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Behavioral response of parasitoid *Acerophagus papayae* Noyes and Schauff to papaya mealybug *Paracoccus marginatus* on different host plants

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Abstract
Acerophagus papayae Noyes and Schauff (Hymenoptera: Chalcidoidea: Encyrtidae), is the efficient parasitoid for the suppression of papaya mealybug *Paracoccus marginatus* Williams and Granara de Willink. Host plant volatiles are important cues in the parasitoid host selection process. Hence, parasitoids orientation behaviour towards different host plants of papaya mealybugs were tested using olfactometer. Results say in the experiment with healthy host plant leaves that parasitoids highly preferred for the papaya host plant followed by mulberry, parthenium, tapioca, brinjal and control with 2.67, 1.72, 0.83, 0.56, 0.39 and 0.11 number of parasitoids attracted, respectively. In addition, when infested host plant leaves with mealybugs were studied, the parasitoids highly preferred for the papaya host plant followed by mulberry, parthenium, tapioca, brinjal and control with 3.61, 2.11, 0.89, 0.61, 0.28 and 0.17 number of parasitoids attracted, respectively. Hence, the identification of volatile compounds in the preferred and non-preferred host plants is of future scope.

Keywords: Papaya mealybug, parasitoid, olfactometer, chemical cues

Introduction
Acerophagus papayae Noyes and Schauff (Hymenoptera: Chalcidoidea: Encyrtidae), an introduced solitary koinobiont end parasitoid is the efficient parasitoid for the suppression of papaya mealybug (PMB) *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcinae). Successful parasitism of parasitoids eventually rests on the host selection process which involves a sequence of phases mediated by physical and chemical stimuli from the host insect and host plants [13, 3]. Since parasitoid foraging time is very much limited and the effective cues available are numerous, the parasitoids have to optimize the time for the exploitation of available cues and discriminate against the potential cues indicating the presence of a suitable host [4]. Plants release blends of volatile organic compounds (VOCs) in response to herbivore damage. Parasitoids use certain VOCs as indirect cues to locate their herbivore hosts [12, 14, 11, 8, 9, 5, 2]. In this study, the parasitoid *A. papayae* was used to test the hypothesis that the host plants of PMB affect the parasitoids response to host-related plant volatiles. Hence, to exploit the behavioral selection of parasitoid *Acerophagus papayae* the six-arm olfactometer with different host plants vi., papaya (*Carica papaya*), brinjal (*Solanum melongena* L.), cassava (*Manihot esculenta*), congress grass (*Parthenium hysterophorus*) and mulberry *Morus alba* L were used.

Materials and Methods
Insect Rearing
PMB from different host plants *viz*., papaya, tapioca, brinjal, and parthenium was collected in September 2017 from farmer field located at 11°37'35.9"N 78°28'41.1"E. Hosts plants *viz*., papaya, tapioca, brinjal, mulberry and parthenium were raised inside the metallic cages under laboratory condition at 33 ± 2 ^{0}C, 40–50% relative humidity and a 14 h light/10 h dark photoperiod. An ovisac from each collected samples was released on to the respective host plants inside the cages and observed for emergence. Mealybugs were allowed to complete one generation and samples for olfactometer analysis were drowned from subsequent generation.

Olfactometer experiment
About 10 g of host leaves were kept in the arm and was firmly closed with a lid. The inlet of the olfactometer on the top center place was connected to an aquarium pump (220-240 volt

AC) to release the pressure. Out of eight arms, one arm leaving as such was treated as control. After five minutes of saturation of different host odors in the olfactometer, ten numbers of one-day-old parasitoids were released in the olfactometer through a central hole, which also served as an odor exit hole. The observation was made on the number of parasitoids settled on each arm at 5, 10, 15, 20, 25 and 30 MAR (minutes after release). This experiment was replicated ten times. Similarly, the experiment (Experiment 2) with the mealybug infested host leaves along with 50 mealybugs was also performed by following the same methodology. Treatment details were listed in Table 1.

Table 1: Treatment details of six arm olfactometer experiment on the behavioural response of *A. papayae* with different host plants of PMB

Experiment 1	Experiment 2
-Parthenium healthy leaves	T1-Parthenium infested leaves
T2-Papaya healthy leaves	T2-Papaya infested leaves
T3-Tapioca healthy leaves	T3-Tapioca infested leaves
T4-Mulberry healthy leaves	T4-Mulberry infested leaves
T5-Brinjal healthy leaves	T5-Brinjal infested leaves
T6-Untreated check	T6- Untreated check

Results and Discussion

Six arm olfactometer analysis with the healthy leaves of different host plants resulted that immediately after the release of parasitoids, the very first preferred host plant was papaya with 0.67 attracted numbers of parasitoids at 5 MAR. At ten MAR onwards parasitoids were observed to spread throughout the olfactometer and they started making its choice. Active host searching behavior of parasitoids was observed till the end of the experiment (30 MAR). The trend of overall preference of parasitoids among the five different host plants subjected was recorded to be papaya>mulberry>parthenium>tapioca>brinjal>control with 2.67, 1.72, 0.83, 0.56, 0.39 and 0.11 number of parasitoids attracted, respectively. The difference in the preference of parasitoids was significant at 5% level of significance (Table 2.).

Experiment with infested host plant leaves along with mealybugs was resulted that immediately after the release of parasitoids; the active participation of parasitoids in the host searching process was more than in the experiment with healthy leaves. The active host searching behavior was observed until the end of the experiment (30 MAR). The significant trend of overall preference of parasitoids among the five different host plants infested with mealybug subjected was recorded tobepapaya> mulberry> Parthenium> tapioca> brinjal> control with 3.61, 2.11, 0.89, 0.61, 0.28 and 0.17 number of parasitoids attracted, respectively (Table 3.).

The outcome of the experiments showed significant variation in the orientation of parasitoid to odors of different host plants. But the trend of host plant preference was similar in both the experiments (healthy leaves and infested leaves); these findings suggest that invariable of the infestation *A. papayae* tend to prefer papaya followed by mulberry and the host plants tapioca and brinjal were least preferred by them. These findings were in accordance with the results of Nisha and Kennedy (2015) [6] who reported the preferred orientation of *A. papayae* towards papaya and mulberry host plants and non-preference of parasitoid to the tapioca host plant.

Compared to the healthy leaves, infested leaves along with the mealybug has attracted the number of parasitoids this may be due to the presence of herbivore-induced plant volatiles along with the host insect and host plant volatiles. This was in concurrence with the report of Nisha and Kennedy (2015) [6] whose results says that the infested leaf with mealybug will attract more number of parasitoids than the infested leaf without mealybugs.

Nisha and Kennedy in the year 2016 [7] resulted that the parasitoid *A. papayae* is well adapted to *P. marginatus* from natal host papaya than the non-natal hosts. Nadel and Van Alphen (1987) [5] found evidence that mealybug-infested cassava plants also release odors that are attractive to the parasitoid *Anagyrus lopezi*. Van Alphen and Visser (1990) [10] also found an attraction to *Phenacoccus manihoti* infested cassava plants by *Apoanagyrus diversicornis*.

In contrary to the present study Bertschy *et al.* in the year 1997 [1] suggested that *A. coccois* may be responsive only to general plant odors and this species obviously did not distinguish between infested and healthy cassava leaves. Hence, it remains to be determined if host plant volatiles play an important role in the specific attraction to infested plants, or if the mealybug and its by-products emit odors that are attractive.

Table 1: Mean parasitoids attracted in six arm olfactometers with healthy host plants

Host plants	Number of parasitoids attracted#												Overall mean**
	5 MAR*		10 MAR*		15 MAR*		20 MAR*		25 MAR*		30 MAR*		
	Mean	SD$	Mean	SD$	Mean	SD$	Mean	SD$	Mean	SD$	Mean	SD$	
Parthenium	0.00	0.00	0.33	0.58	0.67	0.58	1.00	1.00	1.67	0.58	1.33	1.15	0.83(0.94)c
Papaya	0.67	0.58	1.00	0.00	2.33	1.15	4.00	0.00	4.00	1.00	4.00	1.00	2.67(1.65)a
Tapioca	0.00	0.00	0.00	0.00	0.67	0.58	0.33	0.58	1.00	1.00	1.33	0.58	0.56(0.78)d
Mulberry	0.00	0.00	0.67	0.58	1.33	1.15	3.00	0.00	2.67	1.15	2.67	0.58	1.72(1.33)b
Brinjal	0.00	0.00	0.33	0.58	0.33	0.58	0.67	1.15	0.33	0.58	0.67	0.58	0.39(0.66)e
Control	0.00	0.00	0.00	0.00	0.33	0.58	0.33	0.58	0.00	0.00	0.00	0.00	0.11(0.39)f
SE ±m													0.02
CD (p=0.05%)													0.11
CV													8.89

$SD-Standard Deviation
Mean of ten replications
*MAR- Minutes after Release
** Figures in parentheses are square root transformed values
Means followed by the same alphabets are not significantly different at 5 % level by DMRT

Table 2: Mean parasitoids attracted in six arm olfactometer with host plants infested with *P. marginatus*

Host plants	Number of parasitoids attracted#												Overall mean**
	5 MAR*		10 MAR*		15 MAR*		20 MAR*		25 MAR*		30 MAR*		
	Mean	SD$	Mean	SD$	Mean	SD$	Mean	SD$	Mean	SD$	Mean	SD$	
Parthenium	0.33	0.58	1.00	0.00	1.00	0.00	0.33	0.58	1.33	1.53	1.33	1.15	0.89(0.97)c
Papaya	2.33	1.53	4.33	1.53	3.67	1.15	3.67	2.08	4.67	1.15	3.00	1.00	3.61(1.91)a
Tapioca	0.00	0.00	0.33	0.58	0.67	1.15	0.67	0.58	0.67	1.15	1.33	0.58	0.61(0.82)d
Mulberry	0.33	0.58	0.67	1.15	2.67	1.53	4.00	1.00	2.67	0.58	2.33	1.15	2.11(1.47)b
Brinjal	0.00	0.00	0.33	0.58	0.00	0.00	0.67	1.15	0.00	0.00	0.67	0.58	0.28(0.57)e
Control	0.00	0.00	0.00	0.00	0.33	0.58	0.00	0.00	0.33	0.58	0.33	0.58	0.17(0.46)f
SE ±m													0.01
CD (p=0.05%)													0.11
CV													7.79

$SD-Standard Deviation
Mean of ten replications
*MAR- Minutes after Release
** Figures in parentheses are square root transformed values
Means followed by the same alphabets are not significantly different at 5 % level by DMRT

Conclusion

The present study revealed that the potential parasitoid of PMB *A. papayae* highly prefers the papaya and mulberry host plants; whereas, tapioca and brinjal were least preferred by them. Also, parasitoids were highly tended to move towards infested host plants with mealybugs than healthy leaves. Hence it may be concluded that the herbivore-induced plant volatiles may act as a potential cue for host location by parasitoids. In the future, extraction and characterization of volatile compounds using chromatographic techniques will help us to identify the potential kairomonal compound responsible for parasitoids orientation behaviour.

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