

# Searching Behavior of *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) and Its Parasitoid *Tetrastichus coccinellae* Kurd. (Hymenoptera: Eulophidae)

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**Abstract** A current study was established in laboratories of Biological Control Department, Shandaweel Research Station, Agricultural Research Center. Herbivore induced plant volatiles may be attractive to one or more specific natural enemies. Releasing of volatiles from certain pests and host injured plants has a role in host or prey location. Response of newly emerged and starved or fed adults (for three days) for each of *Coccinella undecimpunctata* (Coleoptera: Coccinellidae) and its parasitoid adults, *Tetrastichus coccinellae* Kurd. (Hymenoptera: Eulophidae) to volatile sources were studied. Olfactory orientation was emitted from a combination of host plant and host insect that volatiles from four sources (e.g. infested sorghum plants with aphids (corn leaf aphid (*Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae)), crushed un-infested sorghum leaves and infested sorghum leaves plus the last larvae and newly pupated coccinellid). Results demonstrated the complexity of the use of different volatile info-chemicals generated by the different environmental stimuli. Reactions of the used *C. undecimpunctata* adults and its parasitoids were noticed and different strategies to exploit these signals were evolved.

**Keywords** Olfactometer, Olfactory Orientation Sources, Volatile Info-chemicals

## 1. Introduction

Herbivorous insects are consumed by predatory and parasitic arthropods. These entomophagous arthropods visit plants not only to find hosts or prey, but they may also feed and develop on plants [17]. Coccinellids are

polyphagous insects where they prey many food sources but as far as selective their essential food concerned. Chemical information used by predators of herbivores is adaptively increase predator efficiency by reducing searching time and increasing attack rates on prey [18].

Herbivore induced plant volatiles may be attractive to one or more specific natural enemies and serve as one of several defense options Releasing of volatiles from certain pests and host plant species, alone or associated with their info-chemicals has a role in prey location [8]. Also, these plant volatiles are an important cue used in herbivore location by carnivorous arthropods such as parasitoids and predators to find prey or hosts, where their sensory ability to perceive volatiles and the physical ability to move towards the source differed according to species variation [12] and [1].

From the evolutionary point of view olfaction is one of the oldest senses, allowing for seeking food [4]. When following odor plumes, insects may change their navigational strategy at certain distances from the odor source [3]. Odor sources were caused evident differences in the reactions of the predator ladybeetle, especially as comparing to the reared beetles and the individuals collected in the field [24]. For example, olfactory orientation by aphids is guided by specific volatile blends released from their hosts. Host plants that co-exist with other plants may be less attractive for aphids due to volatile interactions between neighboring plants which can lead to changes in their volatile emissions [7].

The influence of corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) against induced plant volatiles on *Coccinella undecimpunctata* (Coleoptera: Coccinellidae) and its parasitoid, *Tetrastichus coccinellae* Kurd. (Hymenoptera: Eulophidae) searching behavior will be studied. Different volatized odors (e.g. infested sorghum

plants with aphids, crushed un-infested sorghum leaves and infested sorghum leaves plus the last larvae and newly pupated coccinellid) in olfactometer were tested. Strategies to exploit signals on newly emerged and starved or fed adults for the studied prey and its parasitoid were evolved.

## 2. Materials and Methods

### a. Rearing Techniques

**Predator:** The mass rearing of *C. undecimpunctata* was initiated with adult coccinellids collected from sorghum field in Shandaweel Research Station at Sohag Governorate. Corn leaf aphids, *R. maidis* were used as a prey for *C. undecimpunctata* in standardized environmental conditions of ( $26 \pm 2^\circ\text{C}$  and  $65 \pm 10\%$  R.H.). The host plant used was *Sorghum bicolor*.

**Parasitoid:** Collected pupae of *C. undecimpunctata* were introduced individually in vials covered with muslin cloth. Throughout their developmental period, suitable foods were daily provided until emergence of either the predator or the parasitoid. Emerged parasitoids from each coccinellid cocoon were tested for measuring the olfactory responses.

### b. Tests

1. Response of the predator and its parasitoid to odor source flowed from the chambers of an olfactometer device:

Predator and its parasitoid behavior were evaluated in an airflow olfactometer under the temperature of  $26 \pm 2^\circ\text{C}$  and  $65 \pm 5\%$  R.H. The olfactometer consisted of a circular central arena with four arms at 90 degrees, each one with a cylindrical chamber at the end [6]. Ladybeetle and its parasitoid were introduced into the central arena. An odor source was randomly placed in three chambers (active chambers), while the other one was empty (inactive chambers) [14]. One crushed piece of leaf ( $5\text{cm} \times 1\text{cm}$ ) cut from sorghum was used in each treatment. Infested leaves were obtained from sorghum plants infested with *R. maidis* nymphs prior to put in the device chamber.

The olfactometer was placed in a box covered from inside by a black cloth in to avoid the distraction of predator or parasitoid by the other visual stimuli [19].

### 2. Treatment Evaluation

#### a. *C. Undecimpunctata* Behavior

As described by [12] (but with some changes, the odor sources introduced in the olfactometer were: **a.** crushed un-infested sorghum leaf ( $5\text{cm} \times 1\text{cm}$ ) obtained from the apical or flag (the 1<sup>st</sup> leaf on corn plant); **b.** sorghum leaf

( $5\text{cm} \times 1\text{cm}$ ) obtained from an apical branch infested with 30 aphids; **c.** thirty aphids, *R. maidis* and **d.** empty chamber.

To evaluate the *C. undecimpunctata* adult (1 individual) placed in central arena which was: **a.** newly emerged reared adult; **b.** emerged adult starved for three days; **c.** adults fed on aphids for three days.

Number of visits by tested *C. undecimpunctata* adults to each chamber were recorded during a 30 mins period ( $n = 10$  for each treatment). Coccinellids that did not choose a chamber within the treated period were considered to be "non-responders".

#### b. Parasitoid Behavior

Odor sources used were: **a.** crushed uninfested sorghum leaf ( $5\text{cm} \times 1\text{cm}$ ) obtained from the flag leaf; **b.** sorghum leaf ( $5\text{cm} \times 1\text{cm}$ ) obtained from an apical branch infested with 30 aphids; **c.** infested sorghum leaf + a larva of last coccinellid instar + a newly formed pupa and **d.** empty chamber.

- b. 1. Evaluation of the whole parasitoid adults emerged from a predator cocoon and placed in central arena in the form of:

**a.** newly emerged from reared host; **b.** 2 days after emergence from host; **c.** 2 days after emergence from host + honey dew.

Number of individuals visited each chamber were recorded during a 30 mins period ( $n = 10$  for each treatment).

- b. 2. Evaluation of only one parasitoid adult that placed in central arena in the form of: **a.** newly emerged from reared host; **b.** 2 days after emergence from host; **c.** 2 days after emergence from host + honey dew.

Number of visits to each chamber were recorded for a period of 30 min. ( $n = 10$  for each treatment).

Parasitoid individuals that did not choose a chamber within the treated period were considered to be "non-responders".

The olfactometer was cleaned with 96% ethanol and allowed to dry before introducing each of the predator or parasitoid into the chamber.

### Statistical Analysis

Data were analyzed statistically using the PC-STAT software program. Analysis of variance for the mean number of parasitoid adults (of the whole emerged parasitoids from a coccinellid cocoon) that responded to different odors was estimated. These mean numbers were compared by the co-variance analysis to avoid the errors due to the different in numbers of the gregarious emerged parasitoids (introduced in the central of olfactometer) that used in each of the ten replicates.

### 3. Results

#### Olfactometric Response of the Predator, *C. Undecimpunctata* Adults and their Parasitoid Adults to Volatile Info-chemicals Associated with Sorghum Plants and the Aphid Prey:

##### 3.1. Olfactometric Response of *C. Undecimpunctata* Adults to Volatile Info-chemicals Associated with Sorghum Plants and Aphid Prey:

###### 3.1.1. Response of Newly Emerged *C. undecimpunctata* Adults

Data illustrated in Figure 1 are showed the response of newly emerged *C. undecimpunctata* adults towards three odors emitted from crushed un-infested sorghum leaf, sorghum leaf infested with 30 aphids and thirty aphids alone (active chambers) in addition to the empty chamber. Coccinellids that did not choose a chamber within the treated period were considered to be “non-responders”.

Through olfactometer experiments, predator adults were insignificantly visited to the five chambers. *C. undecimpunctata* adults were attracted to volatiles emitted from sorghum plants infested with aphids then crushed un-infested sorghum leaf with 10 and 8 individuals, respectively, whoever, odors emitted from aphid alone

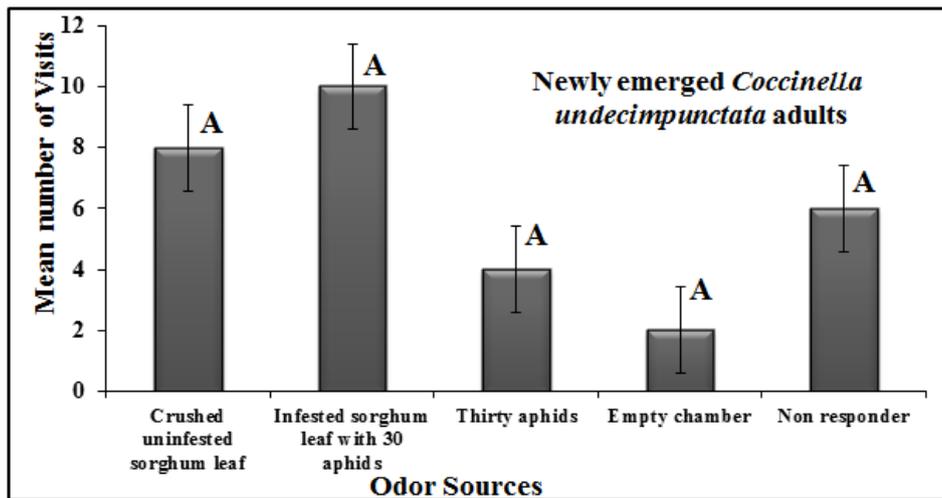
recorded 4 predators and finally, the empty chamber recorded 2 individuals. Furthermore, 6 individuals were not response to any of emitted odors “non-response”.

###### 3.1.2. Response of *C. undecimpunctata* Adults Starved Three Days

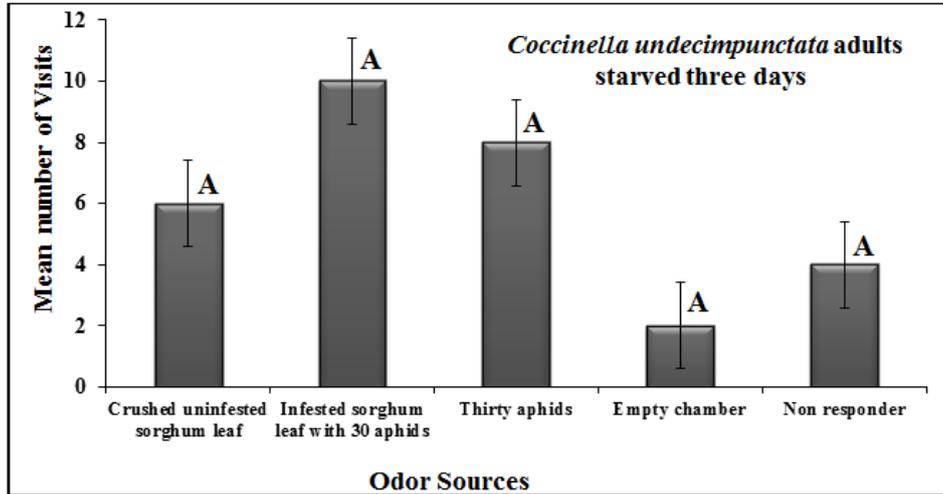
Three days starved ladybeetle tended to the infested sorghum leaf + 30 aphids with 10 predators from thirty ones. Aphid alone and damage sorghum plant treatments received 8 and 6 individuals, respectively. Only 2 individuals were recorded in the empty chamber but 4 ones from the thirty tested individuals not responded to any odors (Figure 2). Mean numbers of visits were non-significantly differences in between the different chambers.

###### 3.1.3. Response of *C. undecimpunctata* Adults Fed on Aphid for Three Days

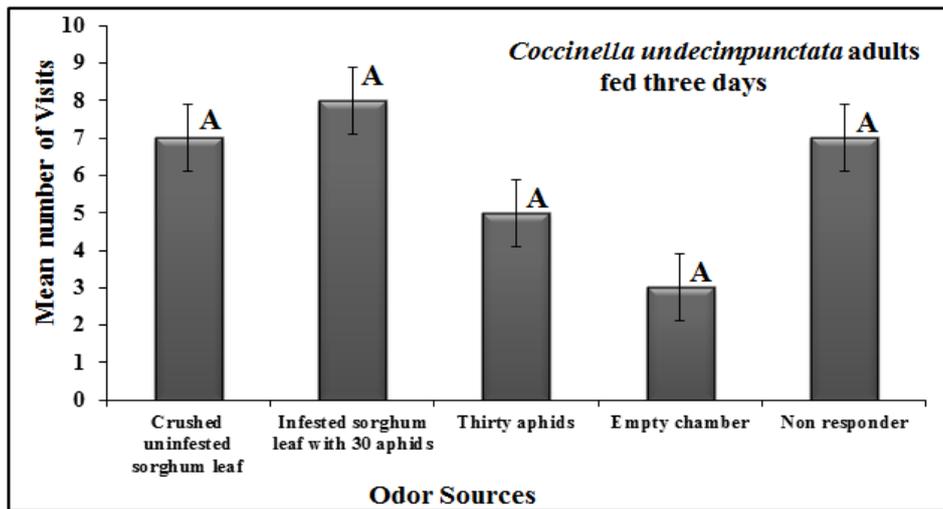
Seven and eight of adults that fed on aphids for three days with sufficient numbers of aphid, responded to crush sorghum leaf and infested sorghum leaf, respectively. In case of the chamber provided with the thirty aphids alone and the empty one, 5 and 3 adults were responded, respectively. On the other hand, 7 adults were found not responded in this experiment (Figure 3). Predators visited insignificantly differences to all chambers.



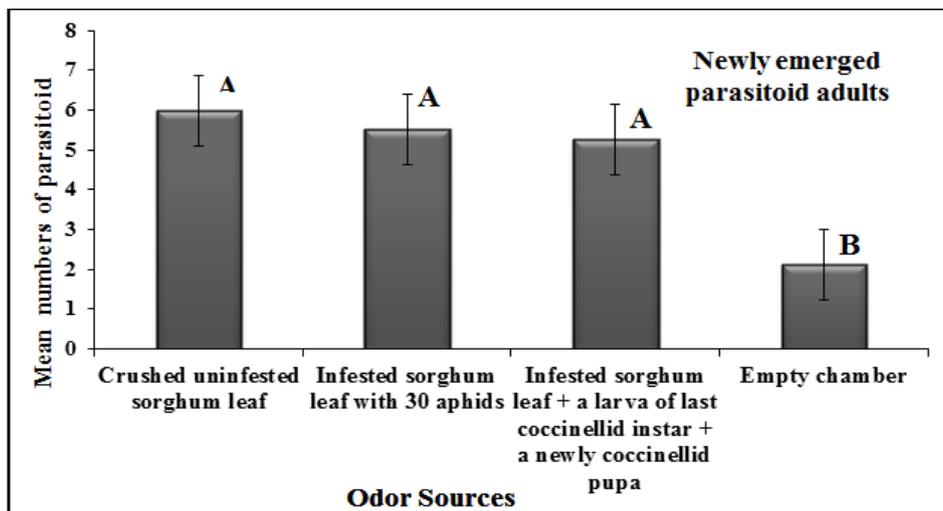
**Figure 1.** Response of newly emerged *C. undecimpunctata* adults towards odors emitted from un-infested sorghum leaf, sorghum leaf infested with 30 aphids, thirty aphids and empty olfactometer chamber. Bars with similar letters are insignificantly different (ANOVA test).



**Figure 2.** Response of *C. undecimpunctata* adults starved three days towards odors emitted from crushed un-infested sorghum leaf, sorghum leaf infested with 30 aphids, only thirty aphids and empty chamber. Bars with similar letters are insignificantly different (ANOVA test).



**Figure 3.** Response of *C. undecimpunctata* adults fed for three days towards odors emitted from crushed un-infested sorghum leaf, sorghum leaf infested with 30 aphids, thirty aphids and empty chamber. Bars with similar letters are insignificantly different (ANOVA test)



**Figure 4.** Response of newly emerged parasitoid adults towards odors emitted from crushed un-infested sorghum leaf, infested sorghum leaf with 30 aphids, infested sorghum leaf + a last coccinellid larva + a newly formed pupa and empty chamber. Bars with different letters are varied significantly (ANOVA test,  $P < 0.05$ )

**3.2. Olfactometric Response of Parasitoid Adults to Volatile Info-chemicals Associated with Sorghum Plants, Aphid and its Host**

**3.2.1. Olfactometric Response of Parasitoid Adults Emerged from One Coccinellid Coccon**

**3.2.1.1. Newly Emerged Parasitoid Adults**

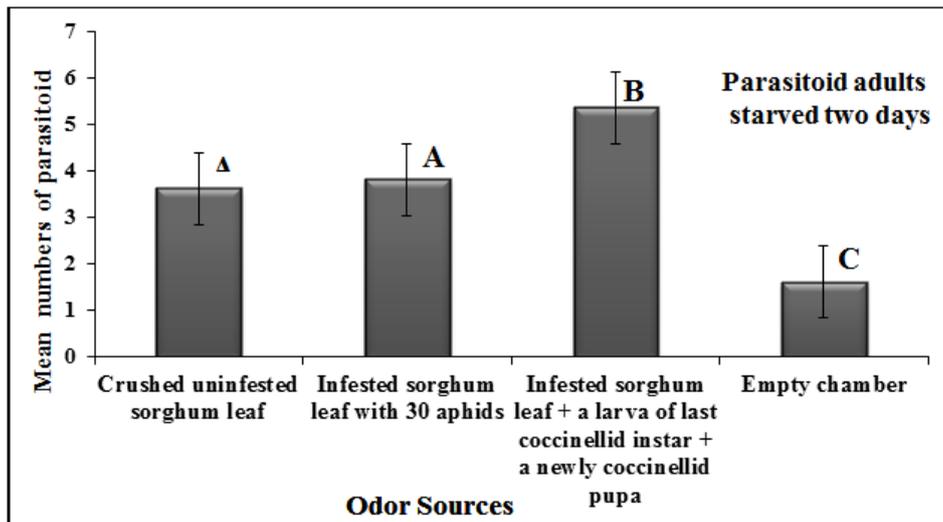
Responding of newly emerged parasitoid adults toward odors emitted from the crushed un-infested sorghum leaf, sorghum leaf infested with 30 aphids, sorghum leaf infested with 30 aphids + a larva of last coccinellid instar + a newly coccinellid pupa, in addition to the empty chamber were illustrated in Figure 4. Parasitoid adults that did not choose a chamber within the treated period were considered to be “non-responders”.

Data showed that the empty chamber recorded the lowest significant number of parasitoid adults of 2.11 adults as compared to the rest chambers. No significant

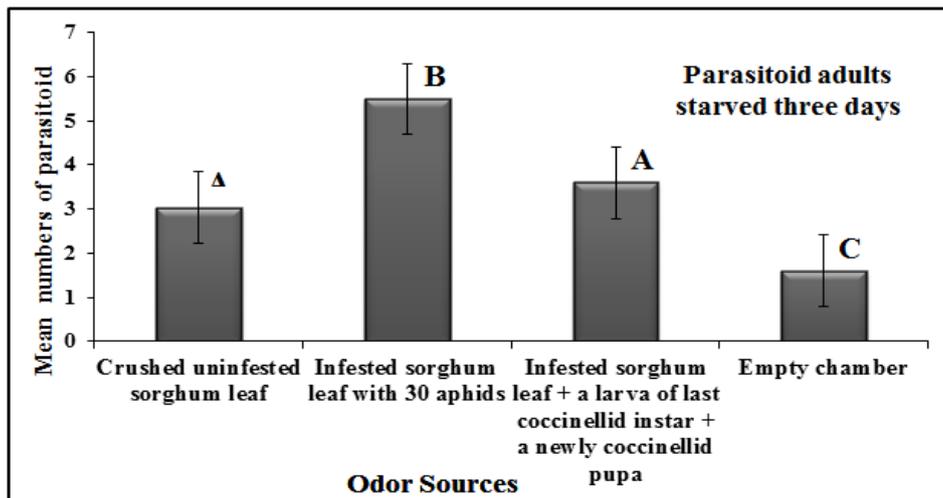
differences were found between crushed un-infested sorghum leaf, sorghum leaf infested with 30 aphids and sorghum leaf infested with 30 aphids + a larva of last coccinellid instar + a newly coccinellid pupa chambers with average numbers of 5.97, 5.52 and 5.26 parasitoids, respectively.

**3.2.1.2. Two Days Starved Parasitoid Adults**

The four chambers were differed significantly in parasitoid response adults. A chamber contained the infested sorghum leaf with a coccinellid host recorded the highest respond with 5.37 parasitoids, then followed by chambers of crushed un-infested sorghum leaf and sorghum leaf infested by 30 aphids with 3.62 and 3.82 parasitoids, respectively, where the last two insignificantly differed. Empty chamber recorded the last response (Figure 5).



**Figure 5.** Response of two days starved parasitoid adults towards odors emitted from crushed un-infested sorghum leaf, infested sorghum leaf with 30 aphids, infested sorghum leaf + a last larvae of coccinellid + a newly formed pupa and empty chamber. Bars with different letters are varied significantly (ANOVA test, P< 0.05)



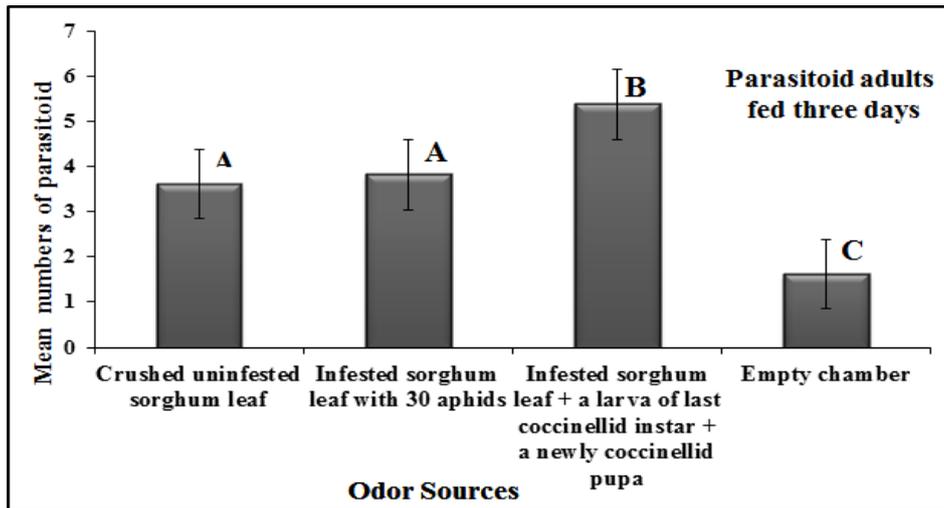
**Figure 6.** Response of three days starved parasitoid adults towards odors emitted from crushed un-infested sorghum leaf, infested sorghum leaf with 30 aphids, infested sorghum leaf + a last larvae of coccinellid + a newly formed pupa and empty chamber. Bars with different letters are varied significantly (ANOVA test, P< 0.05)

3.2.1.3. Three Days Starved Parasitoid Adults

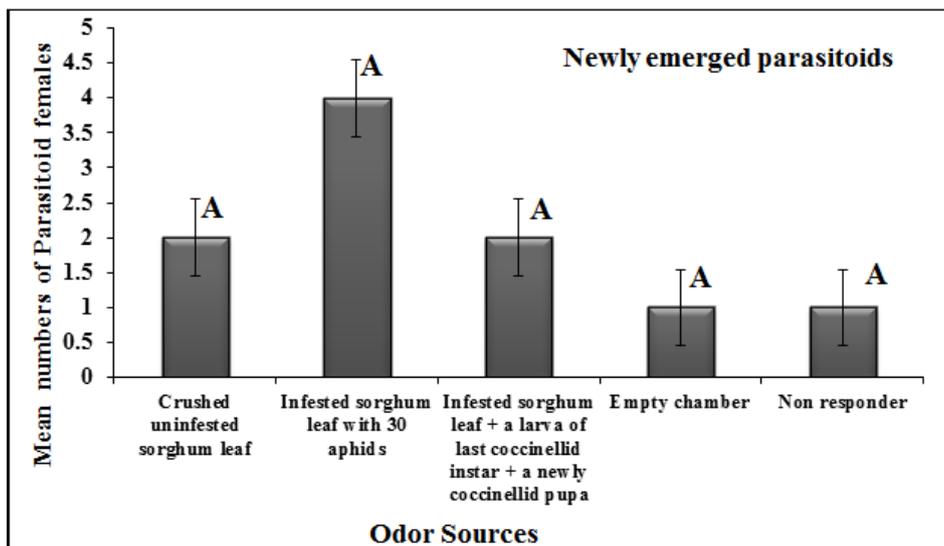
Data showed that the four situations of response could be arranged in ascending order into three significantly groups as follow: (1) chamber of sorghum leaf infested with 30 aphids contained 5.49 parasitoids, (2) chambers of crushed un-infested sorghum leaf and of (a larva of last coccinellid instar + a newly coccinellid pupa) contained 3.03 and 3.59 parasitoids, respectively, and (3) those of empty chamber with 1.60 parasitoids (Figure 6).

3.2.1.4. Response of Parasitoid Adults Fed for Three Days

Chambers contained sorghum leaf infested with 30 aphids + a larva of last coccinellid instar + a newly coccinellid pupa chambers responded by 5.37 parasitoids, but those of crushed un-infested sorghum leaf and sorghum leaf infested with 30 aphids by 3.62 and 3.82 parasitoids, respectively. Empty chamber of non-responded adults was affected by 1.62 parasitoids (Figure 7). Data presented that the four situations of response could be arranged in ascending order into three significantly groups.



**Figure 7.** Response of parasitoid adults fed for three days towards odors emitted from crushed un-infested sorghum leaf, infested sorghum leaf with 30 aphids, infested sorghum leaf + a last larvae of coccinellid + a newly formed pupa and empty chamber. Bars with different letters are varied significantly (ANOVA test,  $P < 0.05$ )



**Figure 8.** Response of newly emerged parasitoid females towards odors emitted from crushed un-infested sorghum leaf, infested sorghum leaf with 30 aphids, infested sorghum leaf + a last larvae of coccinellid + a newly formed pupa and empty chamber. Bars with similar letters are insignificantly different (ANOVA test)

**3.2.2. Olfactometric Response of Parasitoid Females to Volatile Infochemicals Associated with Sorghum Plants, Aphid and its Host:**

**3.2.2.1. Response of Newly Emerged Parasitoid Females**

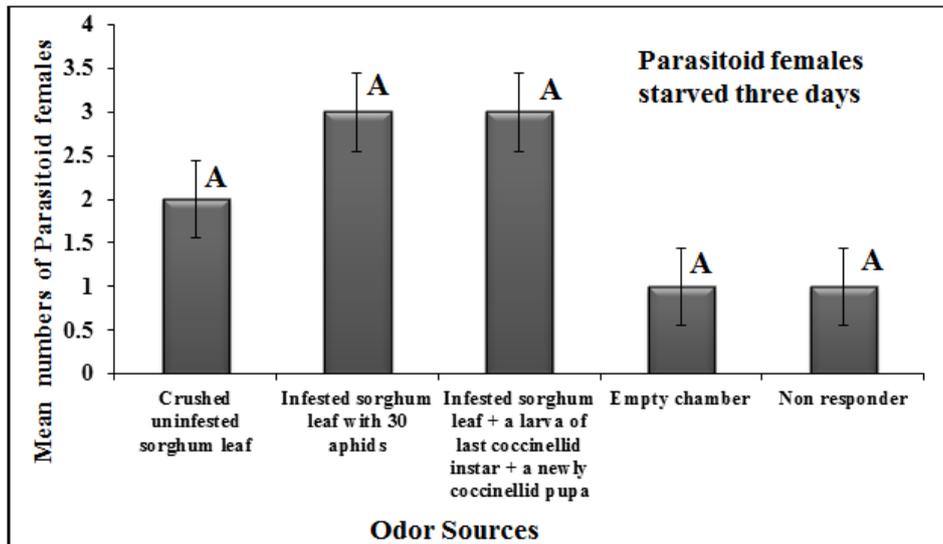
Data in Figure 8 illustrated the response of newly emerged parasitoid females towards the odors emitted from crushed un-infested sorghum leaf, sorghum leaf infested with 30 aphids, sorghum leaf infested with 30 aphids + a larva of last coccinellid instar + a newly coccinellid pupa, in addition to the empty chamber or the non-responded ones.

Results indicated that the chambers of crushed un-infested sorghum leaf, sorghum leaf infested with 30 aphids and (sorghum leaf infested with 30 aphids + a larva of last coccinellid instar + a newly coccinellid pupa) received 2, 4 and 2 parasitoid adults, respectively, from 10

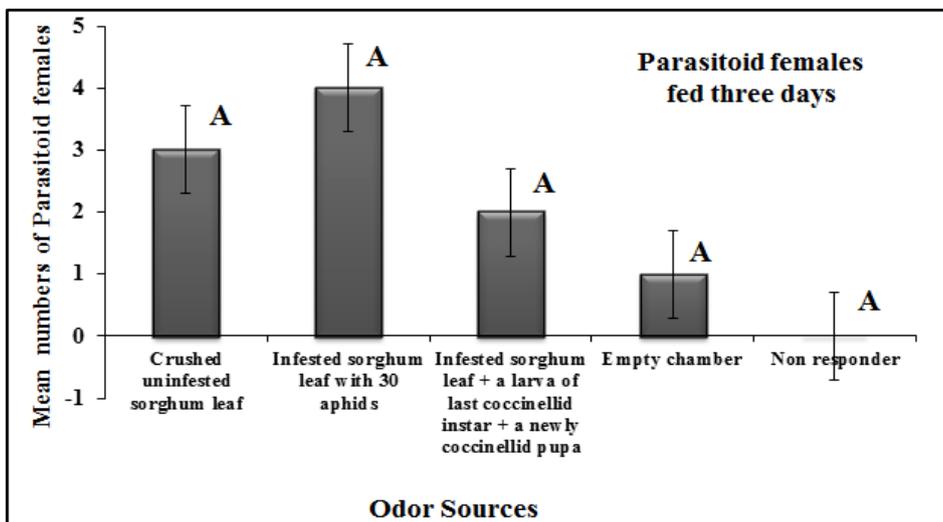
adults. One of the two remained adults was found in empty chamber, which was not responded to any chamber. The response of parasitoid females between all chambers differed non-significantly.

**3.2.2.2. Response of Parasitoid Females Starved for Three Days**

As shown in Figure 9, the parasitoid females of 2, 3, 3 and 1 tended to the chambers contained crushed un-infested sorghum leaf, sorghum leaf infested with 30 aphids, (sorghum leaf infested with 30 aphids + a larva of last coccinellid instar + a newly coccinellid pupa) and the empty chamber, respectively. One parasitoid adult did not respond to any of the others. Parasitoid females were responded statistically non-significant to all contained chambers.



**Figure 9.** Response of parasitoid females starved for three days towards odors emitted from crushed un-infested sorghum leaf, infested sorghum leaf with 30 aphids, infested sorghum leaf + a last larvae of coccinellid + a newly formed pupa and empty chamber. Bars with similar letters are insignificantly different (ANOVA test).



**Figure 10.** Response of parasitoid females fed for three days towards odors emitted from crushed un-infested sorghum leaf, infested sorghum leaf with 30 aphids, infested sorghum leaf + a last larvae of coccinellid + a newly formed pupa and empty chamber. Bars with similar letters are insignificantly different (ANOVA test).

### 3.2.2.3. Response of Parasitoid Females Fed for Three Days

Three of 10 parasitoid females were found on chamber of crushed un-infested sorghum leaf. However, four parasitoid adults were recorded on chamber of sorghum leaf infested with 30 aphids. Also, two parasitoid adults were recorded on chamber of (sorghum leaf infested with 30 aphids + a larva of last coccinellid instar + a newly coccinellid pupa). Empty chamber received one parasitoid adult. No parasitoid adults were found in the non-responder chamber (Figure 10). Parasitoid adults are responded non-significantly differences to all contained chambers.

## 4. Discussion

The olfactometer results indicate that behavior of adult coccinellids was influenced by volatiles emitted from infested sorghum plants and damage sorghum plant. Perhaps, they referred to the existence of shared role of insects and plants to attract predator. These results are supported by [17] observed that volatile chemicals are emitted in response to feeding by herbivorous arthropods and serve to guide predators to their prey. [18] found that coccinellids not receptive to volatiles emitted from aphids and it is less likely that aphid emit kairomonal substances only when feeding. Volatiles emitted signal from infested plant to predators of herbivores that their prey was in the vicinity [22]. [13] suggested that volatiles associated with prey feeding damage on a host and prey waste products may play a more important role in prey-finding of *Harmonia axyridis* than volatiles associated with prey items alone.

Aforementioned results indicated that adult ladybeetles did not visit the chambers randomly. Adults of *Cyclonida sanguinea* were strongly attracted to the odors of infested tomato leaves [12]. Likewise, [13] illustrated that more beetles preferred naturally infested buckthorn over un-infested buckthorn. Beetles preferred the odor of the particular cultivar, after feeding for 24 h on the cultivated aphids. Although in experienced ladybirds preferred odor of an aphid-infested barley cultivar over un-infested plants of the same cultivar, after feeding experience on a different cultivar this preference disappeared [10]. Adult ladybird beetles usually orient their movement towards prey using olfactory cues [23]. In addition, the oldest ladybirds seem to avoid colonies with an accumulation of honeydew [15]. In dissimilarity, [9] revealed that plant leaves alone of (*Vicia faba*, *Brassica napus* and *Salix alba*) or in association with non-stressed of three aphid species did not have any attraction for predator. Also, attraction to herbivory info-chemicals might occur only at certain densities of aphids, which feeding on the plant was given responsiveness in searching and oviposition behavior in coccinellids related to prey densities [23].

In accordance with our findings, [1] noticed that from

perception sensitivity and odor dispersal, the host finding influenced when hosts are heterogeneously distributed. [21] found that predators and parasitoids are attracted by volatiles emitted by plants under attack by herbivores. Also [13] discovered that when herbivores feed on plants, the injured plants respond by producing and releasing odors that are exploited by natural enemies of the herbivores to locate their preys and hosts. On the other hand, [5] mentioned that *Cis*-jasmone were attractive to aphid parasitoids in laboratory bioassays where *Cis*-jasmone is emitted naturally from plants after insect damage and can trigger defensive responses via airborne transport [11]. Finally, [25] claimed that the parasitoid was attracted by a combination of host plant and host volatiles in both the absence and presence of non-host plant volatiles in a Y-tube olfactometer. In dual choice tests the parasitoid preferred the blend of host plant and host volatiles over its combination with non-host plant volatiles.

## 5. Conclusions

The focus of this research was particularly on the olfactometric response of *C. undecimpunctata* and its parasitoid, *T. coccinellae* to volatile info-chemicals associated with sorghum plants and aphid prey. Results demonstrated the complexity of the use of different volatile info-chemicals generated by certain environmental stimuli (e.g. infested sorghum plants with aphids, crushed un-infested sorghum leaves and infested sorghum leaves plus the last instar larvae and newly pupated coccinellid) and showed that the tested predator and parasitoid have evolved different strategies to exploit these signals.

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