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Arbuscular mycorrhiza augments essential oil composition and antioxidant properties of *Ocimum tenuiflorum* L. – A popular green tea additive

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ABSTRACT

The increasing interest and demand of *Ocimum tenuiflorum* L. and its products in different industrial sectors necessitate appropriate cultural practices for cultivation of the plant with increased herbage and essential oil yield. Studies on *O. tenuiflorum* in relation to arbuscular mycorrhiza are restricted to evaluation of diversity of the mycorrhizal fungi in its rhizosphere or assessment of the ability of different species to colonize its roots. Hence, the present study aimed to assess the effect of arbuscular mycorrhizal fungus (*Rhizophagus intraradices*) inoculation on the pharmaceutically desired parameters of two high yielding genotypes of *O. tenuiflorum*. Colonization by *R. intraradices* fungus resulted in significant increase in the leaf biomass and concentration of essential oil in both the genotypes. Formation of arbuscular mycorrhiza resulted in up to 59.01 % and 86.95 % increase in essential oil concentration in mycorrhizal genotype A and B plants, respectively, in comparison with control. The characterization of essential oils by Gas Chromatography-Mass Spectrometry showed significant effect of arbuscular mycorrhiza on their chemical profile. A total of 23 and 37 compounds were identified in essential oils of control genotype A and B, respectively, while 21 and 34 compounds were detected in essential oils of their respective mycorrhizal counterparts. Concentration of eugenol (a valued phenylpropanoid), and other medicinally important terpenoids (β-elemene, β-caryophyllene, germacrene A, and germacrene D) increased in essential oils of mycorrhizal plants.While genotype and mycorrhizal interaction significantly influenced biomass and essential oil concentration in leaves, the effect of *R. intraradices* on concentration of eugenol, β-elemene, and β-caryophyllene did not vary between the two genotypes. Additionally, principal component analysis also highlighted that the volatiles common in the essential oil of the four treatments contributed significantly to the variation in the essential oil composition. Phenol, flavonoid, and polyphenol contents in the leaves were also enhanced on formation of arbuscular mycorrhiza. The study also aimed to evaluate the ability of leaves (fresh and processed) to scavenge reactive oxygen and nitrogen species. Arbuscular mycorrhiza augmented the antioxidant capacity of fresh and processed leaves of both the genotypes. Thus, findings of the study demonstrate that *R. intraradices* inoculation enhances productivity of *O. tenuiflorum* and also improves the quality of the products.

1. Introduction

Ocimum tenuiflorum L. syn. *Ocimum sanctum* (Holy basil or Tulsi) is a perennial shrub belonging to family Lamiaceae, immensely valued for its medicinal properties. The pharmaceutical and therapeutic potential of tulsi is mainly attributed to its essential oil (EO), a concentrated heterogenous mixture of phenylpropanoids and terpenoid derivatives (Joshi and Hoti, 2014; Salles-Trevisan et al., 2006). The EO of tulsi has been used principally in cosmetics and food industries (Viñaand Murillo, 2003), owing to its rich bioactive constituents. The biologically active constituents of tulsi EO include compounds such as eugenol, methylchavicol, β-elemene, β-caryophyllene, camphor, and linalool (Saran et al., 2017; Shasany, 2016; Kothari et al., 2005). Eugenol, owing to its therapeutic potential, is a valued commercial compound. It

Abbreviations: AM, arbuscular mycorrhiza; AMF, AM fungi; ANOVA, analysis of variance; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EO, essential oil; GAE, gallic acid equivalent; GC–MS, gas chromatography-mass spectrophotometer; HSD, honestly significant difference; INVAM, International Culture Collection of (Vesicular) Arbuscular Mycorrhizal fungi; M, mycorrhizal; MANOVA, multivariate analysis of variance; NBPGR, National Bureau of Plant and Genetic Resources; NM, non mycorrhizal; NO, nitric oxide; OH^-, hydroxyl; PC, principal component; PCA, principal component analysis; QE, quercetin equivalent; RNS, reactive nitrogen species; ROS, reactive oxygen species; SD, standard deviation; TAE, tannic acid equivalent

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is traditionally obtained from leaves and barks of cinnamon and buds of clove (Khalil et al., 2017). However, commercial extraction of eugenol from these plants is costly (Saran et al., 2017; Shasany, 2016). Hence, tulsi can be used to replace cinnamon and clove as a cheaper source of eugenol (Mukherji, 1987).

Herbal teas, owing to their antioxidant and anti-inflammatory properties, serve as a new avenue in diet-based therapies. In India, leaves of tulsi are extensively used for herbal tea preparation (Naithani et al., 2006). Leaves of *O. tenuiflorum* are also used as an additive of green tea and are available in commercial market as 'Tulsi-Green tea'. It enhances the pharmacological value of green tea by improving its antioxidant potential. Several bioactive constituents such as phenols, flavonoids, and polyphenols that act as antioxidants have been reported for tulsi (Mahajan et al., 2014; Lee and Scagel, 2009; Wangcharoen and Morasuk, 2007); however, little information on the antioxidant properties of tulsi leaves is available in the literature.

In view of its immense medicinal values, several commercial products of tulsi such as EO, dried leaves, and herbal powder are available (Saran et al., 2017; Kelm et al., 2000). Companies in India such as Organic India and Khadi are the first few companies to introduce products of tulsi in the international market. India also exports tulsi products to countries such as Austria, France, Germany, Indonesia, Netherlands, United States of America, and Turkey (Holy basil exporters and export data, India, 2016). The increasing interest and demand of tulsi and its products in different industrial sectors necessitate appropriate cultural practices for cultivation of the plant with increased herbage and EO yield. In addition, improving the antioxidative capacity along with increased herbage yield would enhance its commercial value.

In this regard, use of arbuscular mycorrhizal (AM) fungi (AMF) offers a promising possibility to enhance the productivity and quality of *O. tenuiflorum*. Arbuscular mycorrhiza represents the most successful and widespread symbiotic association of land plants with soil-dwelling fungi (AMF; subphylum-Glomeromycotina). The fungi colonize roots and bequeath plant with innumerable benefits such as improved nutrient uptake and enhanced resistance and tolerance to abiotic and biotic stress, in exchange of carbohydrates and some fatty acids (Chen et al., 2018; Bravo et al., 2017; Keymer et al., 2017). As AMF interacts with most of the crop, horticultural, and medicinal plants, they receive considerable attention for harnessing their potential in sustainable agriculture to promote plant P-nutrition while reducing fertilizer input (Gosling et al., 2006). This is especially pertinent in the existing time as organic agricultural system is highly appreciated.

The role of AM in improving growth and yield of aromatic and medicinal plants has been increasingly realized. There are several studies on the positive effect of AM on herbage yield and EO concentration of aromatic plants, including plant species belonging to family Lamiaceae (Tarraf et al., 2015; Copetta et al., 2006; Freitas et al., 2004). Most of the studies are limited to evaluating the effect of AM either on EO concentration or on few main components in EO. While concentration of active components is one of the major factors that affect the medicinal plant's quality or its industrial value, the minor compounds may have synergistic effects and contribute in regard to biological activity of EO (Carović-Stanko et al., 2010). Therefore, it is imperative to evaluate the effect of AM on the range of volatiles including compounds present in minor proportions in EO. This study is probably the first one that focuses on the comprehensive effect of AM on all constituents of *O. tenuiflorum* EO.

Different genotypes within a plant species show differences in responsiveness to AMF (Harper et al., 2015; Ortas et al., 2011; Linderman and Davis, 2004). The morphological and genetic differences among genotypes were reported to influence the response of plants to AMF (Linderman and Davis, 2004; Tawaraya, 2003). Therefore, it is imperative to study the effect of genotype and AMF interaction, to distinguish a general response from a genotype-specific response of plant species to AMF colonization.

There have been studies in the past where AMF inoculation in *O. basilicum* L. and *O. gratissimum* L. has resulted in significant improvement in their herbage and EO yield (Hazzoumi et al., 2015; Zolfaghari et al., 2013). However, studies on *O. tenuiflorum* in relation to AMF are limited to evaluating diversity of AMF in its rhizosphere (Chanda and Dey, 2018; Massey and Siddiqui, 2013) or assessing the ability of various AMF species to colonize its roots (Jyothi and Bagyaraj, 2016).

The hypothesis of this study was that AMF inoculation of *O. tenuiflorum* would improve the productivity, EO concentration, and alter composition and bioactivity of EO. Furthermore, this study compares the responses of two high yielding genotypes from north-eastern region of India to *Rhizophagus intraradices* (N.C. Schenck and G.S. Smith) C. Walker and A. Schübler (CMCCWep 319) colonization. The effect of *R. intraradices* colonization on two genotypes of *O. tenuiflorum* was compared in terms of i) industrial features (herbage yield and concentration of EO and eugenol); and ii) therapeutic features (composition of EO and content of phenols, flavonoids, polyphenols, and antioxidant potential of leaves).

2. Material and methods

2.1. Plant material

Seeds of two genotypes of *O. tenuiflorum*; namely accession no. IC 599368 and accession no. IC 589192 were procured from National Bureau of Plant and Genetic Resources (NBPGR), New Delhi, India. Hereafter, accession no. IC 599368 and IC 589192 would be referred to as genotype A and genotype B, respectively. These genotypes, collected from Odisha, India, are reported to be promising lines with high herbage yield and EO content (NBPGR, Annual Report, 2014–2015; 2015–2016). In India, *O. tenuiflorum* is grown in Kharif season (July-October).

2.2. Arbuscular mycorrhizal fungus inoculum

Inoculum of *R. intraradices* was procured from Center for Mycorrhiza Culture Collection, TERI, New Delhi, India. The inoculum was multiplied in sterile soil-sand mix (3:1) as soil-based open cultures using *Sorghum bicolor* L. as trap plants (Kapoor et al., 2002a). Propagation of the inoculum was continued for six months under natural conditions of humidity, light, and temperature. Colonization in the roots by *R. intraradices* was confirmed after the plants were fully established.

In order to spur spore formation, the pots were left to dry without irrigation under shade at room temperature (28 ± 2 °C) to prevent from rapid drying [according to the protocol provided by International Culture Collection of (Vesicular) Arbuscular Mycorrhizal fungi (INVAM)]. After harvesting the dried trap plants, roots were collected separately. The dried roots were later chopped and mixed with the rhizosphere soil (containing approximately 150 spores per 10 g of soil). This root-soil mix was used as AMF inoculum.

2.3. Experimental design

The experiment was conducted in the Botanical Garden, Department of Botany, University of Delhi, Delhi, India. The soil used in the study was collected from the Botanical Garden. The chemical and physical characteristics of the soil were analyzed at Division of Soil Science and Agricultural Chemistry, IARI, New Delhi, India. The texture of the soil was sandy clay loam with a ratio of 48:29:23. Soil pH was 8.3 with high carbon concentration (1.0 %). The available N, P, and K content were 167 kg/ha, 116 kg/ha, and 432 kg/ha, respectively. The content of micronutrients such as Mg, Zn, and Fe were 97.3 mg/kg, 6.6 mg/kg, and 9.4 mg/kg, respectively. Water holding capacity of the soil was 42.5 % and the moisture content was 11.5 %. The soil was mixed with sand in the ratio of 3:1. Henceforth, this mixture of soil and sand will be specified to as soil. The soil was autoclaved at 120 °C and 15 psi for 1 h

to sterilize it. Each pot used in the study was filled with 5 kg of soil.

The experiment consisted of two treatments; control or non mycorrhizal (NM, plants inoculated with mock inoculum) and mycorrhizal (M, plants inoculated with the AMF inoculum) for each genotypes of *O. tenuiflorum* (Supplementary Fig. 1). The mock inoculum provided to NM treatments consisted of soil washing filtered through Whatman filter paper no. 1, so as to introduce microbial populations other than mycorrhizal propagules, along with equal amount of autoclaved (120 °C and 15 psi for 1 h) soil mix. This ensured that the observed effects are only due to mycorrhiza and not any other factor.

The seeds were surface sterilized with 2 % sodium hypochlorite solution for 3 min. The treated seeds were thoroughly washed with distilled water. Nurseries of NM and M plants were separately raised in earthen pots. AMF inoculum (50 g) was spread as a layer below the seeds at the time of sowing. Forty days after sowing, AMF colonization in roots was confirmed. The seedlings (five-leaf stage) were then transplanted into earthen pots (one in each pot) filled with sterile soil. There were 15 pots per treatment. Following transplantation, plants were grown under natural conditions of light and temperature for 90 days. The plants were irrigated twice a day with tap water. During growth period of the plant, the average temperature ranged from 35 °C to 40 °C and average relative humidity ranged from 79 to 96 %. During the growth period, pots were rearranged randomly after every seven days to consider the variations (if any) in environmental conditions.

2.4. Plant harvest

Plants were harvested along with roots after 90 days of transplantation and were washed carefully under running tap water so as to eliminate adhering soil particles. The shoots were then separated from the roots, and leaves were plucked from them. Out of the fifteen plants per treatment, five plants were chosen randomly for determination of leaf yield (fresh weight) and biomass. Leaf biomass was determined after drying the leaves in an oven at 70 °C until constant weight was achieved. From another three random plants, leaves were collected for extraction of EO. The leaves collected from the remaining plants were then divided into two sets for different analyses; set I-Processing of leaves as similar to green tea, and preparation of methanolic extract of the processed leaves; set II-Preparation of methanolic extract of fresh leaves. The plants were randomly chosen for collection of leaves to avoid biasness.

Leaves from set I were processed for preparation of herbal tea or as additive of green tea following the method of Ahmed and Stepp (2013). The leaves were spread overnight on blotting sheets to reduce moisture content, and then steamed for approximately 10 min. The steamed leaves were then dried overnight at 70 °C in oven and were ground using a mortar and pestle into rough powder. The fresh leaves (set II) were chopped into smaller pieces. Subsequently, 10 g of the leaf powder (set I) and chopped leaves were added to a flask containing 50 ml of methanol. The solvent-tissue mixture was incubated for 48 h on an incubator shaker with continuous shaking (300 rpm) at room temperature for extraction of phytoconstituents. The obtained extract was filtered through Whatman filter paper no. 1 and the filtrate was later concentrated on a rotary evaporator under vacuum at 40 °C and stored at 4 °C until further use. Methanolic extracts of known concentration (1 mg/ml) were reconstituted to determine phenol, flavonoid, and polyphenol contents and for analyses of antioxidant properties of both fresh and processed leaves.

2.5. Root colonization by AMF

Root colonization by *R. intraradices* was evaluated at the time of transplant and harvest, following the staining procedure of Phillips and Hayman (1970). Root segments of approximately 1 cm long were cut from the collected fresh roots. The segments were then cleared with potassium hydroxide (10 % w/v) by incubating them for 15 min at 90 °C in a water bath. Trypan blue was used to stain the cleared root segments and the roots were observed under light microscope to visualize colonization and determine the colonization percentage.

2.6. Essential oil extraction

Fifty grams of fresh leaves of each treatment were pulverized in electric grinder using 500 ml distilled water. The ground mass was transferred to a Clevenger's apparatus (1000 ml) for hydro-distillation. The set-up was left undisturbed for 4 − 5 h for extraction of EO. The extracted EO was decanted and filtered over anhydrous sodium sulfate. The filtered EO was then collected and stored in a dark glass vial until further analysis for its chemical composition. The concentration of EO was determined on fresh weight basis (% w/w).

2.7. Characterization of EO by GC–MS (gas chromatography-mass spectrometry)

Chemical composition of EO was analyzed using Shimadzu-QP-2010 Plus (Japan) GC fitted with thermal desorption system TD 20 fused with a DB-5 capillary column packed with 5 % phenyl polysiloxane (30 m, 0.25 mm, and 0.25 µm film thickness). The column oven temperature was programmed initially at 50 °C (held for 2 min) and was then programmed to rise to 210 °C at the rate of 3 °C/min (held for 2 min). Temperature was finally allowed to reach 280 °C with an increase rate of 8 °C/min (held for 4 min). The carrier gas used was helium and its flow rate was 1.21 ml/min. The volume injected was 1 µl with 20:1 as the split ratio. Injection temperature was 260 °C. Flame ionization detector temperature was 300 °C with an ion source temperature of 220 °C and interface temperature of 270 °C. Mass spectra were taken at 70 eV with an EI source having a mass range of 40–650 amu. Identification of the EO components was done based on retention time and MS library search (WILEY8 and NIST14, Adams, 2001). The relative amounts of individual constituents were determined on the basis of the GC peak area.

2.8. Estimation of phytoconstituents

2.8.1. Phenol content

The phenol content of extracts was determined according to Folin-Ciocalteu method (Singleton and Rossi, 1965) with slight modifications. To 1 ml of test sample, 2 ml of Folin-Ciocalteu reagent (diluted 1/10th) was added. The reaction mixture was mixed thoroughly for 3 min and 2 ml of sodium carbonate (20 %, w/v) was added. It was then incubated at 25 °C for 30 min in the dark. Absorbance was recorded at 765 nm. Gallic acid was used to obtain a linear calibration curve for concentrations that ranged from 31.2 µg/ml to 250 µg/ml, with r^2 value of 0.987. Phenol content was expressed as mg GAE (gallic acid equivalent) per plant.

2.8.2. Flavonoid content

Estimation of flavonoid content was done according to the aluminum chloride colorimetric method (Chang et al., 2002). A linear calibration curve was obtained using quercetin for concentrations that ranged from 31.2 µg/ml to 250 µg/ml, with r^2 value of 0.986. Reaction mixture was prepared by adding 1 ml of the extract, 1 ml of methanol, 0.1 ml of 10 % aluminium chloride solution, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. Reaction mixture was incubated for 30 min at room temperature. Absorbance was recorded at 415 nm. The flavonoid content was expressed as mg QE (quercetin equivalent) per plant.

2.8.3. Polyphenol content

Polyphenol content was determined following Folin and Denis (1915). Reaction mixture consisted of 1 ml of test samples and 5 ml of 20 % sodium carbonate. The volume of the mixture was then made up

Table 1
Effect of *Rhizophagus intraradices* inoculation on fresh leaf yield, leaf biomass and essential oil concentration in two genotypes of *Ocimum tenuiflorum*.

Genotypes	AM status	AM colonization (%)		Fresh leaf yield (g/plant)	Leaf biomass (g/plant)	EO concentration (% w/w)
		Transplant time	Harvest			
A	NM	nc	nc	105.03 ± 2.34[b]	67.23 ± 1.92[b]	0.61 ± 0.04[b]
	M	27.70 ± 2.05	63.30 ± 2.33	134.27 ± 6.81[a]	83.46 ± 0.82[a]	0.97 ± 0.02[a]
B	NM	nc	nc	103.32 ± 3.33[b]	69.16 ± 2.57[b]	0.69 ± 0.05[b]
	M	36.70 ± 2.62	74.50 ± 3.15	159.89 ± 2.68[a]	92.12 ± 1.09[a]	1.29 ± 0.03[a]
Significance						
Genotype		***	***	***	***	***
Mycorrhiza				***	***	***
Genotype X Mycorrhiza				***	***	*

All the values are represented as means of five biological replicates (each having three technical replicates) ± SD except for AM colonization and EO concentration (means of three biological replicates; each having three technical replicates ± SD).
Different letters represent significant difference between the treatments within a genotype, derived from Tukey's honestly significant difference post hoc test.
AM-Arbuscular mycorrhiza; EO-Essential oil; NM-Non mycorrhizal; M-Mycorrhizal; nc-No colonization.
*, **, *** represent significance at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively derived from two-way ANOVA.

to 15 ml using distilled water. To this reaction mixture, 1 ml of Folin-Denis reagent was added. Final volume was made up with distilled water to 25 ml and it was incubated in dark for 20 min. Absorbance was read at 660 nm. Tannic acid was used to make a linear calibration curve for concentrations that ranged from 31.2 µg/ml to 250 µg/ml, with r^2 value of 0.992. The results were expressed as mg TAE (tannic acid equivalent) per plant.

2.9. Antioxidant properties

2.9.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging potential

The potency of the extracts to scavenge DPPH was determined according to MacDonald-Wicks et al. (2006) with slight modification. In brief, reaction mixture consisted of 1 ml of extracts and 1 ml of DPPH solution (0.8 mM). Reaction mixture was then vortexed for 10 s and later incubated at room temperature for 30 min. Absorbance was read at 517 nm. Positive control was prepared using ascorbic acid.

2.9.2. Hydroxyl (OH^-) radical scavenging potential

The OH^- scavenging ability of the leaf extracts was determined following the protocol of Klein et al. (1981) with slight modification. To 1 ml of iron-EDTA solution, 0.5 ml of 0.018 % EDTA, and 1 ml of 0.85 % dimethyl sulfoxide [prepared in 0.1 M sodium phosphate buffer (V/V) with pH 7.4] was added. The reaction was then initiated by adding 0.5 ml of 0.22 % ascorbic acid and incubated for 15 min at 90 °C. One ml of ice-cold trichloroacetic acid (17.5 %) and 3 ml NASH reagent were added to terminate the reaction. The reaction mixture was incubated for 15 min at room temperature. Absorbance was recorded at 412 nm and rutin was used as the positive control.

2.9.3. Nitric oxide (NO) radical scavenging ability

The ability of the extracts to quench NO radical was determined by Griess reaction (Sangameswaran et al., 2009). To 1 ml of the extract, 1 ml of 10 mM sodium nitroprusside (prepared in phosphate buffer, pH 7.4) was added and incubated for 30 min at 25 °C. Following this, 0.5 ml of the reaction mixture was taken and to it 0.5 ml of Griess reagent (0.1 % napthyl ethylenediamine dihydrochloride, 1 % sulphanilamide, and 2 % orthophosphoric acid) was added. The absorbance was recorded at 546 nm. The positive control was rutin.

2.9.4. Reducing power assay

Reducing power of the extracts was determined following the method of Oyaizu (1986). In brief, the reaction mixture consisted of 1 ml of the extract, 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6), and 0.25 ml of 1 % potassium ferricyanide. The reaction mixture was incubated at 50 °C for 20 min followed by termination of the reaction by adding 2.5 ml of 10 % trichloroacetic acid (w/v). The reaction mixture was centrifuged for 10 min at 5000 rpm and 2.5 ml of the upper layer was taken and mixed with 0.5 ml of 0.1 % ferric chloride and 2.5 ml of deionized water. Rutin was the positive control and absorbance was read at 700 nm.

2.10. Statistical analysis

The results were analyzed using SPSS software version 21.0 (IBM Corporation, United States). One-way analysis of variance (ANOVA) was performed using Tukey's honestly significant difference (HSD) post hoc test, to study the comparisons between treatments within the genotypes. A multivariate analysis of variance (MANOVA) was performed to include effect of two factors (genotype and mycorrhiza) and their interaction on various parameters. All the values are represented as means of five biological replicates (each having three technical replicates) ± SD except for per cent root colonization by AMF, EO concentration, and GC–MS analysis, which were represented as means of three biological replicates (each with three technical replicates) ± SD.

Principal component analysis (PCA) of the common compounds in the EOs was performed with XLSTAT software version 2019.3.2 (Addinsoft, New York, USA). The analysis was performed as a function of genotype and mycorrhizal treatment to evaluate possible relationship between the samples in terms of genotype and mycorrhizal association based on the composition of EO. The data matrix was based on 156 data points (2 genotypes X 2 treatments X 13 variables common to all treatments, in triplicates).

3. Results

3.1. AMF colonization and leaf biomass

Roots of both the genotypes A and B were successfully colonized by *R. intraradices* at the time of transplantation (Table 1). However, per cent root colonization by *R. intraradices* varied between the genotypes. Genotype B showed significantly ($P \leq 0.001$) higher root colonization at the time of transplant and remained higher than genotype A at the time of harvest too (Table 1). All the structural characteristics of AM such as arbuscules, vesicles, and intra-radical hyphae were observed in the colonized roots of M plants (Supplementary Fig. 1). No colonization by AMF was observed in the roots of NM plants.

Two-way ANOVA showed significant ($P \leq 0.001$) difference in foliage yield (fresh weight as well as biomass) between the two genotypes. Interaction between AMF treatments and genotypes also showed significant effect ($P \leq 0.01$) on leaf yield (Table 1). Mycorrhizal colonization had a significant effect ($P \leq 0.05$) on the leaf parameters analyzed in the present study (Table 1). In response to AMF

Table 2
Effect of *Rhizophagus intraradices* colonization on the chemical composition of essential oils from two genotypes of *Ocimum tenuiflorum*.

Nature of the compound		Compounds of EO	Genotype A		Genotype B	
			Control (%)	Mycorrhiza (%)	Control (%)	Mycorrhiza (%)
Sesquiterpene	V1	α-bergamotene	0.09 ± 0.02	0.10 ± 0.04 ^{ns}	nd	nd
Hydrocarbon	V2	α-copaene	0.42 ± 0.02	0.48 ± 0.03**	nd	0.07 ± 0.07
	V3	α-farnesene	nd	nd	nd	0.02 ± 0.01
	V4	α-humulene	1.68 ± 0.04	1.59 ± 0.02***	nd	nd
	V5	α-silenene	0.47 ± 0.02	0.54 ± 0.03*	0.3 ± 0.01	0.28 ± 0.02^{ns}
	V6	Aromaderene	nd	nd	0.02 ± 0.01	0.13 ± 0.09*
	V7	β-barbetene	0.21 ± 0.04	0.2 ± 0.03 ^{ns}	0.05 ± 0.04	0.06 ± 0.03^{ns}
	V8	β-bisabolenol	nd	nd	0.03 ± 0.02	nd
	V9	β-caryophyllene	20.31 ± 0.38	22.91 ± 1.47*	5.79 ± 0.50	9.12 ± 0.35**
	V10	β-cucurmene	nd	nd	0.03 ± 0.01	nd
	V11	β-duprezianene	0.29 ± 0.08	nd	nd	nd
	V12	β-elemene	23.81 ± 0.49	27.1 ± 0.12***	31.19 ± 0.68	33.02 ± 1.54**
	V13	Bisabolene	0.16 ± 0.04	0.13 ± 0.02 ^{ns}	nd	nd
	V14	δ-cadinene	0.18 ± 0.05	0.16 ± 0.02 ^{ns}	0.04 ± 0.02	0.04 ± 0.01^{ns}
	V15	Eremoligmol	0.16 ± 0.01	0.33 ± 0.02**	nd	nd
	V16	Eremophila	0.42 ± 0.03	0.44 ± 0.01 ^{ns}	nd	nd
	V17	Germacrene A	6.63 ± 0.09	7.95 ± 0.05**	0.2 ± 0.04	0.23 ± 0.02^{ns}
	V18	Germacrene D	1.67 ± 0.05	1.95 ± 0.05*	1.07 ± 0.03	1.09 ± 0.12*
	V19	Humulene	nd	nd	0.44 ± 0.10	0.35 ± 0.07*
Oxygenated	V20	β –eudesmol	nd	nd	nd	0.01 ± 0.0
Sesquiterpene	V21	Cadinol	nd	nd	nd	0.04 ± 0.02
	V22	Caryophyllene oxide	1.91 ± 0.02	1.61 ± 0.04*	0.53 ± 0.06	0.09 ± 0.04***
	V23	Cedroxyde	0.05 ± 0.02	0.05 ± 0.01 ^{ns}	0.02 ± 0.01	nd
	V24	Globulol	nd	nd	0.02 ± 0.01	0.02 ± 0.01^{ns}
	V25	Guaiol	nd	nd	0.03 ± 0.02	nd
	V26	Hedycaryol	0.91 ± 0.03	0.63 ± 0.04***	0.97 ± 0.03	0.55 ± 0.04**
	V27	Isovalencenol	nd	nd	0.26 ± 0.03	nd
	V28	Lanceol	nd	nd	0.19 ± 0.01	nd
	V29	Neo-intermedeol	0.33 ± 0.05	nd	0.58 ± 0.03	0.44 ± 0.06*
	V30	Rosifoliol	0.06 ± 0.01	nd	nd	nd
	V31	Selin-11	nd	nd	0.02 ± 0.01	nd
	V32	Spathulenol	nd	nd	0.02 ± 0.02	0.01 ± 0.03 ^{ns}
	V33	τ-cadinol	nd	nd	0.07 ± 0.02	nd
Monoterpene	V34	α-pinene	nd	nd	nd	0.09 ± 0.04
Hydrocarbon	V35	β-ocimene	nd	nd	0.04 ± 0.01	0.07 ± 0.01*
	V36	β-pinene	nd	nd	0.01 ± 0.0	0.08 ± 0.02**
	V37	Camphene	nd	nd	nd	0.07 ± 0.03
	V38	Isoterpinolene	nd	nd	nd	0.01 ± 0.01
	V39	Limonene	nd	nd	nd	0.05 ± 0.01
	V40	Prenyl limonene	nd	nd	0.09 ± 0.02	0.08 ± 0.04 ^{ns}
Oxygenated	V41	Camphor	nd	0.11 ± 0.01	nd	nd
Monoterpene	V42	Eucalyptol	nd	nd	0.01 ± 0.01	0.06 ± 0.03**
	V43	l-borneol	nd	nd	0.34 ± 0.05	0.40 ± 0.02**
	V44	Linalool	0.07 ± 0.02	0.21 ± 0.03*	0.05 ± 0.01	0.09 ± 0.02*
Diterpenes	V45	Isophytol	nd	nd	0.03 ± 0.01	nd
	V46	Phytol	nd	nd	0.13 ± 0.05	0.07 ± 0.04**
	V47	Phytol acetate	nd	nd	0.11 ± 0.03	0.03 ± 0.01**
Phenylpropanoid	V48	Eugenol	23.88 ± 0.91	27.62 ± 0.53*	42.78 ± 1.90	47.99 ± 0.30**
	V49	Methylchavicol	0.17 ± 0.04	0.06 ± 0.02 ***	0.99 ± 0.04	0.24 ± 0.2 **
	V50	Methyleugenol	15.58 ± 1.22	5.82 ± 0.77 **	13.44 ± 1.74	5.06 ± 1.61***
Others	V51	Furan	nd	nd	nd	0.03 ± 0.01
	V52	Homosalate	nd	nd	0.04 ± 0.02	nd
	V53	Nonanal	nd	nd	0.01 ± 0.00	nd
	V54	Salicylate	nd	nd	0.04 ± 0.03	nd

Values represent the mean of three biological replicates (each with three technical replicates) (± SD).
nd-Not detected; ns-Non significant; EO-Essential Oil; V-Volatile.
*, **, *** represent significance at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$ within a genotype derived from ANOVA.

colonization, there was 27.84 % and 54.75 % increase in the fresh leaf yield of genotype A and B, respectively, in comparison with their respective controls (Table 1).

3.2. Essential oil concentrations and composition

Two-way ANOVA showed significant effects of genotype, mycorrhizal treatment, and their interaction on EO concentration (Table 1). Foliage EO concentration was significantly ($P \leq 0.01$) higher in M plants in comparison with NM plants of both the genotypes (Table 1). In genotype A, AMF colonization resulted in 59.01 % increase in the EO concentration over the NM plants. While in genotype B, the concentration of EO from M plants was 86.95 % higher than that of NM plants.

Analysis of the EOs by GC–MS showed that the composition of the EOs varied between the two genotypes. In EO from NM plants, while 23 compounds were identified from genotype A, 37 compounds were detected from genotype B (Table 2, Supplementary Fig. 2 and 3). Fifteen compounds were common in EOs of both the genotypes; however the main compounds were eugenol, β-elemene, β-caryophyllene, methyleugenol, germacrene A, caryophyllene oxide, germacrene D, and hedycaryol. The concentrations of these components significantly differed

Table 3
MANOVA test showing the effect of genotypes, treatment, and their interaction on the thirteen common compounds present in essential oils of all the four treatments and on antioxidant potential of *Ocimum tenuiflorum* leaves.

Parameters	Genotype	Mycorrhiza	Genotype X Mycorrhiza
α-silenene	***	ns	**
β-barbetene	***	ns	ns
β-caryophyllene	***	***	ns
β-elemene	***	**	ns
δ-cadinene	***	ns	ns
Germacrene A	***	***	***
Germacrene D	***	ns	*
Caryophyllene oxide	***	***	ns
Hedycaryol	ns	***	**
Linalool	***	***	***
Eugenol	***	***	ns
Methylchavicol	***	***	***
Methyleugenol	ns	***	ns
DPPH scavenging activity	**	***	ns
OH^- scavenging activity	ns	***	ns
NO scavenging activity	***	***	ns
Reducing power	***	***	ns

ns- Non significant.
DPPH - 2,2-diphenyl-1-picrylhydrazyl, OH^- - Hydroxyl radical, NO-Nitric oxide.
*, **, *** represent significance at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$.

in both the genotypes except hedycaryol and methyleugenol (Table 3). While eugenol accounted for 23.88 % of the EO in genotype A, it constituted 42.78 % of the EO in genotype B. On the contrary, β-caryophyllene, and germacrene-A were higher in genotype A in comparison with genotype B (Table 2).

Colonization by *R. intraradices* influenced the composition of EOs of both the genotypes. The number of compounds identified decreased to 21 and 34 in EOs of genotype A and B, respectively (Table 2). While β-duprezianene, neo-intermedeol, and rosifoliol were not identified in EO of M genotype A, camphor was exclusively detected in EO of M genotype A (Table 2). In genotype B, mycorrhizal colonization considerably influenced the chemical profile of EO. While 12 compounds *viz.* β-bisabolenol, β-cucurmene, cedroxyde, guaiol, homosalate, isophytol, isovalencenol, lanceol, nonanal, salicylate, seline-11, and τ-cadinol were present in NM genotype B, these were not detected in EO of M plants (Table 2). Nine compounds *viz.* α-copaene, α-farnesene, α-pinene, β-eudesmol, cadinol, camphene, furan, isoterpinolene, and limonene were exclusively found in EO of M genotype B (Table 2).

Thirteen compounds, namely α-silenene, β-barbetene, β-caryophyllene, β-elemene, caryophyllene oxide, *δ*-cadinene, eugenol, germacrene A, germacrene D, hedycaryol, linalool, methylchavicol, and methyleugenol were common in all the four treatments (Fig. 1). These compounds cumulatively contributed to 95.8 % and 97.4 % of the total EO concentration of NM genotype A and B plants, respectively. In M plants of genotype A and B, these compounds contributed to 96.76 % and 97.86 % of the total EO concentration. Multivariate analysis of variance showed highly significant ($P \leq 0.001$) effect of genotype on all the common components except on hedycaryol and methyleugenol (Table 3). While mycorrhizal treatments had a significant effect on concentration of all the compounds except for α-silenene, β-barbetene, and *δ*-cadinene; genotype and mycorrhizal status interaction had highly significant ($P \leq 0.001$) effect on germacrene A and methylchavicol; moderately significant ($P \leq 0.01$) effect on α-silenene, hedycaryol, and linalool; significant ($P \leq 0.05$) effect on germacrene D (Table 3).

In genotype A, AM colonization increased concentration of eugenol by 15.66 % as compared with control plants (Table 2). In addition, concentrations of α-silenene, β-elemene, β-caryophyllene, germacrene A, and germacrene D increased by 14.89 %, 13.88 %, 12.80 %, 19.90 %, and 16.77 %, respectively, in M plants as compared with that of NM plants. On the other hand, concentrations of caryophyllene oxide,

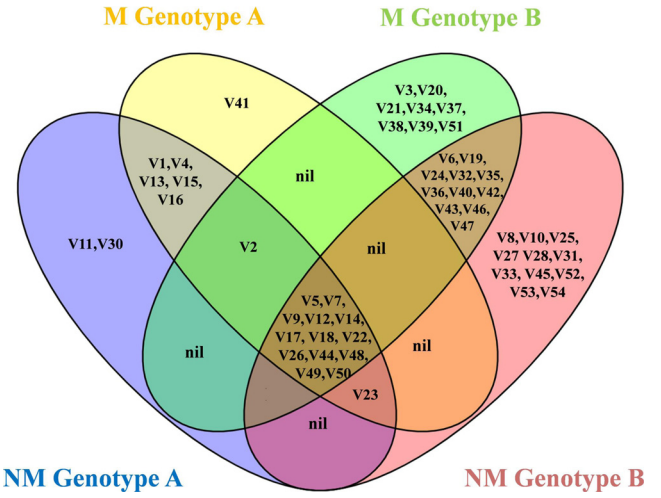

Fig. 1. A Venn diagram illustrating the overlap and distinctiveness of the composition of essential oil extracted from the non mycorrhizal (NM) and mycorrhizal (M) treatments of *Ocimum tenuiflorum* genotype A and B. Of the total 54 compounds identified by Gas Chromatography-Mass spectrometry, while 13 compounds were common in all the treatments, different treatments had between 1–11 distinct compounds identified. (V1- α-bergamotene; V2- α-copaene; V3- α-farnesene; V4- α-humulene; V5- α-silenene; V6- Aromaderene; V7- β-barbetene; V8- β-bisabolenol; V9- β-caryophyllene; V10- β-cucurmene; V11- β-duprezianene; V12- β-elemene; V13- Bisabolene; V14- *δ*-cadinene; V15- Eremoligmol; V16- Eremophila; V17- Germacrene A; V18- Germacrene D; V19- Humulene; V20- β –eudesmol; V21- Cadinol; V22- Caryophyllene oxide; V23- Cedroxyde; V24- Globulol; V25- Guaiol; V26- Hedycaryol; V27- Isovalencenol; V28- Lanceol; V29- Neo-intermedeol; V30- Rosifoliol; V31- Selin-11; V32- Spathulenol; V33- τ-cadinol; V34- α-pinene; V35- β-ocimene; V36- β-pinene; V37- Camphene; V38- Isoterpinolene; V39- Limonene; V40- Prenyl limonene; V41- Camphor; V42- Eucalyptol; V43- l-borneol; V44- Linalool; V45- Isophytol; V46- Phytol; V47- Phytol acetate; V48- Eugenol; V49- Methylchavicol; V50- Methyleugenol; V51- Furan; V52- Homosalate; V53- Nonanal; V54- Salicylate).

hedycaryol, methylchavicol, and methyleugenol significantly decreased upon mycorrhizal colonization (Table 2).

In genotype B, similar trends in alteration of the EO components upon AMF colonization were observed except α-silenene. The concentration of eugenol was increased by 12.18 % in EO of M as compared with their respective NM plants (Table 2). In addition, AM colonization resulted in a significant increase in the concentrations of β-elemene (5.87 %), β-caryophyllene (57.51 %), germacrene D (1.87 %), and linalool (80 %) as compared with EO of NM plants. While the concentrations of caryophyllene oxide, hedycaryol, methylchavicol, and methyleugenol decreased upon mycorrhizal colonization.

Interestingly, though AM colonization significantly enhanced the concentration of eugenol in EO, the concentration of phenylpropanoids taken together decreased in mycorrhizal plants. However, there was increase in the concentration of total terpenoids in EO on AM colonization. This trend was consistent in both the genotypes.

To evaluate and highlight the variability in EO composition due to genotypes and mycorrhizal treatments, the principal component analysis (PCA) was performed on thirteen compounds that were common in EOs obtained from all four treatments. Principal component analysis showed that all the common volatiles of EO contributed significantly to the variation in EO composition. Two principal components (PCs) explained 96.19 % of the total variance in the EO composition, with PC1 and PC2, respectively, contributing 72.17 % and 24.03 % of the total variability (Table 4). Comparison of the PCs showed that PC1 showed positive correlation with α-silenene, β-barbetene, β-caryophyllene, caryophellene oxide, *δ*-cadinene, germacrene A, and germacrene D, whereas PC2 showed a positive correlation with hedycaryol, methylchavicol, and methyleugenol, and a negative correlation with linalool (Table 4).

Table 4
Principal component matrix derived from principal component analysis of the thirteen common compounds obtained in essential oils from the non mycorrhizal and mycorrhizal treatments of *Ocimum tenuiflorum* genotypes A and B.

Compound	Principal Component	
	PC1	PC2
α-silenene	0.982	−0.057
β-barbetene	0.994	0.057
β-caryophyllene	0.988	−0.149
β-elemene	−0.904	−0.409
Caryophylleneoxide	0.953	0.302
δ-cadinene	0.986	0.133
Eugenol	−0.961	−0.277
Germacrene A	0.997	−0.038
Germacrene D	0.978	−0.142
Hedycaryol	−0.024	0.981
Linalool	0.611	−0.688
Methylchavicol	−0.705	0.549
Methyleugenol	0.099	0.992
Eigenvalue	9.381	3.124
Variability (%)	72.159	24.028

Two components having Eigenvalue > 1 were extracted by principal component analysis. PC1-Principal Component 1; PC2-Principal Component 2.

The distribution of compounds on correlation circle of the biplot (Fig. 2) led to the establishment of four groups of compound. Group I consisted of the majority of sesquiterpenes of EO (α-silenene, β-barbetene, β-caryophyllene, caryophellene oxide, δ-cadinene, germacrene A and germacrene D), and group II consisted of two compounds β-elemene- a sesquiterpene and eugenol- a phenylpropanoid. Group III was constituted by a sesquiterpene-hedycaryol and two phenylpropanoids- methylchavicol and methyleugenol, while group IV consisted of a single monoterpene linalool. Among all the groups, grouping of compounds in group III was not as homogenous as that in group I and II as the compounds in group III diffused along the PCs (hedycaryol and methyleugenol along PC2 and methylchavicol along PC1). The groups I and II were representative of PC1 and showed a high negative correlation between them, while, groups III and IV represented PC2 and showed a high negative correlation between them.

The scatter diagram on biplot (Fig. 2) showed that PC1 clearly separated genotype A from genotype B in terms of EO composition. It indicated that sample with a lower value on PC1 will have lower concentration of compounds from group I and a higher concentration of compounds from group II and *vice versa*. When compared with genotype A, genotype B showed higher concentration of group II compounds and lower concentration of group I compounds. Similarly, PC2 clearly distinguished mycorrhizal treatments from their respective non mycorrhizal counterparts in terms of hedycaryol, methylchavicol, and methyleugenol. Mycorrhizal plants having lower PC2 value indicated a lower concentration of group III compounds and higher amount of linalool when compared with their respective NM counterparts.

3.3. Total phenol, flavonoid, and polyphenol content

Total phenol, flavonoid, and polyphenol are among the key indicators of overall antioxidant activity of a plant. Two-way ANOVA showed highly significant influence of genotypes and mycorrhizal treatments independently as well as interactatively, on the contents of phenol, flavonoid, and polyphenols (Table 5). In genotype A, AMF colonization resulted in 60.69 %, 115.38 %, and 70.21 % increase in the total phenol, flavonoid, and polyphenol content, respectively, over control. While, in genotype B, colonization by AMF resulted in 70.48 %, 139.53 %, and 78.03 % increase in the total phenol, flavonoid, and polyphenol content, respectively, when compared with their respective NM plants (Table 5).

3.4. Antioxidant potential

The abilities of fresh and processed leaves to scavenge various ROS such as DPPH and OH^-, and RNS such as nitric oxide were assessed. Reducing ability was also assessed to comprehensively evaluate the antioxidant potentials of both the genotypes of *O. tenuiflorum*.

As expected, fresh leaves of *O. tenuiflorum* showed higher antioxidant activity than processed leaves in both the genotypes under

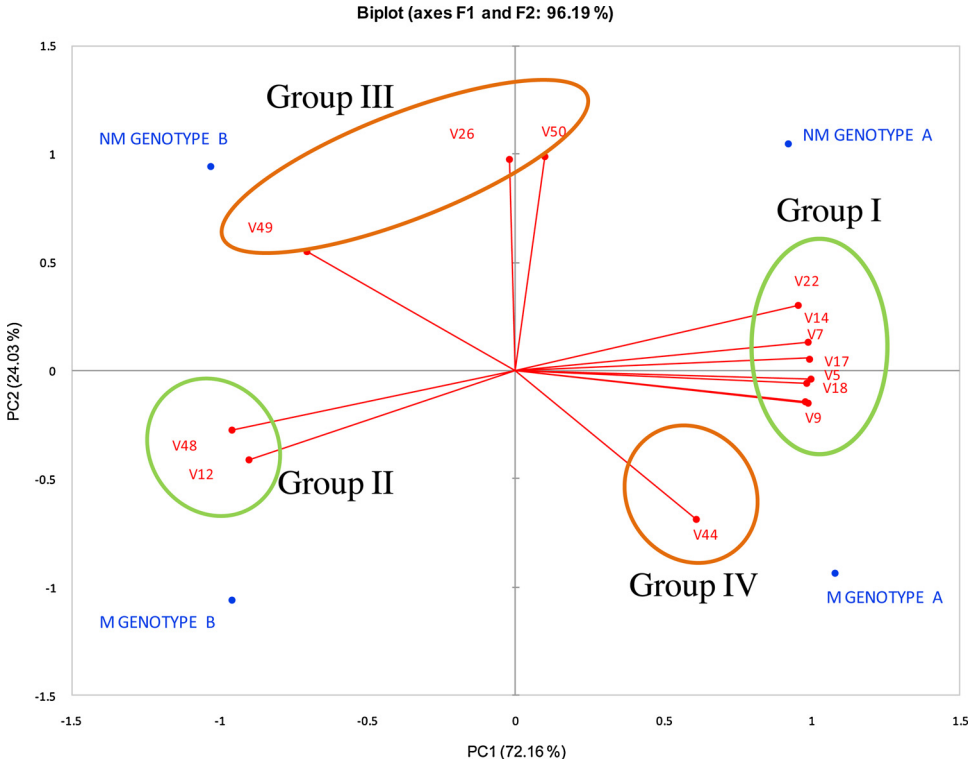

Fig. 2. Correlation biplot illustrating the variation in the thirteen common compounds of essential oils extracted from the non mycorrhizal (NM) and mycorrhizal (M) treatments of *Ocimum tenuiflorum* genotype A and B. The principal component analysis (PCA) established four groups of compound (group I-group IV) distributed on the two principal components (PC1 and PC2) axes. (V5- α-silenene; V7- β-barbetene; V9- β-caryophyllene; V12- β-elemene; V14- δ-cadinene; V17- Germacrene A; V18- Germacrene D; V22- Caryophyllene oxide; V26- Hedycaryol; V44- Linalool; V48- Eugenol; V49- Methylchavicol; V50- Methyleugenol).

Table 5
Effect of *Rhizophagus intraradices* colonization on the contents of phenol, flavonoid, and polyphenol in two genotypes of *Ocimum tenuiflorum*.

Genotypes	AM status	Phenol content (g GAE/ plant)	Flavonoid content (g QE/ plant)	Polyphenol content (g TAE/ plant)
A	NM	1.45 ± 0.07[b]	0.39 ± 0.02[b]	0.94 ± 0.12[b]
	M	2.33 ± 0.13[a]	0.84 ± 0.05[a]	1.60 ± 0.18[a]
B	NM	1.66 ± 0.08[b]	0.43 ± 0.08[b]	1.32 ± 0.09[a]
	M	2.83 ± 0.09[a]	1.03 ± 0.11[a]	2.35 ± 0.07[b]
Significance				
Genotype		***	***	***
Mycorrhiza		***	***	***
Genotype X Mycorrhiza		***	***	***

Values are the means of five biological replicates (each having three technical replicate) ± SD.
Different letters represent significant difference between the treatments within a genotype, derived from Tukey's honestly significant difference post hoc test. AM-Arbuscular mycorrhiza; NM-Non mycorrhizal; M-Mycorrhizal; GAE-Gallic acid equivalent; QE-Quercetin equivalent; TAE-Tannic acid equivalent.
*, **, *** represent significance at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively derived from two-way ANOVA.

study (Fig. 3). The symbiotic association had a positive effect on the ability of the leaves to quench ROS and RNS species. While genotype and mycorrhizal status independently had significant effect on DPPH, and NO scavenging activity and on reducing power, their interaction had non-significant effect on these parameters. In genotype A, DPPH and OH^- radical scavenging potential was found to be higher by 8.4 % and 36.1 %, respectively, in M plants when compared with the NM plants. Similarly, an increase of 15.8 % in NO scavenging ability and 7.1 % in reducing power were reported in M plants with respect to NM plants. The same trend was observed in genotype B. In response to AMF colonization, M plants of genotype B showed an increase in the ability to scavenge DPPH and OH- by 9.8 % and 18.7 %, respectively, over NM plants. Similarly, NO scavenging potential and reducing power were enhanced by 14.7 % and 5.7 %, respectively, upon AMF colonization.

A relatively lower antioxidant activity of processed leaves was observed as compared with fresh leaves. However, AMF colonization enhanced the antioxidant capacity of processed leaves in both the genotypes of *O. tenuiflorum*. The scavenging potential of M genotype A against DPPH and OH^- was 6.67 % and 33.5 % higher than that of NM plants, respectively. The NO scavenging ability and reducing power of M genotype A were 15.2 % and 10.6 % higher as compared with their NM plants. In case of genotype B, DPPH and OH^- scavenging potential were respectively, 8.9 % and 30.3 %, higher in M plants than NM plants. Similarly, 18.4 % increase in the NO scavenging ability and 10.3 % increase in reducing power were reported in M plants over NM plants (Fig. 3).

4. Discussion

To improve the economic yield of holy basil, it would be imperative to increase the leaf biomass per plant, enhance EO production, and increase the concentration of the active components present in the EO. Results obtained from the present study evidently demonstrate the effectiveness of *R. intraradices* inoculation in improving the quantity and quality of the industrially valued products of *O. tenuiflorum*.

The increase in leaf yield and biomass in response to AMF colonization over the NM plants observed in this study are in agreement with several studies that reported the positive effect of AMF in leaf/shoot biomass in plants such as *Coriandrum sativum* L. and *Anethum graveolens* L. (Kapoor et al., 2002a, b); *Mentha arvensis* L. (Freitas et al., 2004); *O. basilicum* (Rasouli-Sadaghiani et al., 2010; Copetta et al., 2006); and *Salvia officinalis* L. (Tarraf et al., 2015). The increase in plant growth and yield in response to mycorrhizal colonization can be attributed to improved nutrient uptake (Zhang et al., 2018). Mycorrhiza forms links between roots and soil. The extrametrical hyphae function analogous to fine root hair, and acquire more nutrients (especially P- a relatively immobile element) by altering the uptake dynamics (Koltai and Kapulnik, 2010). In addition, increased photosynthetic pigments and higher plant photosynthetic rates (Bulgarelli et al., 2017; Zhang et al., 2018) result in higher biomass accumulation in M plants.

Interaction of genotype and AM treatment showed variable effects on biomass and EO concentration. Roots of genotype B enabled higher colonization by *R. intraradices*, and derived more benefit in terms of

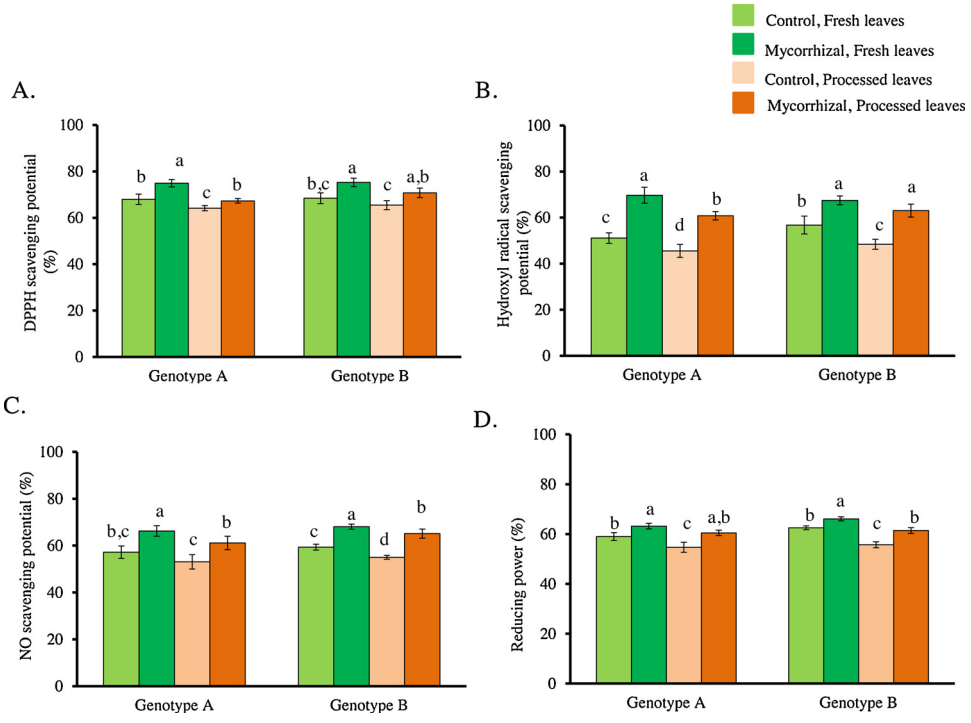

Fig. 3. Effect of *Rhizophagus intraradices* colonization on A. DPPH (2,2-diphenyl-1-picrylhydrazyl), B. OH^- (hydroxyl radical), C. NO (nitric oxide) scavenging potential, and D. Reducing power of fresh and processed leaves of two genotypes of *Ocimum tenuiflorum* L. Values are the means of five biological replicates (each having three technical replicate) ± SD. Different letters within a genotype show significant difference among the treatments within a genotype at $P \leq 0.05$, according to Tukey's honestly significant difference post hoc test.

increase in leaf biomass and essential oil concentration than genotype A. The effectiveness of AM symbiosis is highly dependent on the genotype of host plants (Bazghaleh et al., 2018; Shamloo et al., 2017; Singh et al., 2012). Cultivars of a plant species may differ in their nutrient uptake ability, and the formation and function of root symbioses (Kashiwagi et al., 2006; Bryla and Koide, 1998). Plants with good innate ability to extract soil P are generally less responsive to the AM symbiosis (Bryla and Koide, 1998). The differential response of *O. tenuiflorum* genotypes to AMF colonization may be in parts due to (i) the soil nutrient conditions to which the two genotypes are adapted; and (ii) dependence of a given genotype on AM. It has been reported that adaptations to mycorrhizal fungi can be governed by the resource availability (Collins Johnson et al., 2010). The soil used in the present study was high in plant available P (116 kg/ ha); and probably genotype A is adapted to lower nutrient conditions, and thus displayed lower dependence on AM. Alternatively, there may be differences in other traits that have a pleiotropic effect on AMF colonization, *viz.* root morphology and density. However, the exact reasons for the differences between genotypes of *O. tenuiflorum* to AMF colonization and their response to AM cannot be pinpointed. It warrants more research encompassing wide range of cultivars to identify traits specifically related to difference in responses to AMF.

The EO content in any aromatic plant species varies with its cultivars, plant tissue, and environmental conditions (Verma et al., 2013; Sangwan et al., 2001). Essential oil content in *O. tenuiflorum* varies with the cultivar, and has been reported to range from 0.1 % to 2 % (Verma et al., 2016, 2013). The EO contents of the two genotypes (0.61–0.69 %) in this study were similar to EO content reported for *O. tenuiflorum* grown in other regions of India; 0.53 % in cultivars from northern India (Verma et al. 2013); 0.7 % from eastern India (Kalita and Latif Khan, 2013), and 0.65 % from south India (Kothari et al., 2005).

Inoculation of *R. intraradices* enhanced concentration of EO in both the genotypes of *O. tenuiflorum*. The positive effect of AMF inoculation in improving EO content has been reported in aromatic plants such as *Origanum vulgare* L., *S. officinalis*, and *Thymus vulgaris* L. (Tarraf et al., 2015); *O. basilicum* (Zolfaghari et al., 2013; Copetta et al., 2006); and *C. sativum* and *A. graveolens* (Weisany et al., 2015; Kapoor et al., 2002a, b).

The EOs from the leaves of the two genotypes in this study were mixtures of terpenoids and phenylpropanoids, and their derivatives in different proportions. These are biosynthesized by two different biochemical pathways, namely shikimic acid pathway for synthesis of phenylpropanoids, and methyl erythritol phosphate (MEP) and/or mevalonic acid (MVA) pathways for terpenoids. Thus, increase in the EO contents would be due to cumulative effects of AM on the components of both the pathways. It was observed that AM colonization enhanced the concentration of total terpenoids in EOs as compared with their respective NM plants. Increase in concentration of terpenoids by virtue of mycorrhizal colonization has generally been attributed to improved growth and P nutrition of the plants (Kapoor et al., 2017; Toussaint et al., 2007; Copetta et al., 2006). There are studies that correlate enhanced terpenoid levels in mycorrhizal plants with higher glandular trichome density and altered hormonal balance (Moreno-Fortunato and Avato, 2008; Copetta et al., 2006). Formation of AM results in induction of terpenoid biosynthesis (MEP) pathway by up regulating the transcript levels of genes encoding key enzymes such as DXS1 (1-deoxyxylulose 5-phosphate synthase) and DXR (1-deoxyxylulose 5-phosphate reductoisomerase) (Mandal et al., 2015a, b).

Colonization by *R. intraradices* significantly improved the concentration of eugenol, however the proportion of total phenylpropanoids reduced in EOs of both the genotypes. Formation of AM has been reported to up-regulate the expression of *PAL* gene (Bruisson et al., 2016) that encodes phenylalanine ammonia-lyase- an enzyme that catalyzes the conversion of phenylalanine into trans-cinnamate (first committed step of phenylpropanoid pathway) (Yin et al., 2012). Colonization by *R. intratradices* enhanced the activity of this enzyme in genotypes A and B used in the present study (data not shown). The decrease in total phenylpropanoids in EO observed in present study may be explained by divergence of the precursors of this pathway towards enhanced synthesis of flavonoids, phenols, and other non-volatile phenylpropanoids in *O. tenuiflorum*. A metabolome study covering large array of primary as well as secondary metabolites will provide an overview of the metabolic fluxes in mycorrhizal plant (Kapoor et al., 2017).

The main constituents in EO of both the genotypes were eugenol, β-elemene and β-caryophyllene, in decreasing order. *Ocimum tenuiflorum* has showed considerable diversity in the chemical profile of EOs from different cultivars belonging to various regions of the world. Three chemotypes of *O. tenuiflorum* have been reported, *viz.* eugenol type (Verma et al., 2013; Mondello et al., 2002), methyleugenol type (Kothari et al., 2005; Jirovetz et al., 2003), and methylchavicol type (Brophy et al., 1993). If this classification is followed, the two genotypes used in the present study would be “Eugenol type”. Concentration of eugenol in the ‘eugenol type’ has been reported to be up to 77.5 %, and in most instances above 54.9 %. However, in this study, eugenol concentration in the EOs was much lower ranging between 23.88 % (Genotype A) and 42.78 % (Genotype B). Verma et al. (2013) identified a new chemotype “(E)-caryophyllene/β–elemene/eugenol type” rich in sesquiterpenes and deficient in eugenol content. The two genotypes in this study would be intermediate of eugenol type and (E)-caryophyllene/β–elemene/eugenol type. The difference in the oil contents and chemical composition when compared with earlier reports could be explained by different cultivars used, the difference in harvesting period and climatic conditions, luminosity as well as oil extraction method (Verma et al., 2013; Gobbo-Neto and Lopes, 2007; Kothari et al., 2004). Furthermore, differences in the soil type and nutrient availability among the studies may have had a significant effect on the EO composition of basil (Zheljazkov et al., 2011).

The present study probably reports for the first time the effect of AM colonization on major as well as minor constituents of EO of *O. tenuiflorum*. Eugenol was the most abundant compound in EOs of both the genotypes. Arbuscular mycorrhiza further enhanced its concentration in the EOs. The second most abundant compound identified in EO of both the genotypes was β-elemene followed by β-caryophyllene. In response to AMF colonization, the contents of these compounds were significantly increased in both the genotypes. Interestingly, methyleugenol, a phenylpropanoid derived directly from eugenol, detected in the EOs was found to be present in lower concentration in AMF colonized plants as compared with the NM counterparts of both the genotypes.

Interaction between mycorrhizal treatments and genotypes had no significant effect on the concentrations of main compounds of EO namely β-barbetene, β-caryophyllene, β-elemene, δ-cadinene, caryophyllene oxide, eugenol, and methyleugenol. This suggests that effect of AM on concentrations of main constituents is not variable in the two genotypes, and may be further extrapolated to other cultivars and probably species of *Ocimum*. This is further supported by higher levels of eugenol and caryophyllene reported by Copetta et al. (2006) in *O. basilicum*.

The major components detected in the EOs were common in both the genotypes however the number and concentration of compounds present in minor proportion (less than 1 %) differed. Mycorrhizal colonization further changed the profile, resulting in appearance and disappearance of some compounds, and significantly changed the concentrations of many of other minor compounds. The effect of AM on the minor compounds was variable. The concentration of α-copaene, α-silenene, eremoligmol, and linalool increased while that of α-humulene and hedycaryol decreased in response to mycorrhizal colonization in genotype A. In genotype B, the concentrations of aromadendrene, β-ocimene, β-pinene, eucalyptol, l-borneol, and linalool increased, and that of caryophyllene oxide, humulene, hedycaryol, neo-intermedeol, phytol, and phytol acetate were decreased upon AMF colonization. While effect of AM on total terpenoids and phenylpropanoid, have been explained on the basis of its effect on certain key enzymes, such as

DXS1, DXR, and PAL (Bruisson et al., 2016; Mandal et al., 2015b) that act upstream of the biosynthetic pathways, very few studies have so far been carried out to explain the fluxes within a group of chemicals due to mycorrhizal colonization.

In addition to EO, a number of bioactive compounds, such as phenols and flavonoids that have excellent antioxidant properties have been reported from the leaves of holy basil (Upadhyay et al., 2015; Bravo, 1998; Nakatani, 1997). *Ocimum tenuiflorum* in response to *R. intraradices* colonization showed significant increase in the content of phenols, flavonoids, and polyphenols. Similar to the present findings, Ceccarelli et al. (2010) reported increase in the phenols in leaves and flowers of mycorrhizal *Cynara cardunculus* L., both in greenhouse and field conditions. Oliveira et al. (2013) also reported substantially higher phenols and flavonoids in seedlings of AMF inoculated *Myracrodruon urundeuva* L. The synthesis of flavonoids and phenolic compounds are associated with photosynthetic rates of plants (Santos, 2007), and induction of phenylpropanoid pathway. Inoculation of plants with AMF has resulted in improved photosynthesis (Romero-Munar et al., 2017; Amaya-Carpio et al., 2009).

Processed leaves showed relatively lower radical scavenging ability as compared with fresh leaves. This result of decreased antioxidant capacity in processed leaves is in line with previous study where thermal processing of *Brassica* leaves reduced its antioxidant potential (Lafarga et al., 2018). This could be due to oxidation of the bioactive compounds during processing of the leaves. Nevertheless, leaves of AMF colonized plants were more efficient in scavenging free radicals over NM plants in both fresh and processed samples. Higher antioxidant potential was observed in the leaves of M plants due to its collective effect on concentrations of phenols, polyphenols, flavonoids, phenylpropanoids and terpenoids along with enzymatic antioxidants present in plant.

5. Conclusion

The present study highlights the potential of AMF in organic farming of *O. tenuiflorum* with special emphasis on their contribution to its commercial yield. Inoculation of *R. intraradices* augmented the biomass and essential oil content of leaves in two high yielding varieties of tulsi. The effectiveness of AM symbiosis in terms of growth and EO content was found to be highly dependent on the plant genotypes. This emphasizes on the need to consider AM responsiveness as one of the desired traits for breeding efforts towards high yielding cultivars of *O. tenuiflorum*. Colonization by AMF, not only improves the yield but also enhances the concentration of active principals such as eugenol, β-elemene, and β-caryophyllene, resulting in increase in the commercial value of the EO. Arbuscular mycorrhiza also influences the number and the relative concentration of individual constituent, which together may lead to variation in the therapeutic and biological activity of the EO. Furthermore, AM boosted the antioxidant capacity by enhancing the concentration of flavonoids and phenols in leaves. Thus, the results suggest the benefits of AM inoculation to the consumers of tulsi leaves in form of infusion or green tea.

Credit author statement

The work was designed and planned by RK. TSD executed the experiments. RK, TSD, and SG jointly wrote the manuscript. All the authors have collectively reviewed the manuscript and approved it.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2020.112418.

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