Study the Nectars' Chemical Composition of Apple, Cherry and Pumpkin

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Abstract

It is very important to attract pollinators to proper pollination of specific crops, especially commercial crop production. Plants produce attractive substances to attract pollinators in floral nectar and defenders in extra-floral nectar. Whereas nectars must function in the context of plant-animal mutualisms, their chemical composition makes them also attractive for non-mutualistic, exploiting organisms: nectar robbers and nectar-infesting microorganisms. Carbohydrates and fatty acids in flowers usually function in the attraction of mutualists and appear adapted to the physiological needs of the respective mutualists. Volatiles are a further group of compounds that serves in the attractive function of nectars. In this paper, chemical composition (fatty acids and volatile components) of the nectar of the flowers of apple, cherry and pumpkin were determined by gas-liquid chromatography. Four types of fatty acids such as palmitic (C16:0), stearic (C18:0), linolenic (C18:2), linolenic (C18:3) and arachic (C20:0); and different types of volatile components such as: asarone, hexahydrofarnesyl acetone, phytol, 2-Nonadecanone, decalactone, γ-Dodecalactone, 7-Hexadecenal, 1-Eicosanol, hydnocarpic acid, squalene and some unidentified compounds were extracted from nectars' extracts of apple, cherry, pumpkin flowers. The result of the present study will be helpful to know the visiting pollinators of these flowers, as well as to prepare pollinators attractant, which can be used to entice the pollinators to the target crops, ensuring optimum pollination and productivity. By using these attractants, pollination of the target crop can be achieved at critical time: threatened by poor weather conditions or having less resource in terms of nectar and pollen.

Keywords: flowers, apple, cherry, pumpkin, fatty acids, volatile organic compounds

Introduction

Plants have evolved different types of flowers and a variety of ways to pollinate. Pollination is the process by which pollen is transferred in the reproductive parts of plants, thereby enabling fertilization and sexual reproduction. In spite of a common perception that pollen grains are gametes, like the sperm cells of animals, this is incorrect; pollination is a phase in the alternation. Each pollen grain is a male haploid plant, a gametophyte, adapted to being transported to the female gametophyte, where it can achieve fertilization by producing the male gamete (or gametes, in the process of double fertilization) (Anonymous, 2012a). Self-pollination occurs when pollen is transferred within the same flower or between different flowers on the same plant or cultivar (tomatoes, peppers, and strawberries are examples of self-pollinating plants) (Dana and Lerner, 2002). Cross-pollinated flowers must receive pollen from a different plant or cultivar of the same type of plant for fruit to develop. For example, most apple flowers need to receive pollen from another apple tree and/or different cultivar for fruit set. The

pollen may be carried by insects, wind, etc (sweet cherries, most apples, and most plums, cherry, pumpkin) (Dana and Lerner, 2002).

Pollination by insects is called 'entomophily'. Entomophily is a form of plant pollination whereby pollen is distributed by insects, particularly bees, Lepidoptera flies and beetles. Note that Honey bees are pollinating many plant species which are not native to areas and are often inefficient pollinators of such plants (Table 1; Anonymous, 2012b).

Table 1 Some important crops pollinated by bees. Shaded rows and columns are indicating present studied crops.

Common name	Pollinators	Product	Pollinator impact	Geography of cultivation
Okra	Honey bees (incl. *Apis cerana*), Solitary bees (*Halictus* spp.)	Fruit	2-modest	Temperate
Onion	Honey bees, Solitary bees	Seed		Temperate
Cashew nut	Honey bees, Stingless bees, Bumblebees, Solitary bees (*Centris tarsata*), Butterflies, Flies, hummingbirds	Nut	3-great	Tropical
Strawberry tree	Honey bees, Bumblebees	Fruit	2-modest	Tropical, temperate
Caraway	Honey bees, Solitary bees, Flies	seed	2-modest	Temperate
Chestnut	Honey bees, Solitary bees	nut	2-modest	Temperate
Squash (plant), Pumpkin, Gourd, Marrow, Zuchini	Honey bees, Squash bees, Bumblebees, Solitary bees	fruit	4-essential	Temperate
Cucumber	Honey bees, Squash bees, Bumblebees, Leafcutter bee (in greenhouse pollination), Solitary bees (parthenocarpic gynoecious, green house varieties pollination is detrimental to fruit quality)	fruit	3-great	Temperate
Sunflower	Honey bees, Bumblebees, Solitary bees	seed	2-modest	Temperate
Apple	Honey bees, orchard mason bee, Bumblebees, Solitary bees (*Andrena* spp., *Halictus* spp., *Osmia* spp., *Anthophora* spp.), Hover flies (*Eristalis cerealis, Eristalis tenax*)	fruit	3-great	Temperate
Mammee apple	Bees	fruit	2-modest	Tropical
Mango	Honey bees, Stingless bees, Flies,Ants, Wasps	fruit	3-great	Sub-tropical
Avocado	Honey bees, Stingless bees, Solitary bees	fruit	3-great	
Apricot	Honey bees, Bumblebees, Solitary bees, Flies	fruit	3-great	Temperate
Sweet Cherry	Honey bees, Bumblebees, Solitary bees, Flies	fruit	3-great	Temperate
Sour cherry	Honey bees, Bumblebees, Solitary bees, Flies	fruit	3-great	Temperate
Almond	Honey bees, Bumblebees, Solitary bees (*Osmia cornuta*), Flies	nut	3-great	Temperate
Pomegranate	Honey bees, Solitary bees, Beetles	fruit	2-modest	
Pear	Honey bees, Bumblebees, Solitary bees, Hover flies (*Eristalis* spp.)	fruit	3-great	Temperate
Raspberry	Honey bees, Bumblebees, Solitary bees, Hover flies (*Eristalis* spp.)	fruit	3-great	Temperate
Blackberry	Honey bees, Bumblebees, Solitary bees, Hover flies (*Eristalis* spp.)	fruit	3-great	Temperate
Elderberry	Honey bees, Solitary bees, Flies, Longhorn beetles	fruit	2-modest	Temperate
Rowanberry	Honey bees, Solitary bees, Bumblebees, Hover flies	fruit	4-essential	Temperate
Blueberry	Honey bees, Alfalfa leafcutter bees, Southeastern blueberry bee, Bumblebees, Solitary bees	fruit	3-great	temperate

Source: Anonymous (2012b).

Apples are self-incompatible; they must cross-pollinate to develop fruit. Depending on climate, there are four to seven pollination groups in apples (Table 2; Sansavini, 1986; Polomski and Reighard, 2008; Anonymous, 2012a). During the flowering each season, apple growers usually provide pollinators to carry the pollen. Honey bees are most commonly used. Orchard mason bees are also used as supplemental pollinators in commercial orchards. Bumblebee queens are sometimes present in orchards, but not usually in enough quantity to be significant pollinators (Polomski and Reighard, 2008; Anonymous, 2012a). Various wild bees have been mentioned as important pollinators of apples, including the genera of *Andrena, Bombus, Halictus,* and *Osmia* (Free and Spencer-Brooth, 1964; Glukhov 1955; Kitamura and Maeta, 1969; Loken, 1958; Nicolson and Thornburg, 2007). Some wild bees of *Osmia* visit flowers at lower temperatures than Honey bees. At times and in some areas, wild bees are sufficiently abundant to set an apple crop (Nicolson and Thornburg, 2007).

Prunus avium, commonly called wild cherry, sweet cherry, bird cherry (USDA, 2012), or gean, is a species of cherry native to Europe, western Turkey, northwestern Africa, and western Asia, from the British Islands (British Trees, 2012) south to Morocco and Tunisia, north to the Trondheimsfjord region in Norway and east to the Caucasus and northern Iran, with a small disjunctive population in the western Himalaya (Euro-Med, 2012). This species in the rose family (Rosaceae), has a diploid set of sixteen chromosomes (2n=16) (Tavaud et al., 2004). Most researchers and growers give the primary credit for the pollination of cherries to Honey bees. A heavy pollinator population is needed especially when flowering occurs too early. Hendrickson (1922) noted that cherry crop was greatly increased when several colonies of Honey bees were placed in the orchard. Wind is not a factor in cherry pollination, as has been clearly and repeatedly established over the years (Murneek 1930; Claypool et al., 1931; Nicolson and Thornburg, 2007).

Pumpkin is a gourd-like squash of the genus *Cucurbita* and the family Cucurbitaceae (which also includes gourds) (Anonymous, 2012c). It commonly refers to cultivars of any one of the species *Cucurbita pepo*, *Cucurbita mixta*, *Cucurbita maxima*, and *Cucurbita moschata*, and is native of North America (Anonymous, 2012d). They typically have a thick, orange or yellow shell, creased from the stem to the bottom, containing the seeds and pulp. Pumpkins are widely grown for commercial use, and are used both in food and recreation. Researchers found that the Honey bees are the pollinator of *Cucurbitaceous* plants (like squash, pumpkin and gourd) (Nevkryta, 1953; Thompson et al., 1955; Sanduleac, 1959; Verdieva and Ismailova, 1960; Wolfenbarger, 1962; Battaglini, 1969). Michelbacher et al. (1964) and Hurd (1966) noticed both honey bees and wild bees are responsible to pollinate *Cucurbitaceous* plants.

Therefore, it is very important to attract Honey bees and wild bees to proper pollination of these crops, especially commercial crop production. It is

Table 2 Pollination groups of apple (depending on climate).

Group[1/]	Time of flowering (pollination)
Group A	Early flowering, 1 to 3 May in England (Gravenstein, Red Astrachan)
Group B	4 to 7 May (Idared, McIntosh)
Group C	Mid-season flowering, 8 to 11 May (Granny Smith, Cox's Orange Pippin)
Group D	Mid/late season flowering, 12 to 15 May (Golden Delicious, Calville blanc d'hiver)
Group E	Late flowering, 16 to 18 May (Braeburn, Reinette d'Orléans)
Group F	19 to 23 May (Suntan)
Group H	24 to 28 May (Court-Pendu Gris) (also called Court-Pendu plat)

[1/] One cultivar can be pollinated by a compatible cultivar from the same group or close (A with A, or A with B, but not A with C or D). Varieties are sometimes classed as to the day of peak bloom in the average 30-day blossom period, with pollinizers selected from varieties within a 6 day overlap period.

Source: Sansavini (1986).

well-known that bees chemoreceptor can detect volatile substances, contained in the nectar of crop plants a distance of about 2 km, but the construction of a model of mass transfer of these substances cannot popular, due to lack of information on the chemical composition of the nectar of flowers, which are zoned in the region (Nicolson and Thornburg, 2007).

Determination of the chemical composition in the nectar of cultivated flowers is very urgent task to prepare pollinators attractant, which can be used to entice the pollinators to the target crop ensuring optimum pollination and productivity. By using these attractants, pollination of the target crop can be achieved at critical time. It can save a crop threatened by poor weather conditions or having less resource in terms of nectar and pollen. In many cases the initial attractant will establish foraging patterns that continue the pollination process. In this context, chemical composition of the nectar of flowers in some varieties of apple, cherry and pumpkin were studied in Astrakhan region (Volodarovsky, Ikryanoe), Astrakhan state, Russia.

Materials and Methods

Studied Flowers

Apple (*Malus domestica*) (Figure 1) and cherry blossoms (*Prunus avium* spp.) (Figure 2) were harvested in mid-May, and the flowers of pumpkin (*Cucurbita maxima*) (Figure 3) in September at the gardens near the settlements of the Astrakhan region (Volodarovsky, Ikryanoe), Astrakhan state, Russia.

The extract obtained by extraction of biologically active substances from flowers weighing 150 g of acetone (500 mL) in the apparatus Soksletta. The solvent was removed on a rotary evaporator under reduced pressure (20 mm Hg). The residue was treated with chloroform. After removal of the chloroform, extract was obtained as a reddish-brown viscous mass. Outputs extracts from the flowers (extract sample 100 g) of apple, cherry and pumpkin were 5.7 and 6.5%, respectively.

Figure 1 Flowers of apple.

Figure 2 Flowers of cherry blossoms.

Figure 3 Flowers of pumpkin.

Lipids from the Extract of Flowers

Lipids extraction was carried out by OAAC (2000) method, using 1:1 (by vol.) of chloroform: methanol. Connection nonlipid nature of the extract was removed by washing three times with 1:1 (by vol.) of methanol:water. The chloroform solution of lipids was dried with anhydrous sodium sulfate; the solvent was removed at 60°C under reduced pressure in a vacuum water pump.

Separation of Fatty Acids

Lipid samples were saponified overnight with 20% aqueous ethanol solution of KOH at room temperature. Fatty acid from potassium salts were isolated by acidification of the solution with 5 mol L^{-1} hydrochloric acid and extracted with diethyl-ether. The ether extract was washed three times with distilled water, dried with anhydrous sodium sulfate and filtered (Vogel, 1975).

Determination of fatty acids isolated from the lipid fraction extracts of flowers of apple, cherry and pumpkin was performed by gas-liquid chromatography (Farag et al., 1986), after their conversion into the corresponding methyl esters by treatment with diazomethane. Ethereal solution of diazomethane was prepared from N-nitroso-N-methylurea in a known manner (Bekker et al., 1992).

Column TR-FAME (70% cyanopropylpolysil-phenylenesiloxane) capillary column of 30 m × 0.25 mm film thickness of 0.25. Mode chromatography: injector temperature 200°C, detector temperature 220°C, plasma ionization detector, an initial temperature of 140°C, the retention time of 0 minutes, the rate of 5°C min^{-1}, temperature of 200°C, the retention time of 3 min., the flow rate of gases:nitrogen 30 mL min^{-1}, hydrogen 50 mL min^{-1}, air 350 mL min^{-1}.

Fatty acids (C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C15:1, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C22:0) were derived from lipids and standard samples of fatty acids (chromatographic purity of 99%); which were separately dissolved in a small amount of anhydrous methanol, then added in small portions a solution of diazomethane in diethyl ether until no nitrogen gas. The reaction mass was allowed to stand at room temperature for 10 min and then concentrated to a volume of 10 mL, with an excess of diethyl ether and diazomethane was removed at room temperature using a stream of nitrogen gas. To dissolve the fatty acid methyl ester was added 2 drops of chloroform. The purity of the methyl esters of standard samples additionally controlled by gas-liquid chromatography.

Fatty acid methyl esters obtained from samples of extract and standard materials were analyzed on a gas chromatography Pye Unicam Series 304, equipped with double-ionization detector and a double recording device. To this end, 50 mg of sample extract to 2 mL vial was added internal standard (50 micrograms tridecane solution in hexane). Separation of fatty acid methyl esters was carried out using a glass column twisted into a helix (1.5 m × 4 mm) filled with diatomite (100-120 mesh), to which a 10% polyethylene glycol adipate. Raising the temperature column 8°C min^{-1} from 190°C, then isothermal at 190°C for 25 min with nitrogen at a rate of 30 mL min^{-1}. Content of the individual acids were calculated relative to the internal standard.

Qualitative and Quantitative Composition of the Volatile Components

Qualitative and quantitative composition of the volatile components was performed on extracts of flowers, using chromatography with mass selective detector Shimadzu QP 2010. To identify the components used mass spectra library NIST 02 and Wiley 2007. The content of individual compounds was calculated relative to the internal standard, which was used as a solution of 50 mg tridecane in hexane.

A sample of the extract was dissolved in benzene at a concentration of 0.1% by vol. Column -MDN-1 (metilsilikon, hardly coupled) 30 m, diameter of 0.25 mm. Mode chromatography: injector-180°C, detector-200°C, interface 210°C, carrier gas-helium (99.99999%), 1 mL min^{-1} by dividing the flow 1:10; thermostat -60°C, 1 min, 2 to 70°C min^{-1}, 5 to 90°C min^{-1}, 10 to 180°C min^{-1}, 20 to 280°C min^{-1}, then 1 min isotherm. Registration mode of mass spectra from 39 to 350 m z^{-1} (Tkachev, 2008).

Results

Nectars are aqueous solutions that are secreted by plants to attract and reward animal mutualists. The two major functional groups of these

mutualists comprise pollinators such as insects, birds and bats, which are attracted to floral nectar (FN), and defending arthropods such as ants and parasitoids, which are attracted to extra-floral nectar (EFN). Nectars mainly comprise mono-disaccharides, and fatty acids. However, other compound classes such as proteins, lipids, phenols, alkaloids and volatile organic compounds (VOCs) and their function mainly appears to be related to the varying physiological suitability of consumers and consequently, to their attractiveness to the different visitor guilds. By contrast, secondary metabolites that are commonly associated with herbivore defence, such as alkaloids and phenols, have been also described as nectar components.

Fatty Acid (FA) in the Extracts of Apple, Cherry and Pumpkin Flowers

In our present study, the determination of fatty acids in the chloroform extract of flowers of apple, cherry and pumpkin are presented in Table 3. Four types of FAs such as palmitic (C16:0), stearic (C18:0), linolenic (C18:2), linolenic (C18:3) and arachic (C20:0) were extracted from the flowers of apple, cherry and pumpkin. Among them, in the flowers' of apple were found palmitic FA (C16:0), stearic FA (C18:0), linolenic FA (C18:3) and arachic FA (C20:0) with 43.4, 1.9, 28.2 and 1.1 mg kg^{-1} extract; in the flowers' of cherry were palmitic FA (C16:0), stearic FA (C18:0) and FA arachic (C20:0) with 17.7, 4.4 and 1.1 mg kg^{-1} extract, and the flowers of pumpkin were palmitic FA (C16:0), linolenic FA (C18:2), linolenic FA (C18:3) with 10.1, 1.0 and 15.9 mg kg^{-1} extract. The highest palmitic FA (43.4 mg kg^{-1}) and linolenic FA (28.2 mg kg^{-1}) were extracted from the flowers of apple and the stearic FA (4.4 mg kg^{-1}) were extracted from the flowers of cherry. It was observed that linolenic FA (C18:2) was absent both the flowers of apple and cherry. On the other hand, stearic FA (C18:0) and arachic FA (C20:0) were absent in the flowers of pumpkin (Table 3).

Secondary Metabolites and Volatile Organic Compounds (VOCs)

Results of the determination of quantitative composition of the volatile components are presented in Table 4. Different types of VOCs such as: asarone, hexahydrofarnesyl acetone, phytol, 2-Nonadecanone, decalactone, γ-Dodecalactone, 7-Hexadecenal, 1-Eicosanol, hydnocarpic acid, squalene and some unidentified compounds (M=154, M=156, M=196, Table 4) were extracted from the lipid fraction extracts of apple, cherry, pumpkin flowers. Among these VOCs, flowers of apple contain: asarone, hexahydrofarnesyl acetone, phytol and an unidentified compounds (M=154) with the concentration of 1.75, 1.18, 13.30 and 0.84 mg kg^{-1}; flower of cherry contain: 2-Nonadecanone, hexahydrofarnesyl acetone, decalactone, γ-Dodecalactone, 7-Hexadecenal, 1-Eicosanol and an unidentified compounds (M=154); and pumpkin flowers contain: hydnocarpic acid, squalene and three unidentified compounds (M=154, M=156, M=196). The more identified VOCs were found in the lipid fraction extracts of flowers cherry and more unidentified VOCs were observed in pumpkin. The lipid fraction extracts of flowers pumpkin was the highest VOCs (21.13 mg kg^{-1}), followed by apple (17.07 mg kg^{-1}) and pumpkin (5.43 mg kg^{-1}) (Table 4).

Table 3 Fatty acid (FA) in the extracts of flowers of apple, cherry and pumpkin.

Fatty acid	Retention time (min)	Content of FA in extracts of flower (mg kg^{-1})		
		Apple	Cherry	Pumpkin
Palmitic (C16:0)	12.33	43.4	17.7	10.1
Stearic (C18:0)	15.61	1.9	4.4	-
Linoleic (C18:2)	17.07	-	-	1.0
Linolenic (C18:3)	18.29	28.2	-	15.9
Arachic (C20:0)	21.59	1.1	1.1	-
Total		74.6	23.2	27.0

Table 4 Quantitative composition of the volatile components of the flowers of apple, cherry and pumpkin.

Component	Retention time (min)	Content relative to internal standard (mg kg^{-1})
Extract of apple flowers		
Asarone	4.99	1.75
Hexahydrofarnesyl acetone	6.80	1.18
Phytol	9.836	13.30
M=154[1/]	11.10	0.84
Total		17.07
Extract of cherry flowers		
2-Nonadecanone	6.81	2.08
Hexahydrofarnesyl acetone	7.52	5.09
Decalactone	9.34	3.27
γ-Dodecalactone	9.61	5.42
7-Hexadecenal	10.91	1.40
M=154[1/]	11.13	0.97
1-Eicosanol	14.33	2.26
Total		21.13
Extract of pumpkin flowers		
Hydnocarpic acid	10.57	1.00
M=154[1/]	11.12	2.17
M=156[1/]	13.34	1.16
Squalene	16.29	0.98
M=196[1/]	17.04	0.12
Total		5.43

[1/] Unidentified compound.

Discussions

Floral nectar is considered the most common means by which animal-pollinated plants reward their pollen vectors (Simpson and Neff, 1981). By contrast, EFN is not involved in pollination (Bentley, 1977; Koptur, 1992), but serves plant indirect defence against herbivores (Heil, 2008). EFN has been described for more than 300 plant genera (Bentley, 1977; Koptur, 1992; Heil, 2008; Rico-Gray and Oliveira, 2007) and, thus, also represents an important means by which plants ensure and control mutualistic interactions with animals.

The fraction of soluble solids that can be found in nectars mainly comprises mono- and disaccharides and amino acids. However, other compound classes such as proteins, lipids, phenols, alkaloids and volatile organic compounds (VOCs) have also been reported from various nectars (Kessler and Baldwin, 2007; Nicholson and Thornburg, 2007). The main function of nectar compounds is related to the attraction of mutualistic animals. For example, sugars, amino acids and lipids have been described as nutritionally valuable nectar components (Baker and Baker, 1982; 1986) that contribute to its attractiveness for various mutualistic animals, although VOCs recently have been identified as further attractive constituents (Nicholson and Thornburg, 2007).

Sugars and Fatty Acids

Sugars and fatty acids (FAs) are the compounds mainly associated with the attractive function of nectar. Concentration and composition of nectar sugars has been often correlated with specific responses of nectar visitors (Baker and Baker, 1982; 1986; Baker and Baker, 1990; Baker et al., 1975; Percival, 1961). The attractiveness of sugars and FAs is usually highly correlated with the nutritive value of these compounds for respective

consumers. Because different groups of animals strongly differ in their physiology and, thus, in their nutritive requirements, most authors have assumed that the pollination syndrome of plants is strongly correlated with the composition and concentration of constituents that can be found in floral nectar (Baker and Baker, 1982; 1986; Baker and Baker, 1990; Baker et al., 1975). For example, hummingbirds, butterflies, moths and long-tongued bees usually prefer sucrose-rich nectars, whereas short-tongued bees and flies prefer nectar rich in hexoses, that is, fructose and glucose (Baker et al., 1975; Baker et al., 1998; Dupont et al., 2004; Freeman et al., 1984; Kromer et al., 2008; Lammers et al., 1986).

Similarly, amino acids in FN were found to be related with the pollinator syndrome of the respective plant species. For example, birds and bats do not exclusively feed on nectar and can also gain nitrogen by consuming pollen, insects and fruits, (Inouye and Waller, 1984; Potter and Bertin, 1988), whereas many adult insects feed only on FN. This physiological difference leads to the general expectation that nectar FAs concentration should be higher in insect-than vertebrate-pollinated flowers (Freeman et al., 1985; Gottsberger et al., 1984; Proctor et al., 1996). Indeed, high FAs concentrations have been reported for FNs from flowers that are adapted to pollinators such as butterflies, (Baker and Baker, 1982; 1986) flies (Inouye and Waller, 1984) or bees, (Petanidou et al., 2006) and specific behavioral responses to FAs have been demonstrated for insect pollinators: butterflies and flies can detect single FAs in nectar sugar solutions (Inouye and Waller, 1984; Potter and Bertin, 1988) or showed preferences for nectar mimics containing specific mixtures of amino acids (Alm et al., 1990). By contrast, nectarivorous birds were not found to prefer specific FAs in nectar solutions (Baker and Baker, 1982; Hainsworth and Wolf, 1976; Leseigneur et al., 2007).

Secondary Metabolites and Volatile Organic Compounds (VOCs)

Sugars and FAs shape the nectar visitor assemblage according to its specific composition. Thus, their function mainly appears related to the varying physiological suitability of consumers, and, consequently, to their attractiveness to the different visitor guilds. By contrast, secondary metabolites that are commonly associated with herbivore defence, such as alkaloids and phenols, have been also described as nectar components (Baker and Baker, 1977) and in FNs of at least 21 plant families (Adler, 2000). These compounds are usually regarded as “toxic compounds” (Adler, 2000) that plants secrete into floral nectar in order to achieve a protection from nectar robbers. For example, FN of *Catalpa speciosa* contains iridoid glycosides that fended off nectar robbers but not legitimate pollinators (Stephenson, 1981). Similarly, phenols in FN of *Aloe vryheidensis* lowered its palatability to generalist insects and, thus, the attraction of non-effective pollinators to the flowers of this species (Johnson et al., 2006). By contrast, Adler and Irwin (2005) manipulated contents of gelsemine, which represents the principal FN alkaloid of *Gelsemium sempervirens*, and found that the supplementation of gelsemine to FN deterred not only nectar robbers but also effective plant pollinators, thus decreasing the number of flowers probed and the time spent per flower by both, pollinators and nectar robbers.

Conclusions

From the results and discussion of our present study, it may be concluded that nectar plays a significant role in the functional ecology of plants. In the present study we tried to summarize the major knowledge on functions that certain nectar constituents play as signals of plants to mutualistic animals (especially insects), and as means of nectar protection from exploiters such as nectar robbers and nectar-infesting micro-organisms. This information can be helped to know the visiting pollinators of these flowers, as well as to prepare pollinators attractant, which can be used to entice the pollinators to the target crops, ensuring optimum pollination and productivity. By using these attractants, pollination of the target crop can be achieved at critical time: threatened by poor weather conditions or having less resource in terms of nectar and pollen.

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Manuscript received 22 April 2013, accepted 26 December 2013