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BIOASSAY STUDIES ON THE EFFECT OF ESSENTIAL OILS ON THE FEMALE ORIENTAL FRUIT FLY, *Bactrocera dorsalis* (Hendel) (DIPTERA : TEPHRITIDAE)

ALIOU DIONGUE[1*], TSAIR BOR YEN[2] and PO-YUNG LAI[3]

[1]Ministry of Agriculture, Crop Protection Directorate, Government of Senegal, Dakar, Republic of Senegal

[2]Department of tropical Agriculture and International Cooperation, National Pingtung Unviersity of Science and Technology, Neipu, Pingtung, Taiwan

[3]Office of Research, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu, Hawaii, U.S.A.

E-mail : armigera2002@yahoo.com

ABSTRACT : The semiochemical functions of essential oils extracted from four different plant materials *viz.*, *hinoki*, eucalyptus, cinnamon and *Litsea* were investigated on the females of Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). The GC-MS analysis showed that most identified volatile checmicals from the essential oils were monoterpene with low molecular weights, which included 6-octenal,3,7-dimethyl,(R) (t_R= 21.35, 43.246%); 6-octen-1-ol,3,7-dimethyl (t_R= 23.369, 9.02%); 4-hexen-1-ol,5-methyl-2-(1-methyletheny) (t_R = 24.232, 17.714%); beta-phellandrene (t_R=15.191, 12.616%); eucalyptol (t_R=17.439, 38.216%); (+)-4-carene ($t_{R=}$ 26.82, 18.237%); 1,R-alpha-pinene (t_R=13.875, 29.565%); limonene (t_R=17.18, 19.042%) and fenchyl acetate (t_R=23.096, 10.798%). Bioassay studies on the effects of the essential oils showed that significantly more number of female adults of *B. dorsalis* alighted on filter paper treated with *hinoki* oil, (R)-(+)-limonene, and eucalyptus oil at 400ppm. However, cinnamon was found to inhibit or repel alighting of the female adults at a dosage of 200ppm (p = 0.001), 400ppm (p = 0.0001) and 800ppm (p = 0.0001). A similar repellent activity was observed when filter paper was treated with essential oils extracted from *Litsea* leaves. The calculated preference showed that *hinoki* was the most preferred essential oil (50.91%), which stimulated the alighting response of *B. dorsalis* at 100ppm.

Keywords : *Bactrocera dorsalis*, bioassay, essential oils, semiochemical function

INTRODUCTION

The dipteran family Tephritidae consists of over 4000 species, of which nearly 700 species belong to Dacine fruit flies (Fletcher, 1987). About 250 species are of economic importance, and are distributed widely in temperate, sub-tropical, and tropical regions of the world (Christenson and Foote, 1960). The genus *Bactrocera* contains approximately 440 species distributed primarily in the Southeast Asia, the South Pacific, and Australia (White and Elson-Harris, 1992). One of these species, the oriental fruit fly, *Bactrocera dorsalis* (Hendel), is a very destructive insect pest of many tropical and subtropical fruits and vegetables (Vargas and Carey, 1990; Li and Ye, 2000; Ye, 2001). It was first recorded in Taiwan in 1912, and spread to most countries in the Asia-Pacific region over the next 90 years (Christenson and Foote, 1960; Wang, 1996; Ye, 2001). *Bactrocera* contains most of the world's most serious fruit fly pests. They infest over 100 host plants including many types of commercial fruits, such as citrus (*Citrus reticulata* B.), mango (*Mangifera indica* L.), and peach (*Prunus persica* B.) as well as a wide variety of other agricultural products, such as coffee (*Coffea Arabica* L.), chili pepper (*Capsicum annuum* L.) and watermelon (*Citrullus laratus* M.) (Shukla and Prasad, 1985; Alyokhin *et al*., 2001; Shi and Ye, 2004). In infested areas, fruits and vegetables may be completely or partially destroyed in terms of commercial value based on the levels of infestation. Damage caused by *B. dorsalis* consists of the female puncturing and ovipositing under the skin of fruits. Damage usually consists of breakdown of tissues and internal rotting associated with maggot infestation, but this varies with the type of fruit attacked (Steiner, 1957). Infested young fruits becomes distorted, callused and usually drop; mature attacked fruits develop a water soaked appearance. The larval tunnels provide entry points for bacteria and fungi that cause the fruit to rot. Fruit with high sugar content, such as peaches, will exude a sugary liquid, which usually solidifies adjacent to the oviposition site. When only a few larvae develop, damage consists of an unsightly appearance and reduced marketability because of the egg laying punctures or tissue break down due to the decay (Steiner, 1957). Damage due to larval feeding could also cause direct losses to production of fresh fruits, imposition of trade restrictions by importing countries, and implementation of expensive eradication or suppression programmes to rid countries or parts of countries of the introduced pests. The flying ability of the immature adults which are able to disperse over at least 60 km to find fresh food resources and breeding substrates (Steiner, 1957; Fletcher, 1987) describes the wide geographical dispersion of this pest. In Taiwan, since 1911, attempts have been made to control *B. dorsalis* (Liu, 2002). Most strategies were focused on a field control by the use of protein hydrolysate-based traps, organophosphorous insecticides or male annihilation techniques by irradiation. However, the methyl eugenol attracts only the male (Drew and Hooper, 1981) and the female is responsible for the egg-laying. Other methods like the insecticidal protection by using a cover spray or a bait spray with malathion combined with protein hydrolysate, the sterile insect technique (SIT) or the male annihilation method could not control *B. dorsalis* on a sustainable basis. Biological control has been tried against *B. dorsalis sensu lato*, but introduced parasitoids have had little impact (Wharton, 1989). More environmentally safe methods based on the use of attractants and low toxicity-compounds are currently being investigated. Alternative pest control technology based on the use of essential oils attracted particular attention because of their specificity to pests, their biodegradability and potential for commercial application (Liu *et al.*, 2006). Essential oils are naturally occurring substances, which generally have a broad spectrum of bioactivity because of the presence of several active ingredients that work through several modes of action. Plant essential oils have also been recognized as an important natural resource of insecticides (Adebayo *et al.*, 1999; Gbolade *et al.*, 2000). Their lipophilic nature facitilates them to interfere with basic metabolic, biochemical, physiological and behavioural functions of insects (Nishimura, 2001). The present study reports the systematic laboratory investigations on the repellent and attractant effects of nine essential oils against the oriental fruit fly, *B. dorsalis.*

MATERIALS AND METHODS

Insects

The first parental eggs (F_o) of *B. dorsalis* were initially obtained from the Department of Plant Protection, National Pingtung University of Science and Technology (NPUST). The colony was maintained at the Department of Tropical Agriculture and International Cooperation (DTAIC) laboratory in continuous culture. Eggs were incubated in plastic container (8.1 x 6.7 x 1.8 cm^3) filled with diet composed of a mixture of wheat (160g/ml), sugar (80g/ml), yeast (47g/ml), sodium benzoate (1.3g/ml), HCL (7ml) and RO water (603 ml). The container was placed into a second larger container (14.5 x 11.5 x 4.0cm^3) containing wheat bran bed for the pupation of the 3rd instar larvae. The containers were covered with net to avoid excessive moisture and prevent the escape of larvae. Pupae were collected into glass Petri dishes and put into steel-frame cages (14.2 x 14.2 x 12.7cm^3) enclosed with net on opposite sides for air-ventilation. The sixth side was made of a glass sheet for an easy observation of the flies' behavior. Emerged adult flies were sexed and grouped into the same steel-frame cage. A cotton plug, soaked with distillated water and a solution containing sugar, yeast, peptone and RO water in a ratio of 5:1:1. was laid on the upper side on a white paper and was hung from the top of the cylinder to serve as diet. After mating, eggs were allowed to be laid in plastic tubs (1.5 x 0.5 x 0.5cm^3) which provide several tiny holes at the bottom representing the eggs laying substratum for sexually mature females. Guava juice was placed in the holes to serve as stimulant for ovipositing females. Eggs were collected from plastic tube by for colony maintenance.

Essentials oils

Cinnamon oil extracted from the leaves of *Cinnamonum osmophloeum*, citronella oil from the leaves of *Cymbopogon nardus*, eucalyptus oil from the leaves of *Eucaluptus radiate* and *hinoki* oil from the leaves of *Chamaecyparis obtuse* were purchased from the market. Essential oils from *Litsea* leaves and fruits were routinely extracted by steam distillation in DTAIC laboratory. Citral oil from the fruits of *Litsea cubeba* was purchased from Acros Company. The two (R)-(+)- and (S)-(-)- limonene, which were 98% isomerically pure were purchased from Sigma and ordered via UNI-WARD CORP (Kaoshiung, Taiwan).

The bioassay set-up

The behavioral response of virgin *B. dorsalis* adults toward the essential oils was observed in a commercial conical bug-cage. The cage (BugDorm 2) in a clear plastic net of the size (85 x 60 x 60 cm^3) was used during the test. Bioassay method similar to that described by Khalequzzaman *et al.* (2002) was adopted. A total of 80 to 100 virgin females of 1 to 4 day old, were introduced into the cage as one replicate. For an easy manipulation, flies were freezed for 70 minutes in a refrigerator prior to their introduction inside the cage. Flies were starved for 24 h before the experiment. The essentials oils were dissolved in acetone to make the desired concentrations. Aliquot (1ml) of the test solution of the oils was dispensed separately over a Whatman No. 3 filter paper. Solvent was allowed to evaporate in fume hood under room temperature during 10 min. An appropriate amount of acetone was used as a negative control. The treated filter paper was placed on the upper side of the cage distanced at 55cm from the bottom, the filter paper used as control was dispensed with acetone and placed oppositely at the same distance. During 30 min, the behavior of the females was observed. A prior experiment on the release rate showed that during 30 min more than 50 per cent of the essential oils remain in the dispenser. To avoid any bias in setting up the bug-cage bioassay, two blank filter papers (A and B) without stimulus were exposed to 50 flies, for determining whether or not there is any difference in serving as alighting substrates for *B. dorsalis*. The results showed no significant difference in the alighting of the female on the two blank filter papers; The bioassays were conducted under the room temperature at 26 ± 2°C with a relative humidity of 70 ± 2 %. Each bioassay was replicated four times.

Statistical analysis

Data on the alighting preferences of the female *B. dorsalis* were subjected to unpaired *t*-

test analysis using Graphpad software 2007. The preference percentage was used to characterize the ranking preference of the female towards the essential oils. The Preference of different concentrations was calculated following P = [(Nt - Nc) / (Nt + Nc)*100] (Kramer and Mulla, 1979). Where, Nt is the mean number of flies alighting on filter paper containing the essential oil and Nc is the mean number of flies alighting on the filter paper served as the control. The repellent percentage was calculated by the using the following equation: Repellency (%) = C-E/T*100 (Osmani *et al.*, 1972). C is the number of insect alighted on the negative control; E is the number of insects alighted on the treated filter paper and T is the number of total insects. C, E and T were mean data of 4 replicates.

Chemical analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was carried out on a Hewlett Packard 5890 series II gas chromatograph equipped with a split-splitless injector coupled to an ion-trap 5972 Mass Selective Detector (MSD) system using 70 eV electron impact ionization with the GC capillary column connected directly to the ion source. The mass range was 1-30000 molecular weights. The ion source temperature was 187°C, 54 m torr for a total acquisition time of 69 min. The operating parameters were controlled by an HP series G1701BA computer version B.01.00-ChemStation. Chromatography was performed using a non-polar capillary column HP-5MS (60 m length × 0.25 mm i.d. × 0.25 ìm film thickness). Helium was used as the carrier gas at a linear flow rate of 1.39 ml/min. The samples were heated in the injection port at 250ºC. The oven temperature program was set at 50°C for 2 min, followed by 5°C/min to 250°C for 1 min, then 2°C/min to 280°C for 50 min. Detection was made by comparing their mass spectra with library spectra. Chemical having a match quality of 80 % in the GC-MS library were chosen and confirmed by comparing EI-MS and GC retention indices with synthetic standards under the same operating condition.

RESULTS AND DISCUSSION

Results on the bioassay of essentials oils on *B. dorsalis* showed that the female responded differently to odor emanated from the dispenser in concordance with the emitting essential oil. Based on the mean (± SE) number of alighting flies counted on the dispenser at 100 ppm, highest number of *B. dorsalis* alighted on filter treated with *hinoki* (Fig. 1), (R)-(+)-limonene (68.75±5.54) than on their respective controls (26.75 ±5.28) and (39.5±7.1).When the dosages were increased to 200, 400 and 800 ppm, the response of the female remained same (Figs. 1 and 2). Significant alighting stimulation of the female was observed on the essential oil, eucalyptus, at dosage of 400 ppm (Fig. 3). There was significant alighting response of *B. dorsalis* on treated filter paper (75.00± 3.63) than on its respective control (37.25± 5.44) even though there was no significant difference found on dosages of 100, 200 and 800 ppm. Results on the bioassay of citronella, cinnamon and litsea leaves essential oils showed that both of them inhibited of repelled the alighting of the female *B. dorsalis* as more females alighted on the controls than on treated filter papers. Cinnamon oil was found to inhibit the alighting attractiveness of the female at dosages of 200, 400 and 800 ppm while there was no significant response observed at dosage 100 ppm (Fig. 4). When we calculated the index of repellency converted into percentage, only cinnamon showed a consistent ascending repellency with increased dosages at 7.42 per cent (100ppm) < 27.60% (200ppm) < 52.49% (400ppm) < 80.51% (800ppm). It also demonstrated that cinnamon was the only essential oil which at 400 and 800 ppm showed a repellency percentage over than 50 per cent. *Litsea* leaves essential oil was similarly repellent to the female at the single dosage of 100 ppm (Fig. 5), while citronella oil attempted to repel the female at the 3 dosages of 100ppm, 200ppm (Fig. 6). However, there was no significant difference when alighting counts on treated filter was compared to its control at 800ppm. The calculated preference demonstrated

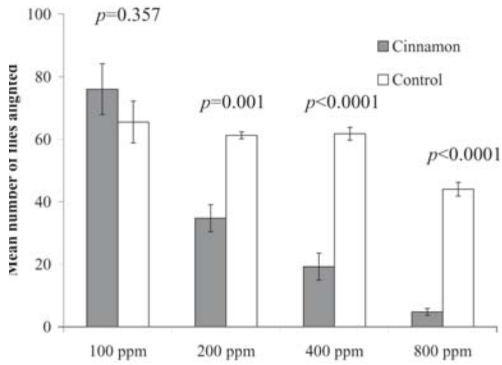

Fig. 1 : Alighting responses of *B. dorsalis* female exposed to different dosages of cinnamon essential oil compared to its control.

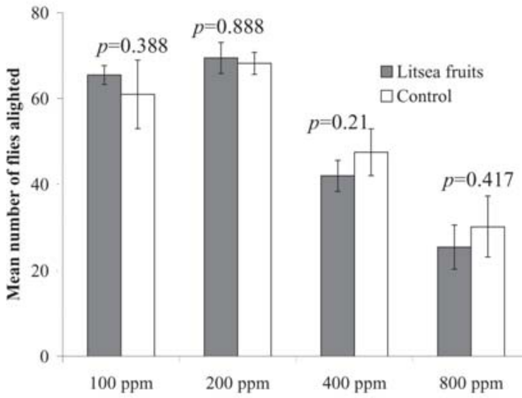

Fig. 4 : Alighting responses of *B. dorsalis* female exposed to different dosages of litsea fruits essential oil compared to its control.

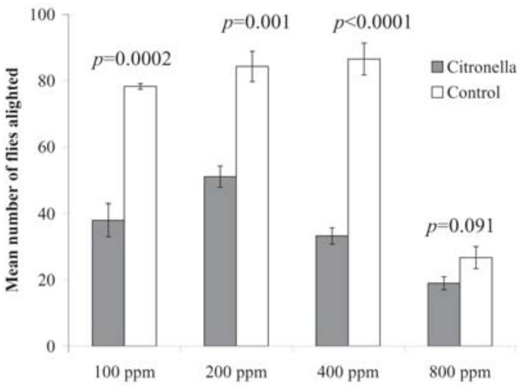

Fig. 2 : Alighting responses of *B. dorsalis* female exposed to different dosages of citronella essential oil compared to its control.

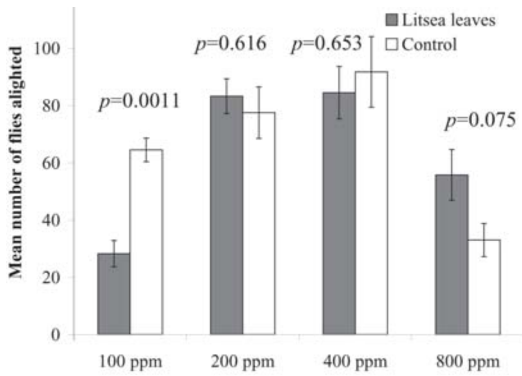

Fig. 5 : Alighting responses of *B. dorsalis* female exposed to different dosages of litsea leaves essential oil compared to its control

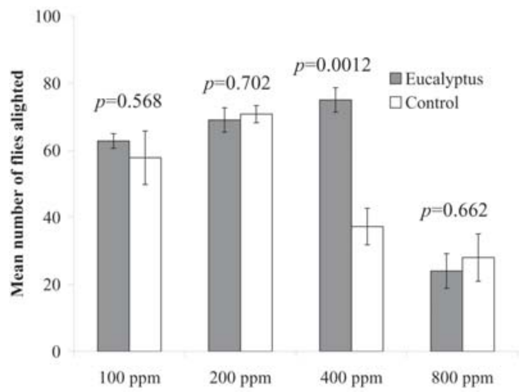

Fig.3. Alighting responses of *B. dorsalis* female exposed to different dosages of eucalyptus essential oil compared to its control.

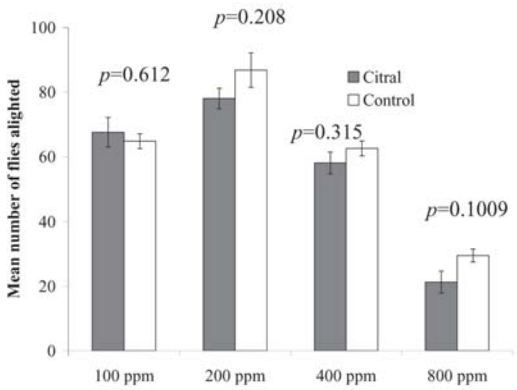

Fig.6. Alighting responses of *B. dorsalis* female exposed to different dosages of citral essential oil compared to its control.

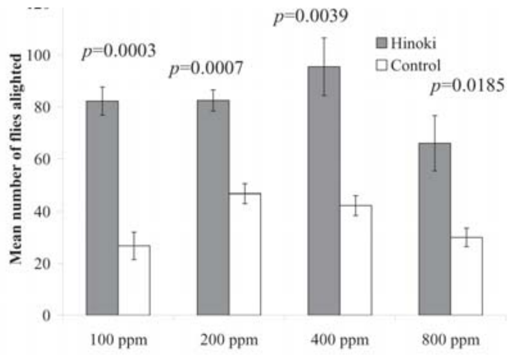

Fig. 7 : Alighting responses of *B. dorsalis* female exposed to different dosages of *hinoki* essential oil compared to its control.

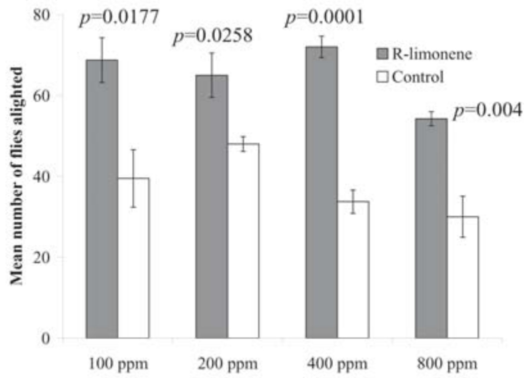

Fig. 8 : Alighting responses of *B. dorsalis* female exposed to different dosages of R-limonene essential oil compared to its control.

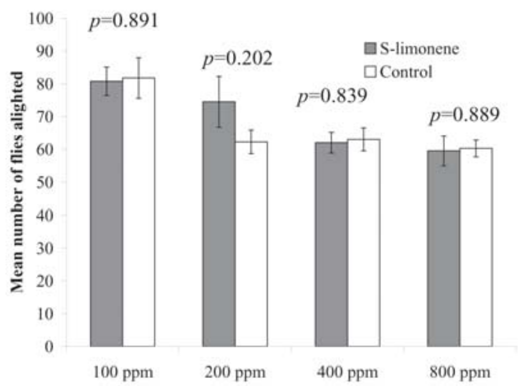

Fig. 9 : Alighting responses of *B. dorsalis* female exposed to different dosages of S-limonene essential oil compared to its control.

that *hinoki* was the most preferred essential oil (50.91%) in stimulating the alighting of *B. dorsalis* at the dosage of 100ppm. Among the nine essential oils tested for behavioral studies, it was found that *Litsea* fruits, citral, (S)-(-)-limonene summarized respectively in Figs. 7, 8 and 9 and certain dosages of eucalyptus did not attempt to influence the behavior of *B. dorsalis* female. The difference in stimulation whether attractiveness in alighting or repellency indicated that the essential oils contain chemical cue that triggered the female fly. Therefore, we investigated the chemical profiles of those essential oils responded whether as attractant or repellant to see whether or not it might have difference in volatile composition regarding the observed behavioral status of the female.

The result on the gas chromatography-mass spectrometry derived from the three essential oils showing the repellent effect of *B. dorsalis* female, identified a total of 20 volatile chemicals (Table 1). The GC-MS which showing good reproducibility was calculated over 3 analyses with the RSD variations d” 10 % in both injected samples. The sample citronella eluted more peak profiles (11) compared to litsea leaves sample which detected 8 volatile compounds. Cinnamon sample seems to be pure sample as only 1 major peak identified as cinnamaldehyde,(E)- at $t_{R=}$ 25.38 min with a high percentage of 75% was detected (Fig. 10). The results from citronella oil showed high concentration for 6-octenal,3,7-dimethyl,(R) ($t_{R=}$ 21.35, 43.246%, MW=154, MQ=96), 6-octen-1-ol,3,7-dimethyl, (R) ($t_{R=}$ 23.369, 9.02%, MW=156 , MQ=94) and 4-hexen-1-ol,5-methyl-2-(1-methyletheny) ($t_{R=}$ 24.232, 17.714%, MW=154 , MQ=83) (Fig. 11). The mean concentration of the major analytes from citronella oil was between 1.015 to 43.246%, while it was between 1.66 to 38.216% for the sample from litsea leaves. The chromatographic results of the sample litsea leaves showed higher concentration for the volatile beta-phellandrene ($t_{R=}$ 15.191, 12.616%, MW=136, MQ=90), eucalyptol ($t_{R=}$ 17.439, 38.216%, MW=154, MQ=98) and (+)-4-carene ($t_{R=}$

26.82, 18.237%, MW=136, MQ=90) (Fig. 12). Most of the compounds identified from the repellent samples were at a retention time d” 31 min with lower molecular weight up to 204. Those parameters showed that most of the volatility of the compounds extracted which are the main characteristics of essential oils. Identification of the volatiles from the essential oil, *hinoki*, which stimulated significant alighting of the female fly showed a total number of nine major compounds. Of that that total number of major compounds, three accounted for more than 58.5 percent of the total concentration. They were listed as 1,R-alpha-pinene ($t_{R=}$ 13.875, 29.565%, MW=136, MQ=96), limonene ($t_{R=}$ 17.18, 19.042%, MW=136, MQ=91) and fenchyl acetate ($t_{R=}$ 23.096, 10.798%, MW=196, MQ=91) (Fig. 13). The isomer (R)-(+)-limonene which also demonstrated significant alighting of the female *B. dorsalis* was an isomeric compound at purity 98 percent. Therefore it was not necessary to inject it in the GC-MS as it was authentic compound.

Our study demonstrated that no bias was detected in the cage tests. The consistency of the female in choosing essential oils that stimulated or inhibited its alighting showed the reliability of our results in cage bioassay event though the flight ability of the female was limited. The results of the bioassay showed that essential oils were able to trigger the behavior of the female *B. dorsalis*. It also demonstrated that certain volatile chemicals present in the essential oil that stimulated the repellency or attraction of the female. The study showed that essential oils of cinnamon, citronella and *Litsea* leaves could be good candidates to repel the *B. dorsalis* as they demonstrated consistency in their repellent effects during the bioassay. The cinnamon showed good repellent effect on the female when the concentration was only >100 ppm while the citronella could repel the female at concentrations d” 400 ppm. That difference in dosage response showed that concentration must be primordial before attempting any behavioral bioassay. Such consideration explained the wide range of dosages employed to discern an appropriate behavior of the female in our study. Hare (1998) reported that concentration is a confounding factor in almost any bioassay and at some levels of exposure the same compound may be attractive, repellent or even toxic. These findings tend to support Dethier’s (1947) threshold concentration theory that a compound which is attractive at one concentration may be repellent at another. According to Khalequzzaman *et al.* (2002), an initially acceptable compound may become more attractive as its concentration is increased until an optimum level is attained; beyond this point the attraction decreases with increased concentration until the nature of response is completely reserved. Thus might partially explain the response of the female towards cinnamon dosages >100 ppm. From such results, we observed that the behavior of insect towards a compound depends not only on the nature of compound but also on its concentration. Liu *et al.* (2006) reported that an allelochemical is assumed to have two complementary attributes, stimulation and inhibition, which are not necessarily to have the same sites of action. As concentration changes, the relative dominance of these attributes alter, so determining the overall property. In the attempt to use essential oils in repellency test there are no findings showing the repellent effect of such oils alone in *B. dorsalis*. The repellent effect of cinnamon may be due to its excellent insecticidal activity demonstrated specially in the control of stored product insects, which is at least documented in Kim *et al.* (2003). They reported that cinnamon oil mixed with mustard oil and horseradish oil produced 100 percent mortality one day after treatment against *Sitophilus oryzae*. In the genus *Bactrocera*, it is controversely reported by Lall and Singh (1969) that citronella oil mixed with palm juice and dried mango juice could trap adult of *B. cucurbitae.* Our finding might be of interest when investigating essential oils or semiochemicals *sensus latus* that could inhibit or repel the female *B. dorsalis* or other sympatric species. Results on the attraction showed the preference of *B. dorsalis* in alighting on treatments composed either *hinoki* or the R-limonene. There was consistency in the results as *B. dorsalis* showed significant preference in both essential oils at 4 different dosages tested. *Hinoki*, which is extracted from *Chamaecyparis obtusa*, conifer in the cypress family Cupressaceae, is known to be effective for soothing and treating bites, stings, custs, rashes, burns, or repelling mosquitoes. The attraction effect of the *B. dorsalis* might be due to the lemon-scented of the oil as most fruit flies are usually attracted to citrus family due to the presence of specific volatile chemicals. There was strong alighting stimulation of *B. dorsalis* female on (R)-(+)-limonene while there was no significant difference when tested with (S)-(-)-limonene. This showed that *B. dorsalis* could detect volatile chemicals at the isomeric level. This specificity in detecting isomer compounds implied the broad olfactory receptivity of the *B. dosalis* in choosing plant compounds. This behavioral discrimination might be due to the fact that female antennae generally appear to be very sensitive to volatile chemicals as they usually bear large populations of olfactory receptor cells and may exhibit chemosensory efficiency during their searching behavior. It is also possible that an insect has saturation in its sensilla and does not attempt to receive other signals during their searching behavior. Collin *et.al.* (2004) reported that insects' olfactory sensilla may have become saturated within the first hour of exposure to the volatiles. However, as our bioassay method was used to screen for the most effective attractants or repellent, it is realistic to expect that the insects responded efficiently within 30 min if the essential oil was effective enough to be attractant or repellent. Therefore the time of 30 min we set up in our observation was considered significant to observe the behavior of the insect and the volatile remains active in the filter paper as demonstrated in the release rate experiment (Table 1).

However, whether the volatile released from *hinoki* or (R)-(+)-limonene essential acted as attractant or contact in stimulating the alighting of the female remains unknown in our study. It was found that when an essential oil seemed to attract *B. dorsalis*, the alighting of the female on the filter paper bearing the essential oil was longer (10.33 ± 2.1 s) and (7.58±3.04 s) for *hinoki* and (R)-(+)-limonene, respectively compared to those previously stated essential oils showing repellency. It was 2.1±1.1 s for cinnamon oil, 1.8 ±0.9 s for litsea leaves oil and 2.9±0.5 for eucalyptus oil. When *B. dorsalis* alighted on the attractant filter paper containing either (R)-(+)-

Table 1. Release rates of essential oils calculated by weight loss

Compounds	Dose applied to filter paper (mg)	Amount released in 30 min(mg)	Release rate mg/hr
Cinnamon	21.3	0.9	2.7
Citronella	11.8	1.4	4.2
Eucalyptus	10.7	2.4	7.2
Litsea fruits	9.8	1.3	3.9
Citral	12.6	0.6	1.8
Litsea leaves	10.8	7.5	22.5
Hinoki	12.5	7.3	21.9
R-limonene	7	5.2	15.6
S-limonene	6.6	5.4	16.2

Table 2. GC-MS characteristics of identified compounds from essential oils

Compounds	Retention time	Percentage in GC-MS	Characteristic MS ions
Litsea leaves oil			
1R-, alpha pinene	13.68	3.6	41(30),77(45),93(100),121(10),136(7)
Beta-phellandrene	15.19	12.61	41(40),77(50),98(100),136(10)
Beta-pinene	15.34	3.63	41(60),69(30),77(30),98(100),136(5)
Eucalyptol	17.43	38.21	49(100),71(50),81(70),108(45),154(20)
-(+)-,alpha,-terpineaol (p-mentol-1-en-8-ol)	22.45	3.23	43(60),59(100),93(70),121(65),136(50)
(+)-4-carene	26.82	18.23	48(100),93(85),121(980,136(45)
Cyclohexane,1-ethenyl-1-methyl-2,4-bis	27.87	1.67	41(95),67(98),81(97),98(100),107(70),189(20)
caryophyllene	28.79	3.71	41(70),69(98),98(100),133(85),204(3)
Citronella oil			
D-limonene	17.04	2.57	41(35),53(35),68(100),79(40),93(80),136(10)
6-octenal, 3,7-dimethyl,(R)-	21.35	43.24	41(100),55(30),69(70),95(25),121(10),154(2)
6-octen-ol,3,7-dimethyl, (R)-	23.36	9.02	41(100),55(45),69(70),81(25),95(20),156(3)
4-hexen-1-ol,5-methyl-2-(1-methyletheny)	24.23	17.71	41(98),53(10),69(100),93(9),123(6),154(2)
2,6-octadienal, 3,7-dimethyl-	24.51	1.05	41(100),53(10),69(98),84(13),94(6),152(3)
Cinnamaldehyde, (E)-	24.84	1.46	51(20),77(50),103(70),131(100)
6-octen-1-ol,3,7-dimethyl,popanoate	26.64	2.66	48(100),55(50),69(70),95(49),123(35),136(15)
Eugenol	26.89	1.01	55(20),77(45),91(55),103(60),149(45),164(100)
2,6-octadien-1-ol,3,7-dimethyl-,acetate	27.44	3.1	41(80),69(100),93(15),121(10),154(1)
Cyclohexane,1-ethenyl-1-methyl-2,4-bis	27.87	1.35	41(95),67(99),81(98),98(100),107(70),204(1)
Cyclohexanemethanol, 4-ethenyl-,alpha-	31.97	4.87	43(60),59(100),93(75),161(30),204(2)
Cinnamon oil			
Cinnamaldehyde,(E)-	25.8	74.21	121(100)
Hinoki oil			
1R-alpha-pinene	13.87	29.56	53(7),67(7),77(45),98(100),121(5),136(3)
Camphene	14.32	2.91	53(10),67(20),79(30),98(100),121(60),136(6)
Beta-myrcene	15.63	2.86	41(100),69(70),93(80)
Limonene	17.18	19.04	41(30),53(28),68(100),79(45),93(80),136(15)
Borneol	21.73	1.09	41(20),55(110),67(10),95(100),110(10),139(5)
(+)alpha-terpineol[p-menth-1-en-8-ol]	22.43	1.40	43(55),59(100),81(40),93(70),121(60),136(50)
Fenchyl acetate	23.09	10.79	41(99),81(100),93(30),121(25),136(30),154(3)
Caryophyllene	28.74	1.88	41(100),69(70),79(80),91(95),133(80),204(5)

limonene or *hinoki*, there were several turns up behavior, exploring upper and the lower surface of the filter paper. *B. dorsalis* drummed the surface with it olfactory senses (e.g. antennae) and searching intensively the source of the essential oil. *B. dorsalis* searched the sides of the filter paper, the net side next to the filter paper and returned to middle several times before taking off and flied to the top of the cage. These flights behavior were significantly affected by the treatments. It showed that the treated filter paper elicited positive klinokinesis indicating that female *B. dorsalis* could be attracted by essential oil which acted as kairomone semiochemicals influencing the arrestment of the females. On treated filter paper, females walked slower and turned frequently resulting in a convoluted walking pattern.

Several volatile chemicals detected in our study were previously described as semiochemicals which elicited responses from insects. D-limonene, eugenol, and cinnamaldehyde,(E) were detected in citronella sample while eucalyptol, beta-pinene, 1-R, alpha pinene, beta phelladrene, (+)-4-carene and caryophyllene were among the detected volatiles chemicals in litsea leaves sample. In *hinoki* samples most of volatile chemicals which could have behavioral effect on insects were know as camphene, limonene, caryphyllene, beta myrcene and 1R-alpha-pinene. Most of the chemicals identified were monoterpenes and had low molecular weights. The isoprenoids limonene, reported to be the most abundant orange-emitted volatiles, induced actively EAG responses in the fruit fly *Ceratitis capitata* (Hernandez *et al.*, 1996). Francis *et al.* (2005) reported that most of the terpenes are infochemicals for herbivore species when emitted from the related host plant. *Diaphania nitidalis* (Stoll.) (Lepidoptera, Pyralidae) is attracted by R-, S-limonene from *Cucurbita pepo* leaves. Limonene was reported eliciting attractiveness of *Helicoverpa armigera* adults (Bruce, 2000) and oviposition of *H. armigera* on several legume crops was correlated with the presence of volatile chemicals including limonene (Blaney and Simmonds, 1990). In other genus of insects, Petterson (2001) found that limonene was attractive to three bark beetle parasitoids, including *Rhopalicus tutela*, *Roptrocerus mirus*, and *Roptrocerus xylophagorum* (Hymenoptera: Pteromalidae). Limonene from *Cinnamomum camphora* was reported to be toxic, repellent or fumigant against insect (Hummelbruner and Isman, 2001; Tripathi *et al.*, 2000, 2003). The volatile chemical eugenol, in synergestic action with other essentials, was demonstrated to have insecticidal activity against cockroaches and ants (Eman, 2001). The monoterpenes alpha-pinene and myrcene have been proven as precursors of aggregation pheromone components of several bark beetle species (Renwick *et al.*, 1976; Klimetzek and Francke, 1980; Byers, 1981, 1989). Our findings demonstrated that when a *B. dorsalis* female encounters an attractant cue on the filter paper, the female varied its locomotory path in such manner to show an arrestment response. The switchover from faster "runway" walking to slower searching pattern or vice versa on the treated filter paper indicated that essential oils might be advantageous in the management of *B. dorsalis.* However, further investigation on the physiological and open-field behavioral responses to those volatiles identified from the essential oils either as an attractant or repellent of *B. dorsalis* could lead to the formulation of an integrated pest management system.

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