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Flower visiting, nectar feeding and oviposition behavioral responses in lepidopteran, hymenopteran and dipteran pollen collecting insect vectors

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Abstract

In the present investigation nectar feeding and ovipositional responses were observed in various pollen collecting insect vectors belong to different orders in vegetable garden. Flower-visiting and nectar feeding behavoiur in insects from three orders of class insect Lepidoptera, hymenoptera and diptera was observed. Both form and function of mouthparts are noted and described in detail. The process of nectar intake is discussed based on significant differences noted in nectar search, foraging time budget and feeding efficiency, choice of plant and floral architecture. With this remarkable differences in maxillary motions, and orientation behavior found in insects during foraging are also discussed. Based on observations made, nectar feeding in insects is a biphasic mechanism, in first phase insects made active searches for finding nectar, while in second phase they were observed trying to establish communication among con-specifics by making different sounds, or through scented marks left on the flowers visited or spread pollens in the air to signal the con-specifics. With this search, operations and communication patterns were found to be influenced by humidity, temperature and photoperiodicity in the territory, while chemical composition of nectar significantly affected the oviposition responses and F1 emergence in *Utetheisa pulcheloides* (Heliotrope Moth).

Key words: Nectar feeding, pollen vectors, hover fly, heliotrope moth, Oviposition behaviour

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1. Introduction

In insects, flower-visiting behavior is an evolutionary adaptive mechanism that solicits them to select specific plant species of its own choice. In turn unknowingly, insects perform nature's job as they transfer pollens among plants and help in cross-pollination [1]. Pollinating vectors rely on nectar to fulfill their essential nutritional requirements. For this purpose, insects made unlimited vast search operations either in groups or with their con-specifics to locate feeding grounds from sunrise to sunset. They invade foraging territories, compete with their con-specifics and communicate to each other by receiving chemical stimulus from volatiles/chemicals released in the air either from flowers, or secreted by flower visiting insects and follow the scented route [2]. However, most of the lepidopteran, hymenoptera and coleopteran insects raid diverse flowering plants, show different behavior patterns during search operations and collection of nectar and pollens in groups [3]. For knowing flower availability on plants and its density, pollen-collecting insects maintain routine checking of foraging grounds [4]. Insects can easily recognize preferred and un-preferred colors of the petals, pigments, size and types of flowers and length and size of nectar tubes [8]. With this, nectar-feeding efficiency in pollen collecting insects depends on amount of nectar and its composition [6] while flower color, shape and floral display [7] determine flower visiting frequency [5]. In addition, pigment color, flowering time and synthesis of sugars, amino acids and alkaloids enhance attraction [9], longevity [10] and oviposition performances in female insects [11, 12]. Further, structural modifications in mouth parts [13] such as long sucking proboscis much ably accelerate nectar feeding [14] thorough food canal taste sensilla [15].. Contrary to this, long foraging distances and presence of predators in the territory affect nectar feeding in insect. It also imposes high cost on offspring production in pollen vectors [16]. This plant-pollinator interaction provides many shared benefits to herbivorous pollen vectors [12] and flowering plants [17]. It regulates progeny production in insects [11] and increases the crop yield in pollinated plants [3]. In the present investigation both field, observations and laboratory experiments were conducted to test the nectar-feeding hypothesis and to determine its impact on reproductive behavior of insects. For this purpose, insects were provided different combinations of amino acids, sugars and honey solution while control insects were served only water to sip. Efforts are being made to compare the frequency of flower visits made by different eight species of insects belong to various arthropod groups on few endemic plant species such as Saunf (*Foeniculum vulgar*), Dania

(*Corundum sativa*) and Mulley (*Raphanus sativus*). Field observations were made to determine the nectar feeding efficiency, time-duration, and effect of temperature and photoperiodicity on nectar feeding.

2. Material and Methods

2.1. Collection, identification and fixation of insects

Adults of different lepidopteran, hymenopteran and dipteran insects were collected from University garden by using an insect catching net in the spring season during month of February, March and April 2011. From observation site specimens of various insect species were collected and chloroformed and observed microscopically for pollen load. Adult specimens of each insect species were preserved for further establishment of their identification and comparison. Insects were mostly identified by using taxonomic keys. They were fixed in 70% ethanol or in FAA solution, i.e. a mixture of 35% formalin, concentrated acetic acid and 80% alcohol (5: 1: 10). Whole mount preparations of the mouthparts were made from dissected heads. They were soaked in diluted lactic acid at 40-50 ^{0}C for 1-2 days, washed in distilled water and embedded on glass slides in polyvinyl lactophenol without dehydration. The preparations were covered with glass slides and dried at 50^0 C.

Serial semi thin-sections of mouthparts were cut to examine its anatomy under inverted light microscope. These were photographed to reconstruct the functional mechanisms of movements. The isolated heads were dehydrated with acidified DMP (2, 2-dimethoxypropane) and acetone, then embedded in ERL-4206 epoxy resin under vacuum impregnation [1] (Parnstich *et al*., 2003). Semi thin sections were cut by using sharp edged knives and stained with a mixture of 1% azure II and 1% methylene blue in aqueous 1% borax solution for approximately 1 min at 80 ^{0}C.

2.2. Insect feeding

For insect rearing clean, un-infected glass jars and covered with muslin cloth for ventilation were used. Culture was maintained in laboratory under controlled temperature (28±2^{0}C), relative humidity (75±5% RH) and a photoperiod of 12: 12 (L: D) h in B.O.D. (Biochemical Oxygen Demand). For knowing nectar feeding and ovipositional responses, insects were provided various feeding solutions such as amino acid mixture (5, 10, 15 mg/ml), amino acid mixture and sugar solution (1:2, 1:4 and 1:8), amino acid mixture and honey bee solution (1:1, 1:2, 1:4) by cotton swabs in separate glass jars. Control insects were served only water, through wet swabs having no ingredient. Nectar fed field collected insects screened and marked as positive control. These were carried to the laboratory, allowed to lay eggs on Whatmann filter paper sheets in separate glass chambers and have not been provided any sugar or amino acid containing diet feeding.

2.3. Behavioral observations

Foraging behaviors of insects were observed on different flowers such as Saunf (*Foeniculum vulgare*), Dhania (*Coriandrum sativum*) and Mulley (*Raphanus sativus*) starting from 5.30 a.m. in morning till evening regularly up to 8.30 p.m. A close watch interaction of insect and flower interaction was recorded for 4 hr each in morning and evening session and 2 hr for afternoon session.

2.4. Preparation of artificial flowers

Artificial flowers of mini size having 10-12 mm diameter were prepared by using crepe paper sheets of yellow, pink, blue and white, in rotating modes of rows with a central gynoecium like cavity having depth of 5-6 mm. Artificial flowers were designed like typical natural flowers having colored elongated petals assembled on artificial base. Paper folds were rotated slightly one over the other to provide shape and arrangement like flower petals. Petal tips were made round in shape and flowers were pasted in a vent position just as natural flowers remain adhere or affix to the stalk. Flowers were stalked very carefully by using cello tape on the plant twigs having moderate number of flowers somewhere on the middle height of the plant. These were handled with care by using forceps to avoid the mixing of any secreted amino acids naturally available on human hands. Various concentrations i.e 0.1 mM, 0.2 mM, 0.4 mM, 0.8 mM, 1.6 mM concentrations of amino acid mixture and sugar solution were applied separately in the bottom of each artificial flower by using small sized dropper and a 50-100 ul volume of each solution was applied very safely in the central cavity resembling to gynoecium. The main purpose behind use of artificial flowers was to know the primary cause of nectar feeding and attraction towards nectar bearing flowers and to compare flower-visiting frequency in nectar insect vectors. Meanwhile, efforts were made more cleverly to find color, odor and nectar based responses in insects by mimicking the effect of natural flowers by using artificial flowers. For this purpose, experiments were designed to differentiate the frequency at three levels, dose, and flower color and nectar ingredients available in the plants. It was found more feasible to differentiate between non-frequent, frequent and most frequent visitors among male and female insect vectors of each plant having flowers of near similar architecture and similar nectar presence.

2.5. Oviposition

For observing the effect of various ingredients on ovipositional responses, different concentrations of each solution were provided to insects in wet cotton swabs. Six replicates were set to determine oviposition inhibition responses in presence of each feeding solution. The number of eggs laid was recorded after 96 hrs in each case.

2.6. Biodiversity and insect behavior

Specimens of different insect species were collected from different sites, identified and preserved for further establishment of their proper identification. A complete list of nectar feeding insects was prepared after observing them more closely, repeatedly, and dissected to confirm their morphology and anatomy for proper identification. Insects those were found performing vital feeding activity were photographed in different orientations with the help of a photographic camera (KODAK).

2.7. Data analysis

Behavioral responses of in different insects were analyzed statistically to conclude the outcome in the field and as well as in the laboratory. The efficacy of the test stimuli was compared with control on the basis of eggs laid by each insect and oviposition induction or suppression index was calculated by using formula, 100 (A- B)/ (A+ B). Here A & B are being the number of eggs in the control and tests respectively.

3. Results and Discussion

3.1. Composition of nectar

Plant nectar contains high amount of carbohydrates mainly arabinose, galactose, mannose, gentiobiose, lactose, maltose, melibiose, trehelose, melezitose, raffinose, and stachyose . The concentration of these natural sugars varies greatly depending on the type and location of the nectar [19]. Nectar also contains amino acids [20] such as alanine, arginine, serine, proline, glycine, iso-leucine, threonine, and valine being the most prevalent. Besides this, plant nectar also contains organic acids [20], terpenes [21], alkaloids [22], flavonoids [23], glycosides [24], vitamins [19], phenolics [25], quercitin [26], and oils ([27]. It possesses many important metal ions. According to Heinrich [28] most plant nectars contain cations like K^+, making up 35 to 74 percent of the total cation content. Averages of other notable cations were Na^+ (17.9%), Ca^{2+} (12.8%), Mg^{2+} (5.9%), Al^{3+} (4.6%), Fe^{3+} (1.2%), and Mn^{2+} (0.8%). Besides this nectarin I is the most abundant of the nectar proteins having molecular mass 29 kDa. Nectarin belongs to germin-like protein (GLPs) conserved among dicotyledenous plants [29].

3.2. Insect diversity

From various observation sites different insect species collected were identified with their nectar feeding behavior, mouthparts, wing venation and color, orientation pattern. A complete list of nectar feeding insects is available in table 1. Key flower visiting insect species noted were *Callidilium* sp. (Long horn beetle) Order: Coleoptera; *Family*: Cerambycidae, *Utetheisa pulcheloides* (Heliotrope Moth) Family: Aganaidae; Order: Lepidoptera, *Syntomis phegea* (Nine spotted moth) Order: Lepidoptera' Family: Arctiidae, *Episyrphus balteatus* (Hover fly) Family: Syrphidae; Order: Diptera, *Ischiodon scutellaris* (Hover fly) Family: Syrphidae; Order: Diptera, *Camponotus hemi* (Carpenter ant) Family: Formicinae; Order: Hymenoptera, *Musca nabulo* (House fly) Order: Diptera; Family: Muscideae, *Syntomis phegea* (Nine spotted moth) Order: Lepidoptera Family: Arctiidae, *Pieris rapae* (White Cabbage fly) Family: Pieridae; Order: Lepidoptera *Episyrphus balteatus* (Hover fly) Family: Syrphidae; Order Diptera. Few insects like heliotrope Moth; *Utetheisa pulcheloides* (family Aganaidae order Lepidoptera) is a colorful insect of medium size 2-3.0 cm in length with a wing span of about 3.5cm (Photograph 1). Another moth *Syntomis phegea* [1], (Lepidoptera-Arctiidae) that feed on *Coriandrum sativum* and *Foeniculum* vulgare nectar was also observed and identified with its nectar feeding behavior and photographed (Photograph 2). Few other insects like *Musca nebula* (Diptera -Muscideae) showing different orientations during nectar feeding on *Coriandrum sativum* and *Foeniculum vulgare* was also identified (Photograph 3). Besides this, Hoverfly, *Episyrphus balteatus* (Family syrphidae Diptera) hovering in midair on blossoming flowers for feeding on nectar and pollens were also identified based on having one pair of wings, spots, bands or stripes of yellow, brown color against a dark-coloured background on the wing and thorax(Photograph 4). These possess spots and yellow bands on the abdomen having spurs behind the hind legs that grow to a length of 1/3 to 1 inch. These possess one pair of true wings and possess dense hairs, covering the body surface and emulating furry bumblebees (Table 1). These took highly active flights to invade flowers; sometimes flew very fast or became motionless. Besides hoverflies, carpenter ants and heliotrope moths were also identified that are involved in nectar feeding on a variety of plants. These are excellent and much needed pollinators. Hover fly on each nectar-gathering trip, visited many different plant species but found less efficient than Carpenter ants. In field experiments, pollen-collecting insects showed significant difference in its behavior when interacted to artificial colored flowers pasted over plant twigs (Photograph 5). Insects have shown rejection responses just after having single visit to such flowers. There was no secondary response made to the color of artificial flowers; exceptionally it was a defaulted no choice response. Even insects never utilized such flowers for a short rest or stay during feeding hours in the territory. Here, insects showed intense nectar feeding on nectar rich flowers in morning hours in comparison to noon and evening session. In morning hours short duration searches occurred with high frequency of nectar feeding in insects, as soon number of insects increases the search periods become longer. Here, flower color, density and nectar availability seems to provide great successes in reproductive behavior in insects

3.3. Flower visiting behavior

Among all the insects, hymenopterans and lepidopteran insects were found to be most frequent flower visitors and nectar feeders (Table 1). These insects usually made search operations much faster than other insects and move very frequently on flowers. Normally search operations start early in the morning but intense feeding occurred between 8-10 a.m. During this period, insects made more vital search operations to explore nectar-bearing flowers and tried to identify such flowers after visual cues obtained from bunches of inflorescence that bears variable number of flowers in group. Nectar feeding took place in two phases, in first phase insects lodge on to a target flower, arrive and orient positively, start and extend their proboscis to suck nectar from the ovary. For this purpose, insects pull down stamens or pollens, and open the nectar tube to reach to the ovary. Insects orient in such a position to sift on a nearer point and position to reach the ovary and turned on and on in clockwise or anti clock wise direction. This mechanism was observed in hover flies during the process of nectar feeding. These insects also exert the bunch of anther/ pollen to strike upon the legs (Photograph 4). After taking 2-3 sips on an average, hover flies flew to collect nectar from neighboring bunch of inflorescence. Same insect apply rotator mechanism when feed on *Foeniculum vulgare* nectar cups, and sip nectar from these cups one by one (Table 2). Contrary to this when bees visit Dhania (*Coriandrum sativum*) flowers they did not require rotating themselves but forage on nectar by sitting in anti parallel or parallel direction. There was a common practice among insects as they do not visit previously visited flowers and kept every visit in the memory. However, their incumbent companions visit the flower and reject it based on scent marks left by previous visitor insect. More specifically, hover flies possess a stiff leg with which they use to intake the nectar and also craft pollens with its mouthparts from the stamen and frequently suck the nectar (Photograph 4). Naturally, their forelegs hold many stamens at a time. Hover flies collect pollens, kept them on the scope of the hind femur, so then pollens are automatically removed off when they search next flower. It is a very rare phenomenon, which helps to know presence of nectar and reason of attraction of number of insects on such flowers. Insects also adjudged the plant species and nectar presence by flower display (Table 2). Insects have shown significantly higher nectar feeding and flower visiting efficiency in morning hours as mean flower visiting frequency noted was very high in *Ischiodon scutellaris* (Hover fly) as they attempted and spent 0.25±0.074 per minutes time/flower/event followed by *Utetheisa pulcheloides* (Heliotrope Moth) which spent 0.38±0.086 per minute time/flower/event (Table 3).. This flower visiting and nectar feeding efficiency was found to be significantly decreased in afternoon and insects have taken longer time to make each nectar search and feeding event successful. There was observed 0.92±0.128 per minute time/flower/event frequency in *Ischiodon scutellaris* (Hover fly) while *Callidilium* sp. (Long horn beetle) spent 0.955±0.395 per minute time/flower/event (Fig 2). Again, in the evening insects achieved almost nearly similar flower visiting frequency as it was in the morning. Contrary to this *Syntomis phegea* (Nine spotted moth) has shown lesser flower visiting frequency in the morning hours in comparison to evening. It has shown flower visiting frequency 0.28±0.1906 per minute time/ flower/visit. Hover fly, *Episyrphus balteatus* have shown 0.08±0.066 per minute time/flower/event (Fig 3), *Utetheisa pulcheloides* (Heliotrope Moth) 0.175±0.141 while *Camponotus hemi* (Carpenter ant) were observed slow feeders as they have taken 1.68±0.651 per minute time/flower/event in morning session (Fig. 3) (Table 3). While in evening session, same insects have taken more time in flower visiting and nectar feeding as it was observed as 0.122±0.0209 per minute time/flower/event in *Episyrphus balteatus* (Hover fly), 0.0465±0.0095 per minute time/flower/event in *Utetheisa pulcheloides* (Heliotrope Moth) (Fig 3). House fly (*Musca nabulo*) usually take more time in nectar finding and feeding than other insects. It showed flower visiting efficiency .e. 2.33±0.58 per minute time/flower/event (Fig 3), visited Saunf, and Dhania flowers one after one and feed on both plants. There was observed a significant effect of temperature, humidity and photoperiod on nectar feeding and flower visiting behavior in insects (Fig 5).

3.4. Nectar feeding behavior

Nectar feeding insects possess highly specialized mouthparts to suck, siphon or sponge the nectar (Photograph 6-7). Each group of insect possesses special architecture of mouth parts as plant floral apparatus possess. Most of the insects, which suck the plant nectar, possess brush shaped laciniae, galae and maxillary palps to form a complete nectar feeding apparatus. Lepidopeternas mainly heliotrope moth possesses a very long proboscis, no mandibles, elongated maxillae modified in to a coiled proboscis with two elongated galae (channels). Hooks and interlocking spines to form a food tube (Photograph 6) hold it together. In these insects lacinia was found to be absent or atrophied, maxillary palpi 5-6 in number most reduced, labrum reduced to form a small plate, but hypopharynx remain present. More especially the cavity of the proboscis communicates with the pharynx. In general, looking mouthparts are modified into a coiled sucking proboscis. Hymenopteran insects possess orthopteroid mouthparts having great flexibility of the maxillary-labium complex highly modified for various purposes (Photograph 7). Mandibles are dentate, and modified for biting, cutting, and foraging purposes. Maxillae consists 6 articulated palpi, cardo well developed, lacinia distinct and labium well developed, bifid gloss (hypo pharynx). Both mandibular and labial glands and mandibular glands were well developed and attached with salivary pump. Moth cavity leads into the pharynx and insects possess a sac-like infra-buccal cavity below the floor of the mouth.

Mainly, nine-spotted moth has shown adaptation to hide from the predators during active feeding (Photograph 1). Insects mainly orient in upside down or down upside when feed on nectar (Photograph 1). They have experienced to hang their body perpendicular to plant stem and leaf structures (Photograph 1), but hymenopterans always feed by sitting over the flowers and adjust mouthparts and its motion according to the sitting posture (Photograph 4). Every time insects have made different orientations in different manner to suck and sip on nectar. In addition, pollens of cultivated plants contain sticky pollens without any side fold or anchored top (Photograph 8). Nevertheless, weed plants possess anchored pollens, which automatically hang or attach with the body of insect just after a slight touch. More interestingly, Hover flies never found on weed plants and rejected the choice of flower visit. They have shown rejection or no choice to the plant, as they have been never stayed for having nectar, contrary to this, heliotrope moth have visited such flowers for nectar search. During active feeding heliotrope moths were observed taking rest on nearby vegetation, but hover flies continuously carried on feeding without any break. They were found involved in flower visit and nectar feeding during maximum part of full session, when they felt tired or saturated at dusk they fly to their hives or shortly.

3.5. Oviposition behavior

Effect of nectar feeding on oviposition and progeny production in female *Utetheisa pulcheloides* the, heliotrope Moth was observed. In pollinating insect vectors, nectar feeding significantly affected oviposition behavior. As results showed that nectar, fed female insects have laid significantly higher number of eggs i.e.126.152±1.47 in comparison to unfed control females 16.66±0.881. They also have also shown significant increase in F1 emergence in tests in comparison to control insects [Table 3]. Female insects, which were, provided different doses (w/v) of amino acid mixture, these also have shown significant enhancement in both egg laying and F1 emergence. It was found extremely significant at $P<0.0001$ in comparison to control insects. Similarly, amino acid mixture and sugar solution was provided in increasing ratios, it also induced oviposition in females and was found to be significant (Table 3). In another experiment, insects that were provided both honey solution and amino acid mixture in feeding showed enormous induction in oviposition, as the number of egg laid was 6.19 times more number of eggs than the controls. It not only induced the egg formation in female ovarian follicles but also significantly increased the emergence of F1 larvae (p<0.01). However, insects, which were provided, honey solution and amino acid fed insects showed higher progeny production. The Turkeys test was applied for knowing the variance among the control and test insects, it was found much higher when results were compared with amino acid mixture (5mg/ml), amino acid mixture + honey solution, and nectar fed insects again it was found to be very significant at P<0.006 (F= 5.78) (Table 4).

3.6. Discussion

In the present study, we have tested the hypothesis that flower visiting and nectar feeding led significant effect on ovoposition behavior in female pollinating insects. It decides reproductive success in them. Therefore, frequency of flower visits made for nectar search and feeding in few pollinating vector insects such as *Callidilium sp*., (Long horn bettle), *Utetheisa pulcheloides* (Heliotrope Moth), *Syntomis phegea* (Nine spotted moth), *Episyrphus balteatus* (Hover fly), Ischiodon *scutellaris* (Hover fly), *Camponotus hemi* (Carpenter ant), *Musca nabulo* (House fly) and *Pieris rapae* (White Cabbage fly) were observed very keenly and carefully. Major events related to flower visits were also noted on cultivated endemic plant species such as Saunf (*Foeniculum vulgare*), Dhania (*Coriandrum sativum*) and Muly (*Raphanus sativus*) and compared with naturally grown flowering weed plants for comparison (Table 2; Fig. 1, 2, 3, 4). During nectar feeding moths have shown rotational movements with varying inverted, hanging and dorsolateral orientations (Photograph 1). Contrary to this, flying lepidopteran *Syntomis phegea* (L) has shown parallel flights over flower clusters in sitting postures with flayed wings. (Photograph 2). *Musca nebula* has shown both downward inverted and upward sitting orientations (photograph 3). There were observed significant differences in nectar feeding in insects on different plant species in comparison to weed plants. More specifically, events made by nectar feeding insects on cultivated plants were significantly more than artificial control flowers (Photograph 8). It proves that flower color is a secondary attractant to the nectar feeding insects. Really, it is nectar composition, which attracts insects for nectar feeding. In field experiments, artificial flowers (un-scented) of various colors could not attract more number of insects in comparison to natural colored flowers but when these flowers (artificial) were coated with various doses of amino acids and sugar solution, insects made significantly more number of flower visiting events. It suggests that insects mainly pollen vectors posses specific quench for nectar constituents rather than color attraction. Besides this, patrolling insects like Hover flies (*Ischiodon scutellaris* and *Ischiodon scutellaris*) more frequently visited natural flowers rather than the control flowers. They were also induced by con-specifics to enhance the flower-visiting frequency on cultivated flowers. Furthermore, large number of pollens found attached to the body of hover fly patrolling males. It suggests that male bees also function as pollen vectors [1]. In addition, insects have also shown rejection responses to flowers foraged by con-specifics or companion insects. A complete rejection response was also observed to weed plants in pollen vector insects, as during their first visit they might have identified these plants because of non-availability of nectar volatile components. Intense and massive nectar feeding observed in insects in morning and evening hors in presence of moderate light and normal temperature in flowering territories. With this, insects were found to be competing with their counterparts to find nectar and reproductive mates.

3.7. Flower morphology and presence of nectaries

The inflorescence in cultivated field crops *Coriandrum sativum* and *Rhaphanus sativus* as well as in uncultivated garden plants were found in form of dense small clusters or small florets. These are highly preferred host plants of lepidopteron, hymenopteran and dipteran insects. However, there was a little size variation among florets according to age but mature florets were found corresponded in size and shape with the head and mouthparts of moths butterflies and bees. Dipteran flies are sponging feeders, which feed on nectar differently. Protendrous florets or nectar cups possess nectarines either united or free anthers outwardly and cylindrically forming a tube around the style. The style growth pushes pollen out of the anthers, and presents agglomeration of pollen attached to the style above the surface of inflorescence. It makes pollens freely available and make nectar harvesting much easier to the insect from above. (Fig.1-4). When feeding on pollen insects raise the labrum and by means of small movements of the distal maxillary structures, the pollen grains are rubbed off the style. High frequency maxillary movements made by the insects persisted for a considerable period to feed on nectraies, which were present at the apex of the ovary. (Fig 1-4). Based on the flower morphology and feeding behaviors of insects it is evident that only pollens are ingested when the head is pushed into a flower. In some cases, honeybees were found clean off pollens from their forelegs by dragging the tarsi through the opened mandibles and raised the labrum. Pollen removed from the legs have been either ingested or collected for preparing the brood royal jelly or honey. Normally pollen grains were found attached to the setae or row of pollen collecting hairs. A comparison of the frequency of flowers in the study site reveals that insects have utilized both *Coriandrum sativum* and *Rhaphanus sativus* as most frequently visited flowers in the area for feeding. The comparison however suggests that inflorescences of a certain floral architecture are preferred.

Feeding observations and identification of the pollen in the gut content honey bees indicate that a wide spectrum of plants are utilizeded belonging to families umbelliferae, Asteraceae, Brassiaceae, Cucurbitaceae (Table 2). The long heads and the partly tubular mouthparts of insects the suggest and adaptation to tubular flowers and the uptake of nectar as well. Therefore, it is not the choice but it is morphological adaptations in insects and preferential flower structure in plants, which are responsible for positive interactions for nectar harvesting by selected insects. It shows the parallel evolution of nectarines and insect mouthparts as well as evolutionarily maintained behavior

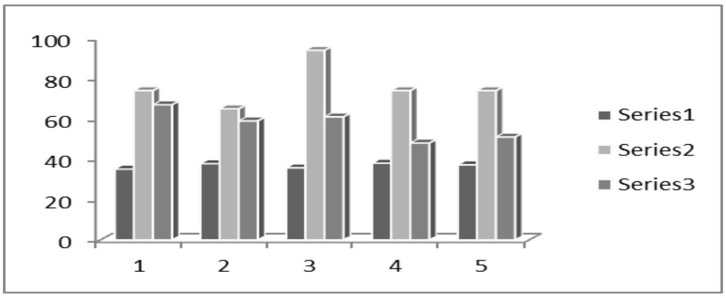

Figure 1. Effect of temperature, humidity and light period on nectar feeding and flower visiting behavior of insects.

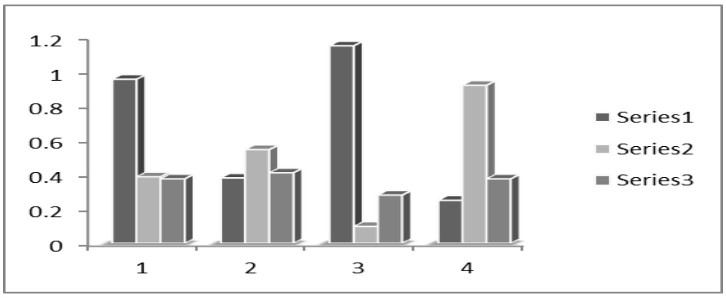

Figure 2. Flower visiting efficiency of various insects on *Coriandrum sativum*.

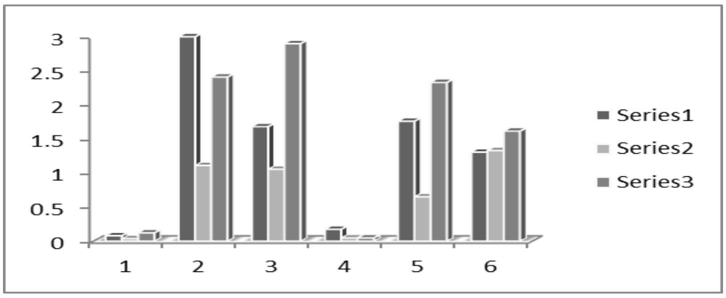

Figure 3. Flower visiting efficiency in various insects on *Foeniculum vulgare*

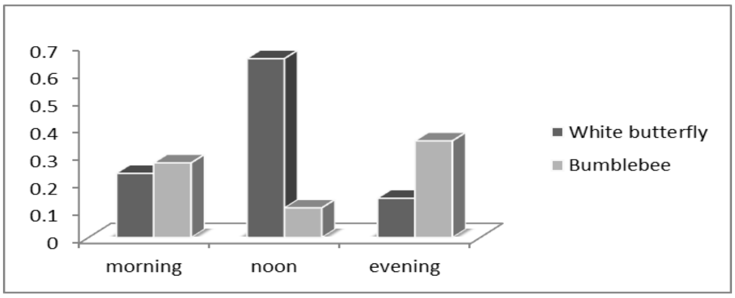

Figure 4. Flower visiting efficiency in heliotrope moth and hover fly on *Rhafanus sativus*

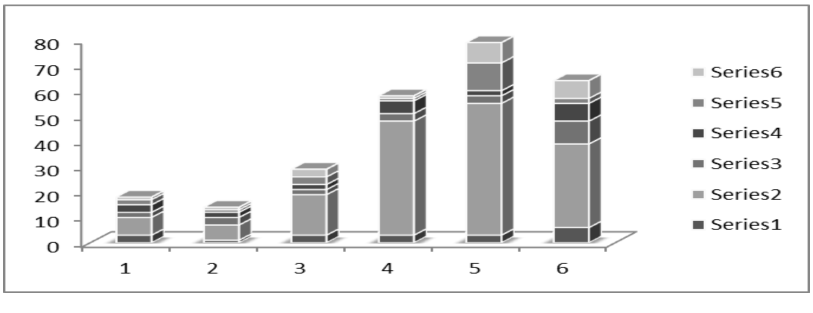

Figure 5. Number of insects from different orders observed and collected on various plant flowers at 6 localities in North Eastern India.

Table 1. Insects identified and collected from 6 localities in North Western and Eastern India.

Order/Family	Genus	Species	No. of individuals	Locality
Lepidoptera				
Galleriidae	*Galleria*	*mellonella* (Fabricus)	2(0)	3
Noctuoidae	*Orthreis*	*fullonia* (Linn)	2(0)	3
	Heliothis	*armigera* (Hubner)	17(3)	6
	Lymantria	*dispar* (Linn	2(1)	6
	Euproctis	*chrysorrhoes* (Linn)	1(0)	4
Pieridae	Pieris	*rapae* (Linn)	3(1)	
	Danaus	*chrysippus* (Linn)	(1)	2,4,5
	Danaus	*plexippus*		
Papiolionidae	*Papilio*	*demoleus* (Linn)	3(1)	1,2
	Papilio	*agamemnon* (Linn)		
	Papilio	*lavassori* (Linn)		
	Papilio	*machaon* (Linn)		
	Spodoptera	*litura* (Fabricus)	5(5)	2,4,5
	Pseudoelatea	*separate* (Walker)		
Arctidae	*Synctomis*	*phegea*	1(0)	1
	Amara	*phegea*		
Arctidae	*Utethesia*	*pulchelloides*	6(1)	
Tortricoodea	*Trotrix*	*phenophoroidea* (Linn)	1(0)	
	Sphenarches	*caffer* (Zeller)		
Pyralidae	*Plodia*	*interpunctatella* (Hubner)	3(1)	2,1
	Diatraea	*Saccharalis(F)*	2(0)	5
	Achrola	*grisela* (Linn)		
	Ephestia	*kuhniella* (Zeller)	1(1)	4
	Leucinodes	*arbonalis* (Guen)		
	Bissetea	*steniellus* (Hmpsn)	1(1)	1
	Scripophaga	*novella* (Fabr)		
Gelichidae	*Gnorimoschema*	*operculella* (Zeller)	1(1)	5
Lycanidae	*Virachola*	*isocrates* (Fabr)	1(1)	1
Metarbelidae	*Inderbela*	*quadrinotata* (Walk)	1(0)	1
Diptera	*Simulium*	*griseifrons* (Bunetti)	2(1)	1,2
Culcidae	*Culex*	*quinquefasciatus* (Linn)	19(4)	1,2,3,4,5,6,
	Anopheles	*gambiae*,(Giles)	21(6)	2,4,5
Muscidae	*Musca*	*domestica* (Linnaeus)	6(11)	2,4,6
Stomoxydidae	*Stomoxys*	*cacitrans* (Linn)	1(0)	2
Drosophilidae	*Drosophila*	*melanogaster* (Meigen)	3(0)	2
Trypetidae	Trypeta	toxoneura (Loew)	1(1)	1
	Ceratitis	*capitata* (Wiedenmann)	2(0)	1
	Batocera	*cucurbitae* (Coquillett)	1(0)	
	Batocera	*rufamaculata*, (Dereer)	1(1)	
	Dacus	*oleae*, (Rossi)	2(1)	
	Dacus	*dorsalis* (Hendel)	1(1)	
	Dacus	*cucurbitae* (Coq)	1(0)	
	Rhagoletis	*pomonellaI* (Walsh)	1(1)	
	Anastrepha	*ludens* (Loew)	1(1)	1,3,5,6
Agromyzidae	*Phytomyza*	*atricarnis* (Meigen)	2(1)	1,2
Tipulidae	*Tipula*	*fulcipenennis* (Curtis)	1(1)	
Muscidae	*Musca*	*domestica* (Linn)	7(3)	
Trypetidae	*Carpomyia*	*vesuviana* (Costa)	1(0)	4
Syrphidae	*Episyrphus*	*bateatus*	1(1)	
	Heliophilus	*pendulus*	1(1)	
	Ischiodon	*scutteris* (Fab)	1(1)	
	Allograpta	*oblique*	1(1)	1
Calliforidae	*Calliforida*	*vicinia*	2(1)	
Hymenoptera				
Apidae	*Apis*	*mellifera* (Linn)	3(2)	1,2,3,4,5
	Apis	*cerana* (Linn)	11(2)	
	Apis	*dorseta* (Linn)	4(1)	
	Apis	*floreae* (Linn)	7(2)	
	Apis	*javana* (Linn)	1(1)	
	Bombus	*pensylvanicus* (De Geer)	1(1)	
	Bombus	pascuorum (Scopoli)	2(0)	1,2
	Bombus	*horacicus* (Spinola)	1(1)	1,3,4
	Bombus	*arcticus* (Dahlbom)	1(1)	
	Bombus	*cognatus* (Stephens)	2(1)	
	Bombus	*teristis*	1(0)	
Vespidae	*Polistes*	*infuscatus* (Linn)	4(1)	2,4,5
	Vespula	*vulgaris* (Linn)	2(1)	1,4,5
	Vespa	*gallica* (Linn)	1(1)	1,2
	Vespa	*maculates*		
	Polistis	*fuscutua*		
Formicidae	*Camponotus*	*hemi*	3(1)	1,2
Thysanoptera				
Thripidae	*Caliothrips*	*fasciatus*	1(1)	2,4,5
	Frankliniella	*occidentalis*	*1(1)*	
Odonata	*Ceriagrion*	*sp*	1(1)	1
Neuroptera				
Chrysopidae	*Chrysoperla*	*rufilabris*	1(1)	2,3
Ascarlidae	*Ascarloptynx*	*appendiculata*	1(0)	2,3
Hemiptera				
Psyllidae	*Diphorina*	*citri* (Kuwayama)	5(0)	3,4
Aphidae	*Lipophis*	*erysimi* (Kaltenbach)	Numerous	1,2,3,4,5,6
Coccidae	Drosicha	*mangiferae* (Green)	1(1)	
Jassidae				
Aleurochidae	*Aleurolobus*	*bordensis* (Mask)	1(1)	1
Coreidae	*Leptocorisa*	*varcunis* (Linn)	2(1)	2,3,5
Coleoptera				
Coccinclinae	*Epilachna*	*vigintioctopunctata* (Fabr)	5(2)	1,2
	Epilachna	*indica* (Linn)		
Cerambicidae	*Callidilium*	*Sp.*	1(0)	2,3
Chrysomelidae	*Aulacophora*	*folveicollis* (Lucas)	1(0)	
	Aulacophora	oveieollis (Lucas)	1(0)	2.4,5

Observation sites: 1. Painda 2. Kheri 3. Barhan 4. Shiwoi 5. Majhua 6. Kusumi

Table 2. Major flowering plant species that are potential food source for insects belong to various orders noted at study site. Total nu flowers in inflorescence was estimated (mean relative frequency) in 8 plots of 7 x 2 meter and number of feeding events was noted. Pre taxa are given in bold letters.

Flowering plants	% of total number of flower	No. of observed feeding events by	% of feeding events
Apocynaceae			
Nerium oleander	<1	0	0
Thivetia nerifolia	<1	0	0
Calotropis procera	2	1	0
Chrysanthemum indicum	<1	0	0
Asteraceae			
Catharanthus oxycanthus	2	1	1
Catharanthus tintorius (Kusum)	2	1	1
Echinops echinatus	<1	0	0
Helianthus annus (Lin)	7	1	1
Tagetes paluta	9	3	5
Sonchus asper	-	-	-
Brassicaceae			
Raphanus sativus	**15**	**5**	**13**
Brassica oleracea	17	6	16
Brassica comprestris (Linn)	13	5	9
Brassica juncea	2	1	1
Caesalpinaceae			
Cassia fistula	<1	0	0
Cassia carandus	<1	0	0
Cucurbitaceae			
Cucurbita maxima,	3	2	5
Cucumis sativus	2	1	4
Euphorbiaceae			
Euphorbia hirta	<1	0	0
Croton sparciflorus,	<1	0	0
Lamiaceae			
Mentha arvensis	<1	0	0
Ocimum sanctum	<1	0	0
Malvaceae			
Hibiscus rosa sinesis	3	3	5
Moraceae			
Ficus glomerata	<1	0	0
Atrocarpus heterophyllus	<1	0	0
Rosaceae			
Rosa centifolia	6	4	3
Solanaceae			
Lycopersicum esculentum	<1	0	0
Solanum melongena	<1	0	0
Solanum nigrum	3	2	5
Umbliferae (Apiaceae)			
Foeniculum vulgare	**7**	**5**	**6**
Coriandrum sativum	**8**	**4**	**16**
Anethum sowa	*7*	*3*	*3*
Daucus carota	2	3	5
Anethis graveolens			
Maliaceae			
Azadiracta indica	<1	0	0
Pedaliaceae			
Sesamum indicum	<1	0	0
Chinopodiaceae			
Chinopodium album	<1	0	0
Polygonaceae			
Rumex maltimus	<1	0	0
Caryophylaceae			
Dianthus chinensis	<1	0	0
Spergula asvensis	<1	0	0
Ranunculaceae			
Clematis paniculata	<1	0	0
Nigella sativum	<1	0	0
Muscaeae			
Musa paradisiaca Linn	<1	0	0

Table 3. Flower visiting behavior of different insect species per minute time/flower/event on different flowering plants.

	Criandrum sativum		
Insects	**Morning session**	**Noon session**	**Evening session**
Callidilium sp. (Long horn beetle)	0.955±0.395	0.387±0.077	0.375±0.1602
Utetheisa pulcheloides (Heliotrope Moth)	0.38±0.086	0.545±0.0601	0.41±0.136
Syntomis phegea (Nine spotted moth)	1.15±0.375	0.0975±0.0808	0.28±0.1906
Ischiodon scutellaris (Hover fly)	0.25±0.074	0.92±0.128	0.375±0.174
Foeniculm vulgare			
Episyrphus balteatus (Hover fly)	0.08±0.066	0.381±0.921	0.122±0.0209
Ischiodon *scutellaris* (Hover fly)	3.0±0.675	1.11±0.161	2.41±0.763
Camponotus hemi (Carpenter ant)	1.68±0.651	1.055±0.56	2.9±0.793
Utetheisa pulcheloides (Heliotrope Moth)	0.175±0.141	0.47±0.185	0.0465±0.0095
Musca nabulo (House fly)	1.76±0.826	0.65±0.164	2.33±0.58
Syntomis phegea (Nine spotted moth)	1.307±0.3402	1.33±0.25	1.615±0.533
Raphanus sativus			
Pieris rapae (White Cabbage fly)	0.234±0.196	0.0652±0.0721	0.143±0.128
Episyrphus balteatus (Hover fly)	0.272±0.223	0.108±0.0703	0.352±0.089

Photograph 1. Showing different orientations of *Utetheisa pulcheloides* (Heliotrope Moth) family Aganaidae order Lepidoptera to feed nectar on *Coriandrum sativum* and *Foeniculum vulgare*

Photograph 2. Showing different orientations of *Syntomis phegea* (Linnaeus, 1758) Lepidoptera-Arctiidae to feed nectar on *Coriandrum sativum* and *Foeniculum vulgare*

Photograph 3. Showing different orientations of *Musca nabulo* Order: Diptera' Family: Muscideae to feed nectar on *Coriandrum sativum* and *Foeniculum vulgare*

Photograph 4. Showing different orientations of *Episyrphus balteatus* Order: Diptera; Family" Syrphidae to feed nectar on *Coriandrum sativum* and *Foeniculum vulgare*

Photograph 5. Mouth parts of *Episyrphus balteatus* (Hover fly)

Photograph 6. Mouthparts of *Syntomis phegea* (Nine spotted moth)

Photograph 7. Showing anthers of cultivated flowers, *Coriandrum sativum*, *Raphanus sativus* and *Foeniculum vulagre*

Photograph 8. Showing artificial flowers made by colored paper and cotton

responses in insects. The seasonal occurrence of both insects and plants is synchronized mechanistic and biologically operated system, which is genus specific. On one side, adaptation arises in habit of insects with significant modifications in mouth parts according similarly habitat develops colorful vegetation with size specific blossoming of flowers in plants mainly nectar tubes and nectarines. It is very similar one is involved in searching another is waiting and both are coexisting mutually for their future progenies. Insects have shown significant nectar feeding and flower visiting efficiency in morning hours as mean flower visiting frequency noted was very high in *Ischiodon scutellaris* (Hover fly) as they attempted and spent 0.25±0.074 per minutes time/flower/event followed by *Utetheisa pulcheloides* (Heliotrope Moth) which spent 0.38±0.086 per minute time/flower/event (Table 3).

However, for knowing frequency of floral visitors and forage efficiency in insects it is highly important to know plant-pollinator interactions. During nectar, search insects utilize scent marks and follow them to find nectar-bearing flowers. Besides this, nectar-feeding insects/pollinators generate chemical signals and cues to respond rest of the insect population. These signals are molded by natural selection to carry specific meanings in specific contexts to operate more appropriately to search the nectar and their con-specifics. Besides this, insects suspected to leave cues at nectar feeding sites or on flower structures may also provide them special information about nectar and companion insect but these are very hard to explain and distinguish. Similarly, scent marks left by the bumblebees *Bombus terrestris* enhance fitness, improve foraging efficiency, and help to locate their nest [30]. In addition, insects follow several visual cues and arrived to detect the available floral resources and nectar presence. Insects easily recognize colored flowers; search them one by one based on nectar amount, and leave scent marks and olfactory cues on the flowers after each visit. This behavior is well known in hymenopterans, which show rejection responses during foraging done by con-specifics. Nevertheless, solitary bees *Colletes patellatus* (Colletidae) and *Osmia orientalis* (Megachilidae) do not show such kind of behavior and do not provide information or signals about flowers that are foraged by con-specifics, but *A. prostomias* and *T. mitsukurii* recognize scent marks left by previous visitors [31]. Thus, pollinating flies take decision to use scent or not to search foraging resources or floral structures in the gardens and orchards [3]. Besides this, such cues perceived during contact with the scent also help finding next insect. Few nectar-feeding insects leave lipid footprints or hydrocarbons, while the next insect could analyze walking on smooth surfaces [32] these. For example, *A. prostomias* and *T. mitsukurii* recognize scent marks left by previous visitors and can make the decisions about richness of resources mainly complexity of floral structure [3]. Besides this, lepidopteron insects show electro-physiological taste responses in the mid-legs [33] that help insects to communicate with their feeding companions [2] by generating odor signals [34] [26] and transfer information to them [34]. In nectar feeding insects, intra-specific interactions were found to be more pronounced and much clarified as con-specifics run neck to neck to find and feed on nectar. It was mostly observed in hover flies and heliotrope moth. Among all the insects' dipterans were found silent feeders that produce no sound but bees have generated buzzing sound when find and feed on nectar.

Besides, above factors, design of mouthparts mechanical factors especially provides extra assistance to insects in nectar feeding. Insects having functional modification in their sucking canals solicit them to select specific plant species of its own choice according to adaptation. It is true that insects on one side possess well-adapted mouthparts to feed on nectar but on other side nature has made nectar bearing flowers more colorful, with palatable nectar composition and sticky pollens to cross pollinate the female flowers (Photograph 5 & 6). For this purpose insects also possess highly specific forage or pollen collecting legs which hold large number of pollens for carrying. These insects possess enormous number of hairs on coxa, trochanter and femur, with enough structural modifications in tibia and tarsus in different insects (Photograph 5 & 6). In worker bees posterior tibia is more dilated and is margined with long hairs, being thus modified to form a corbicula or pollen basket (Photograph 5). The basitarsus (first tarsal segment) flattened on its inner aspect and provided with the several rows of short stiff spines, which form a brush. This brush helps in gathering the pollens by adhering to the hairs present on lateral side of the body and rows present over hind legs of worker flies (Photograph 5). Besides this, mouthparts of worker flies are greatly modified, elongated, and found fit for gathering both nectar and pollen. Similarly, hind legs of worker and queen bees were found specialized for collecting and carrying pollens (Photograph 5). It helps to increase fruit and seed production in crop plants by cross-pollination of flowers, which is an impossible task for any other group of animals. Hover flies are extremely important carrying pollens to one flower to the other and perform enormous cross-pollination among the flowers. These remain traceable as pollinator footprint on natural flowers and can be identified the next insect who visits the same flower for nectar [32]. Besides this, carpenter ant, *Xylocopa* were also found collecting nectar and provides help in cross-pollination of flowers [17]. More especially in vegetable garden different species of insects were observed. Among which lepidopterans were most prominannt and frequent visitors of flowers. Similar features are also observed in insects of family andrenidae mainly in genus *Andrena*. In these insects, hind legs contain pollen gathering apparatus possess hind tibial spurs (Photograph 5). Besides this insects compound eye also contains hairs, which also help to kept away side row of pollens attached over the eye (Photograph 5).

Biological, chemical and ecological factors affect nectar feeding in insects in flowering territories. Nectar components are primary factors, which attract more number of insects. Essentially few important ecological factors such as temperature, humidity, light, water availability, wind velocity and light effect both flower visits and nectar searching behavior in insects. In addition to it chemical components such as volatile chemicals i.e. squalene, cholesterol, and *p*-methoxybenzaldehyde, 12 butyrate esters, alkaloids, sugars and amino acids play very essential role in nectar feeding insects. [19, 21, 23]. These chemicals attract number of insects for nectar feeding and help to display scent stimuli through volatile cues that enable insects to recognize scent marks generated by con-specifics. Similarly, high concentration of quercetin helps to attract number of workers to queen's signals in bees [26]. However, flower color shows positive responses in nectar feeding insects [8] while amino acids and sugars determine frequency of floral visits [6] that also enhance longevity in female insects [10]. Similarly, consumption of nectar alkaloids reduces pathogen load in insects [35] while leaf herbivore and draught stress [36] temperature and light affect floral visits and foraging in bees [37]. Plant nectar contains high amount of carbohydrates, amino acids, proteins and vitamins, which could fulfill nutritional requirements of insects. Nectar feeding also supply nutrients required for egg development, if these are not sequestered with the diet these significantly cut down F1 emergence and effect survival of insect larvae. Flower visiting also help insects to find mating partners, while nectar feeding is to obtain nutrients essentially required for the synthesis of egg lipids and proteins. In lack of these nutrients, insects laid less number of eggs and less F1 larvae were emerged from them. It may also affect post fertilization development of insect eggs (Table 4).

In addition, insects used visual cues for identification of colored nectar bearing flowers. As it is also known that insects are capable of detecting UV and colors using photoreceptors. Bees are able to simultaneously receive information from the wavelength and e-vector (vector representing the electric field of an electromagnetic wave) of incoming light using its receptors. Similarly, flies and moths apply phototactic sensitivity to substantial UV component (UV light) present in sunlight and buzz or hover on colored flowers and attracted towards them because insect eye responds to ultraviolet irradiation [38, 39]. This response leads to several different reactions. When insects are exposed to light they may go toward or away from the source of illuminations (positive or negative phototaxis), they may increase or decrease the rate of their general activity, they may change their posture or move only part of the body [40]. Contrary to this, few insects such as nocturnal moths and ants have been observed to search nectar-bearing plants after dusk or in the dark [41, 42] , but here they, have not been fully identified and studied. However, diverse category of insects they come to search nectar bearing flowers after getting proper sensitization from particular visual light in morning hrs. Possibly hunger may be the second reason of highly frequent visits made on the flowers by vector insects, its reason is still unknown.

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