Journal of Spices and Aromatic Crops
Vol. 32 (1): 80-89 (2023)
10.25081/josac.2023.v32.i1.8151

Indian Society for Spices

Floral biology insights into essential oil yield and chemical composition in davana (*Artemisia pallens* Bess), a high-value aromatic plant of India

R Ravi Kumara[1], P E Keerthi[1] & N D Yogendra[1*]

[1]*CSIR - Central Institute of Medicinal and Aromatic Plants, Research Centre, Bangalore.*

**E-mail: yogendra.nd@cimap.res.in*

Received 12 December 2022; Revised 12 April 2023; Accepted 17 April 2023.

Abstract

Floral organs are identified as the major source of essential oil production in some prime aromatic plants. The quality and quantity of the essential oil are also influenced by the floral developmental stages. The present study was undertaken in *Artemisia pallens* Bess (davana) with respect to its floral biology and floral sequence in relation to the essential oil yield and its chemical constituents for industrial extraction. The findings revealed that floral (inflorescence) biomass is the primary source of essential oil yield in davana, with the highest oil yield (0.90 ml/100 g) recovered during the blooming stage, followed by herbage (stems and leaves) biomass. Furthermore, *cis*-davanone, the major chemical constituent of davana essential oil, was recorded at a higher percentage in the bud stage (66.36±0.60%) followed by the blooming stage (60.56±0.20%) and the seed setting stage (40.54±0.80%). These findings can be used to optimize harvest timing in order to obtain higher quality and quantity of davana essential oil.

Keywords: Davana, *cis*-davanone, essential oil, floral biology, florets, oil glands

Introduction

Artemisia pallens Bess (2n=2x=16) commonly known as davana, is an important and highly prized annual aromatic herb of India that belongs to the Asteraceae family and is commercially cultivated in south India from November to March as a short-duration crop. India is the world's third-largest producer of essential oils and has a monopoly on the manufacture and export of davana oil. Davana is an annual herb that grows 45-60 cm tall and has grayish-white tomentum. Inflorescence is capitulum, axillary, pedunculated or sessile, heterogeneous with yellow glabrous florets; involucre two or more, seriate, oblong to elliptic-linear; inner florets 5-lobed, bisexual; stamens five with free epipetalous filaments; style bifid (Farooqi & Sreeramu 2004). The oil color is orange-yellow to reddish-orange, viscous liquid with a deep, mellow, persistent, rich fruity fragrance. It is recognized as one of the most valuable essential oils for producing natural flavors and is used to flavor cakes, pastries, cigarettes, drinks, sausages, and preserved products. The foliage is commonly used in garlands and bouquets (Narayana *et al.,*1978).

The crop is harvested during February and March, when a large portion of the flower buds start to bloom. The flower-to-plant ratio during harvest time is crucial in determining essential oil yield. The entire plant is cut with a sickle at a height of 10 cm from the ground level for harvesting. The essential oil of davana is extracted from air-dried flowering herbs. The oil contains *n*-alkanes, hydroxydavanone, geraniol, nerol, caryophyllene, cinnamyl cinnamate, linalool, dehydro-linalool, *iso*-davanone, and dihydrorosefuran. The davanafurans are responsible for the oil's distinctive odor (Gulati 1980; Akhila & Tewari 1986). From the vegetative stage through full bloom, the essential oil level is found to vary. The peak levels of the principal constituent, davanone, are seen just prior to anthesis, during anthesis (full blooming stage), and then gradually dropping from the beginning of seed set to the seed maturing stage. The chemical content and floral stage are found to have significant interaction effects. Thus, the complete flowering stage is excellent for harvesting (Mallavarapu *et al.,* 1999). The floral biology, essential oil yield and its chemical composition were studied in palmarosa (Kumar *et al.,* 2021), wild marigold (Kumar *et al.,* 2020), clary sage (Schmiderer *et al.,* 2008), and *Goldenrod caucasian* (Fedotova & Konovalov 2018). On the other hand insight into floral biology generates important information for breeding and seed production programs (Wetzstein *et al.,* 2014). In this context, the current experiment was designed to investigate the floral developmental stages and their roles in essential oil production and chemical composition of essential oil in davana.

Materials and methods

Plant material and growth conditions

The current study was conducted on an important aromatic and cross-pollinated plant, davana (*Artemisia pallens* Bess), with the local cultivar for floral biology studies in relation to essential oil yield and chemical composition. The field experiment was carried out at the CSIR-CIMAP Research Centre, Bengaluru, during the winter season of 2021-2022. The climate of the region is semi-arid tropical with mild temperatures and moderate rainfall, making it ideal for cultivation of davana. The soil was sandy loam with a pH of 6.30 with medium fertility. The davana plants were raised with the recommended package of practices and plant protection measures were ensured throughout the crop's growth.

Floral biology and floral sequence studies

Floral biology studies were carried out from the bud initiation stage to the seed setting stage, and the data was recorded daily. The date of visible bud appearance was recorded, as well as the timing for attaining the bud stage, blooming stage, and seed setting stage. The randomly selected plants (n=100) were tagged for systematic observations. The floral morphological features like floral stages, floral structure, and floral color were recorded by visual observations. The number of capitula/plant, number of florets/capitulum, and number of sepals/capitulum were counted with the help of a hand lens. Under a microscope, the number of oil glands per floret, the number of hermaphroditic disc florets and pistillate ray florets per capitulum were counted during full bloom (n = 50 capitula). The floret length (mm) and pollen grain size (μm) were measured with

the stage micrometer in the microscope. The capitulum diameter (mm) was measured using a digital caliper, and the weight of ten capitula (g) was weighed with the help of a balance. The different developmental stages and sequences of the florets were photographed under the microscope at 4X magnification. In open-pollinated plants, the percentage of seed formation was noted.

Extraction of essential oils

The different floral development stages (bud, anthesis, post-fertilization, and seed setting stage) of davana plants were harvested, and the above-ground whole biomass was separated into floral biomass (inflorescence) and herbal biomass (stem and leaves). To evaluate the essential oil content, the separated plant materials (100g from each sample with 3 replications) from various stages were hydro-distilled in a Clevenger apparatus for six hours. The extracted oil samples were further subjected to the identification of compounds with GC and GC-MS.

GC Method

Gas chromatographic analysis was performed on an Agilent 7890B gas chromatograph equipped with a flame ionization detector. An Agilent HP-5 column of 30 m length, 320 µm internal diameter and 0.25µm film thickness was used for separation. Samples were injected into a split/splitless inlet maintained at a temperature of 250^0C with a split ratio of 1:35. Nitrogen was used as a carrier gas with a 2 mL min^{-1} constant flow rate. The column oven temperature was programmed from 60^0C and increased at the rate of 3^0C min^{-1} till 240^0C and held at 240^0C for 2 min. The FID detector temperature was kept at 280^0C.

GC-MS Method

Gas chromatography-mass spectrometry analysis was performed on a PerkinElmer Clarus 680 model GC and an SQ 8C MS using an Elite-5MS column of dimensions, 30 m x 0.25 mm with a film thickness of 0.25 µm. The injector temperature of GC was kept at 290^0C and helium as carrier gas with 1 mL min^{-1} constant flow rate with a split ratio of 1:100. The column oven was programmed from 60^0C to 240^0C at the rate of 3^0C min^{-1} increase. Samples were transferred from GC to MS through an interline which was maintained at a temperature of 250^0C. The temperature of ionization source of MS was at 250^0C and the compounds were ionized with an ionization potential of 70eV. The mass spectrometer was programmed to scan in the range of 40 to 450 amu with a scan time of 0.8 sec and an interscan delay of 0.01 sec. Compounds were identified by matching the relative retention index calculated using *n*-alkanes, (C_7-C_{30} hydrocarbons) and confirmed by comparing the mass spectrum of the compounds with the mass spectral library.

Results and discussion

The flower head initiation of davana begins at 75-85 days, followed by the full blooming stage (85-87 days) and seed setting stage (90-105 days) after transplanting to the field. The whole crop cycle was completed in around 145-160 days, from seed sowing to seed harvest. In the present study, the reproductive stage of davana was categorized into three different stages: the bud stage, the blooming stage, and the seed setting stage. The floral-metric traits are given in Table 1.

Table 1. Floral metric characteristics of davana

Sl. No.	Parameter		Mean± SE	Range		Sample variance (%)
				Minimum	Maximum	
1	No. of capitula / plant		539.3±53.4	293	816	28.52
2	Capitulum diameter (mm)	Bud stage	3.15±0.157	2.5	4	0.22
		Blooming stage	4.9±0.233	4	6	0.54
		Seed setting stage	7.15±0.131	6.8	8.2	0.16
3	Weight of 10 capitula (g)	Bud stage	0.23±0.03	0.16	0.29	0.03
		Blooming stage	0.36±0.01	0.29	0.44	0.07
		Seed setting stage	0.15±0.01	0.09	0.21	0.06
4	No. of sepals / capitulum		17.1±0.56	16	19	3.21
5	No. of florets / capitulum		39.7±4.57	14	54	29.34
6	Floret length (mm)	Female	3.41±0.14	3	4	0.21
		Bisexual	2.34±0.11	2	3	0.14
7	No. of oil glands /floret	Female	15.9±1.09	8	21	12.04
		Bisexual	10.6±1.24	6	16	15.43
8	Pollen grain size (µm)		19.6±2.31	16	23	8.33

Bud stage

The capitulum initiation in davana was observed between 75 and 85 days after transplanting to the field. Davana's inflorescence is capitulating, with flowers pedunculated/sessile, axillary/farming lax racemes (Farooqi & Sreeramu 2004). An extended bract and numerous imbricate involucral bracts further encircle the inflorescence. The number of capitula per plant ranged from 293 to 816. The average diameter of a capitulum was 3.15 mm, and the weight of 10 capitula was 0.231 g. The developmental stage of inflorescence buds (Fig. 3 A & B) was observed during the 80–90 days after transplanting to the main field. The bud is enclosed by the corolla and is yellowish-white in color. The number of florets per capitulum varied from 14 to 54. The style and stigma are indistinct at this stage. However, the well-developed oil glands were clearly visible on the corolla (Fig. 4 A & B). Aromatic plants' secretory glands occur in a variety of shapes and sizes to ensure that they perform a certain specific function. This function primarily involves the protection of the plant's many organs as well as the attraction of pollinators since it is a cross-pollinated crop. Peltate and capitate hairs are divisions of the secretory glands (Hazzoumi *et al.,* 2019). However, we observed only the capitate type of oil gland with a short-stalked and four-celled head on the corolla of davana. The capitate glands are classified as short-term glands that specialize in the production of non-terpenic compounds (Werker *et al.,* 1993). In the flower buds, these specialized oil glands help in the production of essential oils (Kumar *et al.,* 2020).

The buds contributed about 48 percent of the above-ground biomass and yielded a higher content of oil (0.83 ml/100g) than the herbage (leaves and stem) (Fig. 5). The chemical composition of essential oil extracted from buds

exhibited the highest percentage of *cis*-davanone (66.36±0.60%) content. Davanone, a sesquiterpene ketone that accounts for 24-67% of davana oil (Mallavarapu *et al.,*1999), is the main component. Davanone is odorless, but the fragrance and flavor industries demand a high davanone level. High concentrations of davanone may make the oil smell better (Mallavarapu *et al.,*1999). Thus, harvesting davana plants during the bud stage is ideal to obtain a higher percentage of davanone content. In this stage, initiation and complete development of oil glands is seen along with the development of floral parts. At the end of this stage, before the disc florets open, the bifurcated stigmas of the ray florets start to appear. Only a few central buds begin to open in the mainly closed-core disc florets at this stage.

Blooming stage

The blooming stage started 15-20 days after flower head initiation. At this stage, all the floral parts are easily distinguishable. The capitulum in davana has a heterogamous head with a few pistillate ray florets on the periphery and bisexual disc florets in the center (Fig. 1 A & B), as the salient features of the capitulum in the family Asteraceae. The maximum number of florets per head was 54. The outer 1 to 2 rows were female florets, tubular, three-lobed, yellow-colored, glandular, with a few cottony hairs (Fig. 2A), 3.41 mm in average length, and fertile. Inner bisexual florets were yellow in color, tubular, glandular, with a few cottony hairs, five-lobed (Fig. 2A), 2.34 mm in average length, and fertile. The bisexual florets contain five stamens with free filaments (Fig. 2A), epipetalous filaments, and a dithecous inflorescence. The stamen has syngenesious anthers, awned at the apex, which is connective, prolonged, tapering style, and bifid. The ovary is unilocular and inferior. Ray florets, which open earlier (3-5 days before disk floret anthesis), have fully extended stigmatic arms (Fig. 2A), whereas disk florets with more advanced capitula have florets with exposed stigmas at varied stages of emergence and bifurcation. Anthesis occurred between 8:00 a.m. and 12:00 p.m., with a peak around 10:00 a.m. Prior to the stigma, protandrous disc florets exude pollen with an active pollen presenter. This promotes cross-pollination in the davana. Pollen grains were yellow in color, separate, clumping, and spherical in shape (Fig. 2B), and their average diameter was 19.76 µm. All Asteraceae species with dimorphic florets share the same stigmatic region arrangement and stigmatic papilla shape (Torres & Galetto 2007). Moreover, the female florets had a higher number of oil glands (15.90/floret) than the bisexual florets (10.60/floret).

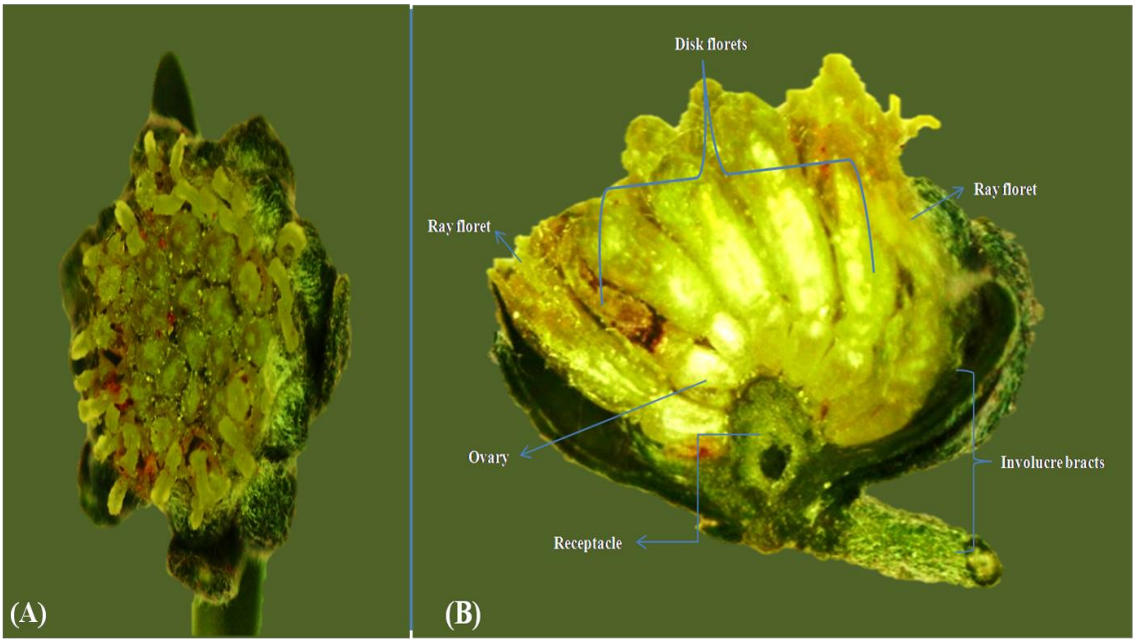

Fig. 1. A) Davana flower during blooming stage; B) Cross section of the davana capitulum.

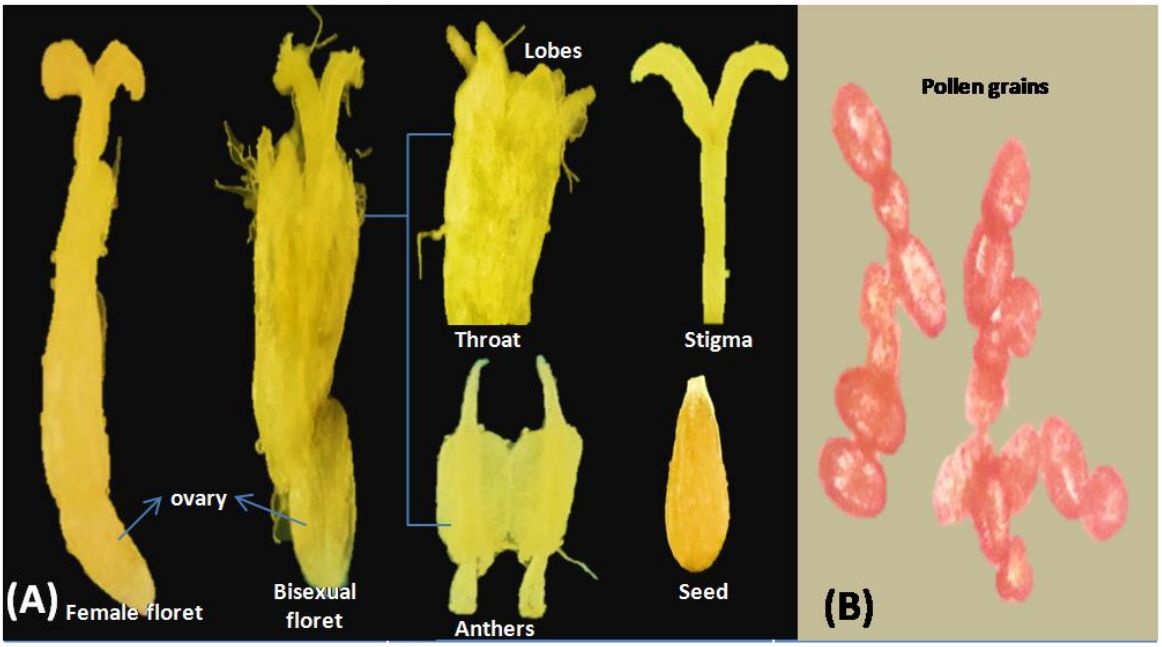

Fig. 2. A) Floral parts B) Pollen grains.

Fig. 3. Morphological view of the floret maturation sequences in the davana capitulum.

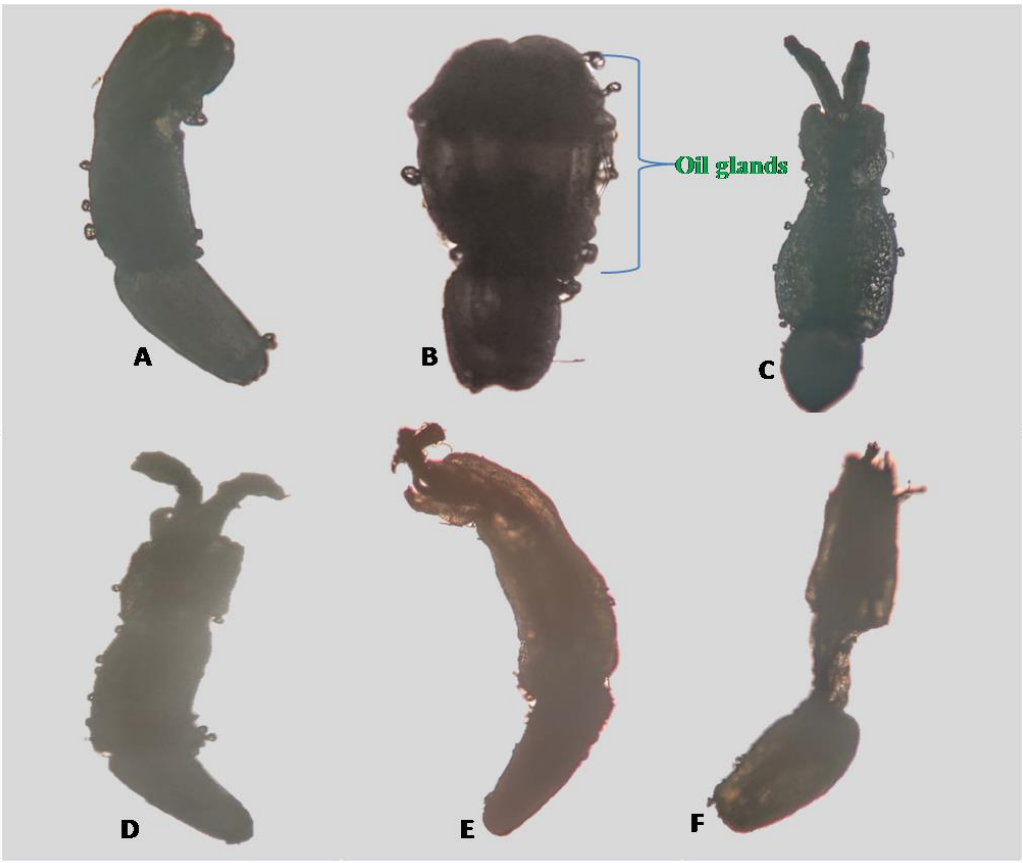

Fig. 4. Morphological view of oil gland sequences on the floret.

One day after fertilization, the oil glands on the corolla bursted (Fig. 4E). After two days of fertilization, stigma color of the fertilized floret changed from yellow to light red, followed by wilting, and the tube color changed from yellow to a light greenish color (Fig. 3F). After five days of fertilization, the corolla tube started shrinking, and oil glands on the tube also started detaching (Fig. 3G). The blooming stage is not only an important stage of reproduction in the davana plant but also plays a pivotal role in determining the quality and quantity of the essential oil. Maximum oil yield (0.90 ml/100 g) was obtained at this stage (Fig. 6). The *cis*-davanone content was 60.56±0.20 percent. These findings support the common practice of harvesting the davana crop when it is at its full bloom stage (Mallavarapu *et al.,* 1999). The ovary began to turn light brown at this point (Fig. 4F). This identifies the stage at which the seed development process has begun (Kumar *et al.,* 2020).

Seed setting stage /maturation stage

The seed maturation stage was attained after 18-23 days of fertilization and the whole flower head started drying (Fig. 3 H). The seed setting percentage was observed to be very low (24%), suggesting that it may be influenced by the season (Farooqi *et al.,* 1990). Under open-pollinated, bagged, and isolated circumstances, there was no variation in the percentage of seed set (Rai & Farooqi, 1990). It was reported that the physiological maturity of davana seeds is attained on the 35th day following anthesis (Jayanthi *et al.,* 2013). During the seed setting/maturation stage the oil yield (0.37%) and the *cis*-davanone (40.54±0.80%) content were drastically reduced in the floral parts (Fig. 7).

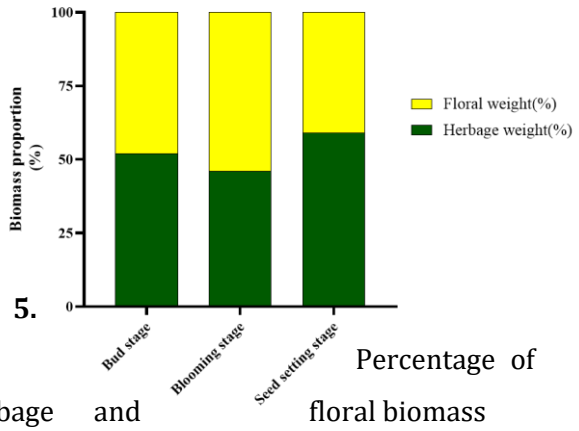

Fig. 5. Percentage of herbage and floral biomass

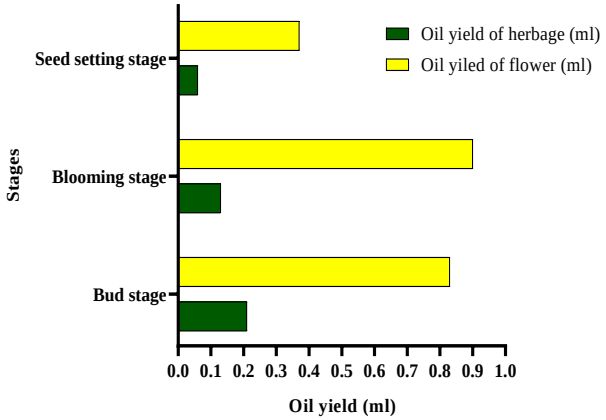

Fig. 6. Oil yield in different developmental stages of davana

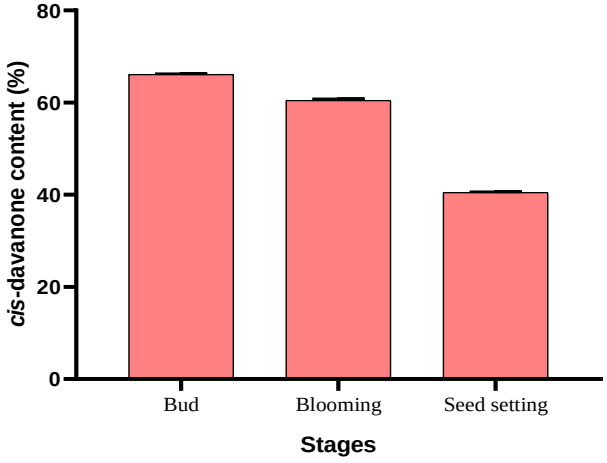

Fig. 7. *cis*-davanone content in different developmental stages of davana

Conclusion

Production and processing of aromatic crops for industrial requirements are essential to

promote the aroma industry across the world. Davana is a seasonal and short-duration aromatic crop which is cultivated exclusively for its essential oil. The quality and quantity of essential oil are highly influenced by the different developmental stages of the davana flower. Present findings suggest that harvesting davana floral biomass at the blooming stage is economical to extract desired chemical compounds with higher concentrations for industrial purposes.

Acknowledgment

The authors thank the Director, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India. The present work was supported by the Council of Scientific and Industrial Research, New Delhi, India (Aroma Mission-II, HCP-0007).

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