FACTORS INFLUENCING INTER AND INTRA-SPECIFIC

PREFERENCES OF HONEYTBEES FOR THREE

CONCURRENTLY FLOWERING VEGETABLE/SPICE CROPS

BY

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2000BS95D

Dissertation submitted to the Chaudhary Charan Singh

Haryana Agricultural University in partial fulfillment

Of the requirements for the degree of

DOCTOR OF PHILOSOPHY

IN

ZOOLOGY AND AQUACULTURE

COLLEGE OF BASIC SCIENCES & HUMANITIES
CCS HARYANA AGRICULTURAL UNIVERSITY
HISAR – 125 004 (HARYANA)

2004

CERTIFICATE-I

This is to certify that this dissertation entitled "**Factors influencing Inter and Intra-specific preferences of honeybees for three concurrently flowering vegetable/spice crops**", submitted for the degree of **Doctor of Philosophy** in the subject of **Zoology** of the Charudhary Charan Singh Haryana Agricultural University, Hisar , is a bonafide research work carried out by **Neelam Chaudhary,** Admn. No. **2000BS95D** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE-II

This is to certify that dissertation entitled "**Factors influencing Inter and Intra-specific preferences of honeybees for three concurrently flowering vegetable/spice crops**", subvmitted by **Neelarm Chaudhary** Admn. No. **2000BS95D** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in the subject of **Zoology** has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.

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DEAN, POST-GRADUATE STUDIES

ACKNOWLEDGEMENT

It is a rare occasion of having an opportunity to work on a research problem when one gets glimpses of the true worth, edition, enthusiasm and innate nobility of his or her guide. I, therefore, take this opportunity to feel an immense pleasure and honour in expressing my heartiest gratitude and sincere thanks to Dr. R.C. Sihag, my major advisor and chairman of my advisory committee, whose expert and creative guidance, keen interest, generous and friendly nature, close supervision, constructive criticism and untiring help encouraged me to successfully complete this piece of research work. The total quantum of work and its presentation has been possible due to great pains taken by him. I am highly obliged to him especially for creating in me the spirit of independent thinking and decision making which will certainly help me to proceed towards a successful and ideal life in the long run.

My sincere thanks are also due to the members of my advisory committee, Dr. K.L. Jain, Professor (Department of Zoology and Aquaculture); Dr. S.S. Sharma, Sr. Scientist (Department of Entomology); Dr. D.S. Tonk, Assoc. Professor & Incharge. (Computer Section); Dr. Avtar Singh, Assoc. Professor (Department of Vegetable); Dr. V.S. Malik, Professor (Department of Entomology) for their kind suggestions and keen interest in my research work and going through this manuscript.

My special thanks are due to all the faculty members Department Zoology and Aquaculture for their constant encouragement and help during this investigation.

I owe my sincere thanks to my friends Pankaj, Ranjana and Ashima whose constant inspiration, encouragement and entertaining nature made the tenure of this investigation interesting and cheerful.

I shall be utterly failing in my moral duty, if I don't record my sincere gratitude to my better half Yash for rendering me emotional security, moral support and keeping a constant attitude of patience and sacrifice during this tenure.

I accord cordial regards from the core of my heart to my parents and in-laws family and my brother (Dr. Arun Chaudhary and Dr. Vikas Chaudhary) whose blessing encouragement and inspiration have gone a long way in the successful completion of this manuscript.

HISAR (NEELAM CHAUDHARY)

December, 2004

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INTRODUCTION

Honeybees being the primary seeker of nectar and pollen visit the flowering crops and bring benefit to a wide variety of crops by pollinating their flowers (Sihag, 2001). Cross-pollination of flowers of entomophilous crops has a great influence on the quality of seed production (Free, 1993). It was investigated that seed set by honeybee pollinated onion increases by 2.7 percent (Hwang *et al.* 1998). Cane and Schiffhauser (2003) observed six fold differences in mean pollen deposition translated into 15-20 fold differences in Cranberry fruit set and size. In onion, by keeping bee colonies in field, seed yield can be increased (Rashid and Singh, 2000). Most of the vegetable crops are cross-pollinated i.e. the flowers of these crops need conspecific foreign pollen for pollination and seed set. This is because of the certain reproductive barriers in these crops which make self-pollination ineffective (Sihag and Chaudhary, 2003). Most varieties of Australian groves improve their fruit set with the presence of pollinators (Ravetti, 2004).

Carrot (*Daucus carota* L.), fennel (*Foeniculum vulgare* L.) and onion (*Allium cepa* L.) are either vegetable or spice crops. The flowers of these crops are protandrous i.e. male part matures first than the female part. This condition necessitates cross-pollination, the transfer of pollen from anthers of one flower to stigma of another flower. Only the potential pollen vectors shall be able to fulfill this job (Sihag, 1985 a, b).

For different crops, different pollinators are responsible to effect cross-pollination. Almost every order of insects has been reported to play a role in the pollination of flowering vegetable and spice crops. Insects which pollinate the cultivated crops include ants, aphids, bees, beetles, butterflies, flies, midges, mosquitoes, moths, thrips and wasps (McGregor, 1976, Free, 1993). Among them, bees are considered to be the most important pollinators because they are the only insects whose immature stages are reared exclusively on pollen and nectar (Crane, 1990). Crailsheim (1991) has reported that the adult worker honeybees feed upon pollen to produce royal jelly for the queen bees, the brood, the adult workers of different age and the drones. The younger bees are the nurse bees which feed upon pollen mainly to produce royal jelly from their highly developed hypopharyngeal glands. Pollen, nectar sugar concentration, quality of nectar, body size and tongue length of pollinators, floral shape, size and colour are known to cause orientation in honeybees to locate food source of their choice (Priti and Sihag, 1997). Colour preferences by insects, particularly the bees, have been explained for a long time. Yellow flowers are often highly reflective and are visited by a variety of insects, as ultraviolet reflection is often found in yellow flowers. But earlier work by Kevan (1983) reveals that there are "highly visited" flowers and "rarely visited" flowers with ultraviolet reflecting patterns, and there are flowers with weak or no ultraviolet pattern and yet are highly visited by insects. In general, higher energy yielding flowers attract more bees. But sometimes more bees are found visiting the lower energy rewarding plants even in the presence of higher energy rewarding ones due to complexity of the flowers (Sihag, 1995). Honeybees have certain idiosyncrasies which are very useful from pollination point of view and generally increase their efficiency as pollinators (Free, 1993). In collecting nectar and pollen, they are known to be “fairly faithful” and “constant” to plants and become “fixed” to small area of crops being worked by them. While working over flowers, pubescent hairs on their bodies may entangle as many as 2,50,000 to 3,00,000 pollen grains (Skrebtsova, 1957). So when visiting various flowers of the same plant species honeybees are instrumental in disseminating pollen and consequently accomplish the job of pollination par excellence. The honeybees establish preference for the host on the basis of award system of a particular crop (Frisch, 1934; Hobbs, 1962; Macior, 1973). But the honeybees are unable to judge the richness of award system without assessing the food system. These bees then choose the appropriate host at random. A balanced interaction of physical, chemical and biological cues displaying a suitable food source in habitat, therefore, appears to control their fidelity to a host flower.

Nectar and pollen are important energy rewards of a flower. Availability of acceptable and unacceptable type of nectar and pollen act as a factor for attracting or restricting bee visits to a particular host plant. The flower-visitor relationship get set heavily between visitor's energy demand and quantity of food it can harvest from flowers (Hainsworth and Wolf, 1972; Heinrich and Raven, 1972), which influences the frequency of visits to flowers (Heinrich, 1976). Nectar from flowers is the only source of energy for activity, maintenance, metabolism, reproduction and growth of certain pollinators. Nectar secretion in flowers tends to be periodic and pollinators show a corresponding periodicity. It may be energetically more profitable for the bees to forage when the rate of secretion is high (Heinrich, 1976a). Honeybees are well known to engage in highly preferential selection of the plant species whose flower they explicitly visit at any one time or location (Corbet *et al.*, 1984). They readily discriminate among various flowers (Levin and Bohart, 1955; Campana and Moeller, 1977). Verma and Rana (1994) have stated inter-specific difference in foraging between honeybee species.

Most important adaptation to suck nectar from flowers in bees has been the development of a highly mobile and flexible labiomaxillary complex, the proboscis or tongue. Tongue length is a determinant factor for floral preference (Bhatt and Jagdish, 1995) and morphometric variation in the proboscis of bees and the corolla tube determines preference for various floral types. Thus short tongued bees are more efficient on short corolla tube flowers and medium tongued bees on medium corolla tube flowers (Inouye, 1980).

Among the local flora, fennel, onion and carrot are grown for vegetable/ spices. The different varieties of fennel, onion and carrot have similar flowering time and share the same pollinators. However, they do not attract the honeybees in equal numbers (Chaudhary, 2000). This was due to the differences in crop parameters/factors which need to be investigated. To develop better pollination strategy and to reduce crop competition for pollinators, an analysis of these factors was important. With this background, the present research was proposed with the following objectives.

1. To study the relative abundance of different honeybee species on fennel, onion and carrot.
2. To study the floral attributes determining their relative attractiveness to different pollinators.
3. To study the pollinator's attributes influencing relative attractiveness of different crops.

Chapter-II

REVIEW OF LITERATURE

2.1 FLOWER PREFERENCES IN BEES

Past investigations revealed that bees preferred certain types of crops/flowers under identical conditions and at the same time, this preference influenced the pollination of crops. Honeybees preference for some competitive plants that are more attractive than other crops have been reported by some workers (Olsen *et al.*, 1979; Bedascarrasbure, 1983; Free, 1993). Honeybees showed preference even for some clones of alfalfa over the others (Boren *et al.*, 1962; Sowa *et al.*, 1980), for sunflower over alfalfa (Cirnnu *et al.*, 1977), and for *B. campestris* over *B. juncea* (Abrol, 1985). Sihag (1990) reported preference of *A. dorsata* and *A. mellifera* for *B. chinensis* over *Eruca sativa.* Differential attractiveness of *B. campestris* and *Cajanus cajan* were studied by Sihag and Rathi (1993) and observed greater preference of two honeybees (*A. dorsata* and *A. florea*) for *B. campestris* and *Cajanus cajan.* Again Sihag (1995) studied the differential attractiveness of two cruciferous vegetable crops viz. turnip (*B. rapa*) and radish

(*R. sativus*) by two honeybees viz. *A. dorsata* and *A. mellifera.* In the above cases, the differences in the attractiveness seemed to be governed by the foraging profitability which in turn seemed to be influenced by the floral structure and foraging behaviour of the visitors and net energy harvest rate. Jain (1992) made a comparative study on alfalfa and sunflower and reported that although on alfalfa foraging all of the three species of honeybee were observed but on sunflower only *A. mellifera* foragers were observed. Dhingra and Jain (1995) studied bee preference on garden plants. Some of the garden plants, such as dahlia, were highly preferred, while petunia was reported as a non-preferred plant. Investigations carried out in past to know inter or intraspecific competition under identical conditions among different types of pollinators have revealed specific preferences in bees for certain types of flowers. There have been many efforts to diminish the effect of some competitive species on commercial crop pollination. (Nye and Mackensen, 1968; Palacio, 1987).

Apart from flower preferences, pollen collection consistency is also observed. In general, pollen is collected by honeybees from the most abundant species (Percival, 1947). But in a study carried by Coffey and Breen (1997) converse was observed. *Taxacum* type, *Endyminon non-scriptus* and *Plantago* spp. were growing abundantly close to colonies of honeybee (*A. mellifera)* yet when pollen samples were analysed, *Ulex* type (plant growing at a distance) was present in greater proportion. Olsen *et al.* (1979) reported preference among four pollen types. They reported apple and strawberry pollen were well represented in the collected samples, but no cucumber pollen was detected in the traps by honeybee in Blueberg area.

Moezel *et al.* (1987) showed that among 44 species exploited by honeybees for pollen only, a few like *Leucopagan* and *Acacia* species were abundantly visited. They further reported that bees had shown a high degree of consistency and 52.9 per cent pollen collected were solely consisted of a single plant species. Inter-specific preference was also observed by Pandey and Prasad (1995) in *B. juncea* and by Kumar and Sharma (1995) in case of ber (*Zizyphus mauritiana*).

Heinrich and Raven (1972) emphasized the role of energetic reward in flower foraging and in evolution of bee flower inter relationship. Nectar and pollen are the major source of food for various vital life processes of honeybees and in turn they pollinate the flowers. The visitation frequency is taken as a measure of pollinator attractiveness to a plant (Sihag 1990, 1993a) and several pollinators and plant attributes have been reported to influence such attractiveness. This review therefore pertains to the literature available on different factors determining the attractiveness of bees to the plants.

2.2 GENERAL FEATURES OF THE CROPS

Carrot (*D. carota*), fennel (*F. vulgare*) among umbelliferous and onion (*A. cepa*) among liliaceous crops are important vegetable and/or spice crops. These crops are grown in different parts of world and their cultivation is becoming popular in Haryana too (Sihag, 1985a; Mangal *et al.*, 1986). Carrot and onion make an important source of vegetable; seeds of fennel and onion are source of condiments (McGregor, 1976; Shelar and Suryanarayana, 1981; Baswana, 1984). These crops are strongly protandrous; self-pollination is largely absent and these depend upon insects for cross-pollination (Thompson, 1962; Martin, 1979; Rao and Suryanarayana, 1983; Baswana, 1984). The blossoms of these crops are highly attractive to both pollen and nectar collecting insects (Glukhow, 1955; Youngken, 1956; Gary *et al.*, 1972; Martin, 1979; Kumar and Rao, 1991; Free, 1993). The receptivity of stigmas in carrot, fennel and onion remain for 5 days, 1-2 weeks, and 4 days, respectively which provides better chance to the plant to get pollinated by insects (Baswana, 1984; Ottosson, 1984) Higher seed yield of these crops may be obtained by the maintenance of a large population of pollinating insects (Sihag, 1986) because these crops are visited by a large number of pollinating species (Hawthron *et al.*, 1956; Deodiker and Suryanarayana, 1972; Martin, 1979; Belleti and Zani, 1981; Rao and Suryanarayana, 1983; Baswana, 1984; Sihag, 1985a, 1986; Free, 1993; Singh and Hameed, 1995).

2.3 RELATIVE ABUNDANCE AND ACTIVITY DURATION

Population density of the entomophilous insects on a blossom depends on several factors like, nectar volume, nectar sweetness and weather conditions (Seeley and Levien, 1987). Within the favourable limits, insect's activity increases with increasing ambient temperature and decreases as it gets cooler (Burill and Dietz, 1981). Daily flight activity varies with time of the day and meteorological variables, especially wind, rainfall, humidity, temperature and illumination. Unimodal activity is low at dawn and dusk and high around noon (Sarviva, 1985).

Different types of insects namely honeybees, solitary bees, wasps, syrphid flies, chrysomelids, beetles and various hemipteran bugs were found to visit the coriander flowers (Shelar and Suryanarayana, 1981; Baswana, 1984; Sihag, 1986). Hymenopterans were the most abundant insects and constituted 67.7 and 90 per cent of the floral visitors (Hussein and Abdel-Aal, 1982; Sihag, 1982, 1986). Honeybees were the important visitors and their peak activity was found between 1100 to 1400h during the flowering season of the crop. During this period temperature was more favourable for the activity of the insects as well as for the anthesis and nectar secretion of the crop (Baswana, 1984). *A. florea* was found to be the most important pollinator of coriander (Priti and Sihag, 1999). Among honeybees, *A. cerana* indica and *A. florea* were common pollinating agents of coriander blossoms. *A dorsata* was not observed on coriander. Beside these honeybees, *Trigona iridipensis* was also observed foraging on coriander blossoms and was considered an important pollinator (Deodiker and Suryanaryana, 1972; Shelar and Suryanarayana, 1981; Rao and Suryanarayana, 1983).

A diverse insect fauna visited the carrot crop in India and abroad (Bohart and Nye, 1960; Hawthorn *et al.*, 1960; Batra, 1967; Wojtowski *et al.*, 1979, 1980; Sihag, 1985a, 1986; Kumar *et al.*, 1989). Three hundred thirty four species of insects representing 71 families were collected from carrot blossoms. Most of the species of visitors were in the super family Apoidea or the Ichneumonidae, Psammocharidae (Pompilidae), Sphecidae and Vespidae families of Hymenoptera and the Bombilidae, Sacrophagidae, Syrphidae and Tachnidae families of Diptera.

Seventy-one species of insects, belonging to 31 families and 8 orders were found to visit the carrot blossom at Ludhiana (Goyal *et al.*, 1989) whereas Kumar *et al.* (1989) reported 20 sp. of bees belonging to 8 genera of 4 families visiting the flowers of carrot in mid hills of Himachal Pradesh. *Halictus splendidulus* and *Allodape* sp., *Nomoides* sp., *H. vachalii* and *H. himalayensis* constituted 32.15, 31.27, 18.47, 10.70 and 7.41 per cent of the total bees, respectively. *Halictus* sp. together constituted 50.26 per cent of the total bees. At different places bees were principal pollinators of carrot; *A. florea* at Hisar (India) (Sihag, 1982), *A. c. indica* in Bangladesh (Alam *et al.*, 1987) and *A. mellifera* in Poland (Wojtowski *et al.*, 1982). Wild bees also visited the carrot field. Of these, *Megachile rotundata, Bombus terrestris*, *Halictus* sp., *Andrena* sp. and *Nomeoides* sp. were more common (Hussein and Abdel-Aal, 1982; Wojtowski *et al.*, 1982; Tepedino, 1983; Alam *et al.*, 1987; Kolensnik, 1987; Kumar *et al.*, 1989). Flies were also observed to be the predominant insect pollinators of carrot (Bohart and Nye, 1960; Tepedino, 1983; Goyal *et al.*, 1989) and they were equally abundant to hymenopterans on carrot blossoms (Sihag, 1982). At Solan, *Halictus splendidulus, H. vachalii, Allodape* sp. and *Nomioides* sp. were recorded as the most common visitors to carrot flowers (Kumar and Rao, 1991). Honeybee activity on flowers of carrot was observed by Alam and his co-workers in 1987. Foraging populations declined as the temperature surpassed 34.9^{0}C and the bees did not resume foraging when the temperature was favorable in the evening. It was possibly because of lack of pollen and nectar at that time.

Fennel crop was visited by many different types of insects namely, honeybees, solitary bees, syrphid files, chrysomelids, hemipterous bugs and wasps (Baswana, 1984). Narayanan *et al.* (1960) reported 8 species of Hymenoptera and Diptera visiting the fennel blossoms at Pusa (Bihar). Hymenoptera constituted more than 90 per cent of the insect visitors (Sihag, 1982). Honeybee *A. florea* was found to be the efficient pollinator of fennel in semi-arid conditions of Haryana (Priti and Sihag, 2000). Honeybees were the main visitors of fennel, and their peak activity was between 1100 to 1400 h when the temperature was more favourable for the insects' activity (Baswana, 1984). *A. florea* (Narayanan, *et al.* 1960; Sihag, 1982) and *A. dorsata* (Baswana, 1984) were recorded as best pollinators (Youngken, 1950; 1956). *A florea* contributed 81 per cent of the total pollinators (Narayanan *et al.*, 1960).

On *Allium* sp. the giant bee *A. dorsata* F. was the most abundant followed by *A. florea* F. followed by dipterous insects (Chaudhary and Sihag, 2003). On *Allium* sp. *A. dorsata* was the most common honeybee species; *A. cerana* and *A. mellifera* were also present (Kumar and Gupta, 1993). Honeybees began foraging on *A. fistulum* flowers at about 0600 h and ceased at about 1900 h. Foraging activity was significantly and positively correlated with solar radiation intensity, and negatively with RH (Choi, 1987).

Insect activity increases sharply after sunshine, decreases gradually through the day and ceases before the sunset (Oh and Woo, 1990). The daily flight activity varies with the time of the day and meteorological variables, especially wind, rainfall, temperature and humidity (Sarviva, 1985; Priti and Sihag, 1997).

Alam *et al.* (1987) observed that honeybee activity was highest between 0900 and 1100 h on carrot when the temperature averaged from 31.8^0 to 34.9^0C; foraging activity of honeybees declined as temperature increased and did not resume again in favorable temperature in the evening, possibly because of lack of pollen and nectar. The most common visitors were the honeybees (*A. florea* and *A. dorsata*) which were most active about 1100 to 1400 h; the peak of anthesis also occurred during this period (Baswana, 1984). Pollen foragers started their activity at 0845 to 0915 h and stopped at 1600 to 1700h (Shelar and Suryanarayana, 1981). On carrot, foraging started at about 0700 or 0800 h, reached a peak at about 1000 or 1100 h and ceased at about 1900 h. Honeybees began foraging on onion, *A. fistulum* flowers at about 0600h and ceased at about 1900 h. Foraging activity was significantly and positively correlated with solar radiation intensity, and negatively with RH (Choi, 1987).

Each bee has its specific ecological threshold below which activity does not occur. The ecological threshold required for normal activity and its maintenance differ inter and intra-specifically depending upon the level of adaptability of a species in a given environment (Jain and Kapil, 1980). The honeybee visitation frequency was low in the morning, then reached a peak between 1100 to 1300 h and again declined in the evening (Sihag and Khatkar, 1999).

Gary (1975) stated that 13^0C appeared to be the minimum threshold temperature for initiation of field activities by the honeybees. The activity, however, continued at extremely high temperatures of 43^0C but at this temperature nectar and pollen foraging ceased, though the foraging for water still continued. Whitcomb (1980) reported that *A. florea* worker bee did not forage at temp below 18^0C. Lerer *et al.* (1982) stated that though ambient temperature plays an important role in the initiation of flight and hence in the pollination activity of *M. rotundata*, but it is the solar irradiance that appears primarily responsible for controlling the pollination activity. Cessation of activity occurred even before the temperature dropped to the level required for initiation of bee activity. Bee activity was found to be uniformly positively and significantly correlated with the ambient temperature and nectar sugar concentration, and negatively and significantly with the relative humidity in all the three honeybee species and on all the cultivars of oilseed crops (Sihag and Khatkar, 1999a).

Jain and Kapil (1980) reported that air temperature appeared to be a key factor influencing the initiation of bee activity but cessation was independent of air temperature. The suitability of atmospheric temperature coupled with relative humidity and light intensity not only favoured the initiation but also led to maximum of bee activity. In the evening temperature and relative humidity remained favourable but cessation of bee activity occurred due to decline in the light intensity.

Burill and Dietz (1981) found that in honeybees, foraging activity increased with increasing air temperature but was not correlated with changes in atmospheric pressure and relative humidity. Kapil and Brar (1971) stated that a combination of 15-18^{0}C temperature and 80-82 per cent RH appeared to be minimum activity peaked at a combination of 22-25^{0}C temperature and 50-65 per cent RH. The cessation of activity seemed to be governed by the fast decline in light intensity.

Sihag (1984) gave a conclusive report that in social bees temperature acted as a stimulus for the commencement and cessation of pollination activity in winter whereas light did it in summer. Winter solitary bees behaved identically alike which social bee did in winter. However, in summer solitary bees, for the commencement of foraging activity, increasing temperature in the morning acted as a stimulus, whereas, cessation in the evening was affected by decreasing light intensity. Flowers of cauliflower were found to be visited by four hymenopterans and five dipteran species which were most frequent and voluntary pollinators among hymenopterans; activity duration of *A. mellifera* was found maximum (Priti and Sihag, 1997).

2.4 FLOWER COLOUR AND SIZE

The flower colour is an important factor in the attraction of pollinators (Leppik, 1977; Kevan, 1983). Stanton (1987) observed that honeybees accounted for almost 90 per cent of all insects in *Raphanus sativus* and visited typically yellow or white flowers. Yellow flowers are often highly reflective and are visited by a large variety of insects (Kevan, 1983).

Honeybees have been found to be highly sensitive to orange, yellow and green colours (Frisch, 1950; Kugler, 1943) and are more striking to yellow and blue colours (Burkhardt, 1964). The visible spectrum sensitivity in bees lies in between 380-700 nm, including the ultraviolet spectrum (Kevan, 1978). This through various shades and patterns of blue-green, provide a distinct conspicuousness to the visiting bees (Daumer, 1958; Eisner *et al.*, 1969; Erickson and Garment, 1979; Wehner, 1975; Wells *et al.*, 1981). Red and blue (Lutz, 1935) as well as yellow and violet (Guldberg and Atsatt, 1975) flowers in general have been found to reflect more UV than white and green flowers.

Carotenoids, xanthophylls, flavonoids and chlorophyll are primary floral pigments. Their differential rate of synthesis, metal complexing and the pH of the medium impart colours to the flowers (Asen *et al.*, 1972; Goodwin and Thomas, 1964; Harborne, 1965). The quantum of anthocyanin present in a flower seems to regulate the reflectance and the absorbance of UV quantities of a flower (Caldwell, 1968). Most yellow coloured flowers owe their colouration to high concentrations of carotenoids in the corolla. Light yellow and lemon yellow coloured flowers usually have xanthophylls in their corolla. Anthocyanins are the subgroups of plant phenolic pigments termed flavonoids accounting for the cyanic colourations (orange-red-blue-purple) in most flowers (Jones and Little, 1983).

Normally large sized blossoms are visited by large sized pollinators and small sized blossoms by small sized pollinators (Priti and Sihag, 1997).

Inouye (1980) examined two plant species *Aconitum columbianum* (corolla depth = 8.44mm) and *Delphinium barbeyi* (corolla depth = 13.96 mm). *Aconitum* was visited primarily by *Bombus flavifrons*, a bee with medium tongue length, but *Delphinium* was visited primarily by *B. appositus* and to a lesser extent by *B. flavifrons*. Short tongued bees were more efficient on short corolla flowers, long tongued bees on long corolla flowers and medium tongued bees on medium corolla flowers. When a bee had a tongue that was too long for a flower, it was less efficient in foraging (Ranta and Lundberg, 1980).

2.5 NECTAR QUALITY AND QUANTITY

Nectar is recognized as the main attractant for pollinators. Many workers have worked out a linear relationship between the quantity and quality of nectar and population of honeybees (Butler *et al.*, 1945; Vansell and Todd, 1946; Wykes 1953; Mommers, 1977 and Corbet, 1978).

Large variations however, have been reported to occur in both quantity and quality of nectar in different flowers, at inter-specific and intra-specific levels (Peter, 1971; Bataglini, 1974; Macior, 1975; Sihag and Kapil, 1983). Some plants secreted large quantity of nectar per flower (Pryce and Jones, 1943; Butler *et al.*, 1943; Shuel, 1955). Silva and Dean (2000) observed that in 9 inbred lines of onion (*Allium cepa)* the average amount of nectar produced by both the umbels and individual florets were significantly positively correlated with the number of bee visits.

Hagler *et al.* (1990) examined nectar characteristics of 6 onion cvs. (*Allium cepa*). Mean nectar amount ranged from 0.54 to 0.84 μl per floret per day. Mohr and Jay (1990) found that mean daily nectar production and sugar concentration for *B. campestris* cultivars were 0.68 μl and 57 per cent respectively and for *B. napus* cultivars were 0.90 μl and 62 per cent respectively.

Haslbachova *et al.* (1986) studied nectar production in self-incompatible lines of cabbage. The results showed that the average total nectar production per flower was 6.2 mg, containing 1.2 mg of sugars.

The type of nectar in a flower is considered to be a specific feature of a plant family or the closely related families and in most of the plants types the composition of the nectar has been found remarkably consistent at species level (Bailey *et al.*, 1954; Butler, 1953; Furgula *et al.*, 1958; Percival, 1965; Wykes, 1952a). The major sugar constituents of various nectar types are sucrose, glucose and fructose (Wykes, 1952b).

Nectar of *Eucalyptus* (Vansell, 1944) and sweet clover (Furgula *et al.*, 1958) contained sucrose and hexoses in a balanced ratio while *Brassica* and dwarf mistetae have the dominancy of a hexose sugar (Brewer *et al.*, 1974). About one-third of the Phillipine species were with fructose and glucose ratio above one (Rowley, 1976).

Citrus sp., *Lavendula* sp., *Robinia* sp., *Trifolium repens, Aesculus hippocastanum* and some species of *Rhododendrone* had the dominancy of fructose (Davies, 1978).

Majority of the plant species investigated by Rowley (1976) in Phillippines had the dominance of sucrose. Sihag and Kapil (1983) studied nectar sugar of 44 plant species and found that sucrose dominated in 13, glucose in 24 and the rest contained equi-proportioned glucose, fructose and sucrose. Generally, pollinators with high energy requirements foraged on sucrose rich flowers whereas those with low energy requirements relied on glucose or fructose rich flowers (Abrol and Kapil, 1991).

2.6 NECTAR SUGAR CONCENTRATION

Nectar sugar concentration is one of the most important factors affecting bee flower interaction. A positive correlation was found between attractiveness to bees and nectar sugar concentration in citrullus, suggesting that this characteristic is one of the parameters responsible for variability in attractiveness to honeybees (Wolf *et al.*, 1999).

Generally high nectar sugar concentration was desirable for attracting the honeybees (Frisch, 1950; Meloyan, 1975). Frisch (1950) reported the threshold value (5 to 40 per cent) of sugar concentration for its acceptance by the bees. Backman and Waller (1971) observed that the bees rejected a solution with sugar concentration less than 20 per cent. It has also been reported that honeybees were able to discriminate a sugar solution with a difference of 5 per cent concentration

(Jamieson and Austin, 1956). Various workers in a variety of plant species recorded floral nectar sugar concentration ranging between 4-87 per cent (Vansell, 1934; Fahn, 1949; Percival, 1965). Some of the species having remarkably high concentration of sugar are: *Astragalus pachypus* (59.2 per cent), *Salix* sp. (60 per cent), *Robinia pseudo-acasia* (63 per cent), *Aesculus* sp. (72.2 per cent) and *Vitis* sp. (75 per cent) (Vansell, 1939; Pryce-Jones, 1943; Shuel, 1955). Gegear and Thompson (2004) observed a strong positive relationship between degree of flower constancy and rate of net energy gain indicating that honeybees were more economic foragers.

In general nectar sugar concentrations have been reported in the range of 33-71 per cent at Ludhiana and Hisar (Kapil and Brar, 1971; Sihag and Kapil, 1983 and Abrol, 1985). Sharma (1958) recorded 49 and 45 per cent average nectar sugar concentration in sarson and toria respectively. Abrol (1985) observed nectar sugar concentration in the range of 35-52 per cent in *B. campestris* var. toria, and 32-53 per cent in *B. juncea.* Rao and Lazer (1983) found that nectar sugar concentration in onion cvs. (*Allium cepa* L.) varied from 67 to 75 per cent during the day.

In case of *Helianthus annuus* nectar sugar concentration was found to vary from 42-60 per cent depending upon geographical location (Montogomary, 1958; Peter, 1971). However, Deodikar (1976) reported that nectar sugar concentration varied from 15-29 per cent and at a sugar concentration of 17 per cent bee started collecting nectar. Fell (1986) observed mean value of 24.2 per cent sugar in nectar of *H. annuus*. Toit and Coetzer (1991) also found variation in the nectar sugar concentration in different cultivars of sunflower.

2.7 POLLEN PROTEIN AND SUGAR CONCENTRATION

Pollen contains all the essential nutrients required for growth and development of honeybees. Chemical analysis of pollen showed that on an average it contains 3-15% moisture (Bell *et al.*, 1983), 1-50% carbohydrates (Todd and Bretherick, 1942); 1-20% fats; 7-35% proteins (Barbier, 1970) while in Switzerland pollen (Wille *et al.*, 1984) mean value of proteins ranged from 18.1-24.7%.

Todd and Bretherick (1942) reported pollen of date palm (*Phoenix dactylifera*) containing 35.5 per cent proteins. In *Populus nigra* var. Ital. the protein contents were 36.5 per cent (Standifer, 1967). In mixed pollen of *Cycas revolute,* the crude protein varied from 32.9 to 33.8 per cent of dry weights. Thus, within a single species, even grown under similar conditions, variation of 5 per cent are common in protein contents of pollen from different plants. Bell *et al.* (1983) reported that crude protein contents varied in 20.6 per cent for *E. marginata* to 27.9 per cent in *E. calophylls* pollens. In *Vicia faba* pollen, 32.3 per cent protein contents were observed (Ibrahim, 1974).

Lee and Chung (1976) reported a variation of protein contents from 2.0 to 20 in pollens. Similarly, Petkova and Ivanov (1977) reported that crude protein contents ranged from 14.00 per cent in *Taxacum officinals* to 31.88 per cent in *Quercus ceris.* Guan *et al.* (1984) reported 26.02 per cent protein contents in bee collected pollen and (Echigo *et al.*, 1986) 21 per cent in bee collected loads. Tabio *et al.* (1988) reported about 19.5 per cent protein contents in multifloral pollens. Serra Bohnvehl *et al.* (1986) reported total protein contents varied from 12.6 to 18.2 per cent in bee collected pollen, while Wille *et al.*, (1984) reported that the mean value for proteins ranged from 18.1 to 24.7 per cent in Switzerland. During the year, mean values for protein contents of pollen showed a typical pattern; low in early spring with a sudden rise in second half of April and increased up to 25 per cent or even more than 30 per cent thus showing that protein contents varied from season to season. Day *et al.* (1990) made a chemical analysis on the bee collected pollen and reported that the protein contents varied from 29 per cent (Pistillate Kiwifruit) to 23.5 per cent (Broom pollens). Youssef *et al.* (1978) reported that the protein content of the bee-collected pollen of *Trifolium alexandrinum, Zea mays, Vicia faba* and *Brassica kaber* ranged from 23.3 to 37.7 per cent. Crude protein content of the bee-collected pollen varied with season. It was lowest in autumn (13.5%) and highest in the rainy season (18.5%) (Sharma and Gupta, 1996). Saa-Otero *et al.* (2000) reported that protein content of corbicula pollen of *Castanea sativa, Erica, Eucalyptus, Halimium alyssoides, Quercus robur, Raphanus raphanistrum, Rubus* and *Cystisus* varied between the 14 per cent and 29.6 per cent.

In most pollen, carbohydrates constitute the major dry matter fraction and many comprise up to 50 per cent of their dry weight. Lunden (1954) reported that *Zea mays* trinucleate pollens were high in total carbohydrates but *Beta* and

Anbrocia pollen contained low carbohydrate contents. The angiospermic pollens collected from bees are generally higher in reducing sugars but low in non-reducing sugars than pollens directly isolated from plants (Todd and Bretherick, 1942) Lunden (1954) attributed this decrease in non-reducing sugars to a high rate of metabolism in bee collected pollens.

Kozma and Mohacsy (1968) reported that grape pollen contained 2.5 to 5.6 per cent total sugars. Similarly, Youssef et al. (1978) reported total reducing and non-reducing sugar contents in four pollen types viz. Egyptian clover, maize, broad been and wild mustard. Total reducing sugar contents varied from 7.5 per cent to 13.0 per cent while total non-reducing sugars contents varied from 0 to 4.5 per cent carbohydrates. Day *et al.* (1990) further carried out nutrient composition studies of nine pollen types and reported that the total carbohydrates ranged from 12.6 (in Matagouri pollen) to 29.6 per cent (Pistillate kiwifruit pollen) but non-reducing sugar contents ranged from 1.1 per cent (Matagouri pollen) to 25.7 (Pistillate kiwifruit pollen). The least preferred petunia flowers constituted about 2.51 per cent of sugars.

Back and Lee (1974) further reported the concentration of glucose and fructose as free sugars 13.78 per cent and 7.52 per cent, respectively in bee collected pollens in hive.

McLellan (1977) observed that pollen collected from hive of honeybees contained about 30 per cent carbohydrates of the dry matter (Glucose and fructose) in 7 pollen types in Scotland.

2.8 MORPHOMETRIC VARIATION OF BODY AND TONGUE LENGTH OF BEES

Most important adaptation to suck nectar from flowers in bees has been the development of a highly mobile and flexible labio-maxillary complex. However, variation in size and shape of glossae among aids at generic and specific level has gained special attention (Michner, 1974). In primitive bees, the glossae are very short and conical whereas in higher forms the glossae are long and covered with distally diverted hair. These long tongued bees are characterized by the presence of labial palp galae and glossae longer tongue stipes (Winston, 1979). Functional length of proboscis is due to glossa (Harder, 1982). Hobbs *et al.* (1961) defined tongue length as combined length of prementum and labial palps. Inouye (1980) and Harder (1982, 1983) emphasized that tongue length of prementum and glossae. Macior (1978) reported that to determine the actual depth from which nectar can be extracted by a bumble bee can be calculated by adding length of head and even part of thorax must be added to proboscis length if mouth of corolla is wide enough to accommodate them.

Tongue length plays an important role for bumblebees in determining the type of flower to be explored (Hobbs *et al.*, 1961; Morse, 1977; 1978 and Harder 1982). Bumble bees, as other flower visitors, appear to partition flower resources primarily on the basis of relationship between proboscis length and length of corolla tubes of flowers available (Knuth, 1906). A direct relationship has been observed by Brian (1957) Heinrich (1976 a,b) and Inouye (1978) between corolla tube length and proboscis length in bumble bees. This relationship has its importance in resource partitioning in nectivores (Macior, 1975 and Inouye, 1976, 1977).

Bhatt and Jagdish (1995) reported absence of short tongued bees on sunflower due to non-accessibility of nectar from these flowers by these bees. Heinrich (1976a) reported that each bee assumes a characteristic posture while lending on flowers for collection of nectar and pollen both. In *Brassica* sp. the bee collecting nectar either enters through its mouth or between the petals at their base if it was difficult to extract nectar from the mouth of flower (Sihag, 1990). In case of sunflower nectar thieving is not possible and only long tongued bees are capable of extracting nectar. Bhatt and Jagdish (1995) and Sihag (1993a) reported complete dissertation of sunflower heads by *A. florea* in favour of *Brassica campestris* due to non-accessibility to sunflower nectar and easy accessibility to that of *B. campestris*.

Holm (1966) observed that short tongued bumble bees and honeybees collected nectar from long tube red clover and blue berry flowers only by cutting a hole in corolla tube. Inouye (1980) observed bees with short proboscis length visiting efficiently on short corolla tubes than those having long proboscis. He also reported that proboscis length is an “important determinant” of the efficiency of nectar extraction during foraging by bumblebees.

Chapter III

MATERIALS AND METHODS

The present study was carried out at the Research Farm of Department of Vegetable Crops and in the Apiculture laboratory of Department of Zoology & Aquaculture of CCS Haryana Agricultural University, Hisar. Three concurrently flowering vegetable/ spice crops viz fennel (*Foeniculum vulgare* L.), onion (*Allium cepa* L.) and carrot (*Daucus carota* L.) were selected for this study. The varieties taken of fennel were Rajendera Saurabh, GF-1, HF-33 and Local Check; of onion were HS-1 and HS-2 and of carrot were HCP-1, HCO-4, HCB-1 and HC-1. These were grown in the adjacent plots as described in figure 1. An account of various methodologies followed during the present study is described under the following heads:

3.1. Relative abundance of different honeybee species as a measure of their preference for different crops/ varieties

The pollinator abundance on a crop/ variety was taken as a measure of its preference for the reference crop/ variety. For this purpose, five plots of 1 x 1 m size were randomly selected in each crop area. On a day, population abundance of different honeybee species on each plot was recorded for one minute at two hourly intervals starting from the commencement to cessation of the bee activity. Observations were repeated at weekly intervals starting from commencement till the cessation of the flowering on the experimental crops/ varieties (Sihag, 1986). The data so recorded were analyzed in Completely Randomized Design (Snedecor and Cochran, 1967) and differential attractiveness of different crops/ varieties were compared.

3.2 Crop parameters responsible for their attractiveness to the pollinators

Following crop attributes were studied to ascertain their role as causative factors for relative attractiveness of different crops/ varieties.

3.2.1 Flower colour

Flower colors of fifty receptive flowers of each crop/ variety were observed.

i. **Visual colour appearance**

The colour of the flowers of different crops/varieties were recorded simply on the basis of their visual appearance.

ii. **Colour pigments**

Petals of flowers of each crop/ variety were used for the estimation of carotenoids and xanthophylls by following the method of Davies (1965), and anthocyanins by following the method of Elliot (1977).

Carotenoids and Xanthophylls

Reagents used

1) 80 per cent acetone in water (v/v)
2) Petroleum ether
3) 90 per cent methanol in water (v/v)

Procedure

Samples of 100 mg of fresh petals of each crop/ variety were homogenized in 10 ml of acetone and repeated extractions were made with acetone till the supernatant was colorless. Total volume of the extract was made to 20 ml with acetone. To 10 ml of this, 10ml of petroleum ether was added. The contents were mixed in a separating funnel and the two distinct layers were obtained by adding few drops of water. After removing the lower layer, 10 ml of methanol was added into the separating funnel. Contents were again shaken well and the mixture was allowed to stand to form two layers. Upper layer contents were examined spectrophotometrically at 424 nm for carotenoids and lower layer contents at 450 nm for xanthophylls against their respective reagent blanks.

<u>Anthocyanins</u>

Reagent used

One per cent Hydrochloric acid (HCl) methanol mixture (v/v)

Procedure

Like above experiment, samples of 100 mg of fresh petals mixed 5 ml of HCl- methanol mixture were kept in darkness for 24 h. Repeated extractions were made to get total extraction of the anthocyanins. The total volume of the extract was made to 10 ml by adding more HCl- methanol solvent. Optical density (OD) was read at 425, 450, 475, 500, 525, 550 and 575 nm to find the absorbance maxima for each extract.

3.2.2 Floral size

Corolla length was measured under a microscope by using the occulo-micrometer. This measurement reflected the actual depth that the bee must reach in order to harvest the nectar [Fig.2 : (a) Sihag, 1985b (b) Sihag, 1985c]. Fifty flowers of each variety were taken for determining the floral size viz. length of the corolla.

3.2.3 Quality of nectar

Types of sugars present in the nectar in the flowers of all the experimental crops/ varieties of fennel, onion and carrot were determined by paper chromatography. Nectar from the flowers of crops/ varieties was collected with the help of micropipette between 0900-1100h. Very small spots (2 or 3) of the nectar taken from the flowers were applied on chromatography paper. The glass chamber, where chromatography could be done, was made saturated with the vapours of the solvent before chromatography was started. Then chromatography was started by placing chromatography paper in the saturated glass chamber taking butanol, acetic acid and water in the ratio of 4:1:5 as solvent. After keeping for 12-15 h, chromatography paper was allowed to dry at room temperature. Then developer ammonical silver nitrate was sprayed and chromatography paper was then kept in oven at 50°C till dried. Spots of different sugars so appeared were characterized by their 'Rf' values in relation to the standard glucose, fructose and sucrose. This experiment was performed on the flowers of all crops/ varieties.

3.2.4 Quantitative estimation of nectar sugar concentration

For this purpose, floral buds which may bloom on the next 1 or 2 days were selected and covered with butter paper bags. Nectar was collected with the help of fine capillaries and the volume of nectar was measured with the help of 5 λ micropipette between 0900-1100h. Total soluble sugars of nectar of the flowers of all the experimental crops/ varieties of fennel, onion and carrot were determined following the standard method given by Yemm and Wills (1954).

Taking 0.1 ml of ethanol extract in a test tube was evaporated to dryness on a water bath. After cooling, the residue was dissolved in one ml of distilled water and then 4ml of anthrone reagent (0.4 per cent anthrone in concentrated sulphu ric acid) was added to it. The mixture was then heated in a water bath for 10 minutes. After cooling, OD was read at a wavelength of 620 nm against reagent blank spectrophotometrically. Standard curve was prepared using graded concentrations of D- glucose.

The calorific value present in the floral nectar was determined following Heinrich and Raven (1972). Knowing the sugar concentration and volume of nectar per floret the total energetic reward/flower was calculated by assuming that 1mg of sugar produced 4 calories (cal.) of energy irrespective of the sugar type by using the following relationship:

Energy/ flower = Nectar volume (µl) × concentration of nectar × 4 cal.

3.2.5 Proteins in collected anthers

Anthers were collected from the flowers of the crops of the study directly in the petri plates with the help of a fine brush. The total protein contents in the collected anthers of all the crops/ varieties were determined following the standard method given by Lowry *et al.* (1951). The fresh anthers weighing 100 mg were separately homogenized in 80 per cent ethanol (v/v) using a pinch of acid washed sand as an abrasive material. The homogenates were reflexed for 15 minutes on a water bath and centrifuged. The residue was further reflexed twice with 80 per cent ethanol. The supernatant and pellet were partitioned further for analysis as per the flow diagram.

Reagents used

1. 2 per cent aqueous Na_2CO_3 (Sodium carbonate)
2. 0.5 per cent $CuSO_4.5H_2O$ in 1 per cent of sodium citrate
3. Alkaline $CuSO_4$ solution. Mixed 50 ml of aqueous Na_2CO_3 with 1 ml of $CuSO_4$. $5H_2O$ in sodium citrate.
4. Folin- Cio-Calteau Reagent (1N).

Procedure

To 1 ml of aliquot, taken from protein extract in 1N NaOH, 5 ml of alkaline $CuSO_4$ (Copper sulphate) was added and mixed well. The mixture was allowed to stand for 10 minutes and 0.5 ml of Folin's reagent was then added to it and mixed vigorously. It was allowed to stand for 30 minutes and OD was read at 750 nm against reagent blank. Standard curve was prepared using graded concentrations of bovine serum albumen.

3.2.6 Quantitative estimation of sugar concentration in collected anthers

Total soluble sugars of collected anthers from the experimental crops/ varieties of fennel, onion and carrot were determined following the standard method given by Yemm and Wills (1954) as explained under point 3.2.4.

3.3 Pollinator parameters influencing their preference for crops

3.3.1 Pollinator size

To ascertain whether the size of the pollinator was a factor in determining its suitability to the flowers of a crop, length and breadth of workers of four honeybee species viz. *A. mellifera, A. florea, A. dorsata* and *A.cerana* were measured. Foragers of each honeybee species were captured from the field and their length were measured with the help of linear scale and breadth of the thorax region with vernier callipers. Observations were taken on thirty bees of each species.

3.3.2 Tongue length

Freshly caught foragers of four honeybee species viz. *A. mellifera, A. florea, A. dorsata* and *A. cerana* were killed by exposing them to the vapours of ethyl acetate so that after death honeybees might have full extended proboscis. The whole proboscis (prementum, glossa, labial palps and maxillary palps, Fig.3, Khatkar, 1996) were removed from the head and stretched on the occulo-micrometer. Its length (prementum + glossa) was measured. Since the proboscis length of dead bee is less than that of live bee by a factor of 10 per cent and length is greater by 1.0 mm than tongue length (Root, 1962; Bhatt and Jagdish, 1995), so the functional tongue length was calculated by using the following relationship given by Bhatt and Jagdish (1995).

$$PL = pl + \frac{pl \times 10}{100} + 1.00mm \text{ (extensions of proboscis)}$$

Where,

Pl = Functional proboscis length

pl = Proboscis length of dead bee

Observations were recorded on thirty bees of each species.

Chapter-IV

EXPERIMENTAL RESULTS

This study was carried out on the honeybees as pollinators of three vegetable or spice crops/ varieties. Therefore, the results are presented accordingly under the following heads:

4.1 Relative abundance of different honeybees as a measure of intra-specific preferences for different varieties

4.1.1 Fennel

All the four species of honeybees (*Apis florea, Apis cerana, Apis mellifera* and *Apis dorsata,* Plates 1, 2, 3 and 4 respectively) were observed to visit the blossoms of four varieties of fennel at Hisar. The variety GF-1 was most preferred by the honeybees as the number of honeybees was found to be maximum on this variety (1.96 honeybees /min/m^2). The least number of honeybees was found to visit the flowers of the variety "local check" (0.90 honeybees /min/m^2). The difference of preference for different varieties was significant ($P \leq 0.05$, ANOVA; Table 1).

4.1.2 Onion

Four species of honeybees (*A. dorsata, A. florea, A. cerana,* and *A. mellifera*, Plates 5, 6, 7 and 8 respectively) were observed to visit the blossoms of two varieties of onion at Hisar. The variety HS-2 was visited by 58.33 percent of the total honeybees whereas on HS-1 this proportion was 41.67 percent. The average number of honeybees was significantly higher on HS-2 (0.98 honeybees /min/m^2) than on HS-1 (0.70 honeybees /min/m^2) ($P \leq 0.05$, ANOVA; Table 2).

4.1.3 Carrot

All the four species of honeybees (viz. *A. mellifera, A. florea, A. dorsata* and *A. cerana,* Plate 9) were found to visit the blossoms of four varieties of carrot at Hisar. The relative percentages of these honeybees were 39.89, 32.64, 4.66 and 22.79 on four varieties of carrot, i. e. HCP-1, HCO-4, HCB-1 and HC-1, respectively. The number of honeybees was significantly higher (0.77 honeybees/min/m^2) on HCP-1 than on other varieties ($P \leq 0.05$, ANOVA; Table 3).

4.2 Relative abundance of different honeybees as a measure of inter-specific preferences for different crops

Four species of honeybees viz. *A. mellifera, A. florea, A. dorsata* and *A. cerana* were found to visit the blossoms of the three crops of this study viz. fennel, onion and carrot. Their relative percentages on the three crops were 53.02, 29.89 and 17.08 respectively. The number of honeybees was significantly higher on fennel (1.49 honeybees /min/m^2) than on onion or carrot ($P \leq 0.05$, ANOVA; Table 4).

Among these honeybees, *A. mellifera, A. florea, A. dorsata* and *A. cerana* comprised 5.55, 83.03, 3.36 and 8.07 per cent, respectively on fennel; 9.76, 24.26, 53.85 and 12.13 per cent, respectively on onion; and 3.12, 90.63, 2.08 and 4.17 per cent, respectively on carrot (Table 4,5) (Fig. 4). The number of *A. florea* was significantly higher on fennel (4.94 honeybees /min/m^2) than on carrot (1.74 honeybees /min/m^2) followed by onion (0.82 honeybees /min/m^2). Whereas, *A. dorsata* preferred flowers of onion (1.82 honeybees/min/m^2).

Average number of all the honeybees, irrespective of their species was low in the beginning of the flowering period. Significant increase in number was observed during the peak flowering, which was obtained at the time of full bloom (5^{th} April, 2004). After that the number decreased significantly and it was again low at the time of cessation of flowering ($P \leq 0.05$, ANOVA; Table 4).At five observation hours (0900, 1100, 1300, 1500 and 1700 h) the numbers of different honeybees visitors, i. e. *A. mellifera, A. florea, A. dorsata* and *A. cerana* were observed. The number of honeybees was maximum at 1100 h and minimum at 1700h ($P \leq 0.05$, ANOVA; Table 5).

4.3 Flower colour as a determinant factor for inter-varietal preferences

4.3.1 Fennel

Flower colour of four varieties of fennel as recorded by visual observations revealed that the corolla of all the varieties were bright yellow.

Calorimetric analysis of the petals showed non-significant difference in colours of various varieties of fennel (Table 6).

Table 6: Flower colour and relative density of carotenoids and xanthophylls in the floral petals of four varieties of fennel

Crop	Colour	OD of the petal extract*	
		Carotenoids (A_{424} nm)	Xanthophylls (A_{450} nm)
Rajendera Saurabh	Yellow	0.1612 ±0.0016	0.0605 ±0.0037
GF- 1	Yellow	0.1614 ±0.0012	0.0607 ±0.0049
HF- 33	Yellow	0.1610 ±0.0025	0.0602 ±0.0032
Local Check	Yellow	0.1609 ±0.0015	0.0602 ±0.0038

* Mean ± S. E. of 10 observations
NS difference was found in carotenoids and xanthophylls of different varieties (F-test)

The absorption spectrum of the acid-methanol extract of the petals showed that quantitatively, the anthocyanins differed non-significantly in all the varieties of fennel (Table 7). In the varieties, the highest amount was that of carotenoids followed by xanthophylls and anthocyanins. But the number of visiting honeybees was not same. Hence it seems that there must be some other factor determining inter-varietal preferences of honeybees.

Table 7: Absorption spectrum for the anthocyanins extract from the floral petals of four varieties of fennel

Wavelength	OD of petal extract*			
	Rajendera Saurabh	GF- 1	HF- 33	Local Check
A_{425}	0.0318 ±0.0123	0.0320 ±0.0002	0.0318 ±0.0056	0.0317 ±0.0089
A_{450}	0.0278 ±0.0012	0.0279 ±0.0021	0.0277 ±0.0012	0.0276 ±0.0005
A_{475}	0.0175 ±0.0113	0.0177 ±0.0105	0.0175 ±0.0125	0.0175 ±0.0082
A_{500}	0.0142 ±0.0041	0.0143 ±0.0023	0.0142 ±0.0036	0.0141 ±0.0020
A_{525}	0.0129 ±0.0001	0.0131 ±0.0005	0.0128 ±0.0012	0.0128 ±0.0007
A_{550}	0.0088 ±0.000	0.0089 ±0.0002	0.0087 ±0.0016	0.0086 ±0.0017
A_{575}	0.0067 ±0.0002	0.0069 ±0.0005	0.0066 ±0.0008	0.0066 ±0.0018

* Mean ± S. E. of 10 observations

NS difference was found in anthocyanins of different varieties (F-test)

4.3.2 Onion

Flower colours of different varieties of onion were depicted simply by visual observation. It revealed that the corolla of two varieties of onion were white coloured. Calorimetric analysis of flower colour of the two varieties of onion was also observed. It was found that amounts of carotenoids as well as that of xanthophylls (Table 8) and anthocyanins (Table 9) do not differ significantly of the two varieties of onion. But the number of honeybees visiting the varieties of onion was not same. Therefore, some other factors seem to play any role for intra-specific preference.

Table 8: Flower colour and relative density of carotenoids and xanthophylls in the floral petals of two varieties of onion

Variety	Colour	OD of the petal extract*	
		Carotenoids (A_{424} nm)	Xanthophylls (A_{450} nm)
HS-1	White	0.0344 ±0.0038	0.0155 ±0.0067
HS- 2	White	0.0346 ±0.0008	0.0156 ±0.0006

* Mean ± S. E. of 10 observations
NS difference was found in carotenoids and xanthophylls of different varieties (F-test)

Table 9: Absorption spectrum for the anthocyanins extract from the floral petals of two varieties of onion

Wave length	OD of the petal extract*	
	HS- 1	HS- 2
A_{425}	0.0320 ±0.0033	0.0322 ±0.0018

A_{450}	**0.0278 $\pm$0.0054**	0.0280 $\pm$0.0002
A_{475}	**0.0176 $\pm$0.0024**	0.0177 $\pm$0.0076
A_{500}	**0.0144 $\pm$0.0035**	0.0146 $\pm$0.0027
A_{525}	**0.0132 $\pm$0.0062**	0.0133 $\pm$0.0096
A_{550}	**0.0088 $\pm$0.0047**	0.0090 $\pm$0.0012
A_{575}	**0.0067 $\pm$0.0034**	0.0068 $\pm$0.0072

* Mean $\pm$ S. E. of 10 observations
NS difference was found in anthocyanin of different varieties (F-test)

4.3.3 Carrot

Flower colours of four varieties of carrot as recorded simply by visual observations revealed that the corolla of four varieties of carrot were white coloured. The calorimetric analysis showed that petals of flowers of four varieties of carrot had non-significant difference in the quantities of carotenoids as well as xanthophylls (Table 10). The absorption spectrum of the acid-methanol extract of the petals of four varieties of carrot was maximum at 575 mm and lowest at 425 nm (Table 11).

Table 10: Flower colour and relative density of carotenoids and xanthophylls in the floral petals of four varieties of carrot

Variety	Colour	OD of the petal extract*	
		Carotenoids (A_{424} nm)	Xanthophylls (A_{450} nm)
HCP-1	White	0.0362 ±0.0029	0.0155 ±0.0007
HCO-4	White	0.0361 ±0.0067	0.0154 ±0.0076
HCB-1	White	0.0360 ±0.0019	0.0151 ±0.0077
HC-1	White	0.0360 ±0.0020	0.0152 ±0.0065

* Mean ± S. E. of 10 observations
NS difference was found in carotenoids and xanthophylls of different varieties (F-test)

Quantitatively, anthocyanins differed non-significantly in all the four varieties of carrot. Flower colour does not seem to be a factor in intra- specific preference as number of honeybees that visited the four varieties was not same. Therefore, there must be other factors that determine intra-specific preference.

Table 11: Absorption spectrum for the anthocyanins extract from the floral petals of four varieties of carrot

Wave length	OD of petal extract*			
	HCP-1	HCO-4	HCB-1	HC-1
A_{425}	0.0322 ±0.0003	0.0321 ±0.0003	0.0320 ±0.0007	0.0321 ±0.0003

A_{450}	0.0279 ±0.0012	0.0279 ±0.0009	0.0276 ±0.0001	0.0277 ±0.0001
A_{475}	0.0175 ±0.0006	0.0174 ±0.0001	0.0173 ±0.0009	0.0174 ±0.0002
A_{500}	0.0144 ±0.0006	0.0144 ±0.0002	0.0142 ±0.0007	0.0143 ±0.0008
A_{525}	0.0131 ±0.0005	0.0130 ±0.0002	0.0130 ±0.0008	0.0130 ±0.0007
A_{550}	0.0089 ±0.0008	0.0088 ±0.0006	0.0086 ±0.0009	0.0086 ±0.0002
A_{575}	0.0068 ±0.0001	0.0067 ±0.0001	0.0066 ±0.0005	0.0067 ±0.0004

* Mean ± S. E. of 10 observations
NS difference was found in anthocyanins of different varieties (F-test)

4.4 Flower colour as a measure of inter-specific preferences for different crops

Flower colours of these crops as recorded simply by visual observations revealed that the corolla of fennel was bright yellow and that of onion and carrot were white. The calorimetric analysis of the petals of fennel, onion and carrot revealed the presence of more carotenoids in fennel than the flowers of onion and carrot (Table 12). The xanthophylls were also more in the flowers of fennel than flowers of onion and carrot.

Table 12: Flower colour and relative density of carotenoids and xanthophylls in the floral petals of vegetable/spice crops

Crop	Colour	OD of the petal extract*	
		Carotenoids (A_{424} nm)	Xanthophylls (A_{450} nm)

Fennel	Yellow	**0.1611[a] ±0.0017**	0.0604 [a] ±0.0020
Onion	**White**	**0.0345* [b] ±0.0005**	0.0156 [b] ±0.0004
Carrot	**White**	**0.0361 [b] ±0.0006**	0.0153 [b] ±0.0003

* Mean ± S. E. of 40 observations
** Mean ± S. E. of 20 observations
Note: Figures with different letters differ significantly (Multiple Range Test)

The absorption spectrum of the acid-methanol extract of the petals indicated absorption maxima for fennel, onion and carrot at 575 nm (Table 13).

Table 13: Absorption spectrum for the anthocyanins extract from the floral petals of vegetable/ spice crops

Wave length	OD of the petal extract		
	Fennel* [a]	**Onion** [a]**	**Carrot* [a]**
A_{425}	0.0318 ±0.0058	0.0321 ±0.0001	0.0321 ±0.0002
A_{450}	0.0278 ±0.0009	0.0279 ±0.0002	0.0278 ±0.0016
A_{475}	0.0176 ±0.0054	0.0177 ±0.0000	0.0174 ±0.0014
A_{500}	0.0142 ±0.0017	0.0145 ±0.0015	0.0143 ±0.0008
A_{525}	0.0129 ±0.0008	0.0133 ±0.0001	0.0130 ±0.0006
A_{550}	0.0088 ±0.0008	0.0089 ±0.0006	0.0087 ±0.0006
A_{575}	0.0067 ±0.0009	0.0068 ±0.0004	0.0067 ±0.0003

* Mean ± S. E. of 40 observations
** Mean ± S. E. of 20 observations
Note: Figures with same letters differ non-significantly (Multiple Range Test)

Quantitatively, the anthocyanins were very less in all the three crops viz. fennel, onion and carrot. But more honeybees visited the yellow flowers having higher amounts of carotenoids and xanthophylls of fennel. Hence flower colour is considered as an important parameter for inter- specific preference.

4.5 Flower size as a measure of intra-specific preferences for different varieties

4.5.1 Fennel

Table 14 depicted the variation in corolla length/nectar depth of different varieties of fennel. The corolla lengths of four varieties of fennel differed non-significant ($P \leq 0.05$, ANOVA). But the preference by honeybees for different varieties was not same. Hence, it does not seem to be a determinant factor for differential preference of fennel varieties by honeybees. Therefore, inter-varietal preference must be determined by some other factors.

4.5.2 Onion

Corolla lengths of two varieties of onion are shown in table 15. In this case also the difference in corolla lengths of the varieties was non-significant ($P \leq 0.05$, ANOVA; Table 15) but honeybees preferred HS-2. Hence, floral size was not a determinant factor for intra- specific preference in onion also and some other factors must be responsible for intra-specific preference.

Table 14: Variation in corolla length/nectar depth of four varieties of fennel

Variety	Corolla length (mm) *
Rajendera Saurabh	**2.66** ±4.34
GF –1	**2.67** ±4.61
HF- 33	**2.62** ±4.58
Local Check	**2.65** ±4.59

* Mean ± S. E. of 50 observations
NS difference was found in corolla lengths of different varieties (F-test)

Table 15: Variation in corolla length/nectar depth of two varieties of onion

Variety	Corolla length (mm) *
HS-1	**5.58** ±2.41
HS-2	**5.57** ±2.39

* Mean ± S. E. of 50 observations
NS difference was found in corolla lengths of different varieties (F-test)

4.5.3 Carrot

Data presented in Table 16 depicted the corolla length/nectar depth of four varieties of carrot. Here too the difference in corolla length of four varieties of carrot was non-significant (P $\leq$ 0.05, ANOVA; Table 16). This factor is not a determinant factor in attracting the honeybees towards the four varieties of carrot because of differences in the choice of preference for different varieties byhoneybees. Therefore, there must be some other factors determining intra-specific preference.

Table 16: Variation in corolla length/nectar depth of four varieties of carrot

Variety	Corolla length (mm) *
HCP-1	**1.72** ±0.66
HCO-4	**1.71** ±0.70
HCB-1	**1.73** ±0.62
HC-1	**1.72** ±0.64

* Mean ± S. E. of 50 observations

NS difference was found in corolla lengths of different varieties (F-test)

4.6 Flower size as a measure of inter-specific preferences for different crops

Data presented in Table 17 depicted the mean corolla lengths/nectar depths of flowers of three crops of this study. Corolla lengths of fennel, onion and carrot differed significantly ($P \leq 0.05$, ANOVA; Table 17). These varied from 1.72 – 5.58 mm respectively. Flower size seems to be a determinant factor for inter- specific preference as flowers of onion with comparatively large corolla lengths were preferred by *A. dorsata*. Whereas, smaller sized florets of fennel blossoms and carrot were visited mainly by *A. florea* having smaller tongue length.

Table 17: Variation in corolla length/nectar depth of vegetable/ spice crops

Crop	Corolla length (mm) *
Fennel	**2.64**^{b} ± 0.33
Onion	**5.58**** ^{a} ±1.69
Carrot	**1.72**^{c} ±2.25

*** Mean ± S. E. of 200 observations**

** Mean ± S. E. of 100 observations

Note: Figures with different letters differ significantly (Multiple Range Test)

4.7 Quality of nectar as a measure of intra-specific preferences for different varieties

4.7.1 Fennel

Plate 11 showed the different types of sugars presented in the nectar of flowers of four varieties of fennel. In the nectar of the different varieties of fennel, glucose, fructose and sucrose were found. But honeybees showed the differential preference for different varieties. Therefore, quality of nectar is not a parameter intra- specific preference and some other factors determine the preference of honeybees.

4.7.2 Onion

Plate 10 showed the different types of sugars presented in the nectar of flower of both varieties of onion viz. HS-1 and HS-2. In the nectar of the two varieties of onion glucose, fructose and sucrose were present. But the preference for HS-2 was more by honeybees. Therefore, quality of nectar does not seem to be a factor in attracting the honeybees towards the varieties of onion. Therefore, some other factors must play a role in intra-specific or inter-varietal preference.

4.7.3 Carrot

Plate 10 showed the different types of sugars presented in the nectar of flowers of different varieties of carrot. In the nectar of the four varieties of carrot glucose, fructose and sucrose were found. Therefore, preference of four varieties of carrot by honeybees was not affected by quality of nectar. Therefore, some other factors must determine the intra-specific preference.

4.8 Quality of nectar as a measure of inter-specific preferences for different crops

Plate 10 and 11 showed the different types of sugars presented in the nectar of flowers of three crops of this study. In the nectar of the fennel, onion and carrot three sugars viz. glucose, fructose and sucrose were common. But differential preference of fennel by honeybees was more than onion and carrot. Therefore, nectar quality does not seem to be a factor determining flower preference by honeybees in these crops and some other factors must play role in attracting the honeybees towards the different crops.

4.9 Nectar Sugar Concentration and Energetic reward as a measure of intra-specific preferences for different varieties

4.9.1 Fennel

Average nectar volume/floret was maximum in the GF-1 (0.0 85μl/floret). There was found non-significant difference among different varieties of fennel ($P \leq 0.05$, ANOVA; Table 18).

Table 18: Variation in nectar volume of four varieties of fennel on different days

Date	Volume of nectar per floret* (μl)				
	Rajendra Saurabh	**GF-1**	**HF-33**	**Local Check**	**Mean**
24.3.04	0.08 ± 0.005	0.08 ± 0.006	0.07 ± 0.003	0.06 ±0.003	0.072 ± 0.004
31.3.04	0.06 ± 0.003	0.09 ± 0.003	0.07 ± 0.007	0.07 ±0.006	0.078 ± 0.005
7.4.04	0.09 ± 0.008	0.09 ± 0.005	0.08 ± 0.003	0.07 ±0.006	0.082± 0.007
14.4.04	0.08 ± 0.003	0.08 ± 0.006	0.07 ± 0.004	0.06 ±0.007	0.072 ± 0.006
Mean ±S.E.	0.082 ± 0.002	0.085 ± 0.001	0.012 ± 0.002	0.06 ±0.001	-

*Mean ±S.E. of three observations
NS difference was found in nectar volume of different varieties and dates (F-test)

Statistically insignificant difference was found between different dates. This means, volume of nectar per floret does not seem to play any role in attracting the honeybees towards the different varieties of fennel.

NSC of four varieties of fennel was observed between 0900-1100 h (4th April, 2004). Statistical analysis indicated a significant difference in the NSC between four varieties of fennel. The NSC of GF-1 was maximum (0.36 mg/μl). Local check was found to have minimum NSC (0.27 mg/μl) ($P \leq 0.05$, ANOVA; Table 19).

Table 19: Variation in nectar sugar concentration (NSC) of four varieties of fennel

Variety	Nectar sugar concentration* (mg/ µl)
Rajendra Saurabh	**0.35** $\pm$ 0.03
GF- 1	0.36 $\pm$0.01
HF- 33	**0.33** $\pm$0.07
Local Check	**0.27** $\pm$0.03

*** Mean $\pm$ S. E. of three observations**
CD values (P≤ 0.05) for
NSC : 0.0185

Energy per floret of varieties of fennel Rajendra Saurabh, GF-1, HF-33 and local check 0.116, 0.122, 0.096 and 0.067 cal respectively were measured ($P \leq 0.05$, ANOVA; Table 20). The number of honeybees visiting the flowers was more on the variety with high nectar sugar concentration and energy per floret. Hence, these can be considered as determinant factor in floral preference. Similarly, sugar per floret of GF-1 (0.031 mg/floret) was maximum (Appendix-I, II).

Table 20: Variation in the energy per floret of four varieties of fennel on different days

Date	Energy/floret (cal.)*			
	Rajendera Saurabh	**GF-1**	**HF-33**	**Local Check**
24.3.04	0.112 ± 0.005	0.115 ± 0.006	0.092 ± 0.003	0.064 ± 0.006
31.3.04	0.112 ± 0.002	0.129 ± 0.005	0.092 ± 0.007	0.064 ± 0.003
7.4.04	0.126 ± 0.008	0.129 ± 0.003	0.106 ± 0.004	0.076 ± 0.006

14.4.04	0.112 ± 0.004	0.115 ± 0.007	0.092 ± 0.003	0.064 ± 0.007
Mean ± S.E.	0.116 ± 0.002	0.122 ± 0.001	0.096 ± 0.002	0.067 ± 0.001

* Mean $\pm$ S. E. of three observations
CD values ($P \leq 0.05$) for
Energy/floret: 0.0074

4.9.2 Onion

Average nectar volume/floret of two varieties of onion was observed. Nectar volume/floret of HS-2 (0.75 μl/floret) was significantly more than that of HS-1 (0.72 μl/floret) ($P \leq 0.05$, ANOVA; Table 21).

NSC of two varieties of onion was measured between 0900-1100 h (4th April, 2004). There was found significant difference among the two varieties of onion. NSC of HS-2 was more (0.49 mg/μl) than HS-1 (0.42 mg/μl) ($P \leq 0.05$, ANOVA; Table 22). High NSC flowers were preferred by honeybees. Therefore, this seems to be an important factor in differential preference.

Table 21: Variation in nectar volume of two varieties of onion on different days

Date	Volume of nectar per floret* (μl)		
	HS-1	**HS-2**	**Mean**
24.4.04	0.72 ± 0.003	0.72 ± 0.006	0.72 ± 0.007
31.4.04	0.68 ± 0.009	0.75 ± 0.006	0.715 ± 0.005
7.4.04	0.75 ± 0.006	0.77 ± 0.003	0.760 ± 0.004
14.4.04	0.69 ± 0.003	0.74 ± 0.003	0.715 ± 0.004
Mean ±S.E.	0.72 ± 0.003	0.75 ± 0.004	-

*Mean S.E. of three observations

NS difference was found in nectar volume of different varieties and dates (F-test)

Table 22: Variation in nectar sugar concentration (NSC) of two varieties of onion

Variety	Nectar sugar concentration* (mg/ µl)
HS-1	**0.42**
	± 0.03
HS-2	**0.49**
	±0.03

*** Mean ± S. E. of three observations**
CD values (P≤ 0.05) for
NSC : 0.0524

Mean energy/floret of HS-1 and HS-2 was 1.192 and 1.460 cal/floret respectively (Table 23). Likewise, sugar per floret of HS-2 was found more (0.365 mg/floret than HS-1 (0.298 mg/floret) (Appendix I, II). As the number of honeybees that foraged the two varieties of onion was different, the nectar volume is not a determinant factor in attracting the honeybees. Whereas, energy and sugar per floret seem to be a determinant factor as bees preferred flowers with high energy.

Table 23: Variation in the energy per floret of two varieties of onion on different days

Date	**Energy/floret (cal.)***	
	HS-1	**HS-2**
24.4.04	1.208	1.411
	± 0.003	± 0.007
31.4.04	1.142	1.470
	± 0.008	± 0.006
7.4.04	1.260	1.509
	± 0.006	± 0.003

14.4.04		1.159 ± 0.003	1.450 ± 0.003
Mean ± S.E.		1.192 ± 0.003	1.460 ± 0.004

* Mean ± S. E. of three observations
CD values (P≤0.05) for
Energy/floret : 0.0045

4.9.3 Carrot

Average nectar volume/floret differed non-significantly on different days. Non-significant differences were found in nectar volume of all the four varieties of carrot (P ≤ 0.05, ANOVA; Table 24).

The nectar sugar concentration of the four varieties of carrot was observed (4th April, 2004). Significant difference was observed between different varieties (P ≤ 0.05, ANOVA; Table 25). The nectar sugar concentration of HCP-1 was maximum (0.29 mg/μl) followed by HCO-4 (0.28mg/μl) and HC-1 (0.25 mg/μl). Nectar sugar concentration of HCB-1 was found to be minimum (0.17 mg/μl) (P ≤ 0.05, ANOVA; Table 25).

Table 24: Variation in nectar volume of four varieties of carrot on different days

Date	**Volume of nectar per floret* (μl)**				
	HCP-1	**HCO-4**	**HCB-1**	**HC-1**	**Mean**
24.3.04	0.05 ± 0.007	0.05 ± 0.012	0.04 ± 0.003	0.04 ±0.012	0.045 ±0.015
31.3.04	0.06 ± 0.009	0.05 ± 0.006	0.04 ± 0.006	0.04 ±0.012	0.048 ±0.014
7.4.04	0.06 ± 0.003	0.06 ± 0.003	0.04 ± 0.007	0.04 ±0.004	0.050 ±0.006

14.4.04	0.06 ± 0.005	0.05 ± 0.006	0.03 ± 0.013	0.04 ±0.007	0.045 ±0.008
Mean ±S.E.	0.058 ± 0.005	0.052 ± 0.001	0.040 ± 0.001	0.040 ± 0.002	-

*Mean S.E. of three observations

NS difference was found in nectar volume of different varieties and dates (F-test)

Table 25: Variation in nectar sugar concentration (NSC) of four varieties of carrot

Variety	Nectar sugar concentration* (mg/ µl)
HCP-1	0.29 ± 0.01
HCO-4	0.28 ±0.04
HCB-1	0.17 ±0.01
HC-1	0.25 ±0.01

*** Mean ± S. E. of three observations**

CD values (P≤ 0.05) for
NSC : 0.0290

Energy/floret of four varieties of carrot HCP-1, HCO-4, HCB-1 and HC-1 were 0.066, 0.058, 0.023 and 0.040 cal/ floret (Table 26).

Table 26: Variation in the energy per floret of four varieties of carrot on different days

Date	Energy/floret (cal.)*

	HCP-1	HCO-4	HCB-1	HC-1
24.3.04	0.058 ± 0.007	0.056 ± 0.013	0.027 ± 0.004	0.040 ± 0.014
31.3.04	0.069 ± 0.009	0.056 ± 0.005	0.027 ± 0.006	0.040 ± 0.005
7.4.04	0.069 ± 0.003	0.067 ± 0.003	0.027 ± 0.007	0.040 ± 0.007
14.4.04	0.069 ± 0.003	0.056 ± 0.006	0.020 ± 0.013	0.040 ± 0.004
Mean ± S.E.	0.066 ± 0.005	0.058 ± 0.001	0.023 ± 0.001	0.040 ± 0.003

* Mean ± S. E. of three observations
CD values (P≤0.05) for
Energy/floret: 0.0084

Similarly, sugar per floret of HCP-1 was found highest (0.017 mg/floret) followed by HCO-4, HC-1 and HCB-1 (0.0145, 0.010 and 0.006 mg/floret) respectively (Appendix I, II). On the basis of relative abundance of honeybees NSC, sugar per floret and energy per floret were found to be determinant factors for intra- specific preference whereas, nectar volume per floret does not play any role in differential preference.

4.10 Nectar Sugar Concentration and energetic reward as a measure of inter-specific preferences for different crops

Nectar volume/floret was observed on different dates. There was found non-significant difference between the dates, but found significant difference between the three crops ($P \leq 0.05$, ANOVA; Table 27). Nectar volume/floret was maximum in onion (0.73 μl) followed by fennel (0.07 μl). Nectar volume/floret was lowest in carrot (0.05 μl).

NSCs of the three crops of the study were observed between 0900-1100 h on 4th April, 2004. Statistical analysis indicated a significant difference in the NSC of the three crops ($P \leq 0.05$, ANOVA; Table 28). NSC of onion was maximum (0.46 mg/µl) followed by fennel (0.33 mg/µl) and carrot (0.24 mg/µl).

Table 27: Variation in nectar volume of vegetable/spice crops on different days

Date	Volume of nectar per floret (µl)		
	Fennel*	**Onion****	**Carrot***
24.3.04	0.06 ± 0.007	0.72 ± 0.050	0.04 ± 0.003
31.3.04	0.08 ± 0.009	0.75 ± 0.050	0.05 ± 0.004
7.4.04	0.08 ± 0.009	0.69 ± 0.052	0.05 ± 0.006
14.4.04	0.07 ± 0.008	0.72 ± 0.044	0.05 ± 0.004
Mean ±S.E.	0.07^{b} ± 0.008	0.73^{a} ± 0.048	0.05^{c} ± 0.005

*Mean S.E. of twelve observations
** Mean S.E. of six observations
Note: Figures with different letters differ significantly (Multiple Range Test)

Average amounts of energy per floret available to the honeybees as pollinators of fennel, onion and carrot were 0.100, 1.327 and 0.048 cal./floret ($P \leq 0.05$, ANOVA; Table 29) and average amount of sugar per floret of fennel, onion and carrot were 0.025, 0.332 and 0.012 mg/floret respectively (Appendix I, II).

In spite of high NSC, nectar volume and energy per floret, flowers of onion flowers were not preferred over fennel. Therefore, these factors do not seem to be determinant factors for inter- specific preference.

Table 28: Variation in nectar sugar concentration (NSC) of vegetable/ spice crops

Crop	Nectar sugar concentration* (mg/ µl)
Fennel	**0.33 [b]** **± 1.08**
Onion	0.46**[a] ±1.51
Carrot	**0.24 [c]** **±1.36**

*** Mean ± S. E. of twelve observations**

**** Mean ± S. E. of six observations**

Note: Figures with different letters differ significantly (Multiple Range Test)

4.10 Sugar and protein concentrations in anthers as a measure of intra-specific preferences for different varieties

4.10.1 Fennel

Sugar concentration in anthers of four varieties of fennel viz. Rajendra Saurabh, GF-1, HF-33 and local check were 43.68, 43.80, 42.00 and 38.30 mg/g respectively. The sugar concentration in anthers of GF-1 (43.80 mg/g) was found significantly higher than Rajendera Saurabh (43.68 mg/g) followed by

HF-33 (42.00 mg/g). The sugar concentration in anthers of local check was least (38.30 mg/g) (P ≤ 0.05, ANOVA; Table 30).

Table 29: Variation in the energy per floret of vegetable/ spice crops on different days

Date	Energy/floret (cal.)		
	Fennel*	**Onion****	**Carrot***
24.3.04	0.096 ± 0.008	1.310 ± 0.060	0.045 ± 0.004
31.3.04	0.099 ± 0.009	1.306 ± 0.052	0.048 ± 0.004
7.4.04	0.109 ± 0.007	1.385 ± 0.045	0.051 ± 0.006
14.4.04	0.096 ± 0.008	1.305 ± 0.044	0.046 ± 0.003
Mean ± S.E.	0.100 [b] ± 0.007	1.327[a] ± 0.047	0.048 [c] ± 0.005

* Mean ± S. E. of twelve observations
**Mean ± S.E. of six observations
Note: Figures with different letters differ significantly (Multiple Range Test)

The protein concentration in anthers of four varieties of fennel ranged from 24.00- 29.52 mg/g. There was found significant difference between all the four varieties. GF-1 was having maximum protein concentration in anthers (29.52 mg/g) followed by that of Rajendra Saurabh (29.40 mg/g) and HF-33 (26.52 mg/g) (P ≤ 0.05, ANOVA; Table 30). The minimum protein concentration in anthers was present in local variety (24.00 mg/g). The varieties with highest sugar and protein concentrations were visited by maximum number of honeybees. Therefore, both sugar and protein concentrations were found to be determinant factors in attracting the honeybees.

Table 30: Variation in sugar and protein concentration in anthers of four varieties of fennel

Variety	Sugar concentration in anthers* (mg/ g)	Protein concentration in anthers* (mg/ g)
Rajendera Saurabh	**43.68**	**29.40**
	± 0.01	**±0.01**
GF- 1	**43.80**	**29.52**
	±0.01	**±0.03**
HF- 33	**42.00**	**26.52**
	±0.01	**±0.01**
Local Check	**38.30**	**24.00**
	±0.01	**±0.00**

*** Mean ± S. E. of three observations**
CD values ($P \leq 0.05$) for
Sugar concentration in anthers: 0.1830
Protein concentration in anthers: 0.3222

4.10.2 Onion

Statistically, significant difference was found between sugar concentrations in anthers of two varieties of onion. HS-2 was having more sugar concentration in anthers (23.86 mg/g) than HS-1 (22.32 mg/g) ($P \leq 0.05$, ANOVA; Table 31).

Similar pattern was found in protein concentration in anthers. The variety HS-2 was found to have more proteins in anthers (22.36 mg/g) than that of HS-1

(19.82 mg/g) (P $\leq$ 0.05, ANOVA; Table 31). Both sugar and protein concentrations were found as determining factors as variety with high sugar and protein concentrations in anthers attracted more honeybees.

Table 31: Variation in sugar and protein concentration in anthers of two varieties of onion

Variety	Sugar concentration in anthers* (mg/ g)	Protein concentration in anthers* (mg/ g)
HS-1	**22.32 ± 0.01**	**19.82 ±0.05**
HS-2	**23.86 ±0.003**	**22.36 ±0.01**

*** Mean ± S. E. of three observations**
CD values (P≤0.05) for
Sugar concentration in anthers: 0.0168
Protein concentration in anthers: 0.0154

4.10.3 Carrot

The sugar concentration in anthers of four varieties of carrot HCP-1, HCO-4, HCB-1 and HC-1 were 21.40, 21.00, 13.33 and 17.60 mg/g, respectively. The sugar concentration in anthers of HCP-1 (21.40 mg/g) was found significantly more than that of other varieties ($P \leq 0.05$, ANOVA; Table 32).

The protein concentration in anthers of four varieties of carrot ranged from 13.68-19.20 mg/g. The protein concentration of HCP-1 (19.20 mg/g) was maximum and that of HC-1 (13.68 mg/g) was minimum ($P \leq 0.05$, ANOVA; Table 32). The variety HCP-1 with highest sugar and protein concentrations was visited by maximum number of honeybees. Therefore, it is to be considered as a determinant factor in differential preference.

Table 32: Variation in sugar and protein concentration in anthers of four varieties of carrot

Variety	Sugar concentration in anthers* (mg/ g)	Protein concentration in anthers* (mg/ g)
HCP-1	**21.40** **± 0.01**	**19.20** **±0.03**
HCO-4	**21.00** **±0.01**	**18.89** ±0.34
HCB-1	**13.33** **±0.01**	**10.35** ±0.02
HC-1	**17.60** **±0.03**	**13.68** ±0.04

*** Mean ± S. E. of three observations**

CD values (P≤0.05) for
Sugar concentration in anthers: 0.5230
Protein concentration in anthers: 0.4468

4.11 Sugar and protein concentrations in anthers as a measure of inter-specific preferences for different crops

Sugar concentration in the anthers of the three crops ranged from 18.34-41.95 mg/g. The maximum sugar was found in anthers of fennel (41.95 mg/g) followed by that of onion (23.24 mg/g) and carrot (18.34 mg/g) ($P \leq 0.05$, ANOVA; Table 33). Statistically, there was found significant difference between protein concentrations in anthers of the three crops. The maximum protein concentration in anthers was present in fennel (27.36 mg/g) followed by onion (21.15 mg/g), whereas, lowest concentration was in the anthers of carrot (15.53 mg/g) ($P \leq 0.05$, ANOVA; Table 33).

Table 33: Variation in sugar and protein concentration in the anthers of vegetable/ spice crops

Crop	Sugar concentration in anthers* (mg/ g)	Protein concentration in anthers* (mg/ g)
Fennel	**41.95[a] ± 0.06**	**27.36[a] ±0.06**
Onion	**23.24**[b] ±0.04**	**21.15**[b] ±0.06**
Carrot	**18.34[c] ±0.09**	15.53[c] ±0.12

*** Mean ± S. E. of twelve observations**

**** Mean ± S. E. of six observations**

Note: Figures with different letters differ significantly (Multiple Range Test)

Both sugar and protein concentrations in anthers seem to be important factors in attracting honeybees as crop with high sugar and proteins in anthers were preferred.

4.5.1 Variation in the body dimensions of honeybees

Table 34 presents the data on body length and breadth of three honeybee species. Maximum body dimensions were of *A. dorsata* followed by that of *A. mellifera, A. cerana* and *A. florea.* Statistically size of all the bee species differed significantly ($P \leq 0.05$, ANOVA; Table 34). The flowers with small corolla lengths of fennel and carrot were visited by small sized *A. florea.* Whereas, large sized honeybees *A. dorsata* preferred large flowers of onion. Therefore, size of honeybees has an important role in inter- specific preference whereas due to similar nectar depth of different varieties of each crop, this does not play any role in inter- specific preference.

Table 34: Variation in the body dimensions of four honeybee species

Bee species	Length* (mm)	Breadth* (mm)
A. dorsata	**18.42 ±0.002**	**4.32** ±0.002
A. mellifera	**13.77 ±0.002**	**4.12** ±0.002
A. cerana	**12.63 ±0.003**	**3.67** ±0.001
A. florea	**9.41 ±0.002**	**2.77** ±0.001

*** Mean ± S. E. of 50 observations**
CD values (P≤0.05) for
Length: 0.0066
Breadth: 0.0048

4.5.2 Variation in the tongue length of three *Apis* species

The data on functional tongue length of three honeybees are shown in Table 35. Longest tongue length was measured for *A. dorsata* (5.76 mm) followed by *A. mellifera* (5.53 mm) *A. cerana* (4.53 mm) and *A. florea* (3.22 mm) ($P \leq 0.05$, ANOVA; Table 35).

Tongue length play great role in inter- specific preference of honeybees for the three vegetable/ spice crops as the honeybee with smaller tongue length preferred flowers with smaller corolla lengths or vice- versa. Whereas, for intra-specific preference, due to similar corolla lengths of different varieties of each crop tongue length does not act as a determinant factor.

Table 35: Morphometric variation in proboscis length of four honeybee species

Bee species	Proboscis length (mm)*	
	P. L. of dead bee	**Functional P. L. of live bee**
A. dorsata	**5.76±0.001**	**7.336**
A. mellifera	**5.53±0.002**	**7.083**
A. cerana	**4.53±0.001**	**5.983**
A. florea	**3.22±0.002**	**4.542**

* Mean ± S. E. of 50 observations
CD values (P≤0.05) for
P.L. of dead bee: 0.0048

Chapter-V

DISCUSSION

The angiosperms are the most beautiful gift of nature; these bear flowers. The variant coloured entities attract a wide variety of visitors; the ultimate attraction is the floral reward constituted by nectar and pollen (Sihag, 1984). The sweet floral reward, the nectar, is the primary attractant and its presentation pattern in the flower determines how easily it is available to a seeker. The floral visitor may harvest it through a legitimate or illegitimate route. The visitor's behaviour has great bearing on the reproductive success of a plant it visits. The latter may be impaired if the flowers are not visited by a suitable pollinator, as well as if their number is not adequate. Furthermore, the reproductive success in plants is often pollinator limited. That is why, characterization of the appropriate pollinators of a plant species is important.

Like many other inputs in agriculture (e. g. fertilizers, pesticides and irrigation), managed pollination is also required as one of these essential inputs. In recent years, there has been an increase in the accumulation of data to indicate that seed yields of insect-pollinated crops may often be lower than the expected, not because of adverse climatic, edaphic or cultural factors, but simply because the number of certain pollinators is inadequate (McGregor, 1976; Free, 1993; Sihag, 1993a). In many entomophilous crops, all the cultural practices would prove useless to affect fruit or seed set if its pollination is neglected. Insect pollinators set a greater proportion of early flowers of the crop and increase quality and quantity of the seed yield. Before the use of managed (bee) pollination, it is necessary to have a basic knowledge of factors which influence the bee activity and preference of the bees for different crops. Therefore, in the present study an attempt was made to know the factors responsible for variation in the foraging activity and the preference of bees for different crops.

5.1 ABUNDANCE (VISITATION FREQUENCY) OF THE POLLINATORS

Abundance of the pollinators presents the full spectrum of different species which visit the flowers of a crop during the full span of its flowering period. Higher the visitation frequency more are the chances of a flower to be visited repeatedly by a species which should ensure more pollination in its flowers and more will be its contribution towards the reproductive success of the plants. The latter is measured in terms of degree of seed set and seed/fruit yield (Free, 1993).

In this study, four honeybee species, *A. mellifera, A. florea, A. dorsata* and *A. cerana* were found to visit the vegetable/spice crops at sub-tropical Hisar. Throughout the flowering period of fennel and carrot, *A. florea* was the most frequent visitor. In onion, *A. dorsata* was the most abundant pollinator.

Among the four varieties of fennel (viz. Rajendera Saurabh, GF-1, HF-33 and local check), GF-1 was the most preferred variety (Table 1). Between the two varieties of onion, the number of honeybees was more on HS-2 than HS-1 (Table 2). Likewise, the number of honeybees was found to be more on the HCP-1 than other varieties of carrot (Table 3).

Pollination process is more efficient when there are more flowers on a plant. At peak flowering, the availability of flowers is more than commencement and cessation and large numbers of insects visit the crops during this period to help maximize the pollination process. Therefore, flower number clearly influence the pollinator abundance and in turn, level of pollination. Plants with many flowers often attract more floral visitors than those with fewer (Free, 1993). In the present investigation, a fluctuation in visits of insect pollinators on different days on different crops was observed. The visits were low at the time of commencement and cessation of flowering but these remained high during mid flowering period (Table 4). This difference should be due to variation in the floral density during the span of blooming on the crops. Kendall and Smith (1975), Willson and Price (1977), Schaffer and Schaffer (1979), Schemske (1980) and Dhaliwal and Atwal (1985) also stated that at the peak flowering, number of flowers was more/maximum and these were visited by larger number of pollinators and helped maximization of pollination in different crops. Decrease in floral density can disrupt mutualistic interactions between plants, their pollinators and decrease reproductive success (Knight, 2003).

If relative abundance of the pollinators on different crops is taken as a measure of their preference, then the pollinators of these crops were found to show their distinct preferences, Or else, these crops showed distinct attractiveness for their pollinators. For example, *A. florea* is more attracted towards fennel and carrot whereas *A. dorsata* is more attracted towards onion (Table 4 and 5). Between fennel and carrot, the former is more attractive to *A. florea* than the latter crop. In the presence of fennel, therefore, onion and carrot are at a disadvantage with respect to *A. florea*. Therefore, fennel, onion and carrot should not be grown in the adjacent/adjoining fields because it seemed to carry less pollination in the target crop(s) (Chaudhary, 2000).

The varieties of the crops, fennel, onion and carrot also showed distinct attractiveness for their pollinators. For example, the four species of honeybees (*A. mellifera, A. florea, A. dorsata* and *A. cerana*) are more attracted towards GF-1 of fennel followed by Rajendera Saurabh, HF-33 and local check (Table1). Between varieties of onion, the variety HS-2 is more attractive to honeybees than the HS-1 (Table 2). Likewise, among varieties of carrot, HCP-1 is more attractive to the honeybees than HCO-4, HC-1 and HCB-1 (Table 3). In the presence of the variety GF-1, HS-2 and HCP-1 of fennel, onion and carrot respectively, the other varieties of fennel (Rajendera Saurabh, HF-33, local check), onion (HS-1) and carrot (HCO-4, HCB-1, HC-1) are at a disadvantage with respect to honeybees. Therefore, different varieties of each crops (fennel, onion and carrot) should not be grown in the adjacent/adjoining fields.

5.2 FACTORS DETERMINING POLLINATOR PREFERENCE

When a pollinator is in foraging flight, it encounters several stimuli presented by the floral source. These stimuli are deciphered by the pollinator in a sequence. Sihag (1984) has presented the diagrammatic representation of these stimuli. Flower colour is the first stimulus to attract the pollinators. This is then followed by the size/ structure of the flower as well as the morpho- metric dimensions of the pollinators it-self. The ultimate stimulus is the floral reward in the form of pollen and nectar. Where nectar make the primary reward due to presence of soluble sweet sugars and other constituents, second choice comes for the pollen types as a source of solid proteins, carbohydrates and lipids along with several other nutritive and fragrant chemicals. According to its own needs and suitability, the pollinator makes strategy for subtractions/ additions of these floral attributes to form a combination which suits best for its efficient and profitable foraging. This contention has been proved in the present study. These results have been supported by Sihag and Khatkar (1999b). They reported that within different cultivars of *B. juncea*, bee visitation followed the same pattern as did the energetic reward.

5.2.1 FLOWER COLOUR

It is an important factor for attraction of pollinators and acts as a long distance advertisement to insects. Yellow coloured flowers are often highly preferred by a large variety of insects (Kevan, 1983). The bees are known to be highly sensitive to ultraviolet reflectance and absorbance patterns of a plant host than those of the visible coloration patterns (Daumer, 1958; Eisner *et al*., 1969; Frisch, 1967; Wehner, 1975).

Analysis of the floral pigments revealed the presence of relatively more carotenoids and xanthophylls in yellow flowers of fennel than in white flowers of onion and carrot (Table 12). The anthocyanins were found less in the flowers of fennel, onion and carrot (Table 13). The anthocyanins are known to be UV absorptive components, whereas, carotenoids as UV reflectants (Caldwell, 1968). Highest carotenoid levels occur in yellow, orange, dark- red and orange-red flowers (Niuwhaf *et.al.*, 1989). The yellow flowers of *Parkinsonia aculeate* L. with ultra violet guide and superior nectar make it preferentially attractive to the megachilid bees than alfalfa (Jones and Buchman, 1974; Jain and Kapil, 1980), which are the sole pollinators of alfalfa (Sihag, 1982). Obviously, differential distribution of these two pigments makes the hosts distinct. In fennel, flowers were having yellow petals due to high contents of carotenoids and xanthophylls than white flowers of onion and carrot (Table 12). The visitation frequency of honeybees is highest on yellow coloured flowers of fennel (Table 4, 5) (Fig. 4). Therefore, flower colour seemed to be an important factor to decide inter-specific preferences of honeybees for three concurrently flowering vegetable/ spice crops viz. fennel, onion and carrot. However, inspite of yellow coloured flowers of fennel, *A. dorsata* preferred onion flowers because of its longer corolla length than that of fennel flowers. The latter floral attribute suited more than the former to the honeybee. That is why, *A. dorsata* ignored the yellow colour of fennel and preferred white flowers of onion.

Floral pigments of different varieties of fennel, onion and carrot were also analysed. The difference among the varieties of same crop were non-significant (Table 6, 7, 8, 9, 10 and 11) for intra-varietals preference. Hence, when there were no significant differences in colours, some other factor must be responsible.

5.2.2 FLOWER SIZE

The relation between pollinator morphology and flower morphology influences the time needed to land on a flower for obtaining the reward. There is definite relationship between proboscis length and time spent by insect pollinators on a flower. Bumble bees with short tongue length foraged more rapidly on flowers of short corolla than did long tongued bees (Morse, 1979). Similarly, Inouye (1980) found that long tongued bees foraged more quickly than short tongued bees on long corolla flowers and flower preference depended, in part, on the relationship between pollinator morphology and flower's morphology. There is also correlation between floral size and size of pollinators. As pointed out above, normally large sized blossoms are pollinated by large sized pollinators and small sized blossoms are visited by small sized pollinators (Priti and Sihag, 1997).

In the present study, the preference of *A. florea* for fennel and carrot could be explained in terms of suitability of this bee for harvesting nectar from flowers of these crops. On the other hand, *A. dorsata* preferred large blossoms of onion with relatively large sized florets over fennel and carrot even ignoring the yellow colour. This could be explained in terms of long proboscis of *A. dorsata* so that it could harvest nectar from flowers of onion more efficiently as compared to those of fennel and carrot. Therefore, in these crops preference of the pollinators seems to be determined by floral size also. Similar results have been reported by Sihag and Khatkar (1999b). They reported that the preference of *A. florea* for *B. campestris* even in the presence of higher energy rewarding *E. sativa* could be well explained in terms of suitability of this bee for harvesting nectar.

But, among varieties of fennel, onion and carrot conspecific floral sizes did not have significant variations. Therefore it can not be the determinant factor to attract pollinators towards the conspecific varieties.

5.2.3 QUALITY OF NECTAR

The type of nectar in a flower is considered to be a specific feature of a plant family or the closely related families and in most of the plant types the composition of the nectar has been found remarkably consistent at species level (Wykes, 1952a ;Bailey *et al.*, 1954; Butler, 1953; Furgula *et al.*, 1958; Percival, 1965). The major sugar constituents of various nectar types are sucrose, glucose and fructose (Wykes, 1952b), however, some variations do exist.

Nectar of *Eucalyptus* (Vansell, 1944) and sweet clover (Furgula *et al.*, 1958) contained sucrose and hexoses in a balanced ratio while *Brassica* and dwarf mistlitre have the dominancy of a hexose sugar (Brewer *et al.*, 1974). About one-third of the Phillipines species were with fructose and glucose ratio above one (Rowley, 1976).

Sihag and Kapil (1983) studied nectar sugar of 44 plant species and found that sucrose dominated in 13, glucose in 24 and the rest contained equi-proportioned glucose, fructose and sucrose. Majority of the plant species investigated by Rowley (1976) in Philippines had the dominance of sucrose.

In the present study, visitation frequency of *A. florea* was maximum on fennel varieties and that of *A. dorsata* on onion varieties. But the nectar of flowers of all the varieties of fennel and also of onion was having the three sugars glucose, fructose and sucrose (Plate 10 and 11). Therefore, in this study nectar quality did not seem to be a factor determining the intra and inter-specific preferences of honeybees.

5.2.4 NECATAR SUGAR CONCENTRATION (NSC)

Concentration of nectar is of much importance for social bees (Butler *et al.*, 1943). In terms of energetic reward, it is profitable for them to have concentrated nectar. This will avoid their energy expenditure involved in concentration of nectar for storage purpose. Sihag and Kapil (1983) reported that *A. florea* visited flowers with low caloric rewards whereas *A. dorsata* preferred those with high rewards. The size of an insect determines its relative energy cost (Wolf, 1975;

Wolf *et al.*, 1975). Therefore, with the increase of body size, energy requirements of an insect increases and this makes the individual more selective in obtaining an energy reward. Wolf *et al.* (1999) found positive correlation between attractiveness to bees and nectar sugar concentration within the genus *Citrullus*. Cultivars of *Brassica napus* produced nectar with more sugar concentration (62%) than cultivars of *Brasssica campestris* (57%) (Mohr and Jay, 1990).

In the present study, in spite of high nectar sugar concentration in onion (Table 25), visitation frequency of honeybees was found more on fennel. Nectar of onion was not preferred by honeybees perhaps because of presence of potassium ion in it (Hagler, 1990; Waller, 1972). Therefore, in this study NSC cannot be taken as a determining factor for inter- specific preference.

Similarly visitation frequency of honeybees was more on the varieties GF-1 of fennel, HS-2 of onion and HCP-1 of carrot (Table 1, 2, 3) respectively. These varieties had nectar with higher sugar concentration, than other respective varieties of fennel, onion and carrot. Therefore, unlike inter-specific preference, for intra-specific preference nectar sugar concentration seems to be a determinant factor.

5.2.5 NECTAR VOLUME/ENERGETIC REWARD

Wolf *et al.* (1999) found no genetic variability in nectar volume among different cultivars within genus *Citrullus*. Different cultivars of *Brassica napus* produced more nectar than did those of *Brassica campestris*. Mean daily nectar production was 0.68µl for *B. campestris* and 0.90 µl for *B. napus* cultivars (Mohr and Jay, 1990). In some self- incompatible lines of cabbage, the average total nectar production per flower was 6.2 mg, containing 1.2 mg of sugars (Haslbachova *et al.*, 1986).

In the present study, nectar volume does not differ on different days (Table 18, 21 and 24). It remains almost similar in crops/varieties. Nectar was available only between 0900-1100 h. Nectar volume per floret differed significantly in the three crops of this study. Nectar volume per floret of onion was found maximum whereas visiting frequency of honeybees was highest on fennel. This was perhaps because of presence of potassium ions in the nectar of onion as has been reported by Hagler (1990) and Waller *et al.* (1972). Hence, again nectar volume is not a determinant factor for inter- specific preference of honeybees for the crops of this study.

Nectar volume per floret did not seem to be a determinant factor for intra-specific preference also. Among varieties of fennel, there difference in nectar volume was non-significant (Table 18). Similarly, the difference in nectar volume of all the carrot varieties was non-significant (Table 24). Likewise nectar volume in onion varieties i.e. HS-2 and HS-1 also did not differ significantly (Table 21).

Therefore, volume of nectar in the flower was not a determinant factor for the varietal preference of honeybees of this study.

In the present study, the energy and sugar per floret varied from a minimum in carrot florets to a maximum in onion florets, reward in fennel florets being the intermediate (Tables 29). The number of honeybees that visited the blossoms of these crops was not proportional to energetic reward. The fennel was visited by maximum number of honeybees whereas in spite of high energetic reward, onion flowers were visited by lesser number of honeybees. Therefore, energetic reward does not act as a determinant factor for preference by the honeybees of this study.

Energetic reward of var. GF-1 of fennel (Table 20), HS-2 of onion (Table 23) and HCP-1 of carrot (Table 26) were also more than other varieties of fennel, onion and carrot respectively. Accordingly, number of honeybees was more on GF-1 of fennel, HS-2 of onion and HCP-1 of carrot than other varieties of fennel, onion and carrot respectively (Tables 1,2,3). Hence, energetic reward acted as a determinant factor in intra-specific preference of honeybees.

5.2.6 SUGAR AND PROTEIN CONCENTRATIONS IN ANTHERS

The sugar concentration in anthers was lowest in carrot and highest in fennel (Table 32). Total proteins were evidently highest in the anthers of fennel followed by onion and lowest range in carrot. The visitation frequency of honeybees was also highest on fennel followed by onion and carrot (Tables 4,5) (Fig. 4). It revealed possibly the role of high nutritional value of anthers as an important determinant factor for honeybees to establish their preference for fennel over onion and carrot.

Wells and Wells (1983) also envisaged that because honeybees made distinct selection among flowers from which they collected pollen, one might suspect that foraging would be concentrated on plants whose pollen was most efficiently collected and richest in nutrients and energy. Pollen is though considered a protein diet and foraging is expected to concentrate much on plant producing better quality pollen. For honeybees, protein is the principal and vital food source. It contains essential nutrients for the production of royal jelly, which nourishes the brood, queen and adult worker bees (Crailsheim, 1991). Pollen sugars along with proteins control the developments of hypopharyngeal glands (Testa *et al.*, 1981). Protein rich pollen is very essential for worker bees. Proteins contained in food/ pollen in their diet substantially increased their longevity (De Groot, 1953). Feeding pollen in their diet enhances development of ovaries in bees significantly (Kropocova *et al.*, 1968). The chemical composition of bee collected pollen from various plant species was studied by various investigators (Kozma and

Mohacsy, 1968; Youssef *et al.*, 1978; Farag *et al.*, 1978). In most pollen, carbohydrates constituted the major dry fraction; its levels varied with the species (Nielsen *et al.*, 1955; Dhingra and Jain, 1995). Honeybees also exhibited differences in feeding preferences and survival when fed pollen of different plant species (Schmidt *et al.*, 1995)

Among varieties of fennel, sugar and protein concentrations of anthers differed greatly. Sugar and protein concentrations of anthers were found highest in GF-1, followed by Rajendera Saurabh, HF-33 and local check, respectively (Table 30). In onion, sugar and protein contents in anthers of HS-2 was more than HS-1 (Table 31). Anthers of HCP-1 of carrot were found to have highest sugar and protein contents than other varieties (Table 32). The varieties GF-1 of fennel, HS-2 of onion and HCP-1 of carrot were also visited by more number of honeybees than other varieties of respective crops (Table 1,2,3). Hence, sugar and protein concentrations in pollen are important factors for honeybees to establish their preferences for these crops.

5.2.7 SIZE OF POLLINATORS

Size of the pollinators determines their suitability for a floral source. Normally large sized blossoms are visited by large sized pollinators and small sized blossoms by small sized pollinators (Priti and Sihag, 1997). In the present study, large sized *A. dorsata* preferred blossoms of onion with large sized florets and blossoms of fennel and carrot with small sized florets were preferred by smaller sized *A. florea* (Table 34). Therefore, the size of honeybees is a determining factor for deciding their suitability to a particular crop of this study. But size of flowers of different varieties of fennel was similar (Table 14). Similarly, corolla lengths of various varieties of onion (Table 15) and carrot (Table 16) were also same. Whereas, the number of honeybees that visited these flowers was different Hence, in this study size of the different species of honeybees did not act as a determining factor in intra-specific (intervarietal) preference.

5.2.8 TONGUE LENGTH

Tongue length is a major factor in determining the insect-flower relationship. Flowers of short corolla length (fennel and carrot) were visited primarily by the short tongued honeybee (*A. florea*) but only scantily by the long tongued honeybee (*A. dorsata*). While flowers with relatively long corolla tube length (onion) were visited by long tongued honeybee (*A. dorsata*) and to a lesser extent by the short tongued honeybee (*A. florea*) (Table 35). These observations find support from Inouye (1980) and Ranta and Lundberg (1980) who reported that short-tongued bees were more efficient on short corolla flowers, long tongued bees on long corolla flowers and medium tongued bees on medium corolla flowers. When a bee had a tongue that was too long or too short for a flower was less efficient in foraging.

Flower size of different varieties of fennel, onion and carrot were similar (Table 14, 15 and 16). Hence, in this study, tongue length of honeybees was not a determining factor in intra-specific (intervarietal) crop preferences.

5.3 CONCLUSION

From the foregoing account, therefore, it is evident that if the relative abundance of the pollinators on different crop/varieties is taken as a measure of their preference to the different crops/varieties, different honeybee species show distinct preference for the crop as well as varieties. This preference is decided by a suitable combination of many factors. Flower colour is considered as one of the primary attractant for the flower visitors. If the colour of the flowers is different in different crops then it will be a determining factor to decide the attractiveness of the crops to honeybees. In the present study, more number of honeybees was attracted towards yellow coloured flowers of fennel than towards white flowers of onion and carrot. This was because yellow colour considered to be more attractive to honeybees. Therefore, it can be taken as a factor determining differential attractiveness of the study crops. However, this factor remained operative if the subsequent stimuli were also favourable. Otherwise, the pollinators changed their primary preference. This was evident from the fact that flower preference subsequently was also influenced by the size and tongue lengths of the honeybees. Due to long tongue length and size *A. dorsata,* seemed to feel it easy in harvesting nectar from the flowers of onion than from fennel and carrot. Here this be sacrificed its first choice of colour stimulus in favour of second more suitable stimulus- the size of the florets and its own tongue length and size. *A. florea*, however, due to its small size and tongue length preferred yellow flowers of fennel (two suitable factors) but not of carrot because of its first stimulus the less preferred white colour as well as lesser nectar sugar concentration, energy per floret, sugar and protein concentration in anthers than that of fennel. Therefore, compatibility of tongue length and size of honeybee species and nectar depth in a flower type was the second determinant factor deciding preference of that bee species to that flower type.

Nectar quality though a very important factor in determining the preference of honeybees was not a determining factor for inter- specific preference in the crops of this study, because nectar of all the crops was having common sugars glucose, fructose and sucrose. Nectar sugar concentration and energetic reward are the third stage and ultimate important attributes of flowers. If the flowers have similar size then this becomes the sole attribute in deciding the preference of honeybees. But if the size of different flowers differs it does not act as an important factor responsible for deciding the differential attractiveness of crops to different bee species. In the present case, nectar sugar concentration and energetic reward were not the determinant factors for the pollinators preference because in spite of more nectar sugar concentration and energetic reward, honeybees preferred flowers of fennel over onion because of better floral size and honeybee size/tongue length compatibility. The above contention becomes more clear from the study on intra varietal preference.

Here, among different varieties of the same crop floral colour and size; pollinator size and tongue length, quality of nectar of all were similar. The only variable was the nectar sugar concentration and energy/ floret which made the important factors determining differential attractiveness of intra- specific preference as honeybees visited varieties with high nectar sugar concentration and energetic reward. Honeybees preferred crops/ varieties with high sugar and protein concentrations in their anthers. Therefore, sugar and protein concentrations of anthers can also be taken as a determinant factor influencing the pollinators' preference to the different varieties of three crops, fennel, onion and carrot.

Fennel was best preferred by *A. florea, A. mellifera* and *A. cerana* because of preferred floral attributes like yellow colour, more suitable floral size compatible with the honeybee tongue length and size, and floral floral reward system. The carrot blossoms with relatively poor floral attributes were at a disadvantage. Onion, however, due to its better floral size versus pollinator size – tongue length compatibility was preferred by *A. dorsata.* The latter honeybee even ignored the repulsive nature of its nectar containing potassium ions. The foraging profitability for *A. dorsata* seemed to be better on onion. Due to similarity of most of the attributes of the conspecific varieties, the lone variable determining the floral preference was the energetic reward.

5.4 RECOMMENDATIONS

This study clearly reveals that blossoms of fennel attracted more number of bees if grown in the vicinity of onion and carrot. Likewise, among the conspecific varieties, GF-1 of fennel, HS-2 of onion and HCP-1 of carrot were more preferred over the other conspecific varieties grown in the vicinity. This preference is based on the floral advertisement (colour) or reward attributes. On the basis of this study, therefore, it is recommended that less preferred crop/ varieties should not be grown in the vicinity of highly preferred varieties by the honeybees. It is also recommended that floral parameters of the plant breeders for evolving highly preferred varieties to attract more number of pollinators.

Chapter-VI

SUMMARY AND CONCLUSION

Insect pollination has great effect on the worlds food production, It's direct effect is on the quality and quantity of crop yield. In entomophilous crops, agricultural production can be increased by many folds simply by providing adequate number of pollinators. All the umbelliferous crops and onion are protandrous and need cross-pollination in their flowers for setting the seeds. Honeybees are considered to be the most effective pollinators of crops. However, several factors are found to influence their orientation to the crop plants, fennel (*Foeniculum vulgare*), onion (*Allium cepa*) and carrot (*Daucus carota*). Therefore, the preference of honeybee species viz. *A. florea*, *A. mellifera*, *A. dosata* and *A. cerana* were studied with respect to four varieties of fennel (Rajendra Saurabh, GF-1, HF-33 and Local check, two varieties of onion (HS-1 and HS-2) and four varieties of carrrot (viz. HCP-1, HCO-4, HC-1 and HCB-1). As these crops/varieties have same flowering time, these provided an opportunity for comparative study. Commencement and cessation of honeybee activity was studied in relation to commencement and cessation of flowering on the crops. Abundance of the honeybees was recorded in 5 plot of 1 x 1m^2 area of each crop/variety. The observations were recorded at two hourly intervals and twice a week. Commencement and cessation of honeybee activity was also studied in relation to floral rewards (viz. flower colour, flower size, quality and quantity of nectar sugars and concentration of sugars and proteins in anthers). Flower colour was observed by simple visual observation and calorimetric analysis of carotenoids, xanthaphylls and anthocyanins in petals of each crops/variety. For flower size, corolla length of each crop/variety was observed by occulo-micrometer. Quality of nectar was observed simply by paper chromatography using butanol, acetic acid and water as solvent and ammonical silver nitrate as sprayer. Volume of nectar was measured with the help of 5 λ micropipette. Sugars in nectar and anthers were observed by the standard method followed by Yemm and Wills (1954). Proteins in anthers of each crop/variety were estimated by the method given by Lowry *et al.* (1951). Flower structure, nectar depth and energetic reward/floret were studied to know their influence on visitation frequency of four honeybee species.

Pollinator size and tongue length were studied for determining their suitability to the flowers of a particular crop variety. These were measured with the help of vernier callipers. On the basis of visitation frequency, the study revealed that different crops/varieties had differential attractiveness to the four honeybee species. Fennel was the most attractive to *A. florea* and onion was most attractive crop to *A. dorsata*. The least number of honeybees visited the flower of carrot.

For inter-specific preference, flower colour of the three crops was not same. Yellow coloured flowers of fennel were preferred by honeybees than white coloured flowers of onion and carrot. Yellow coloured petals of fennel were found to have higher amounts of carotenoids and xanthophylls than in white coloured petals of onion and carrot. Flowers of all the crops also differed in their nectar depth. Corolla length of onion flowers was more than that of fennel and carrot. Smaller sized *A. florea* having smaller tongue length than *A. dorsata* preferred flowers of fennel and carrot with smaller nectar depth and *A. dorsata*, inspite of yellow coloured flowers, high sugar and proteins in anthers of fennel, preferred onion flowers because of their suitability to the flowers. Hence tongue length and size of honeybees was an important factor for their suitability of these crops. All the crops had three common sugars viz. glucose, sucrose and fructose. But the visitation frequency of honey bees was found maximum on fennel followed by onion. The least abundance of honeybees was observed on carrot. Hence, for determining the honeybee preference for three crops of this study quality of nectar did not play any role. Nectar volume, nectar sugar concentration, energy and sugar per floret of onion were higher than fennel and carrot. Inspite of high nectar sugar concentration, energy and sugar per floret, flowers of onion were less preferred to honeybees than that of fennel. This was probably because of presence of potassium ions in the nectar of onion. Anthers of fennel flowers with higher sugar and proteins were foraged in maximum number by the honeybees. The flowers of onion and carrot with lower sugar and proteins in their anthers were less preferred by honeybees. Therefore, flower colour, nectar depth, concentration of sugar and proteins in anthers acted as factors in determining the relative attractiveness of the vegetable/spice crops of this study.

For inter-varietal preference, due to similar flower colour, nectar depth, quality of nectar of different varieties of each crop; these factors did not play any role in relative attractiveness. On the other hand, varieties with high energetic reward and high sugar and proteins in anthers were preferred by honeybees.

Among different varieties of fennel, GF-1 with highest nectar sugar concentration and highest sugar and protein concentrations in anthers were visited in maximum number by the honeybees followed by Rajendera saurabh, HF-33 and Local check. The least preferred variety of fennel was local check because of low energetic reward and low sugar and protein in their anthers. Likewise in onion, of the two varieties (viz. HS-1 and HS-2), HS-2 with higher nectar sugar concentration and higher sugar and protein concentration was preferred over HS-1. Of different varieties of carrot (viz. HCP-1, HCO-4, HC-1 and HCB-1), honeybees preferred HCP-1 followed by HCO-4, HCB-1 and HC-1. This was because of higher nectar sugar concentration and higher sugar and proteins in anthers of HCP-1 than the other varieties (viz. HCO-4, HCB-1 and HC-1). Therefore, intra-specific or inter-varietal preference of honeybees in this study was determined by energetic reward and concentration of sugar and proteins in anthers. Due to the similar flower colour and size of different varieties of each crop, for honeybee preference their tongue length and body size were not the decisive factors.

This study clearly reveals that blossoms of fennel attracted more number of bees if grown in the vicinity of onion and carrot. Likewise, among the conspecific varieties, GF-1 of fennel, HS-2 of onion and HCP-1 of carrot were more preferred over the other conspecific varieties grown in the vicinity. This preference is based on the floral advertisement (colour) or reward attributes. On the basis of this study, therefore, it is recommended that less preferred crop/ varieties should not be grown in the vicinity of highly preferred varieties by the honeybees. It is also recommended that floral parameters of the plant breeders for evolving highly preferred varieties to attract more number of pollinators.

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Appendix – I

Variation in the Sugar per floret of four varieties of fennel on different days

Date	Sugar/floret (mg.)			
	Rajendera Saurabh	**GF-1**	**HF-33**	**Local Check**
24.3.04	0.028	0.029	0.023	0.016
31.3.04	0.028	0.032	0.023	0.016
7.4.04	0.032	0.032	0.027	0.019
14.4.04	0.028	0.029	0.023	0.016
Mean	0.029	0.031	0.024	0.017

Variation in the Sugar per floret of two varieties of onion on different days

Date	Sugar/floret (mg.)	
	HS-1	**HS-2**
24.4.04	0.257	0.353
31.4.04	0.355	0.368
7.4.04	0.315	0.377
14.4.04	0.290	0.363
Mean	0.298	0.365

Variation in the Sugar per floret of four varieties of carrot on different days

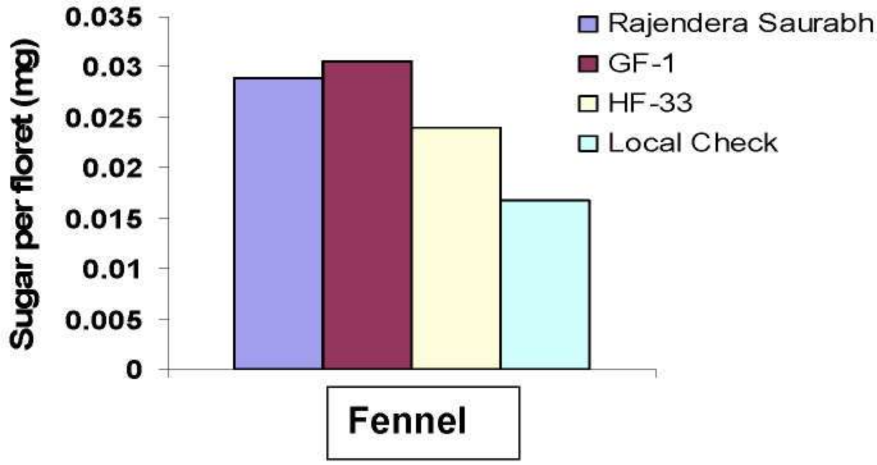
Date	Sugar/floret (mg.)			
	HCP-1	HCO-4	HCB-1	HC-1
24.3.04	0.015	0.014	0.007	0.010
31.3.04	0.017	0.014	0.007	0.010
7.4.04	0.017	0.017	0.007	0.010
14.4.04	0.017	0.014	0.005	0.010
Mean	0.017	0.015	0.006	0.010

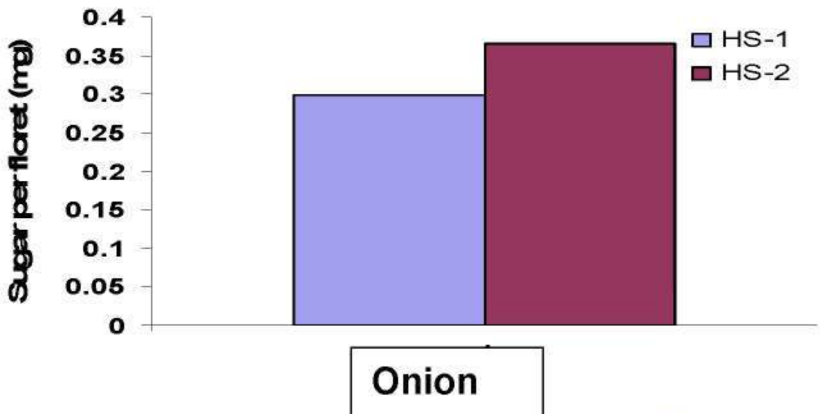
Variation in the Sugar per floret of vegetable/ spice crops on different days

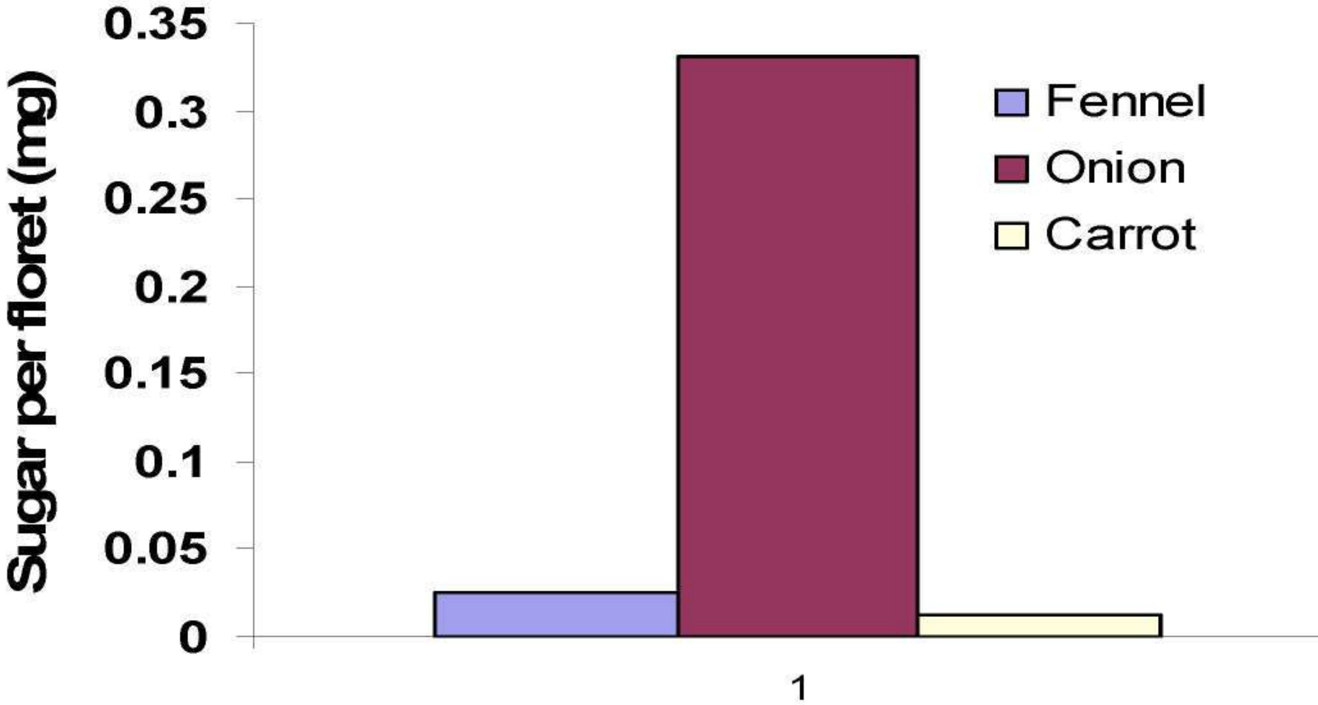
Date	Sugar/floret (mg.)		
	Fennel	Onion	Carrot
24.3.04	0.024	0.328	0.011
31.3.04	0.025	0.327	0.012
7.4.04	0.027	0.346	0.013
14.4.04	0.024	0.326	0.012
Mean	0.025	0.332	0.012

Appendix – II

Variation in the Sugar per floret in different varieties of three vegetable/spice crops







Variation in the Sugar per floret in different varieties of three vegetable/spice crops

ABSTRACT

Title of thesis	**Factors influencing inter and intra specific preferences of honeybees for three concurrently flowering vegetable/ spice crops**
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Admission No.	2000BS95D
Title of degree	Doctor of Philosophy in Zoology
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Year of award of degree	2004
Major subject	Zoology
Total number of pages in thesis	96
Number of words in abstract	600
Keywords	Honeybees, Floral Preferences, Vegetable Crops, Spice Crops, Pollination, Nectar, Energy

Insect pollination has great effect on the worlds food production, It's direct effect is on the quality and quantity of crop yield. Various factors are responsible to determine the preference of a particular bee species for a particular crop. Therefore, the relative preference of four honeybee species viz. *A. florea*, *A. mellifera*, *A. dosata* and *A. cerana* for different varieties of three crops viz. fennel (Rajendera Saurabh, GF-1, HF-33, Local Check.); onion (HS-1 and HS-2) and carrot (HCP-1, HCO-4, HC-1 and HCB-1) to four honeybee species viz. were studied. Commencement and cessation of honeybee activity was studied in relation with commencement and cessation of flowering on the crops. Abundance of the honeybees was recorded in 5 plots of 1 x 1m^2 area of each crop/variety. The observations were recorded at two hourly intervals and twice a week. Commencement and cessation of honeybee activity was also studied in relation to floral attributes viz. flower colour, flower size, quality and quantity of nectar sugars and concentration of sugars and proteins in anthers. Pollinator size and tongue length were also studied for determining their suitability to the flowers of a particular crop/variety. On the basis of visitation frequency, the study revealed that different crops/varieties had differential attractiveness to the four honeybee species. Fennel was the most attractive to *A. florea* and onion was most attractive

crop to *A. dorsata*. The least number of honeybees foraged the flowers of carrot. For inter-specific preference, flower colour of the three crops was not same. Yellow coloured flowers of fennel were preferred more by the honeybees over the white coloured flowers of onion and carrot. Yellow coloured petals of fennel were found to have higher amounts of carotenoids and xanthophylls than the white coloured petals of onion and carrot. Flowers of all the crops also differed in their nectar depth. Corolla length of onion flowers was more than that of fennel and carrot. Smaller sized *A. florea* having smaller tongue length than *A. dorsata* preferred flowers of fennel and carrot with smaller nectar depth and *A. dorsata*, inspite of yellow coloured flowers, high sugar and proteins in anthers of fennel, preferred onion flowers because of their suitability to the flowers. Hence tongue length and size of honeybees was an important factor for their suitability as pollinators of these crops. The flowers of all the three crops had three common sugars viz. glucose, sucrose and fructose. Hence, quality of nectar did not play a role in determining the floral preference of honeybees in this study. Inspite of high nectar sugar concentration, energy and sugar per floret, flowers of onion were less preferred to honeybees than that of fennel. This was perhaps because of presence of potassium ions in the nectar of onion. Anthers of fennel flowers having higher sugar and proteins were foraged in maximum number by the honeybees. Therefore, flower colour, nectar depth, concentration of sugar and proteins in anthers seemed to act as factors in determining the preference of honeybees for different vegetable/spice crops. For inter-varietal preference, due to similar flower colour, nectar depth, quality of nectar of different varieties of each crop; these factors did not play any role in relative attractiveness. On the other hand, due to similar flower colour and size of different varieties of each crop, frequency of honeybees was not influenced by tongue length and size of honeybees. However, varieties with high energetic reward and high sugar and proteins in anthers were preferred by honeybees. Among different varieties of fennel (viz. Rajendera saurabh, HF-33 and Local check), GF-1 was preferred. In onion of the two varieties (viz. HS-1 and HS-2), HS-2 was preferred over HS-1. Of different varieties of carrot (viz. HCP-1, HCO-4, HC-1 and HCB-1), honeybees showed their foraging activities maximum on HCP-1 followed by HCO-4, HC-1 and HCB-1. Therefore, intra-specific or intervarietal preference was determined by energetic reward and concentration of sugar and proteins in anthers. For a higher seed yield, therefore, it should be desirable that less preferred crop/variety should not be grown in the vicinity of more preferred crop/variety. Furthermore, to attract more pollinators, floral reward should also be taken as one of the selection parameters by the crop breeders.

Major Advisor **Signature of student**

Head of the Department