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Determination of n-alkane profile through developmental state of sunflower leaves

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Abstract

The n-hexane extracts of young, mature and senescent sunflower leaves containing a thin layer of epicuticular waxes was analysed by TLC and GC using standard hydrocarbons. The Scanning Electron Microscopy study indicated changes in the deposition of epicuticular wax throughout the developmental stage of the sunflower leaves. The young, mature and senescent leaves contained nine, nine and five long-chain n-alkanes accounting for 86.22%, 99.76% and 92.07% of the hydrocarbons, respectively. The predominant n-alkane was n-C29 for young and mature leaves, and n-C23 for senescent leaves representing 35.85%, 45.37% and 68.54% of the hydrocarbons, respectively; whilst n-C24, n-C32 and n-C19 were present in least amounts in young, mature and senescent leaves indicating 0.59%, 0.83% and 0.53% of the hydrocarbons, respectively.
***Keywords**: Helianthus annuus, young, mature and senescent leaves, n-alkane*

1. Introduction

The leaf surfaces or cuticles are covered with a thin layer of mixture of hydrophobic wax constituents (Baker, 1982). The surface wax typically consist of a variety of long-chain hydrocarbons, alkyl esters, primary and secondary alcohols, fatty acids, etc. (Schoonhoven *et al.*, 2005). Alkanes, the main component of epicuticular wax, serve as an indicator of taxonomic relations between plant species (Maffei *et al.*, 2004; Santos *et al.*, 2005; Medina *et al.*, 2006; Sonibare *et al.*, 2007) and play an important role in plant-insect interactions study (Schoonhoven *et al.*, 2005). Further, n-alkanes of plant cuticular wax have been widely used in nutrition studies of ruminants and proposed as markers for estimating forage intake under free-ranging conditions and that, for this aim, the n-alkane profile and their evolution during different stages of the leaf development have been studied in herbs, shrubs and trees grazed by ruminants (Piasentier *et al.*, 2000).

The sunflower, *Helianthus annuus* L. (Asteraceae) is an important oil seed crop. Several studies have shown numerous compounds with allelopathic activity from sunflower leaf (Anjum and Bajwa, 2005; Gao *et al.*, 2008; Macías *et al.*, 2002 and 2008) and ovipositional stimulant to *Cochylis hospes* (Lepidoptera: Tortricidae) (Morris *et al.*, 2005 and 2009), but there is no report of epicuticular compounds from leaf surface wax which may act as attractant and ovipositional stimulant to the insect pests. The leaf surface provides an enormous variety of microstructures, unicellular and multicellular outgrowths from the epidermis which are usually indiscernible to human eye. The structural as well as chemical composition of the epicuticular wax layer differs among plant species and also within developmental state of the leaves (Hellmann and Stoesser, 1992; Piasentier *et al.*, 2000; Jetter and Schaffer, 2001). Hence, changes in the physical features of the leaf surfaces throughout different leaf developmental state whether causing variations in chemical characteristics of epicuticular leaf surfaces of sunflower are of considerable interest. The plant secondary metabolite, i.e., alkanes is the main component of epicuticular wax (Baker, 1982; Schoonhoven *et al.*, 2005), and for this the alkanes are only considered during this study. The aim of the present study was to determine the changes in adaxial leaf surfaces through Scanning Electron Microscope (SEM) study and n-alkane profile from the epicuticular leaf surface throughout the developmental state of sunflower leaves.

2. Materials and Methods

2.1 Plant Material

Fresh young (1–2 weeks old), mature (2–4 weeks old) and senescent (5–7 weeks old) sunflower cv. PAC-36 leaves were harvested randomly during January, 2011 from sunflower plant growing in the field near Chinsurah Rice Research Center (22° 53' N, 88° 23' E), West Bengal, India. The voucher specimen numbers are Roy & Barik 1 & 2, one of which has been retained by Prof. Ambarish Mukherjee, Ecotaxonomy Laboratory, Department of Botany, The University of Burdwan, Burdwan, West Bengal, India. Sufficient amount of freshly collected leaves (i.e., young, mature and senescent) were rinsed with double distilled water and dried on paper toweling.

2.2 SEM Study

The paper toweled dried adaxial surface of the leaf sample of each type was mounted on aluminum holders (stabs) and coated with gold-palladium (2 nm thickness) using Hitachi made Scanning Electron Microscope (Model: S 530 with IB 2 ion cotter, Japan).

2.3 Extraction

Fresh samples (100g) of each type (i.e., young, mature and senescent) were collected randomly from ten randomly selected plants and paper toweled dried sunflower leaves were dipped in 2L n-hexane for 45 minutes at room temperature for extraction of the surface wax from the leaves, which yielded a straw coloured extract without the trace of chlorophyll. The crude extract was then passed through Whatman (Maidstone, UK) No. 41 filter paper, and the solvent was removed under reduced pressure. The extract was further passed through a column of aluminium oxide (Alcoa, Frankfurt, Germany: F-20 grade, 80–200 mesh size, neutral) and eluted with light petroleum (Barik *et al.*, 2004). The eluent was fractioned by TLC on silica gel G (Sigma St. Louis, MO, USA) layers (thickness 0.5mm), which had been prepared using a Unoplan (Shandon, London) coating apparatus, with carbon tetrachloride as the mobile phase. The single hydrocarbon band was eluted from the silica gel layer with chloroform and subjected to argentation TLC, which showed no absorption of detectable functional groups by IR spectroscopy. The extraction process was repeated three times for each type of the leaf. Total of nine samples were produced for GC analysis.

2.4 GC Analysis

The purified hydrocarbon fractions from each type of sunflower leaf were analyzed directly by GC on a Hewlett Packard (HP: Palo Alto, CA, USA) model 5890 series II instrument fitted with a HP-1 capillary column (25m) and a flame ionization detector. The oven temperature programme was initially 170°C held for 1 min., then raised at 5°C/min to 300°C and finally held for 15 min. The carrier gas was nitrogen with a flow rate of 16.5 ml/min. Components were characterized by co-elution with authentic n-alkanes standard obtained from Sigma, and the compounds were quantified against the internal standards (i.e., n-C15, n-C21, n-C24, n-C28, n-C32 and n-C36) by manually integrating peak areas on the basis of retention times of the standards (Barik *et al.*, 2004).

3. Results and Discussion

The change of adaxial surface wax coating throughout the developmental state of *H. annuus* leaves by SEM study (Figure 1) indicated that the young leaf surface was dominated by a large number of trichomes with short and long tubular structures, and the cellular configuration of epidermis was lost due to superficial deposition of wax. A few shorter trichomes with dilated bases, and predominant furrows and ridges were observed in mature leaf surface; whereas persistent base of trichomes and tertiary deposition of wax granules were seen in senescent leaf. Previous work on other SEM study of leaf surface among plant species and also during leaf developmental state within the plant have shown that there is an extensive variation in their micromporphology, ranging from amorphous films to mixed arrays of wax tubes, rods, granules and plates (Jetter *et al.*, 2000). The cuticular wax deposition increased from young leaf to mature leaf and then decreased in senescent leaf (Jetter and Schaffer, 2001).

The GC analysis of the n-hexane extracts of epicuticular leaf surface wax shows separation of hydrocarbons, representing 86.22%, 99.76% and 92.07% of the n-alkanes present in young, mature and senescent sunflower leaves, respectively (Table 1). Nine long-chain alkanes were identified and quantified both for young and mature leaves (i. e., n-C18 for young leaves, n-C24 to n-C30 and n-C33 for both young and mature leaves, and n-C32 for mature leaves), whereas senescent leaves indicated five long chain alkanes (i.e., n-C19 to n-C23) (Table 1). The determination of n-alkane profile throughout developmental state of sunflower leaves revealed that n-C29 was the major n-alkane followed by n-C27, n-C28, n-C30, and n-C25 representing 35.85%, 31.55%, 6.75%, 4.12%, and 3.23% of the hydrocarbons in young leaves, and 45.37%, 36.62%, 6.20%, 4.32%, and 3.04% of the hydrocarbons in mature leaves, respectively. Whilst n-C23 was the most abundant n-alkane followed by n-C21, n-C20, and n-C22 representing 68.54%, 10.35%, 6.98% and 5.67% of the hydrocarbons in senescent leaves, respectively. n-C24, n-C32 and n-C19 were the least abundant, accounting for 0.59%, 0.83% and 0.53% of the n-alkanes in young, mature and senescent sunflower leaves, respectively (Table 1). The unknown numbers of unidentified branched-chain alkanes were 13.78%, 0.24% and 7.93% in young, mature and senescent sunflower leaves, respectively.

In fact n-alkanes are among the commonest constituents of all plant waxes (Baker, 1982). The results of the n-alkane determination in sunflower leaves throughout the developmental stages are indicative of the fact that the n-alkane profile during the three developmental stages underwent important modifications in the individual and total n-alkane composition. In literature, several reports are available mentioning that changes in n-alkane profile throughout the developmental stages of leaves within a particular species are different (Piasentier *et al.*, 2000; Szafranek and Synak, 2006; Sonibare *et al.*, 2007; Nikolic *et al.*, 2010). There was a progressive decline in total n-alkane concentrations in *Pennisetum glaucum*, *Sorghum* sp. and apple leaves; whereas an increase in total n-alkane content with the aging of leaves was noted in Norway spruce needles (Laredo *et al.*, 1991). Furthermore, an increase in total n-alkane levels for a limited period and then decrease was noted for *Malus hupehensis* leaves (Baker and Hunt, 1981). In the present study, an increase in total n-alkanes was observed from young to mature sunflower leaves and then a decrease from mature to

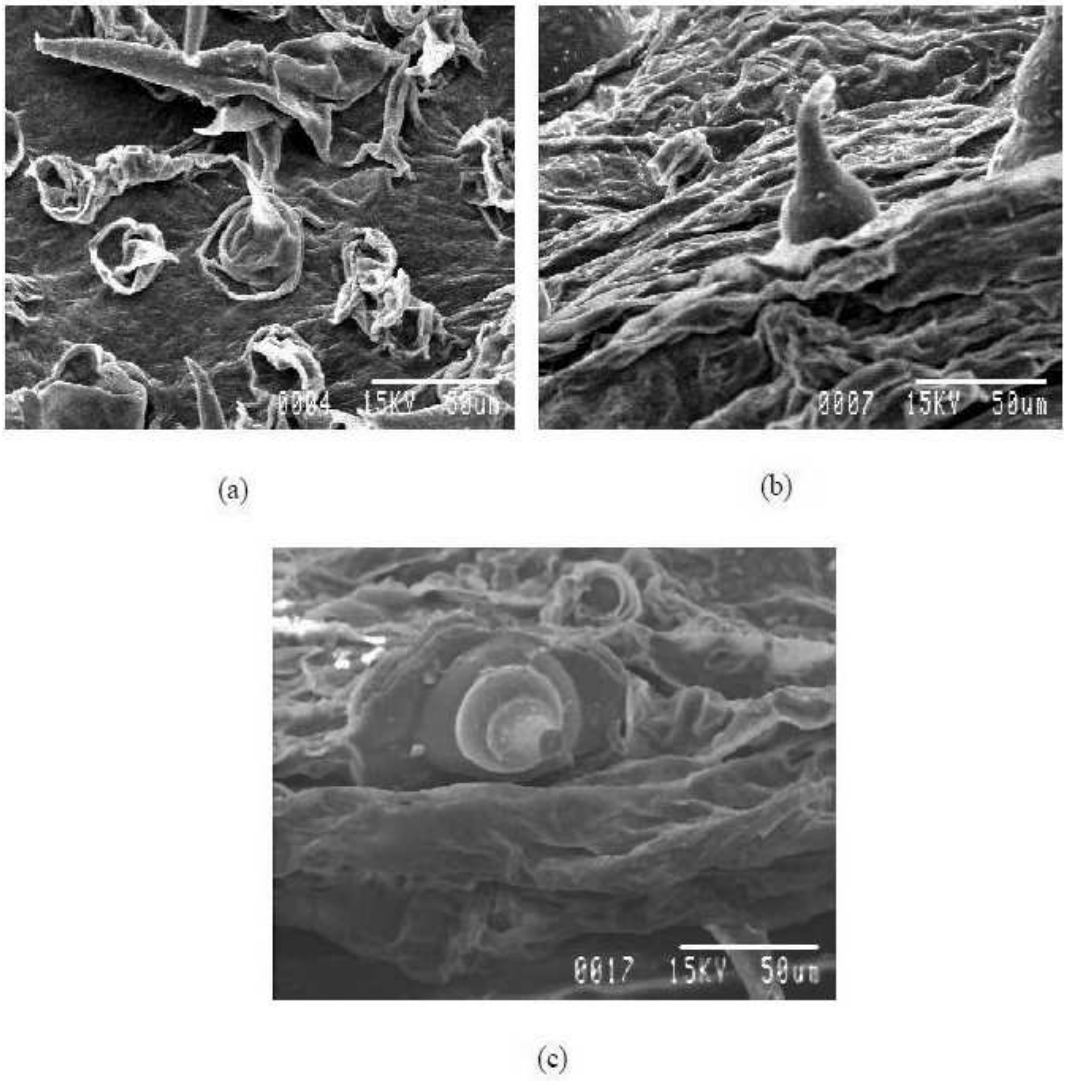

Figure 1. Scanning electron micrographs of young (a), mature (b), and senescent (c) sunflower leaf documenting cuticular wax layer from adaxial surface.

senescent leaves, though the total n-alkane concentrations were higher in senescent leaves than young leaves. The variations in n-alkane content and levels throughout the developmental stages of leaves occur due to change in the rate of wax production after leaf emergence (Piasentier *et al.*, 2000), and the results of the present study are consistent with the hypothesis that the amount and composition of n-alkanes in the leaves change depending on the developmental age of leaves (Hellmann and Stoesser, 1992, Dutton *et al.*, 2000; Jetter and Schaffer, 2001). Further, Piasentier *et al.* (2000), Barik *et al.* (2004), Chowdhury *et al.* (2010) demonstrated that n-C27, n-C25, and n-C31 were the predominant n-alkanes in the epicuticular wax of leaves of beech, *Ludwigia adscendens*, and *Cestrum nocturnum*, respectively; but in the present study, n-C29 was the predominant n-alkane in young and mature sunflower leaves and n-C23 was the most abundant alkane in senescent leaves. The odd number n-alkanes were clearly predominant throughout the developmental state of sunflower leaves, which is in good agreement with the findings of previous observation carried out on six browsed broad leaf trees (Piasentier *et al.*, 2000).

In literature, several instances are known about the role of n-alkanes in plant-insect interactions as attractant or ovipoisition stimulant (Dutton *et al.*, 2000; Schoonhoven *et al.*, 2005; Muller, 2006; Srinivasan *et al.*, 2006; Kotze *et al.*, 2010) and nutrition studies of ruminants as markers for estimating forage intake under free-ranging conditions (Dove and Mayes, 1996; Piasentier *et al.*, 2000). The determination of n-alkanes in sunflower leaves may also serve specific cues to the behavior of herbivorous insect pests (i.e., *Spilosoma obliqua*, *Spodoptera litura*, *Diacrisia obliqua* and *D. casignetum*) (Banerjee and Haque, 1984; Roy and Barik, 2013) in the field. Further, herbivores with narrow host ranges usually prefer young growing leaves, whereas, over all, the larvae of polyphagous species prefer mature leaves of their various host plants (Schoonhoven *et al.*, 2005; Roy and Barik, 2012). So, the determination of n-alkanes throughout the developmental age of sunflower leaves may fulfill

Table 1. Identification and quantification of the n-alkane compounds from n-hexane extract of the young, mature and senescent sunflower leaves.

Compound	Carbon number	Young leaf Amount (mole%)	Mature leaf Amount (mole%)	Senescent leaf Amount (mole%)
Octadecane	n-C18	1.13 ± 0.017	-	-
Nonadecane	n-C19	-	-	0.53 ± 0.017
Eicosane	n-C20	-	-	6.98 ± 0.249
Heneicosane	n-C21	-	-	10.35 ± 0.398
Docosane	n-C22	-	-	5.67 ± 0.185
Tricosane	n-C23	-	-	68.54 ± 0.531
Tetracosane	n-C24	0.59 ± .005	1.14 ± 0.046	-
Pentacosane	n-C25	3.23 ± 0.023	3.04 ± 0.121	-
Hexacosane	n-C26	1.75 ± 0.011	1.12 ± 0.025	-
Heptacosane	n-C27	31.55 ± 0.196	36.62 ± 0.34	-
Octacosane	n-C28	6.75 ± 0.294	6.20 ± 0.133	-
Nonacosane	n-C29	35.85 ± 0.323	45.37 ± 0.514	-
Triacontane	n-C30	4.12 ± 0.121	4.32 ± 0.45	-
Hentriacontane	n-C31	-	-	-
Dotriacontane	n-C32	-	0.83 ± 0.017	-
Tritriacontane	n-C33	1.25 ± 0.133	1.12 ± 0.029	-
Total n-alkanes		86.22 ± 0.456	99.76 ± 0.029	92.07 ± 1.01
Branched chain alkanes		13.78 ± 0.456	0.24 ± 0.029	7.93 ± 1.01
Ratio of normal to branched hydrocarbons		6.26:1	415.67:1	11.61:1
Carbon Preference Index		5.01	6.33	6.28

Mean ± SE of 3 observations.

the purpose of further study on the role of n-alkanes in plant-insect interaction (i.e., attractant and ovipositional stimulant) study of monophagous and polyphagous insect pests. On the other hand, determination of n-alkane profile on the sunflower leaf surface may also be used as diet composition markers of its ruminants.

4. Conclusion

The determination of n-alkane profile throughout the developmental stage of sunflower leaves showed important differences in individual n-alkane and total n-alkane concentration(s) in young, mature and senescent sunflower leaves. Except senescent leaves, where n-C23 was the most abundant alkane, n-C29 was the predominant alkane in young and mature sunflower leaves. The total n-alkane levels increased from young to mature leaf stage and then decreased from mature to senescent sunflower leaves. The results of the present study may be helpful in future plant-insect interaction study including use of n-alkanes as diet composition marker in nutrition studies of grazing ruminants.

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References

Anjum, T. and Bajwa, R. 2005. A bioactive annuionone from sunflower leaves. *Phytochemistry* **66**, 1919-1921.

Baker, E.A. 1982. Chemistry and morphology of plant epicuticular waxes. In: *The plant cuticle*. D.F. Cutler, K. Alvin and C. E. Price (Eds), Academic Press. London. 139-165.

Baker, E.A. and Hunt G.M. 1981. Developmental changes in leaf epicuticular waxes in relation to foliar penetration. *New Phytologist* **88**, 731-747.

Banerjee T.C. and Haque, N. 1984. Defoliation and yield loss in sunflower caused by caterpillars of *Diacrisia casignetum* Kollar (Lepidoptera: Arctiidae). *Indian Journal of Agricultural Science* **54**, 137-141.

Barik, A., Bhattacharya, B., Laskar, S. and Banerjee, T.C. 2004. The determination of n-alkanes in the

cuticular wax of leaves of *Ludwigia adscendens* L. *Phytochemical Analysis* **15**, 109-111.

Chowdhury, N., Ghosh, A., Bhattacharjee, I., Laskar, S. and Chandra, G. 2010. Determination of the n-alkane profile of epicuticular wax extracted from mature leaves of *Cestrum nocturnum* (Solanaceae: Solanales). *Natural Product Research* **24**, 1313-1317.

Dove, H. and Mayes, R.W. 1996. Plant wax components: a new approach to estimating intake and diet composition in herbivores. *Journal of Nutrition* **126**, 13-26.

Dutton, A., Mattiacci, L. and Dorn, S. 2000. Plant-derived semiochemicals as host location stimuli for a parasitoid of leafminers. *Journal of Chemical Ecology* **26**, 2259-2273.

Gao, Y., Zheng, C., Li, Y., Fan, C., Tu, G. and Gao, J. 2008. Chemical constituents from leaves of allelopathic cultivar sunflower in China. *Chemistry of Natural Compounds* **44**, 773-775.

Hellmann, M. and Stoesser, R. 1992. Seasonal, ontogenic and variety specific changes of the surface wax from apple leaves. *Angewandte Botanik* **66**, 109-114.

Jetter, R. and Schaffer, S. 2001. Chemical composition of the *Prunus laurocerasus* leaf surface. Dynamic changes of the epicuticular wax film during leaf development. *Plant Physiology* **126**, 1725-1737.

Jetter, R., Schaffer, S. and Riederer, M. 2000. Leaf cuticular waxes are arranged in chemically and mechanically distinct layers: evidence from *Prunus laurocerasus* L. *Plant, Cell & Environment* **23**, 619-628.

Kotze, M.J., Jürgens, A., Johnson, S.D. and Hoffmann, J.H. 2010. Volatiles associated with different flower stages and leaves of *Acacia cyclops* and their potential role as host attractants for *Dasineura dielsi* (Diptera: Cecidomyiidae). *South African Journal of Botany* **76**, 701-709.

Laredo, M.A., Simpson, G.D., Minson, D.J. and Orpin, C.G. 1991. The potential for using n-alkanes in tropical forages as a marker for the determination of dry matter intake by grazing ruminants. *Journal of Agricultural Science*, Cambridge **117**, 355-361.

Macías, F.A., López, A., Varela, R.M., Torres, A. and Molinillo J.M. 2008. Helikauranoside a, a new bioactive diterpene. *Journal of Chemical Ecology* **34**, 65-69.

Macías, F.A., Torres, A., Galindo, J.L., Varela, R.M., Alvarez, J.A. and Molinillo, J.M. 2002. Bioactive terpenoids from sunflower leaves cv. Peredovick. *Phytochemistry* **61**, 687-692.

Maffei, M., Badino, S. and Bossi, S. 2004. Chemotaxonomic significance of leaf wax n-alkanes in the Pinales (Coniferales). *Journal Biological Research* **1**, 3-19.

Medina, E., Aguiar, G., Gomez, M., Aranda, J., Medina, J.D. and Winter, K. 2006. Taxonomic significance of the epicuticular wax composition in species of the genus *Clusia* from Panama. *Biochemical Systematics and Ecology* **34**, 319-326.

Morris, B.D., Charlet, L.D. and Foster, S.P. 2009. Isolation of three diterpenoid acids from sunflowers, as oviposition stimulants for the Banded Sunflower Moth, *Cochylis hospes*. *Journal of Chemical Ecology* **35**, 50-57.

Morris, B.D., Foster, S.P., Grugel, S. and Charlet, L.D. 2005. Isolation of the diterpenoids, et-kauran-16α-ol and ent-atisan-16α-ol, from sunflowers, as oviposition stimulants for the banded sunflower moth, *Cochylis hospes*. *Journal of Chemical Ecology* **31**, 89-102.

Muller, C. 2006. Plant–insect interactions on cuticular surfaces. In: *Biology of the plant cuticle*. M. Riederer and C. Muller (Eds), Blackwell Publishing. Oxford, 398-422.

Nikolic, B., Tesevic, V., Đordevic, I., Jadranin, M., Todosijevic, M., Bojović, S. and Marin, P.D. 2010. n-Alkanes in the needle waxes of *Pinus heldreichii* var. pančići. *Journal of the Serbian Chemical Society* **75**, 1337-1346.

Piasentier, E., Bovolenta, S. and Malossini, F. 2000. The n-alkane concentrations in buds and leaves of browsed broadleaf trees. *Journal of Agricultural Science, Cambridge* **135**, 311-320.

Roy, N. and Barik, A. 2012. The impact of variation in foliar constituents of sunflower on development and reproduction of *Diacrisia casignetum* Kollar (Lepidoptera: Arctiidae). *Psyche : A Journal of Entomology*. doi:10.1155/2012/812091.

Roy, N. and Barik, A. 2013. Influence of four host plants on feeding, growth and reproduction of *Diacrisia casignetum* (Lepidoptera: Arctiidae). *Entomological Science* **16**, 112-118.

Santos, L.C., Dokkedal, A.L., Sannomiya, M., Soares, M.C.P. and Vilegas, W. 2005. n-Alkanes from *Paepalathus* Mart. species (Eriocaulaceae). *Acta Botanica Brasilica* **19**, 727-732.

Schoonhoven, L.M., Van Loon, J.J.A. and Dicke, M. 2005. *Insect-Plant biology*. Oxford University Press, Oxford, 421pp.

Sonibare, M.A., Soladoye, M.O. and Ekine-Ogunlana, Y. 2007. A chemotaxonomic approach to the alkane content of three species of *Anthocleista* Afzel. (Loganiaceae). *African Journal of Biotechnology* **6**, 1516-1520.

Srinivasan, R., Uthamasamy, S. and Talekar, N.S. 2006. Characterization of oviposition attractants of *Helicoverpa armigera* in two solanaceous plants, *Solanum viarum* and *Lycopersicon esculentum*. *Current Science* **90**, 846-850.

Szafranek, B. and Synak, E. 2006. Cuticular waxes from potato (*Solanum tuberosum*) leaves. *Phytochemistry* **67**, 80-90.

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