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Orientation and Behavioural Responses of *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) Towards Honey and Sucrose

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Abstract: In present study effect of sucrose, honey odour and its different fractions on orientation and behaviour of *Cotesia plutellae* (K) (Hymenoptera: Braconidae), a larval endoparasitoid of *Plutella xylostella* (L) (Lepidoptera: Plutellidae), was studied under laboratory conditions. Orientation responses of naïve, unmated and unfed adults of *C. plutellae* were tested in a T-shaped tunnel. Female wasps showed attraction, antennation and mouth parts extension to honey at a distance in response to its odour. Attraction of females to honey was innate because the wasps derived from sucrose-fed parents showed same level of attraction to honey as those derived from honey-fed parents. Hexane fraction of hexane-extracted honey attracted females, but did not elicit antennation and mouthparts extension. Aqueous fraction of hexane-extracted honey elicited latter two responses but no attraction. Male wasps showed antennation and mouthparts extension but no responses to honey odour. A comparison of Preference Indices of the wasps for different fractions showed that their attraction to the hexane fraction and antennation/mouthparts extension to the aqueous fraction were significantly less than those to the un-extracted honey. The present study suggests that it may be helpful in biological control with *C. plutellae* to use honey odour in the background of an appropriate, nutritionally efficient, food like sucrose or a formulation of various nutrients which may itself be unattractive to the parasitoids.

Keywords: *Cotesia*, Honey, Orientation, Honey fraction, Antennation

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Introduction

Hymenopterous parasitoids have been reported to require adequate nourishment in adult stage to survive, produce eggs, move

about to forage and parasitize their host insects (Jervis and Kidd, 1986; Takasu and Lewis, 1993, 1995; Heimpel *et al.*, 1997; Lewis

et al., 1998; Costamagna and Landis, 2004; Wäckers, 2005). In nature, some adult parasitoids feed on their host insects (Jervis and Kidd 1986; Heimpel and Collier 1996; Jervis *et al.* 1996) whereas many others feed on non-host food sources such as nectar from nectaries and honeydew on various plants (Zobelein, 1955; Leius, 1967; Jervis and Kidd, 1986; Jervis *et al.*, 1993; Idris and Grafius, 1995; Wäckers *et al.*, 1996; Wäckers and Steppuhn, 2003; Wäckers, 2005). Food provided to various parasitic hymenopterans reared or maintained in insectaries and laboratories includes honey and sugars, particularly sucrose. Different food sources have been reported to differ in their influence on longevity, fecundity, mobility and host-foraging activity of various parasitoids (Leius, 1961a, b; Hagley and Barber, 1992; Mitsunaga *et al.*, 2004; Witting-Bissinger *et al.*, 2008). The food sources that specifically enhance these life processes of parasitoids can be manipulated appropriately for boosting their populations on pest-infested crops to increase parasitization and suppression of their host pest populations (Munir *et al.*, 2018).

Trials conducted for manipulating food sources of various parasitoids have been reviewed by Wade *et al.* (2008b), discussing their successes and limitations. Two main approaches have been followed for such manipulations: (i) growing in the vicinity of pest-infested crops such non-crop plants which are rich in floral /extra-floral nectaries or homopteran honeydew which serve as nutritious food sources for adult parasitoids; (ii) applying appropriate artificial food supplements consisting of one or more nutrients like sugars, proteins, etc., to pest-infested crop plants or to neighboring non-crop plants. In both these approaches,

parasitoids can readily move between food sources and pest-infested crops, obtaining nourishment from the former and parasitizing their hosts on the latter. The first of these approaches has a limitation that non-crop plants bearing food sources of parasitoids may not be synchronous in time and space with host pests and their crop plants in appropriate stage (Heimpel and Jervis, 2005; Wade *et al.*, 2008 b). Under such circumstances, applying an artificial food supplement to pest infested crop plants or to non-crop plants in its vicinity is desirable. Artificial food supplements so used in some trials include sugars, particularly sucrose (Canäs and O'Neil, 1998) and honey alone (Takasu and Lewis, 1995), or in combination with other nutrients (Wade *et al.*, 2008 a).

In order to choose an appropriate food supplement for adult parasitoids, its nutritional as well as sensory properties need to be taken into consideration. The nutritional properties include nutrient composition of food and its utilizability for parasitoids whereas its sensory properties include the sensory stimuli. Nutritional properties can play their role only after a food supplement is found and fed upon by parasitoids. Finding and feeding on a food supplement is determined by its sensory stimuli some of which (olfactory, visual) are detected by parasitoids at a distance whereas others (gustatory stimuli) are detected by them after contact with the food. Distance-detectable stimuli from a food source may elicit orientation response of a parasitoid involving its attraction to or repulsion from the food source. If these stimuli attract the parasitoid, it moves to the food source. After contact with the food, if its gustatory stimuli are phagostimulatory, the parasitoid responds by

feeding on it. Thus, a food supplement with attractants and phagostimulants is likely to be found and fed upon by parasitoids earlier, thereby avoiding the risk of death due to starvation, than a food source that lacks attractants and can be found by the parasitoids by chance during their random searching movements.

Nectar-bearing flowers of some plants have been reported to emit odours which attract naïve adults of various parasitoids (Wäckers and Swaans, 1993; Stapel *et al.*, 1997; Wäckers, 2004) as well as those having prior experience of associating those odours with food (Takasu and Lewis, 1993, 1996; Siekmann *et al.*, 2004). Among the artificial food supplements like sucrose and honey, sucrose does not attract any parasitoid but acts as a strong phagostimulant (Wäckers, 1999; Winkler *et al.*, 2006) and is fed upon readily when it comes in contact with the parasitoid.

Males and females of parasitoid, *Pimpla alboannulatus*, were reported (Sato and Takasu, 2000) to show flight response indicating their attraction to honey odour in a wind tunnel after they were conditioned to its odour by feeding on honey for three days. Some naïve females, but not males, also showed flight response to honey odour. Their attraction was not considered to be innate because these females emerged from pupae which developed in host larvae reared on artificial diets with honey as an ingredient. The honey odour could have persisted in the pupae and might have conditioned the experimental wasps during or after emergence.

There is hardly any report of innate attraction of naive hymenopterous parasitoids

to honey odour. Therefore the *Cotesia plutellae* adults were used to study certain aspects of orientation to honey and sucrose.

Materials and Methods

Insect culture

Larvae of diamondback moth (DBM), *Plutella xylostella*, parasitized by *Cotesia plutellae* were collected from cauliflower plants near Delhi (India) and reared on its leaves in the laboratory at 25 ± 1 C under $65 \pm 5\%$ R.H. and 14L:10D photoperiod. The pupae formed by the parasitoid larvae emerging from the DBM were placed in separate jars for emergence. The emerging wasps were transferred to a clear acrylic ventilated chamber (20 x 20 x 20 cm) and given 50 % honey in water as food. These wasps were allowed to mate, and oviposit on late second instar DBM larvae which were transferred to clean jars and reared on cauliflower leaves in the absence of honey.

For each test, required number of parasitoid pupae were drawn from the culture and kept one each in a glass vial (60 mm long, 15 mm dia). Virgin, water satiated and food-deprived wasps were used for various tests within 4 – 8 h of their emergence. For some tests, the experimental wasps were derived from parents that were fed 1 M sucrose solution instead of 50% honey solution, and the parasitoid larvae developed within DBM larvae reared on cauliflower leaves in the absence of honey. All tests were carried out in a room with exhaust facility and maintaining 25 ± 1 C and $65 \pm 5\%$ R.H.

Tests for Orientation Responses

The orientational responses were conducted in a T-shaped glass tunnel employing a modification of the method described by

Saxena and Prabha (1975) (Fig. 1). The T-tunnel consisted of a 75 mm long, vertical arm (10 mm bore) opening perpendicularly into the middle of a 150 mm long horizontal arm (15 mm bore) from below, demarcating it into two side arms. Each side arm was graduated in millimeters starting from the entry arm junction. Each side arm was provided with a snugly fitting plunger which consisted of a glass vial (60 mm long, ≈ 15 mm outer dia.) with a narrower mouth (10 mm inner dia.) at its inner end facing the entry arm junction, and a flat bottom at its outer end, attached to a glass rod (60 mm long, ≈ 15 mm dia) with parafilm. The glass rod partly projected out of the side arm through its open end and served as a handle to slide the vial in and out. The T-tunnel was supported on two cylindrical stands (25 cm ht x 4 cm dia), one under each side arm, with the entry arm projecting vertically downward between the stands. The tunnel was illuminated from above by two 20 W fluorescent tube lights (60 cm each) at a height of 50 cm from the horizontal arm. The light intensity at the level of the tunnel was 450 lux.

Fig.1: Schematic representation of T-tunnel to study the orientation response.

For each test, plungers were taken out of the side arms and their vials were detached, cleaned and re-attached to their rods. A

rectangular strip (55 x 25 mm) of Whatman No. 1 filter paper was rolled up and inserted into each vial through its mouth to form a lining around the inner side of its wall, leaving its bottom and 8-mm length behind the mouth bare. The filter paper in one vial (designated 'A') was impregnated with 0.3 ml solution of honey (50%), its fractions or sucrose (1 M). The filter paper in the opposite vial (designated 'B') was either blank or impregnated with 0.3 ml of distilled water or 1M sucrose solution that served as a control or a second choice against the material in vial 'A'. In tests with hexane fraction of honey in vial A, the control in vial B consisted of filter paper impregnated with 0.3 ml plain 5 % paraffin oil. The mouth of each vial was then covered with a nylon net (12 meshes/cm), and promptly introduced into their respective side arms. The mouth of each vial was kept at a distance of 50 mm from the entry arm junction. A wasp was introduced into the entry arm through its lower open end 60-65 s after positioning the vials in the side arms and its behaviour was observed continuously for 5 min. After this period, the wasp was removed, the plunger vials were cleaned and test was repeated with another wasp, interchanging the position of vials in the side arms so as to eliminate positional effect. The test was repeated with 50 wasps arranged in five replicates of 10 each.

Test Materials

Responses of *C. plutellae* wasps were tested towards the following materials: (i) honey (Dabur, India) as 50% solution in water; (ii) sucrose as 1M solution in water; (iii) two fractions of honey that were prepared as follows: A measured volume of 50% honey solution was shaken with three successive equal aliquots of *n*-hexane in a separating

funnel. The lower aqueous phase of the three lots was drained off and the upper hexane fractions of the three lots were pooled together. The aqueous fraction was used as such. The pooled hexane fraction was used for the bioassays after concentrating it in a rotary evaporator to the initial volume of the original 50% honey solution and adding paraffin oil to it to a concentration of 5%.

Statistical analysis

Oriental responses of wasps to materials in vial A were compared with those to materials in vial B in T-tunnel tests using Student's *t* test for paired samples. Preference of wasps for a response to honey or its fractions over that to water or control in T-tunnel tests was expressed as Preference Index which was calculated as $100[(a-b)/n]$, 'a' being the response to materials in vial A, 'b' that to vial B and 'n' the number of wasps tested in a replicate. Preference Indices for honey fractions were compared using one-way ANOVA. Two-way ANOVA followed by Tukey's HSD test, if required, was used to compare Preference Indices of wasps for honey among males and females derived from honey-fed and sucrose-fed parents.

Results

Responses of Wasps from Honey-Fed Parents

When unmated female wasp was released in the T-tunnel, significantly greater percentage of wasps ($P < 0.001$, *t* value 9.8) turned to move towards honey-bearing vial (74%; Table 1) than those (26%) moving first to the blank vial having dry filter paper. On presenting water-soaked filter paper in vial B against honey in vial A, the percentage of females turning and moving first to the honey-bearing vial was again greater (78%; Table 1) than water-bearing vial (22%), the difference being

highly significant ($P = 0.01$, *t* value 7.48). Similar results were obtained when sucrose solution (1 M) was presented in vial B against honey in vial A, the wasps turned and moved first to the honey-bearing vial in a significantly higher percentage (74 %) than to the sucrose-bearing vial (26%) ($P = 0.001$, *t* value 9.8) (Table 1). However, when sucrose solution was presented in vial A and water in vial B, the percentage (48%) of wasps turning and moving first to the sucrose-bearing vial was not significantly different from that (52%) to water-bearing vial ($P > 0.05$, *t* value -0.54).

Females which moved to honey-bearing vial, 80-84 % of them started antennation by bending their antennae downwards and tapping the surface with their antennal tips (Table 1). At the same time, they extended their mouthparts and probed the net cover. These wasps, being unable to reach on honey, moved back and reached to vial B containing blank, water-, or sucrose-impregnated filter paper. But they did not exhibit antennation and mouthparts extension.

The number of visits on vial containing honey was significantly higher than the vial containing water (*t*-value 5.18) (Table 1). However, the vial containing sucrose or blank did not differ significantly from the honey containing vial. Moreover, the wasp showed more number of visits on sucrose than the water containing vial (*t* value -2.77; Table 1). The duration spent by female wasp on the net cover of honey containing vial was statistically greater than the vials of water or sucrose (*t* value 14.31 and 9.51, respectively; Table 1). There was no such significant difference between the vials of sucrose and water ($p < 0.05$, *t* value 0.66).

Table 1: Orientation responses of adult *C. plutellae*, derived from honey-fed parent wasps.

Sex	Source of stimuli		% Response				
	Vial A	Vial B		First turning & reaching to vial mouth	Antennation & mouthparts extension	Number of visits on vial mouth	Staying on vial mouth during 5 min
Female	Honey	Blank	Vial A	74 ± 2.45***	84 ± 4.0***	7.46 ± 0.68 ^{ns}	45.12 ± 2.53***
			Vial B	26 ± 2.45	0.0 ± 0.0	6.8 ± 0.78	4.54 ± 1.47
	Honey	Water	Vial A	78 ± 3.74**	80 ± 5.48***	15.2 ± 0.88**	27.3 ± 1.92***
			Vial B	22 ± 3.74	00 ± 0.0	12.3 ± 1.15	0.58 ± 0.20
	Sucrose	Water	Vial A	48 ± 3.74 ^{ns}	00 ± 0.0	13.5 ± 0.42*	1.36 ± 0.23 ^{ns}
			Vial B	52 ± 3.74	00 ± 0.0	13.96 ± 0.30	1.22 ± 0.15
	Honey	Sucrose	Vial A	74 ± 2.45***	82 ± 4.90***	13.5 ± 0.22 ^{ns}	25.82 ± 2.54***
			Vial B	26 ± 2.45	0 ± 0.0	12.8 ± 0.37	0.64 ± 0.15
Male	Honey	Water	Vial A	46 ± 2.45 ^{ns}	68 ± 3.74***	10.44 ± 0.32*	14.78 ± 2.01**
			Vial B	54 ± 2.45	0.0 ± 0.0	9.58 ± 0.39	0.86 ± 0.16
	Sucrose	Water	Vial A	42 ± 3.74 ^{ns}	00 ± 0.0	13.78 ± 0.73*	1.3 ± 0.16*
			Vial B	58 ± 3.74	00 ± 0.0	14.54 ± 0.77	1.7 ± 0.19
	Honey	Sucrose	Vial A	56 ± 4.0 ^{ns}	68 ± 5.83***	9.4 ± 0.17 ^{ns}	12.94 ± 1.66***
			Vial B	44 ± 4.0	0.0 ± 0.0	9.2 ± 0.54	1.46 ± 0.12

, * Response significant at p<0.01 and p<0.001, respectively.

^{ns} Difference not significant statistically

Male wasps, derived from honey-fed parents, turned and moved first to honey-bearing vial did not differ significantly with that of water-bearing vial (46%, 54%; Table 1) ($p=0.05$, t value -1.63). However, they did show antennation and mouthparts extension (Table 1, 68 %) on the net cover of the honey-bearing vial. The number of visit on the honey vial was also significantly more than water bearing vial (t value 4.07). But no difference was observed between honey and sucrose vial (t value 0.46; Table 1). The male wasps also stay for longer duration on the honey bearing vial rather than water or sucrose vials (t value 6.51 and 7.36, respectively; Table 1).

Responses of Wasps from Sucrose-Fed Parents

Naïve unmated females derived from sucrose-fed parents, too, turned and moved first to the honey-bearing vial in a significantly greater percentage (68%; Table 2) than to the water-bearing vial (32%) (t value 4.81). However, males turned and moved first to the honey-bearing as well as water-bearing vials in almost equal percentages (50%, 50%; Table 2), indicating lack of their attraction by honey. Sucrose, too, did not attract these males and females (t value -2.45; Table 2). Both female and male wasps showed antennation and mouthparts extension on honey bearing vial but not on sucrose or water bearing vial (t value 11.22 and 7.48, respectively; Table 2). The number of visits on honey bearing vial did not differ significantly with water in both sexes ($p= 0.05$) (Table 2). However, both female and male wasps stayed significantly for longer duration on honey vial than water or sucrose bearing vials (t -test) (Table 2).

Preference of Wasps in Relation to Sex

Preference of the wasps for attraction, antennation and mouthparts extension to

honey in the T-tunnel was compared among males and females derived from honey-fed and sucrose fed parents on the basis of their Preference Index. Two-way ANOVA showed that Preference Index of female wasps derived from honey-fed parents for moving first to the honey-bearing vial was not significantly different from that of sucrose-fed parents (Table 3, F -value 0.563, df 1, $P = 0.464$, 2-way ANOVA). But these Preference Indices were significantly higher than those for corresponding males (Table 3, F -value 39.061, df 1, $P < 0.001$ by 2-way ANOVA).

With reference to antennation and mouthparts extension, Preference Indices showed significant differences between the two sexes (Table 3, F -value 9.389, df 1, $P = 0.007$, 2-way ANOVA) and, also, between the wasps derived from honey-fed and sucrose-fed parents (F -value 84.5, df 1, $P < 0.001$, 2-way ANOVA), both sexes from honey-fed parents showing these responses in a significantly greater degree than those derived from sucrose-fed parents (Table 3).

Preference index of number of visit by wasp on honey vial also differ significantly between the sexes (F -value 17.99, df 1, $P < 0.001$, 2-way ANOVA) and also between the honey fed and sucrose fed parents (Table 3, F -value 24.48, df 1, $P < 0.001$, 2-way ANOVA).

When the wasp reached on the honey vial mouth, the honey odour arrested the wasp and it searched on vial mouth for longer duration. The preference index showed that the duration of stay on the honey vial mouth differ significantly between the sexes (F -value 37.52, df 1, $P < 0.001$, 2-way ANOVA) and also between the honey fed and sucrose fed parents (Table 3, F -value 51.35, df 1, $P < 0.001$, 2-way ANOVA).

Table 2: Orientation responses of adult *C. plutellae*, derived from sucrose-fed parent wasps.

Sex	Source of stimuli		% Response				
	Vial A	Vial B		First turning & reaching to vial mouth	Antennation & mouthparts extension	Number of visits on vial mouth	Staying on vial mouth during 5 min
Female	Honey	Water	Vial A	68 ± 3.74**	42 ± 3.74***	13.34 ± 0.64 ^{ns}	13.22 ± 1.67**
			Vial B	32 ± 3.74	0.0 ± 0.0	12.76 ± 0.61	1.02 ± 0.12
	Sucrose	Water	Vial A	46 ± 4 ^{ns}	0.0 ± 0.0	12.02 ± 0.50 ^{ns}	1.48 ± 0.18*
			Vial B	54 ± 4	0.0 ± 0.0	12.22 ± 0.32	1.08 ± 0.15
	Honey	Sucrose	Vial A	64 ± 2.45**	42 ± 3.74***	12.06 ± 0.43 ^{ns}	11.2 ± 0.76***
			Vial B	36 ± 2.45	0.0 ± 0.0	11.36 ± 0.55	1.16 ± 0.24
Male	Honey	Water	Vial A	50 ± 5.48 ^{ns}	28 ± 3.74**	9.0 ± 0.34 ^{ns}	5.52 ± 0.44***
			Vial B	50 ± 5.48	0.0 ± 0.0	9.8 ± 0.52	0.78 ± 0.11
	Sucrose	Water	Vial A	44 ± 2.45 ^{ns}	0.0 ± 0.0	9.68 ± 0.51 ^{ns}	1.34 ± 0.18 ^{ns}
			Vial B	56 ± 2.45	0.0 ± 0.0	10.44 ± 0.54	1.28 ± 0.16
	Honey	Sucrose	Vial A	48 ± 3.74 ^{ns}	40 ± 3.16***	9.84 ± 0.58 ^{ns}	8.58 ± 1.67**
			Vial B	52 ± 3.74	0.0 ± 0.0	10.06 ± 0.66	1.22 ± 0.18

,* Response significant at p<0.01 and p<0.001, respectively.

^{ns} Difference not significant statistically

Table 3: Preference index¹ of orientation of *C. plutellae* adults towards vial A on different parameters

Test materials in vials		Preference index on vial A at different Parameters					
Vial A	Vial B	Parent fed	Sex	First turn and reaching to vial	Antennation and mouth parts extension	No of visits/ wasp in 5 minutes	Duration of stay/ wasp iin 5 min.
Honey (50% solution)	Water	Honey	Male	-8 ± 4.90 ^{aA}	68 ± 3.74 ^{aA}	8.6 ± 2.11 ^{aA}	139.2 ± 21.39 ^{aA}
			Female	56 ± 7.48 ^{bA}	80 ± 5.48 ^{aA}	29 ± 5.5 ^{bA}	267.2 ± 18.67 ^{bA}
		Sucrose	Male	00 ± 10.95 ^{aA}	28 ± 3.74 ^{aB}	-7.4 ± 0.75 ^{aB}	47.4 ± 4.7 ^{aB}
			Female	36 ± 7.48 ^{bA}	42 ± 3.74 ^{bB}	5.8 ± 3.6 ^{bB}	122 ± 16.3 ^{bB}

¹

Preference Index was calculated as $100[(a-b)/n]$, 'a' being the response to materials in vial A, 'b' that to vial B and 'n' the number of wasps tested in a replicate. Two way ANOVA was performed and means were separated by Tukey's test. Means followed by different upper case superscripts differ significantly ($p < 0.05$) within the same sex. Means followed by different lower case superscripts differ significantly ($p < 0.05$) in between the sexes.

Table 4: Orientation responses of adult *C. plutellae*, derived from honey-fed parent¹ wasps, to two fraction of hexane extracted honey in T-tunnel

Response parameters	Source of stimuli			Mean $\pm$ SEM
	Vial A	Vial B		
% wasps turning and moving first to vial mouth	Aqueous fraction	Water	Vial A	52 $\pm$ 3.74
			Vial B	48 $\pm$ 3.74
	Hexane fraction	Paraffin liquid	Vial A	66 $\pm$ 2.45
			Vial B	34 $\pm$ 2.45
% wasp antennating/ extending mouth parts on vial mouth	Aqueous fraction ²	Water	Vial A	32 $\pm$ 3.74
			Vial B	0.00 $\pm$ 0.0
	Hexane fraction ³	Paraffin liquid	Vial A	0.0 $\pm$ 0.0
			Vial B	0.0 $\pm$ 0.0
Number of visit /wasp on vial mouth in 5 minutes	Aqueous fraction	Water	Vial A	10.26 $\pm$ 0.69
			Vial B	10.06 $\pm$ 0.61
	Hexane fraction	Paraffin liquid	Vial A	12.24 $\pm$ 0.93
			Vial B	11.82 $\pm$ 0.95
Duration of stays (secs) on vial mouth in 5 minutes	Aqueous fraction	Water	Vial A	5.58 $\pm$ 0.56
			Vial B	0.48 $\pm$ 0.09
	Hexane fraction	Paraffin liquid	Vial A	1.50 $\pm$ 0.22
			Vial B	0.56 $\pm$ 0.05

*** Response significant at $p < 0.001$

^{ns} Difference not significant statistically

Table 5: Preference index¹ of adult *C. plutellae* female towards different fraction of honey

Test materials in vials		Preference index on vial A at different Parameters			
Vial A	Vial B	First turn and reaching to vial	Antennation and mouth parts extension	No of visits/ wasp in 5-min.	Duration of stay/ wasp in 5-min.
Honey (50% solution)	Water	56 ± 7.48 ^a	80 ± 5.48 ^a	29.0 ± 5.59 ^a	267.2 ± 18.67 ^a
Aqueous fraction of honey	Water	4 ± 7.48 ^b	32 ± 3.74 ^b	2.0 ± 1.26 ^b	51.0 ± 5.34 ^b
Hexane fraction of honey	Control (5% paraffin liquid)	32 ± 4.90 ^a	00 ± 0.0 ^c	4.2 ± 2.18 ^b	9.40 ± 1.81 ^b

¹Preference Index was calculated as 100[(a-b)/n], 'a' being the response to materials in vial A, 'b' that to vial B and 'n' the number of wasps tested in a replicate

Means followed by different superscripts are statistically significant (p<0.05) in a column at particular parameter

Responses of Wasps to Honey Extract

These females, when presented aqueous fraction of hexane-extracted honey, turned and moved first to this fraction (52%; Table 4) which was not significantly different from that (48%) moving to the water-bearing vial. Thus, water soluble components in honey did not attract the wasps. But hexane fraction attracted these wasps, their percentage turning and moving first to the vial bearing this fraction being significantly greater (66%; Table 4) than that (34%) to the water-bearing vial ($P < 0.001$, t-test). However, antennation and mouthparts extension were shown by the wasps to the aqueous fraction (32%; Table 4), but not to the hexane fraction of honey. The number of visits on vial A and vial B was not differed significantly in hexane as well as aqueous extract (Table 4). The female wasp stayed significantly longer duration on the vial A bearing aqueous extract of honey (Table 4).

A comparison of preference indices of the wasps for different fractions showed that their attraction to the aqueous fraction was significantly lower than unextracted or hexane extracted honey ($p < 0.05$, Table 5). Antennation/mouthparts extension to the unextracted honey was significantly higher than hexane or aqueous extracted honey fraction. ($p < 0.05$, Table 5). The number of visits and duration of stay on unextracted honey was again higher than the rest of the honey fraction ($p < 0.05$).

Discussion

Use of parasitoids to augment biological control programs required careful consideration of ecological factors and of relationship between a parasitoid nutrition and its success as a biological control agents. The importance of parasitoids is based on the

premise that they not only constitute important factor for regulating insect pest population, but are also sustainable and self-perpetuating (Lim, 1986). Use of nutrition in biological control program is necessitated due to lack of synchrony between natural enemy and their host or isolation of entomophagous insects from the natural environment that normally supplies alternate food sources such as nectar and honey dew (Hagan, 1986). Olfaction is the primary sense which is utilized by insect parasitoids to locate their food resources (Roux *et al.*, 2005). Odours emanating from food resources enhance their searching efficiency and reduces time and energy expended by them.

In the present study of orientation response, honey elicited the following three types of responses from naïve unmated *C. plutellae* adults of one or both the sexes at a distance, prior to physical contact: attraction, antennation and mouthparts extension.

Attraction of female adults to honey was indicated in T-tunnel elicited by vapour from honey solution on filter paper in the vial diffusing through its mouth and the side arm to reach the entry arm junction. Males differed from females in showing no attraction to honey and sucrose did not attract any of the two sexes in the T-tunnel. Soyelu (2014) also reported that *Cotesia vestalis* preferred honey odour against moistened air. However, female wasps preferred honey-beebread when provided choice with honey odour in olfactory chamber. Another hymenopterous parasitoid which has been shown to be attracted by honey odour is *Pimpla alboannulatus*, (Sato and Takasu, 2000). But adults of both sexes of this parasitoid, which showed attraction to honey odour in a wind tunnel, had been conditioned by feeding on honey before tests.

Even naïve females, which were attracted by honey odour, were regarded to be conditioned by previous exposure to honey odour remaining on the host pupae from the host larvae reared on artificial diets containing honey. *Cotelis plutellae* females thus differ from *Pimpla alboannulatus* in that their attraction to honey odour in T-tunnel tests was innate as suggested by the following facts: (a) they were reared on DBM larvae in the absence of honey so that there was no chance of their experiencing its odour prior to tests. (b) the females derived from honey-fed as well as sucrose-fed parents showed more or less the same level of attraction to honey odour; the Preference Index values of the two being not significantly different. Males of *Cotelis plutellae* were, however, similar to those of *Pimpla alboannulatus* in that they did not show any innate attraction to honey odour in the T-tunnel, regardless of whether their parents were fed on honey or sucrose. Even the odour of a chemical e.g., 1-octanol, to which the parasitoid *Microplitis croceipes* was conditioned to associate with sucrose as food, was reported to elicit its antennal bending (antennation) and mouthparts extension responses (Wäckers *et al.*, 2002).

The present work shows that honey odour elicited both these as innate responses from naïve males and females of *C. plutellae* at a distance, without physical contact in the T-tunnel. The distance from the honey at which the wasps showed these responses was much shorter viz. ≈ 10 mm from the honey-bearing vial in the T-tunnel, than the distance at which they were attracted viz., 50 mm from the entry arm junction in the T-tunnel.

The antennation and mouthparts extension responses elicited by honey odour from females and males derived from sucrose-

fed parents were almost one half of those from wasps derived from honey-fed parents, as indicated by their Preference Index values although no such differences between the two groups of wasps were observed in respect of their attraction. It is not conceivable how feeding the parent wasps on honey would enhance these two responses but not attraction when the experimental wasps had no exposure to honey prior to tests. An alternative explanation may be that differences in the food of parent wasps might have caused certain changes, possibly physiological, which could be responsible for the differences in the levels of their antennation and mouthparts extension responses to honey odour. In any case, these two responses of the wasps derived from sucrose fed parents were evidently innate.

The attractiveness of honey odour can be determined by the number of times a wasp reached on the odour source. In the present study, the adult wasp derived from the honey fed parents show higher number of visits towards the honey bearing vials. The adults derived from the sucrose fed parents did not differ in their number of visits on either of the test solution tested in the T-tunnel.

Honey odour shows the attraction and arrestment behaviour in the *C. plutellae* wasps derived from sucrose fed or honey fed wasps. The wasps stayed for the longer duration on the nylon net cover wrap around the honey bearing vial than water or sucrose in T-tunnel. The wasp continued search and antennate on vial mouth and tried to enter into the honey containing vial.

The broad categories of honey constituents that might be involved in eliciting

attraction, antennation and mouthparts extension responses from *C. plutellae* adults at a distance may also be considered on the basis of T-tunnel tests with two fractions of hexane-extracted honey. The aqueous fraction containing water-soluble constituents of honey did not attract the wasps entering the horizontal tube at the entry arm junction but elicited antennation and mouthparts extension from them when they arrived near or on the net cover of the mouth of the honey-bearing vial. The level of these responses was slightly less than half of that to the unextracted 50% honey solution. The hexane soluble constituents of honey elicited attraction but not antennation and mouthparts extension responses from these wasps. The attraction of the wasps to this fraction was almost one half of that to 50% honey solution. Thus, the distance at which the odour of water-soluble constituents of honey elicited antennation and mouthparts extension responses of the wasps was much shorter than that at which the odour of hexane-soluble constituents attracted them.

Conclusion

The present study suggests that it may be helpful in biological control with *C. plutellae* to use honey odour in the background of an appropriate, nutritionally efficient, food like sucrose or a formulation of various nutrients (Wade *et al.*, 2008 b) which may itself be unattractive to the parasitoids. The honey odour would then attract and bring the parasitoids to the proximity of the food. In order to avoid aggregation of ants on honey in the field, it will be desirable to use hexane soluble fraction of honey which attracts the parasitoids but not ants.

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