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Synergistic effect of mulch and nitrogen management on growth and essential oil yield of *Salvia sclarea* L.

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Balanced plant nutrition and optimal micro-climate are critical for achieving higher production sustainably. Substituting mineral fertilizers with organic amendments under water-conserving strategies like mulch can enhance the quality and yield and improve soil health. Therefore, a two-year study was conducted to examine the synergistic effects of mulch and reducing inorganic fertilizers and partially substituting organic amendments on essential oil (EO) yield and its composition, and soil properties in *Salvia sclarea*, an industrially important crop. Two mulching practices (M_1: without mulch; M_2: with mulch) and five nitrogen (N) management practices [N_1: control; N_2: 100% recommended dose of N i.e.,120 kg/ha; N_3: 25% N through chemical fertilizer (CF) + 75% N through farm yard manure (FYM); N_4: 50% N through CF + 50% N through FYM, N_5: 75% N through CF + 25% N through FYM] were taken as experimental treatments, with a total of ten treatment combinations in a split-plot design during 2021-22 and 2022-23. The results showed that applying mulch increased the plant height, leaf area index (LAI), and EO yield. Among N management practices, N_2 outperformed for all the morpho-physiological and yield parameters studied except stem diameter and produced at par outcomes with N_5 in case of flower spike per plant, LAI, chlorophyll a and b, flower spike yield, EO content and yield. Optimum soil temperature and moisture under mulching modulate the nutrient concentration; similarly, N being necessary for photosynthesis, translocation of nutrients, enzymatic activity, and vegetative and reproductive growth, synergistically enhanced the EO yield and altered the composition. The heat map displayed an inconsistent pattern of the treatment combination's impact on EO components. In soil properties, treatment N_5 recorded highest value of available N at 0–0.15 m soil depth; however, at 0.15–0.30 m, N_2 registered a higher value and produced at par results with N_5. In case of potassium and soil organic carbon, T_3 recorded higher value, followed by T_4 and T_5 at 0–0.15 m. Considering the soil health and without compromising the EO yield much, the present study suggested replacing 25% of recommended chemical N with organic manures under mulch for sustainable and economical production of *S. sclarea*.

Keywords Mulch, Nitrogen management, *Salvia sclarea*, Spike yield, Soil properties

Approximately 900 species of *Salvia*, the most prominent genus in the Lamiaceae family, are found worldwide. One such species, *Salvia sclarea* L., an important medicinal and aromatic plant native to central Asia and southern Europe, is commercially grown in Russia, France, Morocco, and Bulgaria[1]. It is a biannual/perennial herbaceous plant commonly known as clary sage. It is also known as Bear's ear owing to the presence of a curly and hairy leaf surface. The entire plant, especially the inflorescence, has a strong aromatic scent. Essential oil (EO) of *S. sclarea* is extracted from fresh spikes in the full blooming stage as calyces have by far the most number (no.) of EO glands per unit area[2], and its concentration ranges from 0.01–0.83%[3]. It is a highly-appreciated medicinal herb in pharmacy, owing to its EOs antioxidant, anti-bacterial, anti-inflammatory, anti-fungal and analgesic properties. *S. sclarea* has an intensive demand in perfumery and cosmetics industries[4]due to germacrene D, linalyl acetate, linalool, α-terpineol, and sclareol compounds in its EO[5]. The latter biochemical compound, sclareol is the most

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interesting one, which is utilized as a precursor in a semi-synthetic process to synthesize fragrance molecules with ambergris overtones (a translucent waxy substance synthesized in the digestive tract of sperm whale and the fundamental element of one of the priciest animal extract essences available in the fragrance industry)[6]. The juice of fresh parts of clary sage is used to treat eye diseases; therefore, it is often called 'clear eye or eye bright'. Apart from industrial uses, *S. sclarea* is one of the most economically and ecologically significant plants for phytoextraction and phytostabilization of cadmium (Cd) and zinc (Zn) contaminated soil[7,8]. Realizing the uses and industrial demand of *S. sclarea*' EO, there is a growing interest in cultivating this herbaceous plant.

It is well known that numerous species belonging to the family Lamiaceae show a level of chemical variations in their EO content and profile owing to certain exogenous factors (viz., soil, altitudes, latitude, climate, agronomic practices, post-harvest handling, etc.) and endogenous factors such as genetic properties, life span of plant, growth stage and plant parts, etc[9–12]. Similarly, soil temperature and moisture content significantly affect plants' vegetative growth, photosynthetic and net assimilation rate. Mulching improves the soil micro-environment by optimizing the soil temperature and reducing soil compaction, erosion, evaporation, and weed growth. These variations in the soil micro-environment promote root growth, which consequently improves plant growth rates by causing increased water and nutrient absorption[13]. De Falco et al.[14] reported higher EO production in *Rosmarinus officinalis* when mulching was applied, suggesting that the higher soil moisture content maintained by the mulching can boost the EO yield. Similarly, nitrogen (N), a primary macronutrient, plays a vital role in the overall development of plants by affecting the formation of proteins, chlorophyll, co-enzymes and nucleic acid, which directly or indirectly determines the production of secondary metabolites in plants.

However, both yield and quality are to be looked very carefully in medicinal and aromatic crops, as overuse of nitrogenous fertilizers may decrease the accumulation of important chemical compounds, while increasing the yield[15]. Contrary to this, N deficiency could result in poor return by diminishing the produce' yield, thus, adequate supply of N is a vital factor for crop productivity. N applications often boosts the oil yield by increasing the biomass yield per unit of land, leaf area development, and rate of photosynthetic activity in aromatic plants[16,17]. However, over usage of fertilizers (especially nitrogenous) also harms the ecosystem, contributing to eutrophication and excessive greenhouse gas emissions, in addition to their limited supply, higher price and being produced from non-renewable energy sources[18,19]. Efficient nutrient management practices can affect the plant quality and EO composition as well[20]. Organic manures are a good source of essential plant nutrients; however, the primary issue with organic amendment is that it does not provide enough nutrients to support the high yield of crops[21,22]. The mineralization rate of organic manures depends on various factors such as the type of manures and environmental conditions; organic manures with a low C: N ratio provide nutrition in the first year of application and with a high C: N ratio release nutrients to the lesser extent but over the years[23]. Therefore, the ideal proposition is to manage crop growth by integrating inorganic fertilizers and organic manures[24]. The pine (*Pinus roxburghii*), a major evergreen conifer species in the western Himalayan region, creates a carpet of dry needles on the forest floor under these trees in the spring season. This enormous biomass is usually burned. Therefore, many opportunities exist to use this natural biomass as mulch[25]. In case of *S. sclarea*, the effect of mulching, N availability, and their interaction under mid-hill sub-humid conditions are still poorly understood. Keeping the above in view, an experiment was conducted to test whether mulching and N availability through different sources could influence the plant growth, flower spike yield, EO yield, and volatile compounds of EO, and also to standardize the optimum combination of organic and inorganic fertilizers for attaining higher productivity of clary sage under mid-hill sub-humid conditions of western Himalayas.

Results

Analysis of variance (ANOVA) and Bartlett's test

The ANOVA showed that mulch addition significantly (Table 1) affects only plant height, leaf area index (LAI) and EO yield of *S. sclarea* ($P = 0.05$). On the other hand, all the morphological parameters, physiological characteristics, and yield were significantly affected by N management through fertilizers and farm yard manure (FYM). The interaction of mulch with N management significantly affected the no. of branches, stem diameter, inflorescence length, chlorophyll a & b, and flower spike yield as compared to other parameters. Before beginning any data analysis, the homogeneity of the variances of the mean of both the cropping years was checked by using Bartlett's test ($p < 0.05$) (Table 2). The results concluded that the χ^2value of all the measured growth and yield attributes viz., plant height (0.0414), no. of branches (0.2908), stem diameter (0.4749), flower spike per plant (0.0033), inflorescence length (0.5479), LAI (0.0158), flower spike yield (0.0183), EO content (0.0213), EO yield (0.0393), chlorophyll a (0.0027), chlorophyll b (0.0762), and soil properties such as soil pH (0.0277, 0.1792), electical conductivity (EC) (0.0003, 0.1110), available N (0.0066, 0.0026), phosphorus (P) (0.0217, 0.0013),

Source of variation	Degree of freedom	Plant height	No. of branches	Stem diameter	Flower spike per plant	Inflorescence length	LAI	Chlorophyll a	Chlorophyll b	Flower spike yield	Essential oil content	Oil yield
Mulch (M)	1	**	NS	NS	NS	NS	*	NS	NS	NS	NS	*
Fertilizer (F)	4	**	**	**	**	**	**	**	**	**	**	**
M × F	4	NS	**	**	NS	**	NS	**	**	*	NS	NS

Table 1. Analysis of variance for effect of mulch and nutrient management on growth and yield traits of *Salvia sclarea*. **Indicates significant at $P = 0.01$; * indicates significant at $P = 0.05$; NS indicates not significant at $P = 0.01$ and $P = 0.05$.

	Plant height	No. of branches	Stem diameter	Flower spike per plant	Inflorescence length	LAI	Flower spike yield	Essential oil content	Oil yield	Chlorophyll a	Chlorophyll b
χ^2 value	0.0414	0.2908	0.4749	0.0033	0.5479	0.0158	0.0183	0.0213	0.0393	0.0027	0.0762
DF	1	1	1	1	1	1	1	1	1	1	1
p-value	0.8388	0.5897	0.4907	0.9540	0.4592	0.8998	0.8925	0.8840	0.8428	0.9583	0.7826

	0–15 cm						15–30 cm					
	pH	EC	N	P	K	OC	pH	EC	N	P	K	OC
χ^2 value	0.0277	0.0003	0.0066	0.0217	0.0360	0.0071	0.1792	0.1110	0.0026	0.0013	0.0343	0.0566
DF	1	1	1	1	1	1	1	1	1	1	1	1
p-value	0.8677	0.9855	0.9351	0.8829	0.8494	0.9940	0.6718	0.7390	0.9351	0.9713	0.8531	0.8120

Table 2. Bartlett's test for homogeneity of variances.

Treatment	Plant height (cm)	No. of branches	Stem diameter (mm)	Flower spike per plant	Inflorescence length (cm)	LAI	Chlorophyll a	Chlorophyll b	Flower spike yield (t/ha)	Essential oil content (%)	Oil yield (kg/ha)
Mulch											
Without mulch	103.84^{b}	6.13^{NS}	10.68^{NS}	216.68^{NS}	44.95^{NS}	13.04^{b}	1.20^{NS}	0.34^{NS}	11.43^{NS}	0.175^{NS}	20.04^{b}
With mulch	112.99^{a}	6.44^{NS}	11.11^{NS}	221.18^{NS}	49.67^{NS}	13.88^{a}	1.23^{NS}	0.36^{NS}	11.67^{NS}	0.180^{NS}	21.10^{a}
Nitrogen management											
Control	97.81^{e}	5.09^{e}	9.79^{b}	192.62^{e}	43.38^{de}	10.20^{c}	1.08^{d}	0.31^{cd}	10.79^{de}	0.160^{de}	17.19^{e}
100% N (CF)	117.74^{a}	7.05^{a}	11.63^{a}	237.73^{ab}	52.76^{a}	15.25^{a}	1.32^{ab}	0.40^{ab}	12.34^{ab}	0.194^{ab}	23.87^{ab}
75% N (CF) + 25% N (FYM)	112.03^{bc}	6.64^{bc}	11.77^{a}	229.23^{abc}	49.55^{b}	15.03^{a}	1.27^{ab}	0.38^{abc}	11.89^{abc}	0.186^{abc}	22.12^{abc}
50% N (CF) + 50% N (FYM)	108.63^{bcd}	6.44^{bcd}	11.21^{a}	221.54^{bcd}	46.54^{cd}	14.14^{a}	1.21^{bc}	0.35^{bcd}	11.55^{bcd}	0.177^{bcd}	20.46^{bcd}
25% N (CF) + 75% N (FYM)	105.84^{cd}	6.20^{cd}	10.05^{b}	213.53^{cd}	44.33^{cde}	12.67^{b}	1.17^{c}	0.34^{bcd}	11.19^{cde}	0.172^{cde}	19.20^{cd}

Table 3. Effect of mulch and nutrient management on growth and yield traits of *Salvia sclarea* (pooled data of two years). SEm indicates Standard Error of Mean; LSD indicates Least Significant Difference; NS indicates not significant at $P = 0.05$; CF indicates chemical fertilizer; FYM indicates farm yard manure.

potassium (K) (0.0360, 0.0343) and organic carbon (OC) (0.0071, 0.0566) at 0–0.15 m and 0.15–0.30 m soil depth, respectively, is less than that of tabulated χ^2 value (3.841). Furthermore, the p-value for all the variables was > 0.001, indicating the homogeneity of variances for all measured traits; consequently, pooled analysis was performed for the data of all the traits of both the cropping years.

Morpho-physiological traits and flower spike yield

The analyzed data (Table 3) demonstrated that growth parameters like plant height and LAI were affected considerably by applying mulch with the greatest enhancement; however, all other growth and yield traits remain unaffected. The mulch application recorded significantly higher plant height (112.99 cm) and LAI (13.88) than those without mulch. Irrespective of mulch treatments, N management practices also significantly affected morphological parameters. The significantly highest plant height (117.74 cm) was recorded in treatment N_2, where recommended dose of N was applied only through chemical fertilizer (CF), followed by treatment N_5, where 75% of N was applied through CF and 25% of N through FYM. Treatments N_2 and N_5 recorded 20.4% and 14.53% higher plant height over control, respectively. A similar trend under N_2 was observed for no. of branches (7.05) and inflorescence length (52.76 cm) and LAI (15.25), except for stem diameter, where N_5 [75% N through CF + 25% N through FYM] recorded higher diameter, though N_2 and N_4 were at par with N_5. In case of flower spike per plant, N_2 produced significantly higher value (237.73), and produced non-significant differences with N_5 (229.23).

The outcomes of physiological parameters conveyed significant changes by applying different N management practices. The results concluded that chlorophyll a (1.32 µg/ml) and b (0.40 µg/ml) showed a significant increase in treatment N_2, whereas the lowest values were recorded in control (1.08 and 0.31 µg/ml, respectively). Moreover, treatment N_5 was at par with N_2. The results depicted in Table 3 interpreted that the application of mulch had no significant effect on flower spike yield of *S. sclarea*. Irrespective of mulch, a significant increase in flower spike yield was observed when full dose of N was applied through CF (N_2), being at par with N_5 (75% N through CF + 25% N through FYM), whereas the treatment N_1 recorded 23.4 and 19.0% lower yield as compared to treatment N_2 and N_5, respectively.

EO content, yield and composition

The mulch application did not significantly affect EO content; however, it produced a significantly higher EO yield (21.10 kg/ha) than no mulch (20.04 kg/ha). The results also demonstrated that the application of N through

CF alone produced significantly higher EO content (0.194%) and EO yield (23.87 kg/ha), which produced non-significant differences with N_5 (0.186% and 22.12 kg/ha, respectively). The percent increase in the EO yield under N_2 and N_5 was 42.46 and 28.67% over control (N_1). All the treatments were studied for the qualitative analysis of EO. The GC and GC-MS analysis of *S. sclarea* observed 17 major chemical compounds constituting about 79.0% of the total EO. The primary chemical constituents of the EO were linalool acetate (25.30–29.41%), linalool (18.92–23.52%) and sclareol (4.78–7.80%) (Table 4). These three major compounds represented 50–58% of the total identified chemical constituents of different treatments. Linalool acetate and linalool were two major compounds belonging to the class of oxygenated monoterpenes, and sclareol belongs to the class of diterpenes. Geranyl acetate (4.01–5.72%) and neryl acetate (2.30–3.28%) were also found in slightly higher amounts and associated with the class monoterpene ester.

Application of mulch and N fertilizer along with FYM reported different compositions and quantities of EO. Application of mulch recorded significantly highest concentration of spathulenol (1.86%), caryophyllene oxide (2.13%), and sclareol (7.07%); however, linalool (22.80%), α-terpineol (3.70%), endo-fenchol (2.41%), and linalool acetate (29.16%) were maximum under no-mulch conditions. Irrespective of mulching treatments, chemical constituents i.e., linalool (23.52%), linalool acetate (29.41%), endo-fenchol (3.02%), neryl acetate (3.28%) and geranyl acetate (5.72%) were accumulated maximum under N_1 (control) which was at par with N_4 (50% N through CF + 50% N through FYM) in case of former two compounds. Similarly, trans-linalool oxide (2.77%), spathulenol (1.67%), caryophyllene oxide (2.65%), and sclareol (7.80%) were collected significantly higher in treatment N_3 (25% N through CF + 75% N through FYM). Furthermore, N_5 (75% N through CF + 25% N through FYM) recorded significantly higher concentration of α-terpineol and linalool oxide, which was at par with N_4 in case of former compound, and with N_3 in case of latter one.

Principal component analysis (PCA)

PCA was undertaken to analyze the variations among all the treatments and their relationship with the chemical components of EO (Fig. 1). The PCA demonstrated that the first three principal components (PC1, PC2 and PC3), out of a total of 9 PCs with the eigenvalue > 1, interpreted 83.01% of the total variation (Fig. 1a). However, the PCA biplot was created using only PC1 and PC2 because these components contributed 44.26 and 21.48% of the overall variation, respectively (Fig. 1c). The combined contribution of these two PCs was 65.74% variance, and their respective eigenvalues were 5.3 and 2.6. The variable linalool was differentiated at the positive end of the PC1 with a correlation coefficient value 0.42. However, variables endo-fenchol, linalool acetate, neryl acetate and geranyl acetate were separated at the positive end of both the PC's (PC1 and PC2) with the loading values 0.41 and 0.10, 0.35 and 0.01, 0.14 and 0.19; and 0.25 and 0.21, respectively. PCA bi-plot depicted that PC1 has a negative correlation coefficient for variables linalool oxide (−0.30), caryophyllene oxide (−0.16), β-eudesmol (−0.29) and sclareol (−0.37). Variable spathulenol (0.49) was located at the positive end, while α-terpineol (−0.47) was at the negative end of PC1. Association among the factor loadings of PC1 and PC2 with variables (chemical composition of EO) also represented in PCA bi-plot (Fig. 1b), which indicated that the component trans-linalool oxide and linalool oxide were presented at the negative end of both the PCs. However, components endo-fenchol, linalool acetate, neryl acetate and geranyl acetate were present at the positive end of PC1 and PC2. The angle between the vectors represented the correlation between different traits/ variables. The angle between the two vectors was less than the acute angle, then the vectors were positively correlated. On the other hand, a negative correlation was exhibited if the angle was obtuse[26]. Therefore, PCA bi-plot indicated that the linalool,

Treatment	trans-Linalool oxide	Linalool	α-Terpineol	endo-Fenchol	Linalool acetate	Linalool oxide	Neryl acetate	Geranyl acetate	Spathulenol	Caryophyllene oxide	β-Eudesmol	Sclareol
RT	15.23	16.25	19.26	19.70	21.40	24.63	24.92	25.57	31.98	32.17	34.20	47.87
RI Cal	1079	1104	1193	1204	1255	1352	1362	1381	1592	1599	1672	2244
Mulch												
Without mulch	2.60^{NS}	22.80^{a}	3.70^{a}	2.41^{a}	29.16^{a}	0.81^{NS}	2.64^{NS}	4.64^{NS}	1.16^{b}	1.66^{b}	1.16^{NS}	4.78^{b}
With mulch	2.18^{NS}	19.51^{b}	3.15^{b}	1.86^{b}	25.46^{b}	0.79^{NS}	2.86^{NS}	5.00^{NS}	1.86^{a}	2.13^{a}	1.27^{NS}	7.07^{a}
Nitrogen management												
Control	2.15^{cd}	23.52^{a}	3.04^{cd}	3.02^{a}	29.41^{ab}	0.60^{cd}	3.28^{a}	5.72^{a}	1.31^{bd}	1.70^{bcd}	1.05^{b}	4.49^{c}
100% N (CF)	2.26^{bcd}	20.25^{b}	3.36^{bcd}	2.00^{bc}	27.03^{bcd}	0.76^{bcd}	2.55^{bcd}	4.60^{c}	1.62^{abc}	1.99^{bc}	1.46^{a}	5.86^{b}
75% N (CF) + 25% N (FYM)	2.63^{abc}	19.79^{b}	3.80^{ab}	1.70^{cd}	25.30^{cd}	1.04^{ab}	2.76^{bc}	4.47^{c}	1.40^{bcd}	1.50^{cd}	1.14^{b}	5.80^{b}
50% N (CF) + 50% N (FYM)	2.14^{cd}	23.28^{a}	3.64^{abc}	2.25^{bc}	28.44^{abc}	0.73^{bcd}	2.89^{bc}	5.28^{b}	1.55^{abcd}	1.64^{cd}	1.00^{b}	5.69^{b}
25% N (CF) + 75% N (FYM)	2.77^{ab}	18.92^{b}	3.29^{bcd}	1.72^{cd}	26.37^{cd}	0.88^{abc}	2.30^{cd}	4.01^{d}	1.67^{ab}	2.65^{a}	1.43^{a}	7.80^{a}

Table 4. Variation in EO composition of *Salvia sclarea* due to mulch and nutrient management (pooled data of two years). RT indicates Retention time; RI Cal indicates Retention Index Calculated; SEm indicates Standard Error of Mean; LSD indicates Least Significant Difference; NS indicates not significant at $P = 0.05$; CF indicates chemical fertilizer; FYM indicates farm yard manure.

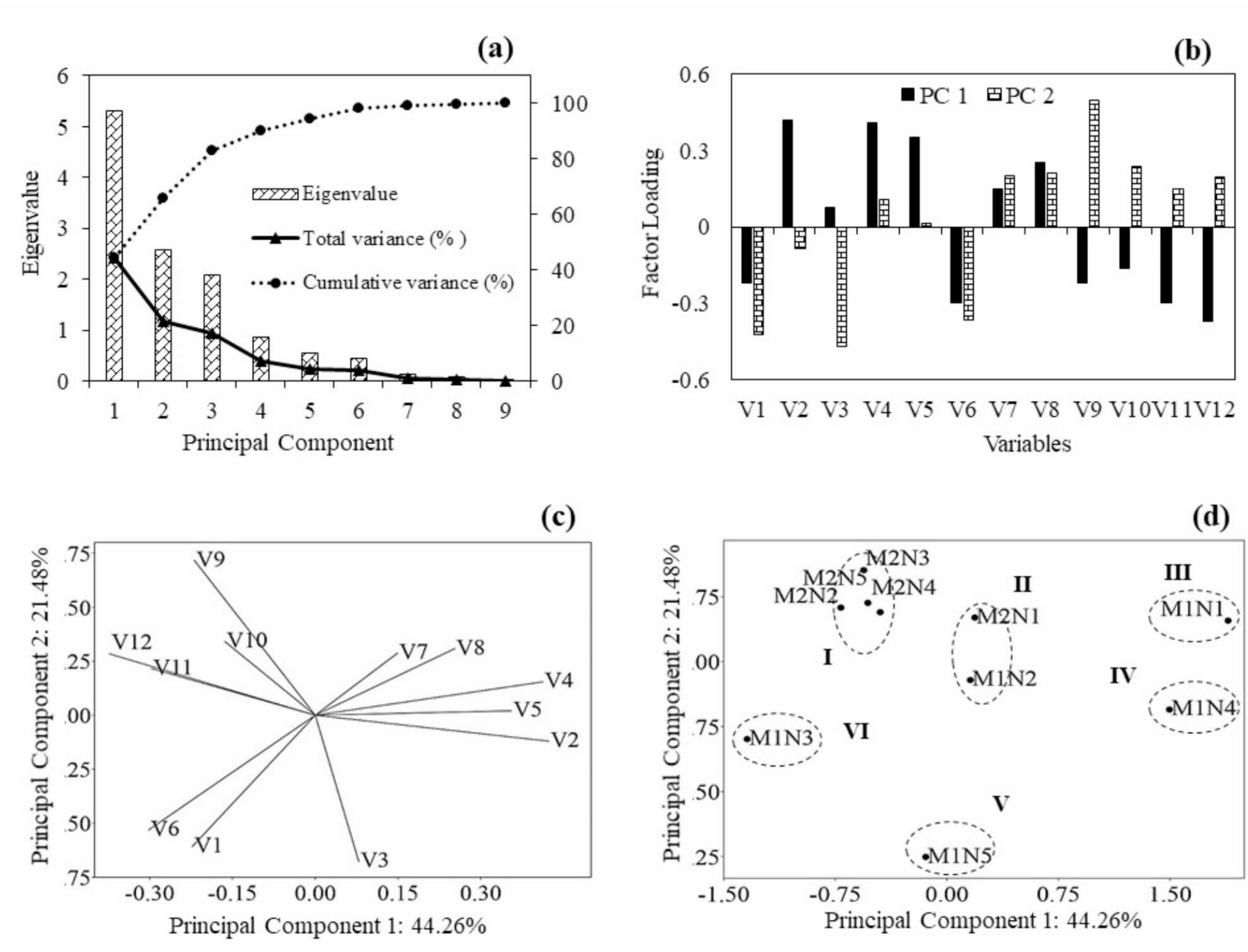

Fig. 1. Multivariate analyses of (**a**, **b**) presented eigenvalues and loading scores of the variables with PC1 and PC2 (**c**) biochemical constituents of essential oil {V1 = trans-Linalool oxide, V2 = Linalool, V3 = α-Terpineol, V4 = endo-Fenchol, V5 = Linalool acetate, V6 = Linalool oxide, V7 = Neryl acetate, V8 = Geranyl acetate, V9 = Spathulenol, V10 = Caryophyllene oxide, V11 = β-Eudesmol, V12 = Sclareol}; (d) treatments' distribution based on biochemical constituents of essential oil.

linalool acetate, endo-fenchol, neryl acetate and geranyl acetate showed a strong positive correlation (Fig. 1c). Whereas all these chemical compounds were negatively correlated with trans-linalool oxide, linalool oxide, spathulenol, caryophyllene oxide, β-eudesmol and sclareol, showed a positive correlation among themselves.

PCA analysis based on the treatments represented the separation of all the treatments at PC1 and PC2 in the PCA bi-plot (Fig. 1d). Except for the control where no fertilizer was added, other treatment combinations having mulch i.e., M_2N_2 (−1.64), M_2N_5 (−1.22), M_2N_3 (−1.29) and M_2N_4 (−1.03) were placed in cluster I and separated in the negative end of PC1. Meanwhile, treatment combinations M_2N_1 (0.43) and M_1N_2 (0.36) were placed under cluster II and isolated in the positive end of PC1. However, the other treatment combinations i.e. M_1N_1 and M_1N_4, having score values of 4.36 and 3.45, respectively, separated in clusters III and IV at the positive end of PC1.

Correlation matrix

A correlation matrix graph was plotted to assess the correlation between the morpho-physiological parameters and EO yield. Stem diameter and no. of branches showed a significant positive correlation with each other ($r = 0.74$, $p < 0.05$), and both of them were also found positively correlated with EO yield ($r = 0.85$, $p < 001$; $r = 0.72$, $p < 0.05$, respectively), LAI ($r = 0.75$, $p < 0.05$; $r = 0.81$, $p < 0.01$, respectively), no. of flower spike per plant ($r = 0.70$, $p < 0.05$; $r = 0.82$, $p < 0.01$, respectively), and plant height ($r = 0.75$, $p < 0.05$; $r = 0.77$, $p < 0.01$, respectively) (Fig. 2a). Stem diameter was also found to be significantly and positively correlated with flower spike yield ($r = 0.67$, $p < 0.05$); however, no. of branches was found to be positively correlated with inflorescence length ($r = 0.66$, $p < 0.05$). Plant height and no. of flower spike per plant documented a significantly positive correlation with EO yield ($r = 0.86$, $p < 0.01$; $r = 0.90$, $p < 0.001$, respectively), flower spike yield ($r = 0.70$, $p < 0.05$; $r = 0.80$, $p < 0.01$, respectively), inflorescence length ($r = 0.71$; 0.75, $p < 0.05$, respectively), and LAI ($r = 0.88$; 0.95, $p < 0.001$, respectively) in addition to, significant positive correlations among themselves ($r = 0.85$, $p < 0.01$). Furthermore, LAI and flower spike yield were positively correlated with each other ($r = 0.71$, $p < 0.0$), and

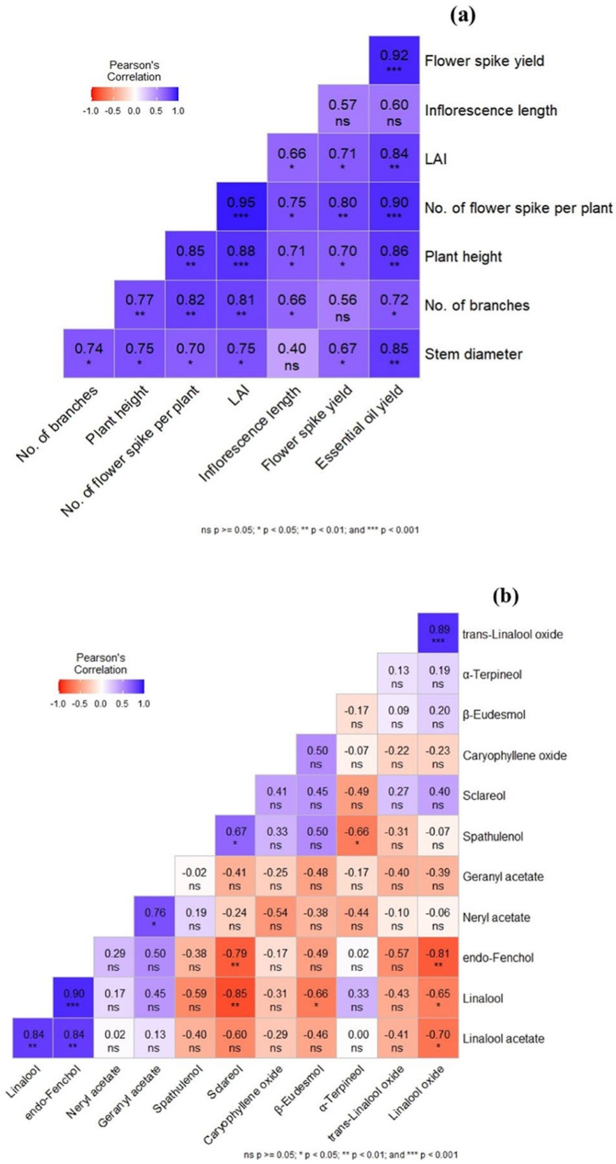

Fig. 2. (**a**) Correlation matrix between agronomic traits of *Salvia sclarea*. *, **, *** indicates significant at $P=0.05$, $P=0.01$ and $P=0.001$, respectively 'ns' indicates not significant at P = 0.05. (**b**): Correlation matrix between 12 major compounds of essential oil of *Salvia sclarea*. *, **, *** indicates significant at $P=0.05$, $P=0.01$ and $P=0.001$, respectively 'ns' indicates not significant at P = 0.05.

both of them also showed a significant positive correlation with EO yield ($r=0.84$, $p<0.01$; $r=0.92$, $p<0.001$, respectively). Moreover, LAI also witnessed a positive correlation with inflorescence length ($r=0.66$, $p<0.05$). These results suggest that higher plant height, stem diameter, no. of flower spikes per plant, and flower spike yield through agronomic practices could result in higher EO yield of *S. sclarea*. Similar results were also found in *Matricaria chamomilla* by Pirkhezri et al.[27]. and *Tanacetum parthenium* by Hamisy et al.[28].

Another correlation matrix graph was plotted between the chemical constituents of EO (Fig. 2b), and the graph illustrated that linalool acetate and linalool were positively correlated with each other ($r=0.84$, $p<0.01$); however, both of them showed a significantly negative correlation with linalool oxide ($r=-0.70$; -0.65, $p<0.05$, respectively), and positive correlation with exo-α-frenchol ($r=0.84$, $p<0.01$; $r=0.90$, $p<0.001$, respectively). In addition, linalool was also found negatively correlated with β-eudesmol ($r=-0.66$, $p<0.05$) and sclareol ($r=-0.85$, $p<0.05$). Similarly, exo-α-frenchol documented a negative correlation with linalool oxide and sclareol ($r=-0.81$; -0.79, $p<0.01$, respectively); however, neryl acetate witnessed a positive correlation with geranyl acetate ($r=0.76$, $p<0.05$). Furthermore, spathulenol had a significant negative correlation with α-terpineol ($r=-0.66$, $p<0.05$) while, positive with sclareol ($r=0.67$, $p<0.05$). Moreover, trans-linalool oxide had a significantly strong positive correlation with linalool oxide ($r=0.89$, $p<0.001$). The positive correlation among the volatile components of EO signifies that the quantity of such components varied synergistically, which means that by increasing the synthesis of one component, the synthesis of another component also increased, or the reduction of one variable caused a decrease in the other. Conversely, a negative coefficient indicates that compounds are inversely correlated with one another[29].

Heat map analysis

The cluster heat map analysis represented all the chemical constituents of EO with different combinations of treatments and divided the heatmap into two-way hierarchical clustering (Fig. 3). The data from the heat map observed that among all the treatment combinations, higher α-terpineol content was found in M_1N_5 where 75% of N was applied through CF and 25% of N was supplemented through FYM without mulching. However, the treatment combinations of M_1N_3 (25% N through CF + 75% N through FYM without mulching) recorded a higher concentration of trans-linalool oxide. Furthermore, treatment combination control without mulching (M_1N_1) depicted a higher concentration of endo-fenchol; a moderate concentration of linalool, linalool acetate and neryl acetate; however, a lower concentration of sclareol. Likewise, the highest caryophyllene oxide content was recorded in the treatment combination M_2N_3, where 25% of N dose was applied through CF and 75% of N through FYM without mulching. Moreover, among all the treatment combinations, M_1N_4 (50% N through CF + 50% N through FYM) witnessed the lower concentration of β-eudesmol. The present research demonstrated that the effect of different combinations of treatments on chemical composition of EO was not uniform. A cluster of treatment combinations of M_1N_3 and M_1N_5 had a poor impact on all the chemical constituents except linalool oxide, trans-linalool oxide and α-terpineol than other treatment combinations.

Soil characteristics

Table 5 shows that mulch application did not significantly affect the different soil parameters at both soil depths (0–15 cm and 15–30 cm). Except for soil pH, slightly higher values were observed for EC, available N, P, K and OC where mulch was applied. Irrespective of the mulching treatments, among different N management treatments, the soil pH values ranged from 5.55 to 5.61, and 5.59 to 5.64 in surface (0–15 cm) and sub-surface soil (15–30 cm), respectively. The numerically lowest and highest soil pH values were recorded under N_2 and N_3, respectively, though all treatments produced non-significant differences. Likewise, EC alters non-significantly among different N management practices at both soil depths. In this study, available N content was significantly influenced by the application of different doses of N fertilizer and FYM. Significantly highest available N content (303.99 kg/ha) was recorded in treatment N_5, followed by N_2 (292.94 kg/ha) in the surface soil. Unlike 0–0.15 m soil depth, at 0.15–0.30 m, available N was recorded significantly higher in N_2 (255.11 kg/ha), which was at par with N_5 (251.73 kg/ha). In case of available P, though all the treatments produced non-significant differences with each other at both soil depths, the higher values were found under N_3 i.e. 32.05 and 22.41 kg/ha in surface and

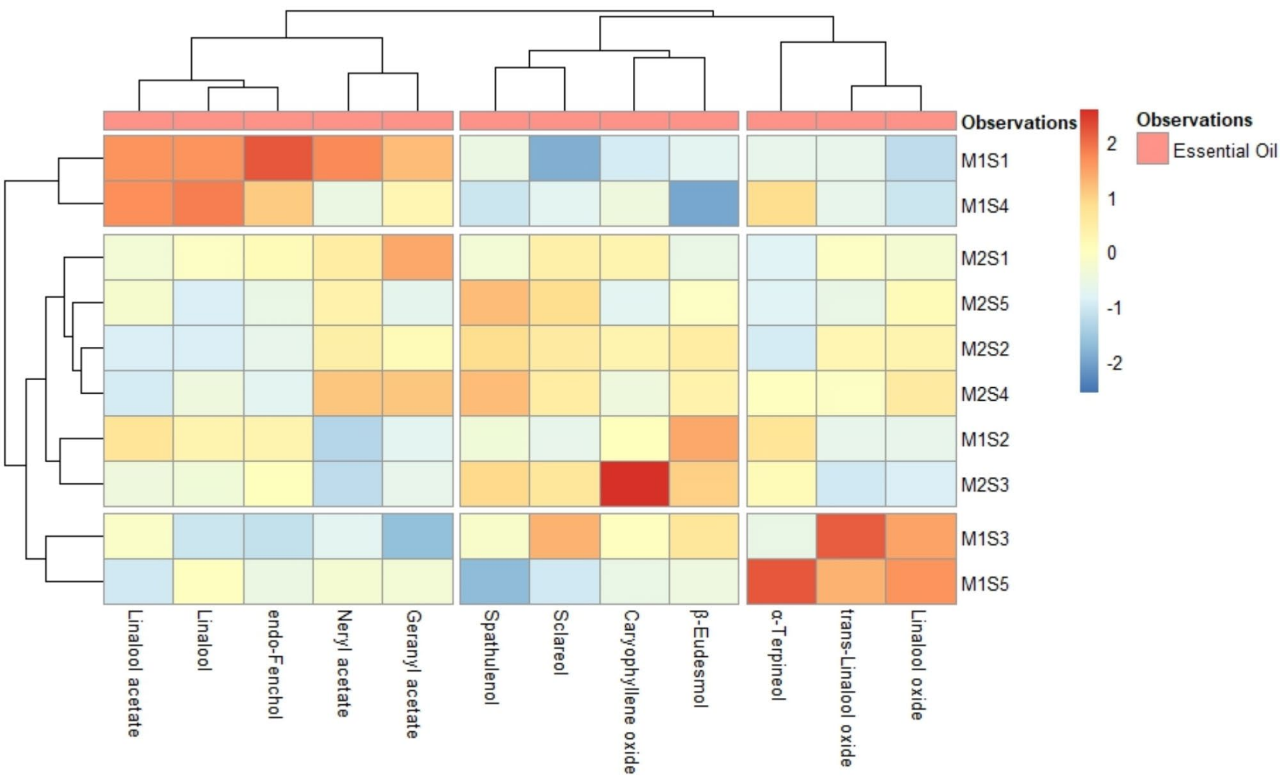

Fig. 3. Heat map representing the variation of chemical compounds identified in the essential oil of *Salvia sclarea* in response to mulch and nitrogen management. M_1 and M_2 represent without mulch and with mulch, respectively, while S_1, S_2, S_3, S_4, S_5 represent nitrogen management Control, 100% N (CF), 25% N (CF) + 75% N (FYM), 50% N (CF) + 50% N (FYM) and 75% N (CF) + 25% N (FYM), respectively.

Treatment	0–15						15–30					
	pH	EC	N	P	K	OC	pH	EC	N	P	K	OC
Mulch												
Without mulch	5.60^{NS}	0.16^{NS}	278.26^{NS}	30.42^{NS}	210.49^{NS}	0.62^{NS}	5.62^{NS}	0.13^{NS}	233.00^{NS}	21.09^{NS}	200.29^{NS}	0.51^{NS}
With mulch	5.58^{NS}	0.19^{NS}	283.75^{NS}	32.60^{NS}	214.24^{NS}	0.65^{NS}	5.60^{NS}	0.16^{NS}	240.05^{NS}	21.90^{NS}	203.19^{NS}	0.52^{NS}
Nitrogen management												
Control	5.61^{NS}	0.15^{NS}	251.63^{e}	30.79^{NS}	203.58^{e}	0.53^{cde}	5.63^{NS}	0.12^{NS}	207.09^{d}	21.34^{NS}	200.30^{NS}	0.50^{NS}
100% N (CF)	5.55^{NS}	0.21^{NS}	292.94^{b}	31.09^{NS}	209.63^{cd}	0.61^{bcde}	5.59^{NS}	0.17^{NS}	255.11^{a}	20.92^{NS}	201.30^{NS}	0.51^{NS}
75% N (CF) + 25% N (FYM)	5.58^{NS}	0.20^{NS}	303.99^{a}	31.75^{NS}	212.80^{bcd}	0.63^{abcde}	5.59^{NS}	0.15^{NS}	251.73^{a}	21.15^{NS}	201.87^{NS}	0.51^{NS}
50% N (CF) + 50% N (FYM)	5.59^{NS}	0.18^{NS}	281.58^{c}	31.89^{NS}	215.09^{bc}	0.68^{abcd}	5.61^{NS}	0.14^{NS}	240.91^{b}	21.65^{NS}	202.01^{NS}	0.53^{NS}
25% N (CF) + 75% N (FYM)	5.60^{NS}	0.16^{NS}	274.87^{d}	32.05^{NS}	220.74^{a}	0.72^{abc}	5.64^{NS}	0.14^{NS}	219.94^{c}	22.41^{NS}	203.23^{NS}	0.54^{NS}

Table 5. Effect of mulch and nutrient management on soil properties after harvest of *Salvia sclarea* (pooled data of two years). SEm indicates Standard Error of Mean; LSD indicates Least Significant Difference; NS indicates not significant at $P = 0.05$; SEm indicates Standard Error of Mean; LSD indicates Least Significant Difference; NS indicates not significant at $P = 0.05$; CF indicates chemical fertilizer; FYM indicates farm yard manure.

sub-surface soil, respectively. The treatment-wise pattern for available P at the sub-surface layer was analogous to the surface layer. Contrary to this, N management practices significantly affected the available K at 0–0.15 m depth only, and significantly highest value was recorded with N_3 (220.74 kg/ha), followed by N_4, which was at par with N_5 in surface soil. The significantly higher OC content was registered under N_3 (0.72%), which produced at par results with N_4 and N_5, while the lowest (0.53%) was observed under absolute control (N_1).

Discussion

ANOVA key premise is the homogeneity of variance. Equality of variance is tested using a variety of test statistics. Certain traditional assessments, like the Bartlett's test, are highly susceptible to deviations from normality[30]. The highest plant height and LAI were recorded with the application of mulch. The observed improvement in plant growth with organic mulch might be due to increased moisture availability, reduced weed growth, optimum soil temperature, and improved soil nutrient status. It is confirmed from the various studies that the optimum soil temperature and sufficient moisture positively affect the microbial activities in soil, which leads to increased nutrient concentration, organic matter and nutrient retention capacity by accelerating the decomposition process of plant residue[31,32]. Besides this, high moisture conditions under mulch conditions make soil macropores more hydraulically active, developing an expanded macropore network and, hence, greater conductance[33,34]. Panwar et al.[35], Kalachaveedu et al.[36]. and Niu et al.[37] also experienced the significant response of mulch on growth and yield attributes of *Andrographis paniculata*, *Stevia rebaudiana* and *Rosa damascena*, respectively.

Similarly, application of recommended dose of N also recorded the significantly higher plant height, no. of branches, inflorescence length and LAI over control. As N is an essential nutrient for plant growth, it aids in synthesizing the amino acid tryptophan, a precursor to the plant growth hormone IAA that stimulates plant height[38]. These results were in accordance with the findings of Singh et al.[39] in *Ocimum basilicum* and Pooja et al.[40]. in *Ocimum sanctum*. Nisarata et al.[41] also found similar results where the growth attributes, such as plant height and no. of branches per plant showed improvement with applying CF in *Coriandrum sativum*. N is the building block of proteins, including the growth, multiplication, and construction of cells, which ultimately enhance photosynthesis and the translocation of photosynthates to the different parts of plants that are developing[42]. Hence, morphological parameter increase was recorded when full dose of N was applied through CF due to readily available nutrients for plants, ultimately leading to more vegetative growth[43]. These results were consistent with the observations recorded by AL-Mansour et al.[44]. and Guo et al.[45]. A heat map analysis was done to visualize data of chemical constituents of EO with treatment combinations and their key features in clusters, which were further used to identify the similarities and differences between data clusters.

Application of 75% of N through CF and 25% of N through FYM also recorded improvement in morphological traits of *S. sclarea* over control. Application of CF with small quantity of FYM shows at par results with N_2, which might be because FYM acts as a nutrient reservoir for plants, preventing leaching losses, keeping high cation exchange capacity (CEC) and enhancing the long-term availability of nutrients through its slow decomposition process[46]. Furthermore, combined use of inorganic and organic fertilizers enriched the nutrient status of soil, enhanced the synthesis of plant growth hormones, encouraged the plants' metabolic activities and positively affected morphological characteristics and yield[41]. AL-Mansour et al.[44]and Vijayabharati[47]also confirmed these findings. Similarly, the highest value of chlorophyll content and flower spike yield was recorded where full dose of N was applied through CF followed by the application of 75% of N through CF and 25% of N through FYM. This might be because the availability of N is directly proportional to the chlorophyll contents of the plant[48]. The application of FYM enhances the physico-chemical properties and microbial activities of the soil, increasing the nutrient-holding capacity of soil and conserving moisture content, which could promote more nutrient absorption, hence leading to improvement in chlorophyll content[49,50]. Application of N through CF looks forward to enhancing the available nutrient content. Thereby, the adequate supply of nutrients elevates the rate of metabolic processes and nutrient uptake, leading to an increase in the growth characteristics, which in turn increases the inflorescence yield of the plant[51]. On the other hand, applying FYM along with N fertilizer also regulated the supply of nutrients, thus increasing the yield[51,52].

It is confirmed from this study that the EO content and yield was substantially influenced by the application of N through CF further followed by the application of 75% of N through CF and 25% of N through FYM. These results indicated that the production of secondary metabolites and other metabolic activities of plants were affected by abiotic factors such as climatic conditions, plant available nutrient content in soil, soil type, etc[53]. As N plays a vital role in increasing photosynthesis, translocation of nutrients and photosynthetic products, enzymatic activity, and vegetative and reproductive growth of a plant, further enhancing the EO yield of medicinal and aromatic plants[54]. Availability of N also regulates the expression of genes involved in phenylpropanoid biosynthesis, a metabolic pathway integral to both flavonoids and terpenes synthesis[55]. Moreover, applying FYM along with N fertilizer improved the supply of nutrients, thus increasing the yield[51]. In addition, FYM increases soil microbial activities, improving the antioxidant enzyme activity in crops, promoting balance between production and scavenging of reactive oxygen species in crop cells and enhancing crop stress resistance so as to promote growth and yield[56,57]. Previous researchers also reported the enhancement in EO content by applying nitrogenous fertilizers in *Ocimum americanum*[58] and *Salvia rosmarinus*[59]. The three primary chemical constituents of the EO i.e. linalool acetate, linalool and sclareol, represented 50–58% of the total identified chemical constituents. The results of previous studies confirmed that the principal constituents of EO of *S. sclarea* were linalool (20.6%)[1], linalool acetate (49.1%)[60], sclareol (5.2%) and geranyl acetate (7.5%)[61].

These results of the chemical composition of *S. sclarea* were corroborated by the findings of various researchers; however, Karayel[62] reported that spathulenol (23.75%), caryophyllene oxide (19.41%) and linalool (10.10%) were the major volatile oil constituents which might be due to the diverse climatic conditions and soil characteristics. N helps synthesize terpenes by promoting leaf photosynthesis and electron transport rate, which fulfill the ATP and carbon substrate requirements for the synthesis of isoprene units. There is a connection between nutrients and stored terpenoids since all carbon-based secondary metabolites ultimately rely on CO_2 fixation[63]. Cruzatty & Vollmann[64] also reported that the N application methods affect the quantity and quality of the EO of *Thymus vulgaris*. PCA studies indicated that changes in EO composition of *S. sclarea* were mainly affected by N application rather than mulching. These changes were probably governed by varying rates of chemical application and organic sources of N. Besides this, the positive association between morphological traits, flower yield attributes, and flower yield might be due to the complex interplay between plant vegetative and reproductive stages in response to nutrient sources. Similar findings were reported by Qayyum et al.[65]. for *Zinnia elegans* and Pathania et al.[66] for *Dendranthema grandiflora* in response to organic and inorganic sources of nutrients.

No significant effects were observed in soil properties with mulch application; however, slight improvements in content were recorded in the second year. Lower values of OC under no mulch conditions might be due to more aerobic conditions in soil, which promotes carbon mineralization[67]. The results further indicated that the duration of mulch application was insufficient for enough decomposition to have a noticeable impact on the soil parameters[68]. Moreover, lower pH under mulch might be due to the acidic nature of pine needles used as mulch, and their little decomposition might result in a slight decrease in the soil pH. Application of recommended dose of N through CF recorded lowest pH value and highest was recorded in treatment where application of 50% of N was applied through CF and 50% through FYM. Since FYM increases the buffering strength of soil, the pH of the soil did not significantly change with the application of FYM along with N[69].

In this study, available N content of soil increased by application of N through CF in combination with FYM. These results may be due to the application FYM improved the soil conditions, which could assist in mineralizing the organic form of N and result in the accumulation of more available N in soil[70]. Similar results were obtained by Singh et al.[71] in wheat. Similarly, application of 50% of N through CF and 50% of N through FYM recorded highest available K and OC content. Adding organic manure such as FYM released organic acid, leading to increased microbial activity, chelation of K fixing cations and release of cations from the exchangeable sites into available form[72]. Reddy et al.[73]also found that the addition of organic matter enhanced the K availability and decreased its fixation in soil. Likewise, the soil OC of surface soil was significantly affected by different N management practices. These results might be due to the direct application of organic matter through FYM, which positively influences the OC content of soil[68,69]. However, different N management practices did not significantly alter the OC content of sub-surface layer of soil. The results also demonstrated a decrease in nutrient content with increasing depth, which might be because, in the subsoil, these changes are broadly influenced by the parent material of soil rather than the agricultural activities[67,74]. However, higher nutrients in the topsoil could be due to tillage, fertilization and other agricultural practices followed.

Conclusion

The observations of this experiment depicted that the short-term incorporation of mulch expressed no substantial differences in most of the morpho-physiological parameters and soil health; however, it improved the plant height, LAI and EO yield of *S. sclarea*. Among N management practices, the crop response was superior under the application of N largely through fertilizer, which produced at par results with the application of small quantity of FYM along with chemical N in most of the growth, physiological and yield parameters studied. Moreover, incorporating FYM with nitrogenous fertilizer improved the soil parameters of the surface and sub-surface layer as well. Considering the EO yield and soil health, it was concluded that a small quantity of the recommended dose of N *via* chemicals could be replaced with organic manures under mulching without significantly decreasing the EO production.

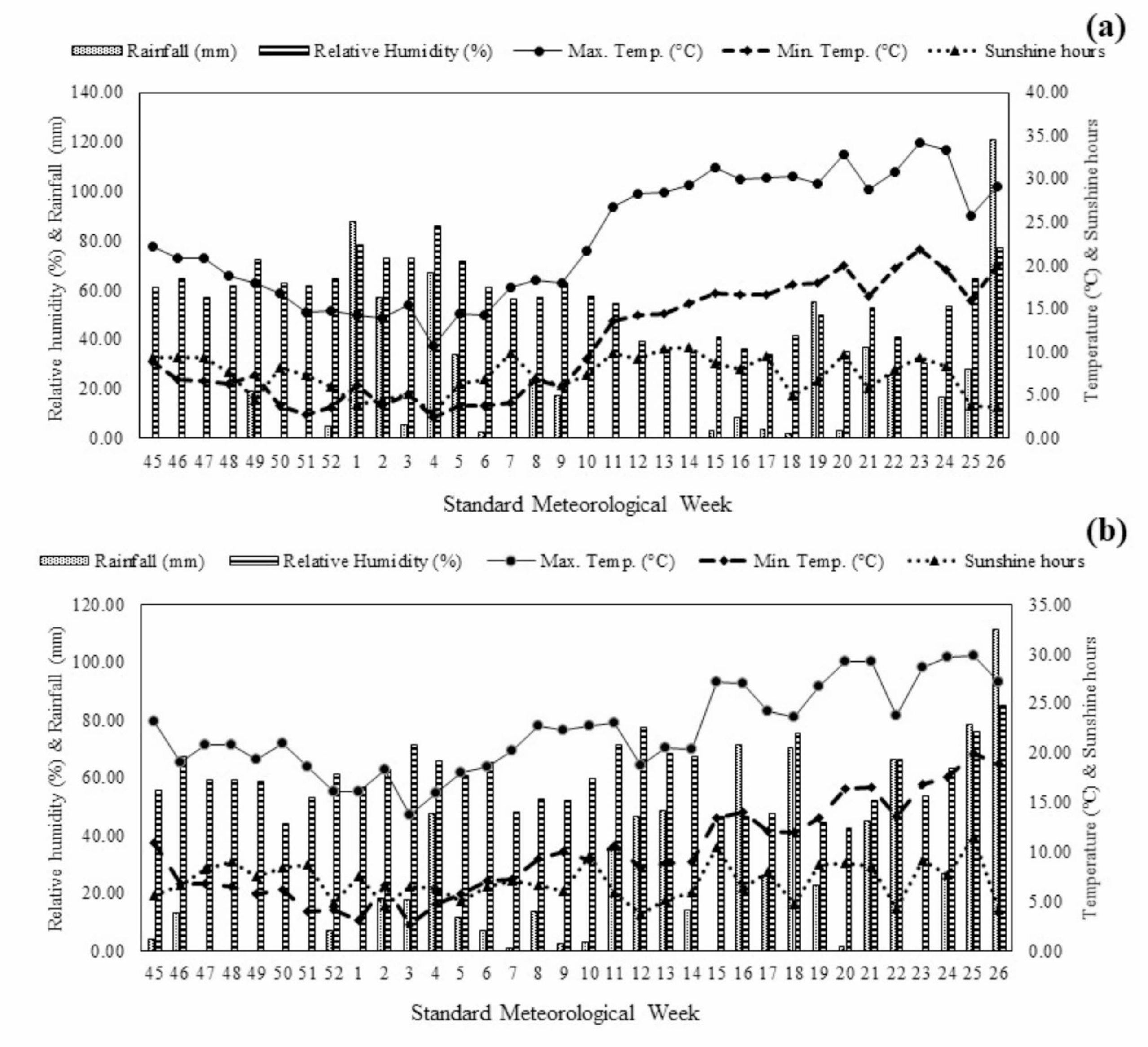

Fig. 4. Weekly meteorological conditions of the study area during the cropping seasons of (**a**) 2021-22 and (**b**) 2022-23.

Materials and methods
Experimental site
The study area was the research farm of CSIR- Institute of Himalayan Bioresource Technology, Palampur, India, situated at an elevation of 1393 m above mean sea level, having latitude 32˚06'47" N, and longitude 76˚33'46" E. The research trials were conducted from October to July over two cultivation cycles i.e. 2021-22 and 2022-23. Before the initiation of the experimental trial, the soil samples from 0 to 0.15 m and 0.15–0.30 m depth were collected for analysis of initial physico-chemical properties. In the initial phase of the experiment, the soil was slightly acidic in reaction with 5.49 soil pH. Similarly, the OC, EC, available N, P and K content in the surface soil layer (0–0.15 m) were 0.50%, 0.13 dS/m, 239.42, 24.13, 178.54 kg/ha respectively. The FYM utilized in the experiment had a nutritional composition of 0.8%, 0.4%, and 0.7% for N, P, and K, respectively. The experimental site is classified as a mid-hill sub-humid zone of Himachal Pradesh with high annual mean rainfall, i.e.~2500 mm. The cropping seasons (2021-22 and 2022-23) recorded a total rainfall of 619.50 and 810.90 mm, with relative humidity ranging from 30.50 to 85.64% and 42.64 to 85.00%, respectively (Fig. 4). A similar variation was recorded in the mean minimum and maximum temperature in both the cropping seasons, ranging from 2.43 to 21.84 °C and 10.76 to 34.16 °C, respectively.

Plant material, treatments and experimental layout
The research trail was laid out in a split-plot experimental design with a two-factor arrangement, i.e. two levels of mulching (M_1: without mulch; M_2: with mulch (pine needles), and five levels of N [N_1: control (no fertilizer);

N_2: recommended dose of N i.e. 120 kg/ha; N_3: 25% N through CF + 75% N through FYM (N = 0.45%); N_4: 50% N through CF + 50% N through FYM and N_5: 75% N through CF + 25% N through FYM. There were ten treatment combinations, each replicated thrice; therefore, thirty plots were established. In case of mulch, dry pine needles (*Pinus roxburghii*) were applied after 30 days of plantation to maintain a 2-inch layer. The seeds of *S. sclarea* were taken from the germplasm repository[75] of aromatic crops at Agrotechnology farm, CSIR-Institute of Himalayan Bioresource Technology, Palampur (H.P.), India. The voucher specimen, PLP 22355, was identified and deposited at CSIR-IHBT, Palampur herbarium. For raising nursery, the seeds were sown in the well-prepared nursery beds in 36th meteorological standard week (the standard meteorological week is a seven-day period used by meteorologists for arranging and evaluating weather and climate data) during both the years and sixty days old seedlings were used for transplantation to the experiment trial in both the cropping years i.e. 2021-22 and 2022-23. The crop was planted in a crop geometry of 0.45 × 0.30 m. The dimensions of each plot were 2.25 m × 2.40 m (5.40 m^2). The full dose of P i.e., 60 kg/ha and K i.e., 40 kg/ha were applied as a basal dose at the time of transplantation, whereas N was given in three splits as per the treatments. Half dose of N *via* chemical including full dose of FYM as per the treatments whereas the remaining dose of chemical N was applied after 30 and 60 days of transplanting into two equal splits. As the sources of N, P and K, urea (N @ 46%), single super phosphate (P_2O_5 @ 16%) and muriate of potash (K_2O @ 60%) were used. The other agronomic techniques, including irrigation (flood irrigation) and weed control (hand weeding), were carried out following the needs of the crop in order to ensure healthy growth and development.

Growth, yield and EO extraction

Ten plants from the experimental unit of each treatment were randomly selected at the time of harvest (full blooming at 23rd − 24th metereological week) for growth and yield observation. Plant height at maturity was determined using a ruler from the ground surface to the inflorescence tip. Treatment-wise stem diameter per plant was recorded using Vernier caliper, and total no. of branches per plant and flower spike per plant were counted manually. Individual plant leaf area was measured according to treatment, and the result was expressed as a LAI. Besides this, the pigments involved in photosynthesis i.e. chlorophyll a and b, were identified using the method described by Lichtenthaler and Buschmann[76]. The values of photosynthesis pigments were expressed in µg/g of fresh leaf weight. For yield estimation, yield attributes viz., inflorescence length, flower spikes per plant, and flower spike yield were recorded at harvest time. Treatment-wise, flower spikes of clary sage (0.500 kg) were harvested and hydro-distilled in a Clevenger-type apparatus (water: flower, 2:1 v/w) for six hours, yielding transparent light yellow oil. The EO was filetrated, dried with anhydrous sodium sulfate (Merck), and then kept in a dark tightly sealed vial in a refrigerator at 4 °C for further gas chromatography–mass spectrometry (GC–MS), and gas chromatography (GC) to identify and quantify the volatile constituents of clary sage oil, respectively. The EO productivity was calculated by multiplying the oil content with the specific gravity (0.9) and the spike yield of particular treatments.

GC and GC-MS analysis

The GC–MS analysis of clary sage' EO was done using Schimadzu GCMS-QP2010 (Shimadzu Corporation, Japan) system, fitted out with an SH-RX-5Si/MS having dimensions 30 m × 0.25 mm × 0.25 µm film thickness. The oven temperature was set in such a way that it gradually increased from 70 to 220℃ (slope of 4℃ per minute), with detention time of 5 min. The temperature of ion source, and the interface was fixed, being 240 and 250℃, respectively. The sample volume was 2 µL, and ionization voltage was 0.85 eV. The flow rate of carrier gas, N was 1 mL/min. The EO was examined with Shimadzu GC 2010 gas chromatograph with a flame ionization detector (FID). The SH-RX-5Si / MS capillary column having dimensions of 30 m × 0.25 mm × 0.25 µm, and auto-injection system with a volume of 2 µL from 4 µL oil in 1.5 ml of CH_2Cl_2 was equipped with gas chromatography. The initial pressure was nearly about 65.3 kPa, and the approximate linear velocity was 37.6 cm/s.

Soil physico-chemical properties analysis

After harvesting *S. sclarea*, the soil samples were taken from the surface (0–0.15 m) as well as sub-surface layer (0.15–0.30 m) of each experimental unit, after that these samples were dried in shade and sieved with 2 mm sieve, except for OC where the soil is sieved through 0.5 mm sieve. Soil pH and EC of soil: water suspensions of 1:2.5 and 1:2 (w/v) were determined using a compound electrode[77]. For determining soil OC content, Walkley & Black[78]procedure was carried out. Soil available N, P, and K were measured as per the method given by Subbaiah & Asija[79], Bray & Kurtz[80]and Mehlich[81], respectively.

Statistical analysis

To determine the significance of the treatments and their interaction, the growth, yield and soil data collected were evaluated through ANOVA for the split-plot design. The significance of the difference between treatment means for the growth, yield and soil properties data was assessed using least significant difference (LSD) values at the P = 0.05 level. Bartlett's homogeneity test was applied to determine the homogeneity of variance of all the recorded parameters across the cropping years. Multivariate PCA was carried out using Past 4.06b software[82] to evaluate the impacts of treatment combinations on secondary metabolites of EO. The correlation coefficient value, determined by the cosine of the angle between any two vector variables in the PCA biplot, indicated the strength of the correlation between any two variables. To ascertain relationships between growth, yield and secondary metabolites, two correlation matrix were also developed and the significance levels of the observations were shown at P = 0.05 and P = 0.01. However, R-software (version 4.3.2)[83] was used to create the heat map to evaluate the effect of different treatments on the quality of EO.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions
D.D. recorded the data and wrote the manuscript, S.V. wrote the manuscript, S.B. recorded the data, S.S. provided the planting material and edited the manuscript, D.K. did the chemical profiling, validation and edited the manuscript, A.K. edited the manuscript, R.C. conceptualized, supervised, edited the manuscript and finalized the original draft.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
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