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Assessment of Phytochemicals and Antioxidant Properties of Lavender under Apple-Based Agroforestry in Kashmir

Talib Nazir*, Megna Rashid, Anayet Gull, GM Bhat, Maqbool Rather, JI Mir, Mansha Manzoor and Sufiya Shabir

CAFRI AICRP (Central Agroforestry Research Institute - All India Coordinated Research Project on Agroforestry), India

***Corresponding Author:** Talib Nazir, CAFRI AICRP (Central Agroforestry Research Institute - All India Coordinated Research Project on Agroforestry) India. E-mail: wanitalib99@gmail.com

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Abstract

The present study, titled "Assessment of Phytochemicals and Antioxidant Properties of Lavender Under Apple-Based Agroforestry in Kashmir," was conducted during 2021–2023 at the Silviculture and Agroforestry Division, Faculty of Forestry, Benehama, Ganderbal. The research aimed to evaluate the impact of different spatial arrangements of apple-lavender intercropping on the growth, phytochemical composition, and antioxidant potential of lavender. Eight treatments were established: T1 (Control, sole lavender), T2 (5.2m × 5.2m), T3 (2m × 3m), T4 (1.5m × 3m), T5 (2.5m × 3m), T6 (1m × 3m), T7 (1m × 2m), and T8 (1m × 1m), using a Randomized Block Design. Statistical analysis revealed that apple tree growth parameters (height, collar diameter, and branch number) and lavender yield attributes (spikes per plant, flowers per spike, and flowers per plant) were significantly influenced by the treatments. The highest essential oil content was recorded in T1 (1.40%), while the lowest was found in T7 and T8 (1.25%). Gas chromatography analysis of lavender essential oil identified twenty compounds in T1, with linalool (37.92%), eucalyptol (19.72%), α-terpineol (5.37%), and linalyl acetate (14.93%) as major constituents; T8 had nineteen compounds. Phytochemical assays showed that T1 had the highest total phenolic (32.29±1.76 mg TAE/g DW) and flavonoid content (25.87±1.07 µg QE/g DW), whereas T8 recorded the lowest values (19.56±0.97 mg TAE/g DW and 12.45±0.94 µg QE/g DW, respectively). Antioxidant activity, measured by DPPH assay, was strongest in T1 (67.22%) and weakest in T8 (38.63%). Overall, the findings indicate that sole lavender cropping yields superior phytochemical and antioxidant profiles compared to denser agroforestry systems, emphasizing the importance of spatial arrangement in optimizing both tree and understory crop performance.

Keywords: Agroforestry, Lavender, Oil content, Flavanoid and antioxidant activity

Introduction

Medicinal and aromatic plants (MAPs) are vital to healthcare systems worldwide, particularly in developing countries. Before the rise of modern medicine, people relied entirely on plants to treat diseases in both humans and animals. Over generations, communities across the globe have developed extensive traditional knowledge about the medicinal uses of plants. This knowledge also includes their use in fishing, hunting, water purification, and managing pests and diseases in crops and livestock. Even today, nearly 80% of the population in many developing nations continue to use plant-based traditional remedies for treating illnesses [1]. Agroforestry has become one of the most promising strategies for sustainable land use. The rising human and livestock populations have led to a significant imbalance between the supply and demand of essential resources. In recent years, the cultivation of medicinal and aromatic plants (MAPs) within agroforestry systems has increased, mainly due to the growing demand from the pharmaceutical industry. Apple has emerged as a preferred tree species in temperate agroforestry systems, valued for its fruit production and consistent market demand. However, selecting suitable intercrops under apple-based systems can be challenging due to suboptimal conditions such as shade and competition. This presents an excellent opportunity to integrate medicinal and aromatic plants as intercrops in apple orchards, which can improve land productivity and generate additional income for farmers.

Lavenders (Lavandula spp.), members of the family Lamiaceae, have been valued for centuries for their therapeutic and cosmetic properties. Native to the Mediterranean, Arabian Peninsula, Russia, and Africa, lavender has a rich history of use in both traditional medicine and modern [2]. Today, its essential oil is widely incorporated into aromatherapy, cosmetics, baked goods, candles, detergents, jellies, massage oils, perfumes, shampoos, soaps, and teas. The genus Lavandula comprises 39 species, predominantly of Mediterranean origin, with Lavandula angustifolia, Lavandula intermedia (lavandin), and Lavandula spica (spike lavender) being of particular commercial importance [3]. Lavender is renowned for its resilience to drought and temperature fluctuations [4], making it suitable for diverse agro-climatic conditions. Its essential oil is highly esteemed in aromatherapy due to its antibacterial and antifungal activities, primarily attributed to components such as linalool, linalyl acetate, lavendulol, geraniol, and eucalyptol (Bialon et al., 2019). Medicinally, lavender oil is utilized in formulations for nervous system stimulation, sedation, stress relief, and dermatological applications, including the treatment of sunburn and rashes [5,6]. While several studies in the Kashmir Himalayas have explored climate change, economic valuation, and cropping systems [7-10], systematic research on medicinal plants, specifically lavender, within horticultural crop combinations remains lacking. To address this gap, the present study, "Assessment of Phytochemicals and Antioxidant Properties of Lavender Under Apple-Based Agroforestry in Kashmir," represents the first comprehensive assessment of lavender's performance under various spatial arrangements in an apple-based agroforestry system in the region. This research aims to evaluate the growth, phytochemical composition, and antioxidant potential of lavender, thereby informing the feasibility and optimization of medicinal plant integration in Himalayan agroforestry systems.

Material and Method

The experiment was conducted at the research field of the Division of Silviculture and Agroforestry, Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKUAST-K), Ganderbal, Jammu & Kashmir, India. The site is geographically situated at 34°16′44″ N latitude and 74°46′31″ E longitude, at an elevation of 1,783 meters (5,850 feet) above mean sea level (amsl).

Table

Agroforestry system	Horti-medicinal
Structural components	1. Apple (Malus domestica Borkh.)
	2. Lavender (*Lavendula angustifolia*)
Treatments	Tree Spacing
T_1	Control
T_2	5.2m × 5.2m
T_3	2m × 3m
T_4	1.5m × 3m
T_5	2.5m × 3m
T_6	1m × 3m
T_7	1m × 2m
T_8	1m × 1m
Planting direction	East-West
Spacing for intercrop	1.5m ×1.5m
Number of treatments	8
Replications	3
Design	RBD (Randomized Block Design)

Oil Content Determination (ml per kg of flowers)

Oil content was assessed using hydro-distillation. For each replicate, 200 grams of fresh lavender stem flowers were collected and combined with 1.5 liters of water. The mixture underwent hydro-distillation for three hours using a Clevenger apparatus, following the standard protocol outlined in the European Pharmacopoeia to determine oil yield (expressed as v/w %).

Total Flavonoid Content

To estimate total flavonoid content, 200 µL of sample was placed in a test tube and the solvent allowed to evaporate. The resulting residue was mixed with 5 mL of 0.1 M aluminum chloride and thoroughly shaken. After a 40-minute incubation at room temperature, absorbance was measured at 415 nm using a UV-visible spectrophotometer. Quantification was performed using a standard curve of quercetin at different concentrations, with results expressed as mg quercetin equivalents (QE) per gram dry weight (D-W).

Total Phenolic Content

Total phenolic content (TPC) was measured spectrophotometrically according to Sakat et al. (2009). Briefly, 0.2 mL of extract (1 mg/mL) was mixed with 1.0 mL of 10% Folin-Ciocalteu's reagent. After 10 minutes, 0.8 mL of 7.5% (w/v) sodium carbonate solution was added. The mixture was incubated at room temperature for 30 minutes, and absorbance was recorded at 743 nm. TPC was calculated from a gallic acid standard curve (25–400 µg/mL) and expressed accordingly.

Antioxidant Activity

The antioxidant potential of the extracts was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay, as described by Dordevic et al. (2010).

Identification of Bioactive Compounds by GC/MS

Bioactive compounds were identified using gas chromatography-mass spectrometry (GC-MS). Extracted oils were stored in glass vials at 4°C, dried with anhydrous sodium sulfate, and centrifuged at 3,000 g for 15 minutes. The clear supernatant was transferred to 2 mL vials and stored at –80°C until analysis. GC-MS was performed on an Agilent 7890A gas chromatograph with a 5975C mass spectrometer and 7683B autosampler, using a polar DB-WAX-etr column (30 m × 0.25 mm, 0.25 μm film thickness, PEG stationary phase). Helium served as the carrier gas at 3 mL/min. The injector was set to 250°C, with 1 μL splitless injections. The oven temperature was programmed from 65°C to 170°C at a rate of 1.5°C/min.

Results and Discussions

The data presented in Table 1 shows the oil content (%) in different treatments. The interaction effect of plant spacing and harvesting age did not have a significant impact on essential oil yield/ha. The highest percentage of essential oil content (1.40%) was obtained from the control treatment (T1), while the lowest value (1.25%) was observed in samples from treatments T7 and T8. Similar results were reported by [11] for Artemisia.

The density of upper storey vegetation, location, and spacing had a highly significant influence on the percentage of essential oil content. This can be attributed to an increase in fresh leaf and flower yield/ha, which contributes to an increase in essential oil yield/ha. This finding is supported by studies conducted on various plants such as Rose Scented Geranium, Spearmint, Lemongrass, Chamomile, and Sage [12, 13, 14; 15; 16].

Different studies have reported varying ranges of essential oil content in lavender and other plants, influenced by factors such as environmental conditions, genetic factors, and extraction methods. The values obtained in the present study were lower than some previous studies but were consistent with others [17, 15, 18, 19, 20, 21]. Spacing and plant density have also been found to affect essential oil yield in lavender and other plants, with higher densities generally resulting in increased above-ground biomass, fresh flower yield, dry flower yield, and essential oil yield [22, 23, 11, 24, 25, 26].

The chemical composition of the essential oil was analyzed using GC/MS and various compounds were identified in Table 3. The composition of essential oil can vary depending on factors such as climatic conditions, season, location, genetics, and extraction methods. The maximum (20) compounds were found in (T8, T1, T3, T4, T5, T6 and T7). The minimum (19) compounds were found in T2 treatment. The highest concentration of Linalool (37.92%), Eucalyptol (19.72%), Alpha.-Terpineol (5.37%) and Linalyl acetate (14.93%) was observed in T1 while as minimum was registered by T8 (Table 3).

The analysis of total phenolic content and total flavonoid content (Table 1) revealed significant differences among the treatments. The control treatment (T1) had the highest total phenolic content (32.29±1.76 mg TAE/g DW), while the lowest content (19.56±0.97 mg TAE/g DW) was observed in treatment T8. Similar findings have been reported in studies on peppers, Citrus unshiu pomaces, Glycyrrhiza glabra, Eryngium foetidum, and other plants (27, 28, 29, 30, 31).

The data presented in Table 2 shows the antioxidant activity of dried L. angustifolia flowers under an apple-based agroforestry system. The antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, which measures the ability of a substance to scavenge free radicals. Higher levels of DPPH antiradical activity have been correlated with the presence of plant polyphenols [32].

The results indicate that all the treatments exhibited significant differences in antioxidant activity. Treatment T1 showed the strongest antioxidant activity with a scavenging percentage of 67.22%, followed by T2 with 58.31%. The weakest activity was observed in treatment T8, with a scavenging percentage of 38.63% (Table 2).

Based on the results, it can be concluded that the different treatments in the apple-based agroforestry system influenced the antioxidant activity of the dried lavender flowers. The highest antioxidant activity was observed in treatment T1, which suggests that this treatment may have resulted in a higher content of phenolic compounds or other antioxidants in the flowers.

Table 1: Oil content, total phenolic content and total flavonoid content of different lavender oil samples.

Treatments	Oil Content	TPC ±SD	Flavonoid ±SD
	(%)	(mg GAE/g d.w)	mg QA/g d.w)
T_1(control)	1.40 [a]	32.29± 1.*76*	25.87±*1.07*
T_2	1.34 [b]	28.22± 0.78	23.87± 2.85 23.87± 2.85
T_3	1.33 [b]	28.07± 1.07	21.34±2.*11*
T_4	1.30 [c]	27.79± 2.*04*	20.17±*1.23*
T_5	1.28 [cd]	26.84± 1.03	20. 11± 1.94
T_6	1.27 [cd]	25. 76± 1.06	18.94±0.99
T_7	1.25 [d]	20.87± 1.54	17.48± 1.56
T_8	1.25 [d]	19.56± 0.97	12.45± 0.94

T1 (control), T2 (5.2m×5.2), T3 (2m×3m), T4 (1.5m×3m), T5 (2.5m×3m), T6 (1m×3m), T7 (1m×2m), T8 (1m×1m) Where, TPC= Total Phenol Content, mg GAE/g d.w= milligrams of gallic acid equivalent per gram dry weight and mg QA/g d.w= milligrams of Quercetin equivalents per gram of sample in dry weight.

Table 2: Antioxidant activity of dried L. *angustifolia* flowers.

Treatments	DPPH (%)
T_1(control)	67.22
T_2	58.31
T_3	56.98
T_4	53.66
T_5	51.74
T_6	48.61
T_7	41.52
T_8	38.63

T1 (control), T2 (5.2m×5.2), T3 (2m×3m), T4 (1.5m×3m), T5 (2.5m×3m), T6 (1m×3m), T7 (1m×2m), T8 (1m×1m) Where, DPPH= 2,2-diphenyl-1-picrylhydrazyl

Table 3: Gas Chromatography-Mass Spectrometry of lavender oil.

S. No.	R time	Name of chemical	Area %							
			T1	T2	T3	T4	T5	T6	T7	T8
1	4.115	Alpha.-Pinene	0.73	-	-	0.92	0.76	0.69	-	-

2	4.368	Camphene	0.37	-	-	-	0.37	-	-	-
3	4.82	Bicyclo[3.1.1] heptane, 6,6-dimethyl-2-methylene-, (1S)-	1.01	-	-	1.19	1.01	1.01	-	-
4	4.943	Beta.-Myrcene	0.6	1.13	-	0.72	0.6	0.6	-	1.13
5	5.67	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	1.04	-	-	-	1.04	-	-	-
6	5.748	Eucalyptol	19.72	15.32	16.59	17.33	17.745	17.62	14.43	11.14
7	6.937	Linalool	37.92	41.51	37.03	37.31	37.92	37.92	37.03	41.51
8	7.783	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	1.19	-	0.83	0.46	1.43	1.39	0.88	-
9	8.117	Endo-Borneol	1.59	2.25	1.88	1.49	1.59	1.59	1.88	2.25
10	8.293	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	0.46	-	0.43	-	0.46	-	0.43	-
11	8.521	Alpha.-Terpineol	5.37	5.7	5.43	5.19	5.27	5.35	5.05	5.41
12	9.063	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	0.59	1.11	0.82	0.53	0.59	0.59	0.82	2.32
13	9.496	Linalyl acetate	14.93	14.63	13.05	12.61	13.17	12.86	13.45	12.58
14	10.002	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, acetate	1.24	3.86	2.96	1.2	1.24	1.24	2.86	3.86
15	11.272	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	1.06	-	2.18	1	1.06	1.06	2.18	1.11
16	11.641	Geranyl acetate	2.07	4.19	4.31	1.97	2.07	2.07	4.31	4.19
17	12.746	Caryophyllene	1.66	1.07	0.83	1.67	1.66	1.66	0.83	1.07
18	16.141	Caryophyllene oxide	0.75	0.86	1.75	0.66	0.75	0.75	1.75	0.86
19	17.058	Tau.-Cadinol	0.54	0.22	0.69	0.46	0.54	0.54	0.69	0.22
20	17.678	Alpha.-Bisabolol	0.76	-	-	0.66	0.76	0.76	-	-
21	7.083	3-Octanone	-	0.23	-	-	-	-	-	0.23
22	7.849	Trans-.beta.-Ocimene	-	1.18	-	-	-	-	-	-
23	7.766	D-Limonene	-	1.47	-	1.09	-	1.04	-	`1.47
24	8.795	2-Carene	-	0.36	-	-	-	-	-	-
25	10.775	Terpinen-4-ol	-	0.48	-	0.44	-	0.46	-	0.48
26	10.959	2-Cyclohexen-1-one, 4-(1-methylethyl)-	-	0.89	0.37	-	-	-	0.37	0.89
27	11.927	Benzaldehyde, 4-(1-methylethyl)-	-	0.34	-	-	-	-	-	-
28	12.138	11,6-Octadien-3-ol, 3,7-dimethyl-, formate	-	1.39	-	-	-	3.14	-	-
29	7.798	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	-	-	-	0.46	-	-	-	0.31
30	11.273	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	-	-	-	0.34	-	0.37	-	-
31	6.417	2-Furanmethanol, 5-ethenyltetrahydro-.alpha.,.alpha.,5-trim	-	-	0.38	-	-	-	0.38	-

32	6.692	Trans-Linalool oxide (furanoid)	-	-	0.42	-	-	-	0.42	-
33	7.007	1-Octen-3-yl-acetate	-	-	0.49	-	-	-	0.49	0.73
34	8.84	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-	-	-	0.71	-	-	-	0.71	-
35	10.04	Bornyl acetate	-	-	0.86	-	-	-	0.76	-
36	4.366	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1S)-	-	-	-	-	-	0.46	-	-
37	8.025	Beta.-Ocimene	-	-	-	-	-	-	-	1.39
38	10.098	(+)-2-Bornanone	-	-	-	-	-	-	-	0.61
39	10.475	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-	-	-	-	-	-	-	-	0.25

T1 (control), T2 (5.2m×5.2), T3 (2m×3m), T4 (1.5m×3m), T5 (2.5m×3m), T6 (1m×3m), T7 (1m×2m), T8 (1m×1m)Where, S. no. = Serial number, R time= Retention time.

Conclusion

The research findings indicate that wider plant spacing promotes better airflow and light penetration within the lavender canopy, leading to improved plant vigor and enhanced essential oil production. On the other hand, narrower plant spacing can result in more vigorous competition for resources, potentially leading to reduced plant growth and lower yields. Therefore, it is crucial to strike a balance between plant density and resource availability to achieve optimal lavender growth and productivity.

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