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RESPONSES OF NAIVE FEMALE DBM (*PLUTELLA XYLOSTELLA*) TO VOLATILE ORGANIC CHEMICALS OF SELECTED BRASSICACEAE PLANTS

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ABSTRACT

Plants respond to insect feeding damage by releasing a variety of volatiles from the damaged site. The profile of the volatiles emitted from undamaged and mechanically damaged plants is markedly different. The diamondback moth (DBM), *Plutella xylostella L*, which feed on the plants of the family Brassicaceae is a major pest of *Brassica* crops in Malaysia and other part of the world where these crops are grown. It is also the most serious insect pest of crucifers worldwide. This study has been conducted to evaluate the effect of volatile organic compounds emitted by three different crucifer plants damaged in four different ways on the naive DBM female by using a Y-olfactometer-based analysis. The DBM females were provided with a dual choice of damaged or undamaged cabbage (*Brassica oleracea*), Chinese mustard (*Brassica nigra*) or Indian mustard (*Brassica juncea*) plants. The results suggest that DBM females attracted to the volatiles organic compounds of the cabbage plant damaged by the DBM's own larvae and to plants damaged by aphids. The female DBM adult is attracted more to Chinese mustard plants damaged mechanically or by the cabbage head caterpillar *(Crocidolomia binotalis* Zeller*)* than to the plants damaged by DBM larvae. Also, the number of female DBMs that responded to undamaged Chinese mustard plants was significantly higher than the number that responded to the plants attacked by DBM larvae or by aphids. The naive DBM female adult also showed a significant preference for undamaged Indian mustard plants compared to Indian mustard plants colonised by aphids. From our results it can be concluded that the naive female DBM adult chooses not to land on host plants either damaged by its own larvae of other insect species to oviposit in order to secure a better food supply for her offspring. Probably also, on undamaged plants, they will face less exposure to their natural enemies - the parasitoid and predators- but this needs further investigation.

Keywords: *Plutella xylostella*, *Crocidolomia binotalis,* crucifer plants, Y-olfactometer, volatile organic compounds.

INTRODUCTION

Plants produce different volatile organic compounds (VOCs) after being attacked by different herbivorous insect species [10]. The chemical identity of these VOCs differs with the plant species and the herbivorous insect species infesting it [18]. In general, odours from infested and mechanically damaged plants are easier to be detected by parasitoids than cues from an undamaged host, but such odours are a weak predictor of insect host presence [9] The emitted VOCs could also be used as cues by predators foraging for their prey and parasitoids looking for host plants [12] [16] because most natural enemies base their foraging decisions on information from their herbivore victims and their food plants [26]. Although the emission of volatiles has been hypothesized to be beneficial to the plant, it is still debated whether this is also the case under natural conditions because other organisms such as herbivores also respond negatively or positively to the emitted volatiles [20].

These volatiles can serve to promote or deter interactions between plants and insect herbivores [18]. However, the herbivore-induced plant volatiles are often very complex blends, differing in composition quantitatively and qualitatively between plant species and qualitatively within plant species when attacked by different herbivore species [2] [6] [10].

The diamondback moth (DBM), *Plutella xylostella* represents one of the greatest threats to crucifer production in many parts of the world including Malaysia [5] [11]. Besides the cost of crop wastage, globally, it is estimated that US$3 billion is spent annually to control infestation [29]. The DBM's ability to develop resistance to all insecticides applied against it, is forcing growers to either increase their usage of the same insecticides or use a mixture of insecticides, both of which further aggravate the resistance problem, particularly in Southeast Asia and the Far East [7] [27] [28].

Clearly, alternative strategies to curb overuse of pesticides and slow down the development of insecticide resistance need to be investigated. One area of research that warrants especial attention is the tri-trophic interaction in which VOCs play an important role. Understanding this mechanism has important implications for the efficacy of integrated pest management (IPM). Insecticide resistance in DBM remains the most difficult, but not intractable, problem to manage in cruciferous crops [5] [24]. To this end, in this study, we investigate the effects of VOCs emitted by three crucifer plant species on the foraging behaviour of the naive DBM female by using a Y-olfactometer-based analysis. The aim of this work is to gain a fuller understanding of the role of these crucifer plants' VOCs in the repellence and attraction of DBM female adults and how this mechanism might be used in IPM programmes to control the activities of the DBM.

MATERIALS AND METHODS

Materials

Plants

The three plant species from the Brassicaceae family used in this study were cabbage (*Brassica oleracea),* Chinese mustard (*Brassica nigra)* and Indian mustard (*Brassica juncea)*. The Brassicaceae seeds were sown in plastic pots (12 cm diam), filled with soil and grown in separate insect-proof cages (2. 5 x 2 x 8 m) watered twice per day and fertilised no more than three times during the study (one time per two weeks). The plants were planted in a greenhouse at the Malaysian Agricultural Research and Development Institute (MARDI) Serdang, Selangor, Malaysia and experiments were performed on 6-week-old plants (i.e. at the 7-8 leaf stage).

Insects

Diamondback Moth (DBM) (*Plutella xylostella* (L.) Lepidoptera: Plutellidae)

The DBMs (adults and larvae) were collected from small farms in Danau Desa at Kampong Batu Muda in Kuala Lumpur, Malaysia. No specific permits were required for the described field studies and permission was provided by the landowners. The field studies did not involve endangered or protected species. The insects were reared on cabbage plants grown in small netting cages (40 x 35 x 45 cm) in the entomology laboratory at the MARDI under environmental conditions of 27±2°C, 60±5% RH and a photoperiod of 16:8 (L:D). Almost 100 newly emerged males and females were mixed for 2–3 days in four separate glass containers (30 cm high x 12 cm diam.) covered with mosquito netting for mating (25 insect par container). The insects were given a 20% honey solution as food through a cotton swab kept in a small sterilized petri plate placed inside the container. Fresh mustard leaves were provided for oviposition. The leaves with the eggs were transferred to small plastic cages (20 x 15 x 30 cm) covered with mosquito netting. The newly hatched larvae were given fresh leaves of cabbage when the mustard leaves were completely consumed. This was replenished daily until the pupal stage. The pupae were kept in the same plastic cages until emergence of the adults. The emerged DBM adults were transferred to the rearing screened cages (40 x 35 x 45 cm) by using an insect aspirator.

All experiments were conducted using 4-5-day-old mated females. The tested adult female DBMs were kept in cages without a host plant, from emergence, to ensure that they had no previous experience with plant volatiles and had no oviposition experience. At least 30 minutes before conducting the olfactometric tests, the females were placed individually into small glass tubes (5 ml) closed by cotton for use in the experiments to acclimatize with experimental arena.

Cabbage Leaf Webber *Crocidolomia binotalis* Zeller (Lepidoptera: Pyralidae)

Larvae of the cabbage leaf webber, were collected from MARDI premises at Jalan Kebun and from the SENR Research Centre of MARDI, Serdang, Selangor, Malaysia. They were than reared on cabbage leaves in small cages (20 x 15 x 30 cm) covered with mosquito netting, as above, until pupation. The pupae obtained were kept in a wooden cage (45cm^3) until emergence of the adults. When the adult moths started emerging, they were transferred into a rearing cage (35 × 40 × 45 cm) and kept in small groups (45-50 insects) with a cotton plug wetted with 20% honey solution placed on the cage floor to serve as food. Small cabbage plants were provided for oviposition. Plants with eggs were removed to cages (40 x 30 x 25 cm) for larvae rearing and 5-7-day-old II instar larvae were then used for bioassay [17].

Aphids (*Lipaphis erysimi (Kltb*) (Homoptera: Aphididae))

Aphids were collected from Chinese mustard plants grown in glasshouses at MARDI, Serdang, Selangor, Malaysia. The insects were kept on Chinese mustard plants in small screen cages (45 × 45 × 50 cm) in the entomology laboratory at MARDI for use in the experiments.

Y-olfactometer

The Y-shape olfactometer was made of transparent Plexiglas (2.5 cm diam; stem 13 cm, arms 10 cm; angle of stem-arms 120°) (Figure-1). Its arms were connected to two 22.2 L glass containers. An electric pump (Cole-Parmer Air cadet vacuum/pressure station, Illinois, U.S.A) was connected to the olfactometer to push the air into both containers through silicon tubes. Air was filtered through an activated-charcoal filter before being pushed into the odour source chamber. Airflow in each arm of the Y-tube was adjusted with a flow meter to 600 ml/min.

Method

To investigate whether DBM females respond differently to different type of damage, the DBM female individuals were tested separately per treatment. In this experiment we used three different plant species and each species has had four treatments namely the undamaged plant versus infested with approximately 25-30 DBM II and III instar larvae, undamaged plant versus infested with 25 *C. binotalis* II instar larvae, undamaged plant versus infested with 150-200 aphids and undamaged versus mechanically damaged plants. We used three different plant species cabbage (*Brassica oleracea*), Chinese mustard (*Brassica nigra*) or Indian mustard (*Brassica juncea*) making the total treatment were 12. Each treatment were repeated 5 times and experiment were following complete block design.

To test the DBM female's response to the VOCs of these plants, 3-day-old mated DBM females were released individually into the end of the stem of the Y-tube and each of them was given 8 minutes to move towards the ends of tube's arms (Figure-1). Choice was recorded when a female DBM passed more than halfway along of one of the two arms and stayed there for more than 30 seconds. The DBM female was considered as having a zero response if it failed to pass beyond the halfway point of the arm in 8 minutes. A total of 25 females were tested in each experiment. After testing five insects per treatment, plant odour sources (damaged or intact plant) were replaced by a different plant that had been given the same treatment. After five runs, the apparatus was rotated 180° to exclude directional bias. After every three runs, all materials (Y-tube, tubes and glass containers) used in the test were thoroughly washed in soap and water, rinsed in 70% ethanol and dried.

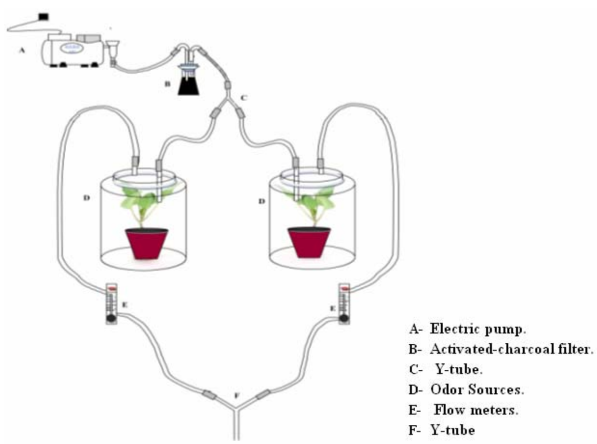

Figure-1. Perspective view of the Y-Olfactometer.

The plants were used in the olfactometer experiment 2 days after the damage had occurred. The DBM larvae, *C. binotalis* larvae and aphids were left on the plant during the test. For the mechanical damage treatment, 3-4 leaves per plant were injured by cutting a quarter of the leaf with scissors 24 h before the test, and were damaged 10 minutes prior to the experiment. The response of the DBM females was tested by using only one host plant per treatment. All observations were conducted in the entomology laboratory at the MARDI in a controlled environment under conditions of 26±2°C, 60±5% RH between 14.00 and 19.00 on 6 June -10 October 2012.

RESULTS

Our results indicated that the volatiles from the cabbage, Chinese mustard and Indian mustard attracted DBM female adults in an olfactometer. Each of the plant species is discussed in turn below.

Cabbage

There was no significant difference (F=2.4, df= 1.48, P=0.12) betwen the mean number of DBM females attracted to the VOCs of the cabbage plant damaged by the DBM's own larvae and the mean number attracted to the undamaged plant (control). (Figure-2)

Similar results were obtained for the response of the DBM females to the cabbage plant damaged by aphids (F=2.13, df=1.48, P = 0.151) (Figure-3).

However, relatively fewer DBM females were attracted to the undamaged plant than to the cabbage plants damaged by the other two methods (*C.binotalis* larvae and mechanical). The mean number of DBM females attracted to the undamaged cabbage plant (control) was significantly higher (F=17.7, DF=1.48, P=0.000) than that attracted to the cabbage plant damaged by the larvae of *C. binotalis,* Zeller (Figure-4).

In the case of the cabbage plant damaged mechanically, while the majority of the females that responded, tended to prefer the volatiles from the damaged plant, we found no significant difference (F= 3.49, DF=1.48, P=0.09) in the mean number of DBM females that responded to the VOCs emanating from the mechanically damaged cabbage plant and that for the undamaged plant. (Figure-5)

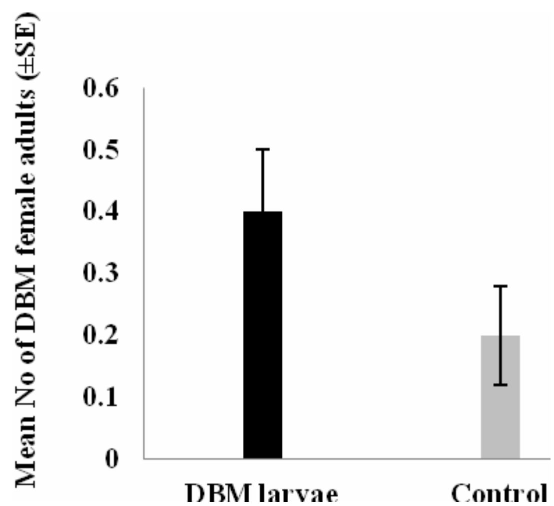

Figure-2. Mean number (±SE) of DBM female adults attracted to VOCs from undamaged (control) Cabbage plants (*Brassica oleracea)* and damaged by DBM larvae tested in Y-olfactometer.

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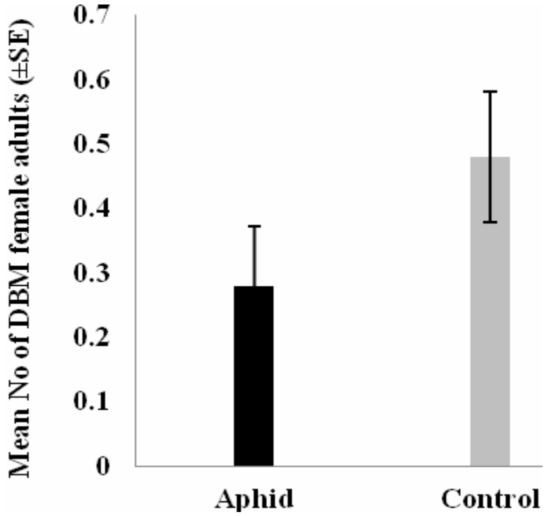

Figure-3. Mean number (±SE) of DBM female adults attracted to VOCs from undamaged Cabbage plants (*Brassica oleracea)* and damaged by aphids tested in Y-olfactometer.

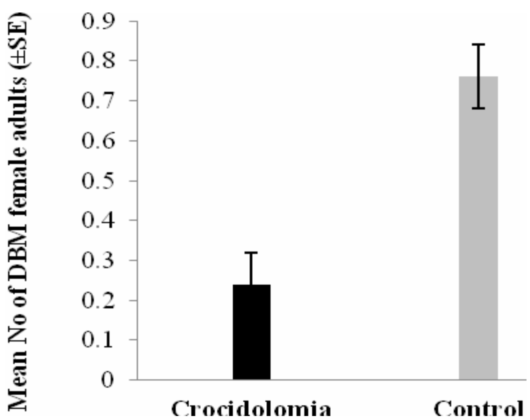

Figure-4. Mean number (±SE) of DBM female adults attracted to VOCs from undamaged Cabbage plants (*Brassica oleracea)* and damaged by Crocidolomia larvae tested in Y-olfactometer.

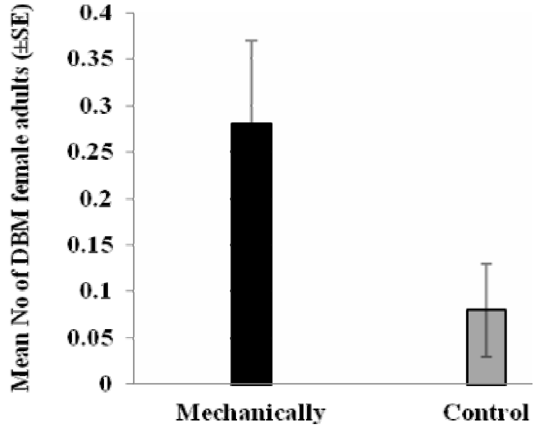

Figure-5. Mean number (±SE) of DBM female adults attracted to VOCs from undamaged Cabbage plants (*Brassica oleracea)* and damaged mechanically tested in Y-olfactometer.

Chinese mustard

The mean number of DBM female adults attracted to the volatiles from the Chinese mustard damaged by DBM larvae and to the undamaged plant was not differed significantly (F=0.32, df=1.48, P=0.57). (Figure-6).

The difference in the mean number of DBM females attracted to the plant damaged by *C. binotalis* larvae and to the undamaged plant was highly significant (F=8.1, df=1.48, P = 0.006) (Figure-7).

There was very high significant difference in the mean number of DBM females that responded to mechanically damaged as compared by undamaged Chinese mustard plant (F=9.72, df=1.48, P = 0.003). (Figure-8).

There was no significant different in the mean number of DBM femailes attracted to the Chinese mustard plant damaged by aphids as compared to the undamaged plant (F=0.32, df=1.48, P=0.573). (Figure-9).

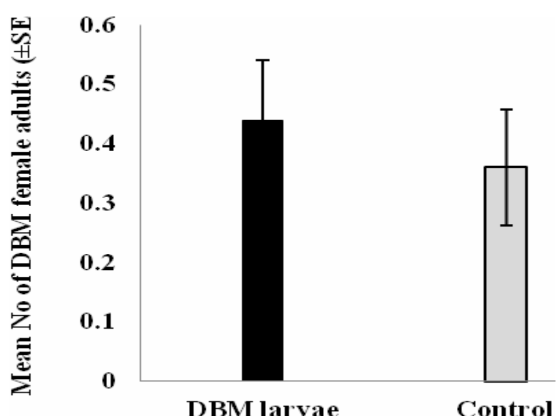

Figure-6. Mean number (±SE) of DBM female adults attracted to VOCs from undamaged Chinese mustard plants (*Brassica nigra)* and damaged by DBM larvae tested in Y-olfactometer.

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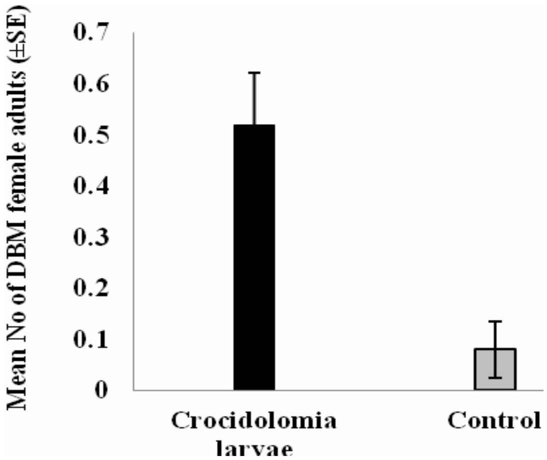

Figure-7. Mean number (±SE) of DBM female adults attracted to VOCs from undamaged (control) Chinese mustard plants (*Brassica nigra)* and damaged by Crocidolomia larvae tested in Y-olfactometer.

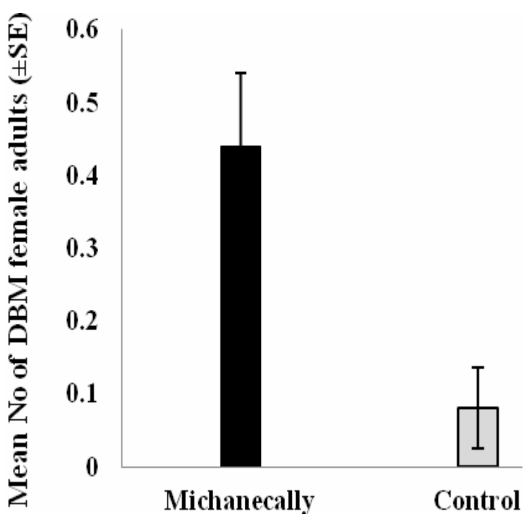

Figure-8. Mean number (±SE) of DBM female adults attracted to VOCs from undamaged (control) Chinese mustard plants (*Brassica nigra)* and damaged mechanically tested in Y-olfactometer.

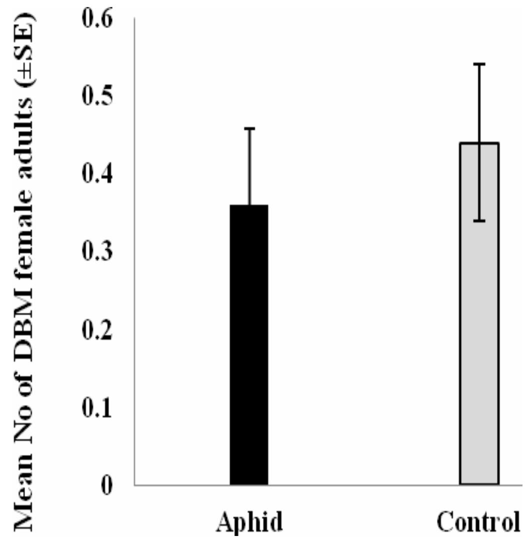

Figure-9. Mean number (±SE) of DBM female adults attracted to VOCs from undamaged (control) Chinese mustard plants (*Brassica nigra)* and damaged by aphid tested in Y-olfactometer.

Indian mustard

The difference in the olfactory response of the DBM females to following treatments was not significant ($P > 0.05$). There was no significant difference in the mean number of DBM females attracted to the plant damaged either by the DBM's own larvae (F=2.40, df=1.48, P= 0.128), larvae of *C. binotalis* (F=0.00, df=1, P=1.000), or by the mechanical method (F=2.24, df=1.48, P = 0.141). (Figures 10, 11, 12).

However, the DBMs response was significantly higher (F=8.10, df=1.48, P =0.006) towards the undamaged plant than to the plant damaged by the aphids. (Figure-13).

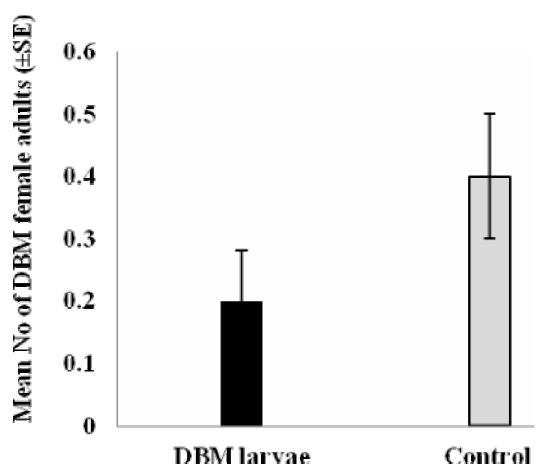

Figure-10. Mean number (±SE) of DBM female adults attracted to VOCs from undamaged (control) Indian mustard plants (Brassica juncea) and damaged by DBM larvae tested in Y-olfactometer.

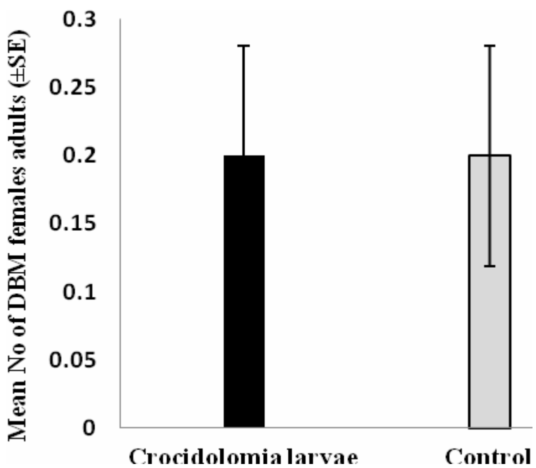

Figure-11. Mean number (±SE) of DBM female adults attracted to VOCs from undamaged (control) Indian mustard plants (*Brassica juncea)* and damaged by Crocidolomia larvae tested in Y-olfactometer.

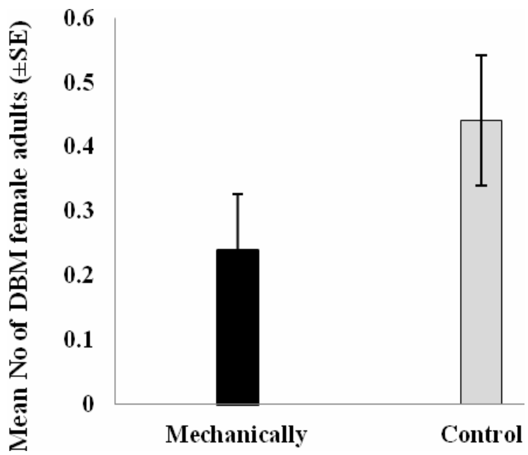

Figure-12. Mean number (±SE) of DBM female adults attracted to VOCs from undamaged (control) Indian mustard plants (*Brassica juncea)* and damaged mechanically tested in Y-olfactometer.

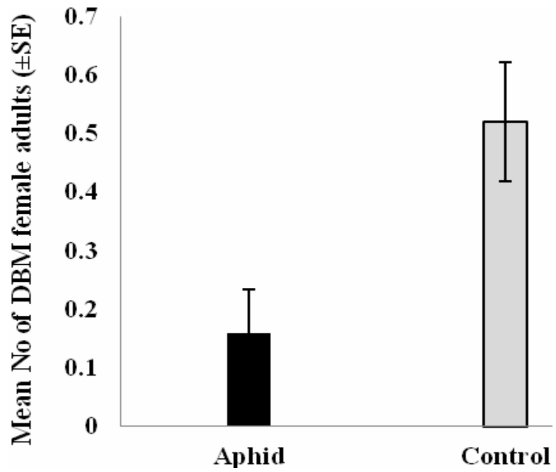

Figure-13. Mean number (±SE) of DBM female adults attracted to VOCs from undamaged (control) Indian mustard plants (*Brassica juncea)* and damaged by aphids tested in Y-olfactometer.

DISCUSSIONS

In general, when all three species of *Brassica* plant were damaged by aphids, the naive DBM females did not show any response to the VOCs that the plant emitted. However, they responded differently to the plants damaged by the three other damage types. The DBM females responded positively to the cabbage and Chinese mustard plant damaged mechanically, but they did not show a significant response to the odour from these two plants damaged in this way. When the tested plants were injured by the *C. binotalis* larvae, the DBM females responded significantly more to the injured Chinese mustard plant compared to the undamaged plant, while the response was found to be significantly higher towards the undamaged plant in the case of cabbage.

Result of olfactometer studies indicate that differential in VOCs produced for different treatment as such it is confirmed that the VOCs emitted by the treated plants were involved in causing differential responses of DBM adult females toward the orders sources. Such VOCs released by the host plant are the main attractant for DBM females in the search of a suitable site for oviposition [14]. It is also clear that the untreated or undamaged plants were generally more attractive to naive DBM females than crucifer plants damaged by different methods, these results tend to agree with Lu *et al*. (2004) who found that Chinese cabbage (*Brassica campestris*) damaged by DBM larvae was less attractive than undamaged Chinese cabbage to ovipositing DBM females. It is assumed that all plant species have a certain degree of specificity in their responses to herbivore attack [19]. Indeed, some plants respond differently to different herbivore species or types of damage [8]. The VOC chemicals emitted by the plants as a result of an herbivore attack appear to be easily detected and no doubt serve as a very clear signal to parasitoids and predators [23].

In the cabbage plant trial, it was observed that DBM females tended to orient themselves towards the cabbage plant infested (damaged) by larvae from their own species more than towards the plant injured by other tested species, mechanically or the undamaged plant, although they did not show a significant response. These results tend to agree with Reddy *et al*., 2003 [21], who report that when two types of cabbage plant are available to adult DBMs, female oviposition is significantly greater on damaged than on undamaged plant leaves. Specifically, Reddy and colleagues found that DBM females lay most of their eggs on cabbage plant leaves damaged by DBM larvae rather than on the leaves of intact plants. The obtained result supported by LU 2004 [15].

Results of our study on plants colonised by aphids or injured by the larvae of the cabbage leaf webber showed that most DBM females prefer the undamaged plants. In contrast, Shiojiri *et al*. (2002) [22] report that DBM females prefer to oviposit on cabbage plants infested with cabbage white butterfly, *Pieris rapae* (*L*). (Lepidoptera: Pieridae) larvae rather than on uninfested plants.

The results of our experiments on Chinese mustard in the olfactometer bioassay showed that adult female DBMs responded differently according to the type of plant damage. It was observed that most female DBMs oriented towards the damaged plants, which supports the finding of Shiojiri *et al*. (2002) [22]. It seems that this preference by naive female DBMs is mediated, at least in part, by the different levels of attraction to the VOCs produced by the tested plants

On the other hand, our results showed that the undamaged Indian mustard plant was always preferred by the DBM females over the damaged one, although they were no significant differences in their responses to the damaged and undamaged plants for each of damage. These preferences for the undamaged Indian mustard may be because of different VOCs are produced by the undamaged plants. Alternatively, perhaps the VOCs produced by the damaged plants actually repelled the DBM females, or their lack of response to the damaged plants could be attributed to the low reliability of the signal in informing the DBM females of the host's presence [25] [26]. Bender (1999) [1]., report that Indian mustard does not appear to be more attractive than cabbage to any lepidopterous pests, which differs from the findings of [3] [4], who found that DBM females have a strong ovipositional preference for Indian mustard over cabbage. Thus, base on this laboratory study, result showed the Indian mustard has a very high value as conventional trap crop. However, in the field Indian mustard is always injured or fed by many insects and this multiple feeding may or may not attract the DBM females to lay eggs. As such field study need to be conducted to evaluate and verify the possible use of Indian mustard as trap crop although report on successful integration of this plants in DBM management in Indian continent.

CONCLUSIONS

Based on our results and analysis, we conclude that the induced reactions of DBM females to repellent or attractant VOCs emitted by undamaged or damaged crucifer plants is a major mechanism that leads to differential behavioural responses of this moth towards her host plants. Further work is essential to gain a fuller understanding of how these different responses can be used to devise an attractant or repellence strategy to protect these Brassica plant species from attack by this pest so as to improve crop yield and reduce the use of pesticides.

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