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Discrepancy in essential oil yield & root structure of vetiver across different storage periods

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Abstract

The aromatic root of vetiver has immense potential for essential oil extraction, making handicraft products, and providing ecosystem services. Harvesting time and storage duration of harvested roots play a crucial role to obtain maximum quantity and superior quality essential oil. Moreover, the vetiver growers often store the harvested roots for up to four months. However, there were no standard recommendations with scientific evidence regarding the optimum storage duration for harvested vetiver roots. In this context, the present experiment was undertaken at the CSIR- CIMAP Research Centre, Bengaluru, to optimize the storage duration for economically viable production of essential oil. The experiment results revealed that roots stored for 7 days (from the harvesting day) yielded maximum quantity of essential oil (1.74±0.10) and that oil recovery decreased dramatically as storage days increased. Hence, distillation of vetiver roots within 30 days of harvest is advisable to obtain maximum returns. Further, the root anatomical observations over different storage durations revealed that the root tissue color changed from 7 days of storage to 90 days with tissue layers progressively loosening and detaching after 60 days of storage.

Keywords: *Chrysopogon zizanioides,* essential oil, root anatomy, storage, vetiver

The vetiver (*Vetiveria zizanioides* (L.) Nash syn. *Chrysopogon zizanioides* Roberty, member of Gramineae/Poaceae family) is also known as Khus grass, a tall perennial important aromatic grass that grows in a wide range of climatic conditions. Vetiver essential oil is extracted from the roots through steam distillation. The dry aromatic roots are also used to construct curtains, mats, fans, and other high-end products because they release a sweet, calming fragrance when they are moistened. Vetiver roots are both biologically and commercially important as they improve physical elements, break down organic matter, and permit the absorption of dangerous substances, chemical fertilizers, pesticide residues, and heavy metals in addition to absorbing water and maintaining soil moisture. As a result, vetiver plantations are excellent for preserving soil health, water and soil conservation (Lavania, 2000).

The essential oil of vetiver, celebrated for its intricate fragrance and therapeutic benefits, plays a pivotal role across diverse sectors such as cosmetics, perfumery, pharmaceuticals, and flavors, thereby driving significant global market demand. Its multifaceted applications in enhancing fragrances, formulating skincare products, and developing medicinal remedies highlight its indispensable role in modern industries (Burger *et al.*, 2017). Vetiver known for its cooling properties, thrives in marginal lands and wastelands where conventional agriculture is unviable. Its remarkable resilience enables it to withstand drought and prolonged submersion, making it valuable asset in soil improvement. Cultivating vetiver through several cycles enhances soil fertility, thereby facilitating more profitable crop production on sandy soils.

Vetiver oil is utilized in the flavor and fragrance sectors due to its distinct aroma; vetiver oil is in high demand in both Indian and the global market. Worldwide it is estimated that 300–350 tonnes of vetiver oil is produced annually. Both the yearly demand and consumption are probably going to rise. Approximately planted over 5000 hectares of land and 100 tonnes of vetiver essential oil is produced annually in India; however, this is not enough to meet the country's needs for oil in the soap, masticatory, perfumery, and attar sectors (Singh *et al.,* 2019; Lal *et al.,* 2021). Vetiver grows naturally in northern India and is also cultivated in the coastal regions of Karnataka, Tamil Nadu, Andhra Pradesh, and Kerala. Vetiver oil from northern India is particularly valued for its high quality (Maheshwari *et al.*, 1986; Pareek, 1994). Recently, farmers in North India have started large-scale cultivation of vetiver in response to the growing demand for the oil. This increase in demand is further driven by the fact that vetiver oil cannot be synthesized or replaced with reconstituted oil. It is primarily used in flavor and fragrance base notes, with usage ranging from 20-50% to 60-70%.

The yield of roots and the proportion of oil varies with changes in the environment and types of varieties; thus, the timing of the vetiver root harvest is crucial. After 15 to 24 months of cultivation, roots are matured. However, if high-quality oil is desired, they should be harvested at 18 months (Belhassen *et al.*, 2015). Crop is normally harvested by digging out the clumps and their roots by hand between December and February. Roots extended up to 35 cm deep can be removed using a tractor-drawn moldboard plough. Before distillation, the collected roots are cleared without aerial components, thoroughly washed, and then sliced into shorter lengths of 5-10 cm to make drying easier. After drying the oil is recovered from the roots by hydro- or steam distillation process. Fresh roots are able to yield the oil and require less distillation time (Aggarwal *et al.,* 1998). Oil obtained from dried vetiver roots is more viscous and has a slightly superior scent compared to oil from freshly harvested roots. Typically, vetiver roots are harvested and stored for a period before being distilled in the field as per the convenience of farmers. However, the yield decreases with longer storage durations. Currently, there is no standard recommendation for the optimal storage time to maximize oil yield. In this context, the present experiment was conducted using the early-maturing vetiver cultivar, CIM-Vridhi (maturing in 10-12 months), to determine the ideal storage duration for maximizing oil recovery.

The vetiver root samples of the variety CIM-Vridhi were collected from the ongoing field experiment plot at CSIR-CIMAP, Research Centre, Bengaluru. Roots of twelve months old plants were collected by digging manually, thoroughly washed with water, and transferred to the laboratory to conduct the storage experiment. Each set of roots was stored from 7 to 90 days at room temperature separately (250g each, n=6), in a clean polythene bag to avoid moisture and dust. Ideally, vetiver roots stored in a cool and dry environment, with temperatures ranging between 25°C±3°C to avoid excessive heat, and the relative humidity maintained at 41-53% to prevent the roots from drying out too quickly, which could damage the cellular structure and lead to the loss of volatile oils. As per the treatment schedule on a particular day's roots anatomy was examined using sections taken by microtome in ten replicates. The sections of roots were envisioned under a light microscope at 4x magnification and the images were captured using a digital camera connected with the microscope. The root anatomical structure and color were recorded for differential analysis. The essential oil was extracted from air-dried and minced roots by hydro-distillation for 24-30 hours in Clevenger-type equipment. Before distillation, the vetiver roots were pre-soaked in water for roughly 6-9 hours to increase yield (Sabila *et al.*, 2021). The essential oil was dried over anhydrous sodium sulfate and stored for further usage in a dark amber bottle.

The fresh roots samples yielded 1.33±0.05 % essential oil. The roots stored for 7 days (from the harvesting day) yielded the maximum essential oil (1.74±0.10 %) and the oil recovery decreased dramatically as storage days increased (Fig. 1). The increase in essential oil recovery was due to loss of excess moisture in the root samples. Further, after 15 days of storage there was a slight decrease (1.49±0.08) in the essential oil recovery. It may be due to the dehydration of the roots (Fig. 1; Table 1). Cell wall damage in vetiver grass roots can occur with prolonged drying, leading to moisture loss and weakening of cell wall integrity. This structural breakdown allows volatile compounds, such as essential oils, to escape more easily. Since vetiver oil has a low melting point (0°C to 20°C), damage to root cells can cause the oil to evaporate or degrade at higher temperatures before distillation, reducing both yield and quality. To preserve the oil and ensure high-quality production, it is essential to dry the roots under controlled conditions, avoiding excessive heat or air exposure that could lead to the loss of valuable volatile compounds (Sabila *et al.*, 2021).

Table 1. Microscopic analysis of structural changes in root and oil recovery over a storage period

Storage duration	Anatomical observation	Oil recovery %
Fresh	Fresh roots with intact cell structures.	1.33±0.05
7 days	Slight dehydration observed, minimal impact on cell integrity.	1.74±0.10
15 days	Optimal balance of dehydration and cell integrity, facilitating maximum oil extraction.	1.49±0.08
30 days	Beginning signs of cell wall breakdown and initial stages of decay.	1.41±0.02
45 days	Noticeable decay and significant structural degradation, leading to lower oil yield.	0.87±0.02
60 days	Initiating the disappearance of oil glands in the cortex, the cells of each layer started to degrade as a result of variations in the cell's shape and size.	0.51±0.09
75 days	The loss of moisture content and oil caused the epidermis to shrink. The initially round epidermal layer transformed into an uneven, elongated shape.	0.45±0.09
90 days	Epidermal and cortex regions started to rupture leading to maximum loss of stored oils in cells, aerenchymatous layer was also completely detached affecting internal cells including the pith (shrunken).	0.38±0.08

The root is the sole part of vetiver grass for extracting essential oil; therefore, understanding the root's anatomical structure and characteristics is of paramount importance in the commercial oil production sector. The root anatomy of different storage days samples is depicted in Fig. 2. which reflects the cause of reduction in oil yield. Like the majority of monocotyledonous roots, vetiver roots exhibited no normal secondary growth. In the radial cross-section, there was typically no cuticle or root hairs. Several tissue sections were clearly discernible in the cross-section of a fresh root (freshly harvested) at 12 months maturity. The epidermis and hypodermis consisted of a single cell layer, while the exodermis and cortex were composed of two to three cell layers, with the cortex containing air spaces. The endodermis, pericycle (visible only in younger roots), and vascular cylinder were also present. The vascular cylinder featured clearly visible metaxylem elements arranged in a single ring, with primary phloem groups alternating with protoxylem groups in some roots, and a parenchymatous pith. Anatomy of fresh roots studies by Linggawati *et al.* (2022) align with our finding. Both studies agree on the epidermis being a single cell layer interacts with the environment. The cortex, in both cases, consists of specialized tissues, including the exodermis, aerenchyma, and parenchyma cells, with air spaces in the aerenchyma aiding gas exchange and buoyancy, and parenchyma cells serving in storage and support. While in both the cases the endodermis regulates nutrient and water movement and the oil storage is happening inside the cortical regions only.

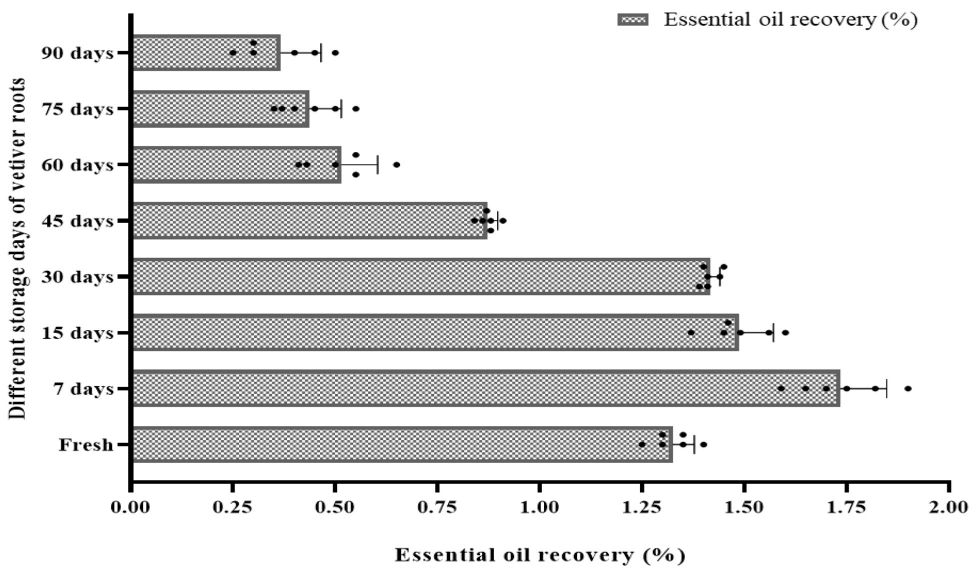

Fig. 1. Essential oil yield as influenced by storage duration

The base region (extraxyllary secondary phloem) in the root is the major source of essential oil, while the vascular cylinder (secondary xylem) supports physical and tensile strength to the penetration of roots. Further, the root anatomical observation in different storage days, *viz.*, 7, 15, 30, 45, 60, 75 and 90 days, showed unique characteristic features in their tissue color and structure, such as the root color being yellowish in 7 days, brownish in 15 days, light blackish in 30 and 45 days, dark black in 60 days, blackish yellowish in 75 days, and black in the pith and yellow in surroundings in 90 days stored roots. The stuff of the root structure is similar at 60 days, and the epidermal layer shrank in 75 days while the aerenchymatous layer was detached in 90 days. Maffei *et al.*, (2007) reported that understanding the root structure, including the epidermis, cortex, and vascular tissues, helps in developing effective harvesting and drying methods to preserve volatile compounds in essential oil yielding plants. Improper drying or storage can lead to the evaporation or degradation of these oils, reducing both yield and quality. By examining root anatomy, it is possible to improve post-harvest handling, minimizing oil loss and maximizing net returns. Research indicates that controlled drying and proper storage are critical to maintaining the integrity of volatile compounds in oil-yielding plants (Kholiya *et al.*, 2022).

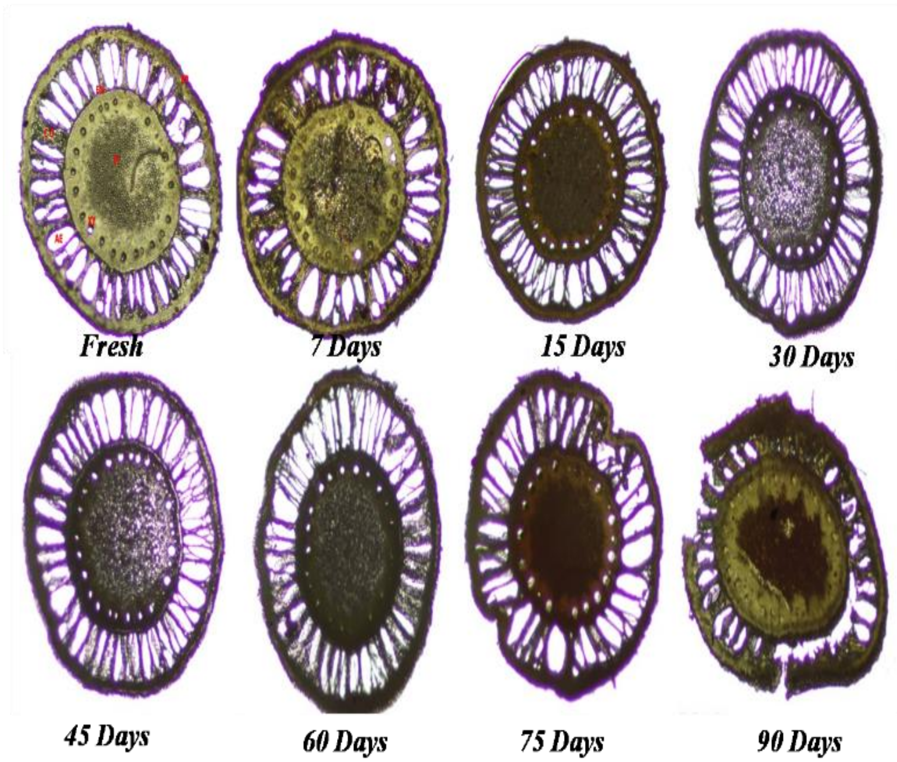

Fig. 2. Root anatomical variation in vetiver during storage

(EP: epidermis, EN: endodermis, CO: cortex, XY: xylem, AE: aerenchyma, OG: oil glands, PI: pith)

Systematic studies of root anatomical variations across different storage durations endeavors to uncover correlations between storage conditions and the physiological responses of vetiver roots. This deeper understanding promises to inform strategies for optimizing storage practices, thereby potentially enhancing the essential oil yield of vetiver. Farmers are advised for distillation of vetiver roots within 30 days for optimal essential oil yield, with the highest recovery achieved when roots are preserved for 7 days before distillation. The execution of the distillation process after 45 days of storage drastically decreases essential oil content and this information is valuable for avoiding the risk of loss in net returns while cultivating vetiver.

Conclusion

Vetiver (*Vetiveria zizanioides*), a robust aromatic grass known for its ecological and commercial value, is widely used in soil conservation and essential oil production. This study examined the effect of root storage on essential oil yield, focusing on the CIM-Vridhi variety. Based on the results obtained, it was concluded that roots stored for 7 days after harvest yield highest oil content (1.74%), while extended storage beyond 30 days leads to a decline in oil recovery due to root tissue degradation. Root anatomical changes, such as darkening and tissue shrinkage were observed over time. The research emphasizes the importance of distilling vetiver roots within

30 days of harvest to maximize oil yield and ensure economic viability for farmers engaged in large-scale vetiver cultivation.

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