Behaviour and evolutionary response of parasitoid Acerophagus papayae Noyes and Schauff using different olfactometers

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Abstract- The host selection process of parasitoid, eventually leading to successful parasitism. The influence of host plant odour was studied using the different type of olfactometers like eight arm olfactometer, six arm olfactometer and Y- tube olfactometer. The olfactometer studies revealed that there were more number of parsitoid attraction events towards the infested hosts than healthy host plants over time. While observing on eight arm olfactometer with mealybugs as standard check, mealybugs attracted more number of parasitoids in the healthy leaves, but at the same time, papaya and cotton leaves infested with mealybug attracted more number of parasitoids than mealybug standard. The parasitoid orientation in Y - tube olfactometer of the present study revealed that, infested leaves attracted more number of parasitoids, when they are tested with healthy leaves. Infested leaves with mealy covering attracted even a more numbers than in infested leaves alone.

Index Terms- parasitoid, Acerophagus papayae, olfactometer, papaya mealybug, Paracoccus marginatus

I. Introduction

The host selection process of parasitoid involves a sequence of phases mediated by physical and chemical stimuli from the host, the substrate, and/or associated organisms, eventually leading to successful parasitism (Vinson, 1985 and Godfray, 1994). Because parasitoid foraging time is limited and the potential cues available are numerous, the parasitoid faces the need to optimize exploitation of available cues and discriminate those most reliable in indicating the presence of a suitable host (Hilker and Meiners, 2006). In the current study, behavioral selection of parasitoid *Acerophagus papayae* was exploited using the different olfactometers like Y – tube olfactometer, six arm and eight arm olfactometers *vis-à-vis* host plant influence.

II. Methodology

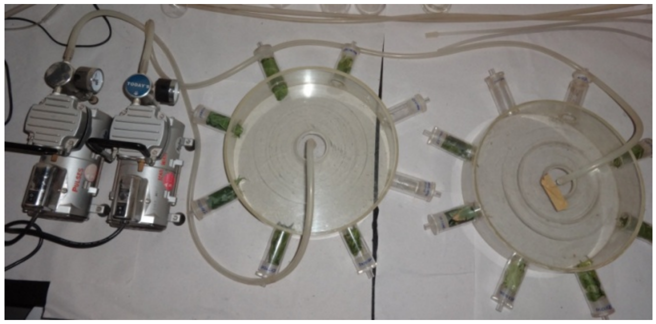
Behavioural bioassay of the parasitoid *Acerophagus papayae*

The adult parasitoids were subjected to a behavioural bioassay for the influence of host plant volatiles using eight arm olfactometer, six arm olfactometer and ‘Y’ tube olfactometers (Ranjith (2007); Hao *et al*. (2012)). In eight arm olfactometer, six host plants were compared against mealybugs as standard to check the preference level of adult parasitoids to host plants over the mealybug. To create a natural condition and to check the odour preference of parasitoids, only host plants were compared against control by taking economically important plants (except hibiscus) in six arm olfactometer. In natural environment, all the plants may or may not be available in a same area. Hence, host plants were compared with each other in Y- tube olfactometer by using pair wise combination of all permutations matrix of host plants.

Eight arm olfactometer

About 10 g of healthy 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 was treated as control and twenty mealybugs were kept in the one arm. The medical air was passed from aquarium pump at the rate of 4 lit/ min in to the olfactometer. After five minutes of saturation of different host odour in the olfactometer, twenty numbers of one day old virgin female parasitoids were released in the olfactometer through a central hole, which also served as odour exit hole. Observation was made on number of female parasitoids settled on each arm at 0, 5, 10, 15 and 20 MAR (minutes after release) for their host preference. This experiment was replicated ten times. Similarly, the experiment was conducted using the mealybug infested host leaves following the same methodology. The data on number of female parsaitoids settled on each arm were recorded (Plate 1).

Plate 1. Eight arm olfactometer experimental set up for host crop influence study



Treatment details

	Experiment 1		Experiment 2
T1-	Papaya healthy leaves	T1-	Papaya infested leaves
T2-	Cotton healthy leaves	T2-	Cotton 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-	Hibiscus healthy leaves	T6-	Hibiscus infested leaves
T7-	Mealybug alone as standard check	T7-	Mealybug alone as standard check
T8-	Untreated check	T8-	Untreated check

Six arm olfactometer

As per the above procedure, experiments were run on six arm olfactometer. Healthy leaves with control (only economically important plants except hibiscus) for first experiment and mealybug infested leaves (only economically important plants) with control for the second experiment were used. The data on number of female parsaitoids settled on each arm was recorded for 5, 10, 15 MAR for infested leaves and 5, 10, 15, 20 and 30 MAR for healthy leaves.

Treatment details

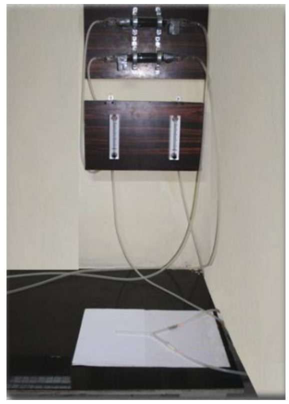
Experiment 1	Experiment 2
T1-Papaya healthy leaves	T1-Papaya infested leaves
T2-Cotton healthy leaves	T2-Cotton infested leaves
T3-Tapioca healthy leaves	T3-Tapioca infested leaves
T4-Mulberry healthy leaves	T4-Mulberry infested leaves
T5-Brinjal healthy leaves	T5-Brinjal infested leaves
T6-Untreated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

'Y- tube' olfactometer or two arm olfactometer

The 'Y- tube' olfactometer had a release chamber (100 ml capacity) at one end and two test chambers (100 ml capacity) connected by a glass runway of length 1 m. For creating pure air current one end of the test chambers will be connected to a blower through an air inlet chamber fitted with a charcoal filter and an air-flow meter. The blower unit will consists of a battery-operated mini-fan fitted in a glass tube to generate an air current at the rate of 2 m/s. The release chamber also has a provision for air exit. The movement of the test insect (female parasitoid) in the test chamber was restricted using a net (Plate 2).

Parasitoids were released in release chamber and the host plants were placed in the test chambers (healthy leaves in one chamber and infested leaves in another chamber). The air released by the air-flow meter was purified using the charcoal filter and was allowed to pass on the glass tube. The passing air absorbed the odour of host plants and reached the adult parasitoid in the releaser chamber. The position and movements of the female parasitoids towards odour source (host plant) was observed for a maximum of 10 minutes. Once it reached the end of the Y arm (i.e. the porous glass plates), the experiment was stopped, the females were removed and observations were recorded in its choice. Likewise the all the host plants were analyzed for the attractiveness of the parasitoid, running the experiments in a same sequence. Mealybug infested leaf sample and uninfested leaf sample will be used as treatment and control respectively for one experiment. For the second experiment, mealybug infested leaf samples and leaf samples with mealy covering were taken as treatment and control respectively. The third experiment was conducted by selecting the different permutations of the host plants.

Plate 2.'Y' tube olfactometer set up for host crop influence study



Treatment details for first experiment

Treatment	Treatment combination		
H1T1	Papaya infested leaves	X	Papaya healthy leaves
H2T2	Cotton infested leaves	X	Cotton healthy leaves
H3T3	Tapioca infested leaves	X	Tapioca healthy leaves
H4T4	Mulberry infested leaves	X	Mulberry healthy leaves
H5T5	Brinjal infested leaves	X	Brinjal healthy leaves
H6T6	Hibiscus infested leaves	X	Hibiscus healthy leaves

Treatment details for second experiment

Treatment	Treatment combination		
H1T1	Papaya infested leaves	X	Papaya leaf with mealy covering
H2T2	Cotton infested leaves	X	Cotton leaf with mealy covering
H3T3	Tapioca infested leaves	X	Tapioca leaf with mealy covering
H4T4	Mulberry infested leaves	X	Mulberry leaf with mealy covering
H5T5	Brinjal infested leaves	X	Brinjal leaf with mealy covering
H6T6	Hibiscus infested leaves	X	Hibiscus leaf with mealy covering

The adult parasitoids being phototrophic, to avoid visual cues and interference, the experiments were conducted in diffused light. Each experiment was repeated at least ten times. Insects once used in the bioassay were not used again. This was to prevent prior exposure to the stimulus influencing the response. The entire olfactometer excepting the blower and the air inlet tube was washed thoroughly with hexane the unit was oven dried between experiments to make the olfactometer odour-free. The observed parasitoid decisions therein were pooled and stastically analysed against even distributions using the goodness of fit test.

III. Results and Discussion

The Generalized Linear Model is an extension of the General Linear Model to include response variables that follow any probability distribution in the exponential family of distributions. The exponential family includes such useful distributions as the Normal, Binomial, Poisson, Multinomial, Gamma, Negative Binomial, and others. Hypothesis tests applied to the Generalized Linear Model do not require normality of the response variable, nor do they require homogeneity of variances. Hence, Generalized Linear Models can be used when response variables follow distributions other than the normal distribution, and when variances are not constant. Hence, data on parasitoid attraction count would be appropriately analyzed as a Poisson random variable within the context of the Generalized Linear Model. Exponential of the regression coefficient is also present in order to examine the likelihood of the parasitoid attracted to the plant.Thus the non-normal data was subject to Generalized Linear Multivariate Model (GLiMM) with data on number of parasitoids attracted as the dependent variable, host plants as independent variables and time as a covariate. A log linear poisson regression model was fit for the count data.

Influence of host plants on the parasitoids using olfactometer studies

The orientation experiment of parasitoid was carried out with eight arm, six arm and 'Y' tube olfactometer to cram the influence of host crops on parasitoid efficiency. The outcome of the orientation of *A. papayae* towards the odours of different host plants were given below. An attempt has been made to find the parasitoid attraction pattern between host plants, when they are healthy and when they are infested with mealybugs over time (5, 10, 15, 20 and 30 minutes). The results showed significant variation in the orientation of *A. papayae*. The parasitoids made more positive movements towards the odours from the infested leaves than the healthy leaves of plants. Insects respond to different olfactory cues like volatiles from plants (e.g., phytophagous insects), host odours (e.g., parasitoids and predators), and pheromones for mate searching and aggregation. In the present study, it was observed that there were more number of parsitoid attraction events towards the infested hosts than healthy host plants over time. This was supported by the findings of Zhang *et al*. (2004), who reported that female parasitoids were attracted to *Phenacoccus solenopsis* infested cotton leaves.

It may be observed that there were more number of parsitoid attraction events in the infested study than the study with healthy host plants over time. Mealybug alone is used as standard arm, to check the presence of any tropic interaction between the pest, parasitoid and host plants against control arm. It may also be observed that there was a uniform trend of more parasitoids visiting the plants over time that there was an increase in the attraction of parasitois.

Host crop influence studies - Eight arm olfactometer

Eight arm olfactometer using healthy host plant leaves

Time variable had been introduced as a covariate and hence it was not presented in the table. The 'B' value signifies the weightage given to each healthy host plant based on the number of parasitoids attracted and the p value provides the statistical significance of the poisson loglinear estimate when time (minutes after release) is a covariate (Table 1).

Table 1. Generalized Linear Model (GLiM) – Loglinear Poisson Regression Model in eight arm Olfactometer using healthy host plant leaves

Parameter	B	p value	Bonferroni Test
Papaya	1.2	<0.001	<0.001
Cotton	0.6	0.056	0.368
Tapioca	0.0	-	-
Mulberry	0.2	0.506	1.000
Brinjal	0.0	-	-
Hibiscus	0.0	-	-
Standard	3.4	<0.001	<0.001
Untreated	0.0	-	-

It was observed that there is a significant difference between the arms. However, standard arm showed statistically significant difference among the eight arms by attracting more number of parasitoids, followed by papaya, cotton and mulberry. The post hoc Bonferroni test reveal that papaya and the standard arm differed significantly from the untreated arm (control) in terms of number of parsitoids attracted (Table 2). Others host plants did not show any trend of attraction over the time. Weightage (B value) was higher for the standard arm (3.4) followed by papaya (1.2). Cotton (0.6) and mulberry (0.2) weightage, whereas no weightage for other plants.

Table 2. Mean parasitoids attracted in eight arm olfactometer with healthy host plants

Healthy host leaves	Number of parasitoids attracted (numbers)*									
	5 MAR		10 MAR		15 MAR		20 MAR		30 MAR	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Papaya	0.6	0.5	0.9	0.7	1.1	0.6	1.4	0.7	1.4	0.7
Cotton	0.4	0.5	0.5	0.5	0.6	0.5	0.7	0.7	0.7	0.7
Tapioca	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mulberry	0.3	0.5	0.3	0.5	0.4	0.5	0.5	0.5	0.5	0.5
Brinjal	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hibiscus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Standard	4.1	1.4	4.8	1.5	9.0	1.2	12.9	1.4	15.5	1.7
Untreated	0.0	0.0	0.2	0.4	0.4	0.5	0.4	0.5	0.6	0.7

*Mean of ten replications
MAR – minutes after release

Eight arm olfactometer using infested host plant leaves

Table 3. Generalized Linear Model (GLiM) – Loglinear Poisson Regression Model in eight arm olfactometer using infested host leaves

Parameter	B	p value	Bonferroni Test
Intercept	-30.6	<0.001	<0.001
Papaya	32.3	<0.001	<0.001
Cotton	31.8	<0.001	<0.001
Tapioca	0.0	-	
Mulberry	31.3	<0.001	<0.001
Brinjal	30.8	<0.001	<0.001
Standard	31.9	<0.001	<0.001
Hibiscus	30.0	<0.001	<0.001
Untreated	0.0	.	

Table 3 exhibited that there was a significant difference among arms. However, standard arm and papaya leaves showed significant difference among the eight arms by attracting more number of parasitoids, followed by cotton and mulberry. Five minutes after release infested papaya leaves and standard arm (mealybug) attracted same number of parasitoids (2.6 ± 0.5) followed by cotton (2.2 ± 1.0) and there was no attraction to tapioca leaves. 10 minutes after release (MAR), the attraction was increased in papaya leaves (4.7 ± 0.5) than standard (4.2 ± 0.6). At 15 MAR, 20 MAR and 30 MAR there was a substantial increase in the number of parasitoids attracted to infested papaya leaves (5.9, 7.4 and 8.3 numbers respectively) than standard arm (4.1, 4.0 and 3.9 numbers respectively). Infested cotton leaves also attracted more number of parasitoids as standard arm on 15 MAR and 20 MAR (4.0 numbers in both) and it was increased after 30 minutes which was higher than standard arm (4.1 numbers) (Table 4).

This finding was supported by many authors, who reported the tropic interaction of pest, plants and parasitoids. Volatiles emitted by mealybug-infested plants are also suspected to attract natural enemies of the mealybug (Nadel and van Alphen, 1987). This has been supported by Calatayud *et al*. (1994), who reported that changes in chemicals produced by the cassava plant due to *Phenacoccus manihoti* infestation resulted in the emission of volatiles and it attracted *Apoanagyrus lopezi* and *A. diversicornis* (sexual strain) towards them. Females of various parasitoids of herbivores make use of plant odours induced by insect feeding to locate host plants that may carry their hosts (Vet and Dicke, 1992; Turlings and Benrey, 1998; Turlings and Wäckers, 2004).

Table 4. Mean parasitoids attracted in eight arm olfactometer with infested host plants

Infested host leaves	Number of parasitoids attracted (numbers)*									
	5 MAR		10 MAR		15 MAR		20 MAR		30 MAR	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Papaya	2.6	0.5	4.7	0.5	5.9	0.7	7.4	0.8	8.3	0.5
Cotton	2.2	1.0	3.3	0.7	4.0	0.7	4.0	0.7	4.1	1.0
Tapioca	0.0	0.0	0.2	0.4	0.2	0.4	0.2	0.4	0.0	0.3
Mulberry	1.0	0.8	2.5	0.5	2.3	0.5	2.3	0.5	2.0	0.5
Brinjal	0.9	0.7	1.5	0.7	1.4	0.7	1.5	0.7	1.3	0.7
Hibiscus	0.6	0.5	0.8	0.6	0.6	0.5	0.6	0.5	0.4	0.5
Standard	2.6	0.5	4.2	0.6	4.1	0.6	4.0	0.8	3.9	0.0
Untreated	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Mean of ten replications
MAR – minutes after release

The trend of parasitoid in the present study was supporting the report of Vinson (1981) and Weseloh (1981), that many parasitic hymenoptera use olfactory cues and responses to orient first towards a potential host habitat and second towards their host. The present findings were in accordance with result of Reddy *et al*. (2002) who indicated that the sex pheromone and larval frass volatiles from the diamondback moth, as well as volatile compounds from cabbage, may be used to locate their diamondback moth host by the parasitoids *Trichogramma chilonis, Cotesia plutellae* and predator *Chrysoperla carnea*.

Host crop influence studies - Six arm olfactometer

Six arm olfactometer using healthy host plant leaves

It was observed that there is a significant difference between the arms. However, papaya showed statistically significant difference among the six arms by attracting more number of parasitoids (weightage 0.2), followed by cotton and control. The post hoc Bonferroni test (Table 5) revealed that the brinjal, mulberry and tapioca were differed significantly from the untreated arm (control) in terms of number of parsitoids attracted (Table 6).

Table 5. Generalized Linear Model (GLiM) – Loglinear Poisson Regression Model in six arm olfactometer using healthy host leaves

Parameter	B	p value	Bonferroni Test
Papaya	0.2	0.368	0.368
Cotton	-0.5	0.012	0.012
Tapioca	-0.2	<0.001	<0.001
Mulberry	-0.7	<0.001	0.001
Brinjal	-0.6	<0.001	0.001
Control	0.0		

Table 6. Mean parasitoids attracted in six arm olfactometer with healthy host plants

Healthy host leaves	Number of parasitoids attracted (numbers)*									
	5 MAR		10 MAR		15 MAR		20 MAR		30 MAR	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Papaya	0.6	0.5	0.8	0.4	1.2	0.4	2.0	0.7	2.4	0.5
Cotton	0.2	0.4	0.2	0.4	0.6	0.5	1.0	0.7	1.6	0.8
Tapioca	0.0	0.0	0.0	0.0	0.6	0.8	0.6	0.8	0.8	0.8
Mulberry	0.0	0.0	0.0	0.0	0.6	0.8	0.8	0.8	1.4	0.5
Brinjal	0.0	0.0	0.0	0.0	0.6	0.8	1.2	0.8	1.0	0.7
Control	0.3	0.5	0.7	0.7	1.3	0.5	1.8	0.9	2.0	0.8

*Mean of ten replications
MAR – minutes after release

Six arm olfactometer using infested host plant leaves

The table 7 exhibited a significant difference among the arms having different host leaves. Bonferroni test revealed higher weightage (B value) to papaya (5.86) followed by cotton (3.14). However, while comparing 20 number of parsitoids released to host plants, infested papaya leaves attracted 2.2 ± 0.8 parsitoids after five minutes and it was followed by cotton with that of 1.4 ± 0.5 number of parasitoids. There was no attraction to tapioca leaves. After 10 minutes the attraction was increased in papaya leaves (3.7 ± 1.3) and tapioca also attracted 1.1± 0.7 numbers. At 15MAR, 20 MAR and 30 MAR there was a substantial increase in the number of parasitoids attracted to infested papaya leaves (5.4, 9.1 and 10.1 numbers respectively) than other plants. On the other hand at 30 MAR, tapioca (0.7) had very lesser number of parsitoids attracted (Table 8).

Table 7. Generalized Linear Model (GLiM) – Loglinear Poisson Regression Model in six arm olfactometer using infested host leaves

Parameter	B	p value	Bonferroni Test
Papaya	5.86	<0.001	<0.001
Cotton	3.14	<0.001	<0.001
Tapioca	0.86	<0.001	<0.001
Mulberry	1.82	<0.001	<0.001
Brinjal	1.02	<0.001	<0.001
Control	0		

Table 8. Mean parasitoids attracted in six arm olfactometer with infested host plants

Infested host leaves	Number of parasitoids attracted (numbers)*									
	5 MAR		10 MAR		15 MAR		20 MAR		30 MAR	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Papaya	2.2	0.8	3.7	1.3	5.4	0.8	9.1	0.7	10.1	0.6
Cotton	1.4	0.5	2.1	0.7	3.2	0.6	5.2	0.8	5.0	0.8
Tapioca	0.6	0.5	1.1	0.7	1.7	0.7	1.4	0.5	0.7	0.5
Mulberry	1.1	0.6	1.9	0.7	2.5	0.5	2.4	0.5	2.4	0.5
Brinjal	0.4	0.5	1.0	0.5	1.6	0.7	1.7	0.7	1.6	0.7
Control	0.0	0.0	0.4	0.5	0.4	0.5	0.2	0.4	0.2	0.4

*Mean of ten replications
MAR – minutes after release

In the present study using eight arm olfactometer, mealybug alone was tested against host plant leaves as standard. However, this situation may not occur naturally under any circumstances. Hence economically important plants except hibiscus were tested in the six arm olfactometer against control. The results revealed that mealybug infested papaya and cotton leaves attracted more number of parasitoids over other plants tested against the healthy leaves. This results indicated that parasitoid have associated learning as inborn character. Since, they chose the plants that the mealybugs evolved naturally such as papaya, which is the natal host of this pest. This is in accordance with the ability of females of many parasitoid species that are able to learn to associate specific plant-produced odours with the presence of hosts. This is supported by Tamo *et al.* (2006), who concluded that *Cotesia marginiventris* and *Campoletis sonorensis* chose the odour of the plant species that they had evolved, but *Microplitis rufiventris* showed an even stronger preference for maize odours, independently of the plant they had experienced earlier. Zurcher (2006) reported the plant odour preferences and learning ability of three solitary endoparasitoids of *Spodoptera* species.

Host crop influence studies – 'Y' tube olfactometer Studies

The count data of 'Y' tube olfactometer studies followed the normal distribution (Kolmogrov Smirnov, p>0.05) and hence one-way ANOVA was employed with the three readings recorded in each experiment that includes the number of parasitoids moving towards healthy arm, the infested arm and the number of parasitoids that made no choice among the host plants.

Experiment on healthy and infested host plant leaves

The results exhibited that there was a significant difference between the arms of 'Y' tube. However, the arm having infested leaves attracted more number of parasitoids and showed statistically significant difference than healthy leaves. The parasitiods made more positive movements towards the odours from the infested leaves over healthy (Table 9) Comparing the preference of the 20 parasitoids towards the healthy and infested host leaves, papaya recorded more number of parasitoids attraction (18.0 ± 0.7) to infested leaves than healthy leaves (2.4 ± 1.2) and 0.2 ± 0.4 recorded no response to either. Papaya was followed by cotton by having an attraction of 16.6 ± 0.5 parasitoids. Mulberry and brinjal were grouped in attracting 16.0 ± 0.7 and 15.7 ± 0.8 number of parasitoids respectively. The parasitoids that were not chosen any leaves was higher in tapioca (2.9 ± 0.9) followed by hibiscus (2.0 ± 0.8).

Table 9. Parasitoid movement in 'Y' tube olfactometer with healthy and infested leaves of host plant

Host plants	No. of parasitoids released	Attraction of parasitoids (numbers)*		
		Towards infested leaves	Towards healthy leaves	No Response
Papaya	20	18.0^{a} ± 0.7	2.4^{c} ± 1.2	0.2^{d} ± 0.4
Cotton	20	16.6^{b} ± 0.5	3.5^{a} ± 0.8	0.1^{d} ± 0.3
Tapioca	20	14.1^{d} ± 1.0	3.1^{b} ± 1.3	2.9^{a} ± 0.9
Mulberry	20	16.0^{c} ± 0.7	3.4^{a} ± 0.8	1.0^{c} ± 0.9
Brinjal	20	15.7^{c} ± 0.8	3.3^{b} ± 1.1	1.4^{c} ± 0.5
Hibiscus	20	13.9^{d} ± 0.6	4.3^{a} ± 0.7	2.0^{b} ± 0.8
One Way ANOVA	F	45.8	3.8	24.3
	p	<0.0001	0.005	<0.0001

*Mean of ten replications
Post hoc test – Duncan's homogenous subsets.

Experiment on infested leaves Vs infested leaves with mealy covering

The results (Table 10) exhibited that there was significant difference between the arms of 'Y' tube. However, the arm having infested leaves with mealy covering of mealybug attracting more number of parasitoids and showed statistically significant difference than infested leaves alone. The parasitiods made more positive movements towards the odours from the infested leaves with mealy covering over the infested leaves alone. Comparing the preference of the 20 parasitoids towards the host leaves, papaya recorded more number of parasitoids attraction (15.6 ± 1.1) to infested leaves with mealy covering than infested leaves alone (4.4 ± 1.1). However, there was no choice for parasitoids to select 'no response'. Papaya was followed by cotton that attracted 14.4 ± 0.7 parasitoids. Tapioca and hibiscus were grouped in attracting 12.1 ± 1.0 and 11.7 ± 0.5 number of parasitoids respectively. The parasitoids that has not chosen choice was not often and observed in tapioca (0.3 ± 0.5), brinjal (0.2± 0.4) and hibiscus (0.1± 0.3).

Table 10. Parasitoid movement in 'Y' tube olfactometer with infested leaves and infested leaves with mealy covering of host plant

Host plants	No. of parasitoids released	Attraction of parasitoids (numbers)*		
		Towards infested leaves	Towards infested leaves with mealy covering	No Response
Papaya	20	$4.4^{c} \pm 1.1$	$15.6^{a} \pm 1.1$	$0.0^{b} \pm 0.0$
Cotton	20	$5.6^{b} \pm 0.7$	$14.4^{b} \pm 0.7$	$0.0^{b} \pm 0.0$
Tapioca	20	$7.6^{a} \pm 1.0$	$12.1^{d} \pm 1.0$	$0.3^{a} \pm 0.5$
Mulberry	20	$6.1^{b} \pm 0.7$	$13.8^{c} \pm 0.6$	$0.1^{a} \pm 0.3$
Brinjal	20	$6.3^{b} \pm 0.7$	$13.5^{c} \pm 0.7$	$0.2^{a} \pm 0.4$
Hibiscus	20	$8.3^{a} \pm 0.5$	$11.7^{d} \pm 0.5$	$0.0^{b} \pm 0.0$
One Way ANOVA	F	45.8	3.8	24.3
	p	<0.0001	0.005	<0.0001

*Mean of ten replications

The parasitoid orientation in Y - tube olfactometer of the present study revealed that, infested leaves attracted more number of parasitoids, when they are tested with healthy leaves. Infested leaves with mealy covering attracted even a more numbers than in infested leaves alone.

Experiment on combination of infested leaves of host plants

The outcome of the experiments showed significant variation in the orientation of parasitoid to odours of different host plants. In natural environment, all the selected host plants will not exhibited together. So, combination of the host plants was tried using the correlation matrix and they were presented in the table 11. The table showing the mean number of parasitoids attracted to the plants when they combined with each other. The infested leaves were taken as the treatment. The upper diagonal of the matrix shows the mean number of parasitoids attracted to the primary arm that the first host of the paired combination and the lower diagonal is data on attraction to the paired plants.

Papaya engrossed more number of parasitoids, when it combined with other host plants, while tapioca with other combinations, attracted lowest number of parsitoids than the paired host. When cotton combined with other plants, it enthralled more number of parasitoids except in combination with papaya. Infested mulberry leaves attracted marginally higher than other plants except with papaya and cotton. Brinjal recorded more parasitoids than hibiscus. Hibiscus was observed to be higher in attracting parasitoids with tapioca only. These results indicated that the papaya is highest preferred host for parasitoid, while tapioca is the least preferred host. The upshot of the experiment revealed that the preference level of parasitoid to infested leaves has been in the order 'papaya > cotton > mulberry > brinjal > hibiscus > tapioca'.

Table 11. Matrix on attraction of parasitoids in 'Y' tube olfactometer using combination of infested host leaves

Host leaves	Papaya	Tapioca	Cotton	Mulberry	Brinjal	Hibiscus
Papaya	*	7.8	8.2	7.2	8.8	8.6
Tapioca	1.6	*	1.6	1.2	0.4	2.6
Cotton	1.8	6.0	*	6.2	6.2	6.8

Mulberry	1.6	5.8	2.6	*	6.0	6.6
Brinjal	1.2	4.2	3.6	3.2	*	4.6
Hibiscus	0.8	1.4	3.2	3.2	4.4	*

All the six host plants tested will not be occur together in the natural environment. So to test the efficiency of parasitoid simulated in a natural system, the plants were tested in different permutations in Y-tube olfcatometer. The plants attracted different number of parasitoids at different permutations of the plants. Infested papaya leaves attracted more numbers, whereas tapioca leaves attracted less numbers than others, when combined with other plants. Next to papaya, cotton attracted more parasitoids in all the permutations except in papaya – cotton combination. This might be due to the volatiles emitted by different plants vary in their dominance over others, as discussed above.

IV. Conclusion

The olfactometer studies revealed that there were more number of parsitoid attraction events towards the infested hosts than healthy host plants over time. Finally the present investigation on adaptive plasticity of parasitoid on mealybug from different host plants has revealed the importance of host plant odour on the parasitoid efficiency. Future research should be focused on capitalizing phenotypic plasticity as an adaptive mechanism in generalist parasitoids living in changing environments, determining the effect of high plastic parasitoids on the efficiency of pest control, and quantifying the relative frequency and dynamics of these *A. papayae* and mealybug-host populations in the field.

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