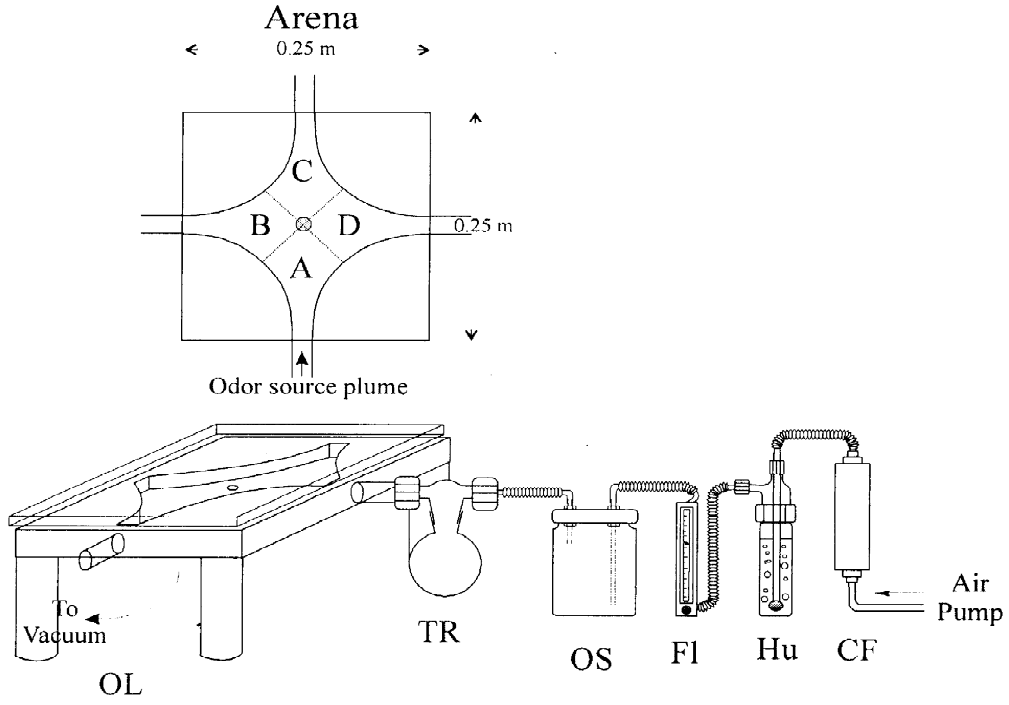

Figure-1: Diagram of four-arm olfactometer. The main arena of the olfactometer consisted of four odor zones. Source: Suazo, et al., 2003

- OL = Olfactometer, TR = insect trap, OS = odor source, Fl = flowmeter, Hu = humidifier and CF = Charcoal filter. Air flow was calibrated with ammonium chloride smoke.

