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Influence of lemongrass (*Cymbopogon flexuosus* (Nees ex Steud.) essential oil yield under inter - cropping with pomegranate (*Punica granatum* L.) with special reference to the plant - soil relationship

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Abstract
The rain - fed/oasis areas, where lemongrass and pomegranate are grown as intercropping crops, are considered as the best ecosystem with particular properties. No research has been made to investigate the relationship between lemongrass essential oil and pomegranate fruit quality in such an environment. A comparison between pomegranate trees was intercropped with lemongrass crop and regular orchards with full sun conditions have been investigated in terms of yield of lemongrass essential oil and pomegranate fruit quality. The intercropped microclimate was more favourable for the production of lemongrass essential oil and pomegranate arils with attractive red colour and high total anthocyanin content. Pomegranates under these conditions contained total volatile content that was about two times higher than that in separate fruits. Particularly, hexanal and limonene were the most abundant compounds characterizing the aroma profile of fruits cultivated under intercropped, and their arils were the most appreciated in terms of colour, odour, and taste by Indian consumers. However, fruits from partial shade oasis provided the sweetest juices with high concentrations of glucose and fructose. Titratable acidity and total organic acid content were also higher in fruits grown under the two conditions. Red colour intensity, monoterpenes, hexanal, citric and quinic acids were useful to discriminate full shade oasis. These compounds seem to contribute to the typical organoleptic properties of oasis pomegranate fruits. The correlations between intercropped varieties of pomegranate and lemongrass, irrigated water, soil, and manure factors, elevated moisture in the soil in the intercropping impaired the expression of high yield of fresh herb and lemongrass oil of the good quality and pomegranate fruits production.

Keywords: correlations, essential oil, intercropping, lemongrass, pomegranate

1. Introduction

Lemongrass (*Cymbopogon flexuosus*) is perennial aromatic grass, which yields an essential oil used in perfumery and pharmaceutical industries. It is a very hardy, drought - tolerant crop, adapted to a wide variety of soils and climatic conditions. The crop is grown mainly as a rainfed crop on poor marginal lands. It is cultivated commercially in many parts of India for aromatic oil present in leaves. Lemon - like odour containing 75 - 80% citral which is used for the manufacturing of vitamin A. Its oil is useful for the manufacturing of soap, cosmetics, and essences.

The intercropping of the annual or perennial crop in between tree spacing is not a new practice it's an old age practice with a new term called agroforestry, which is the Collective name for land - use systems and technologies where woody perennials (trees, shrubs, bamboos, palms, etc.,) are deliberately used on the same land management units as crops and or animals, in some form of spatial arrangement or temporal sequence (Lundgren and Raintree 1982) [26]. Modern Agro forestry establishes a symbiosis among annual or perennial crops, woody species, and livestock.

Pomegranate (*Punica granatum* L.) is an ancient and important fruit crop of tropical and subtropical regions of the world. It belongs to the family Punicaceae with two species *viz. granatum* and *protopunica*. The origin of pomegranate is in southwest Asia probably in Iran. It is commercially cultivated in Iran, Afghanistan, Russia, Israel, North and Latin American countries, Africa and India. India ranks first in the world in area (2.34 lakh hectares) and production (28.45 lakh tonnes), (GOI, 2018). In India, it is commercially cultivated in Maharashtra, Karnataka, Gujarat, Rajasthan, Uttar Pradesh, Andhra Pradesh, and Tamil Nadu. Maharashtra is the main pomegranate - producing state and accounts for 78% of the total production of the country. It is also cultivated in semi - arid and arid regions but requires assured irrigation for better quality fruits. The climatic condition of the Bundelkhand region of central India is almost similar to pomegranate growing areas of Maharashtra and therefore, is suitable for pomegranate cultivation. Meager information is available on the integration of aromatic plants with fruit trees on the same land management unit under the organic regime as compared to the other forms of agroforestry (Mirdehghan and Rahemi, 2007; Lal *et al*., 2017a, b; Boussaa *et al*., 2018) [2, 23, 24, 28, 29]. The integration of aromatic plants provides another opportunity to study diversification of existing land use systems for beneficial environmental impacts and higher returns as compared to sole cropping systems (Chaturvedi 1991; Mars and Marrakchi M, 1999) [6, 27].

2. Material and Method

2.1 Experimental site: The experiment was conducted in a four - year - old pomegranate orchard at the central research farm of ICAR - Central Agroforestry Research Institute, Jhansi w.e.f. 2016. Geographically Jhansi is located at 25^0 27' N latitude and 78^0 35' E longitude at an altitude of 271 meters above mean sea level and has a semi - arid and sub - tropical climate with extreme hot in summer. There is a gradual increase in daily temperature from April to May. The average rainfall of this region is 750 to 1100 mm, the maximum means temperature 32.5^0 C (48^0 C in May) and the minimum mean temperature 17.7^0 C (1.5^0 C in January). The main sources of irrigation water are reservoirs, canals, and tube wells which are dependent on rainfall.

2.2 Experimental details: The experiment was designed in CRBD with two cultivars of pomegranate (V_1 - Ganesh & V_2 - Bhagwa), Three levels of fertilizer (T_1 - Vermi - compost 30Kg/pl, T_2 - FYM 30Kg/pl, and T_3 - T_1 + T_2/pl along with control of pure lemongrass. The experiment was replicated thrice and each treatment is having four plants of each cultivar. During July - August 2016, Krishna variety of lemongrass, procured from CIMAP Lucknow, was covered in plant basins. Since every plant basin is covering a 1.0sqm area and not planted in between the pomegranate plants at the spacing of 50×40cm in a plot size of 60sqm with the area lemongrass in such area, therefore, the net plot size is 56sqm. The pomegranate was planted at 5×3m spacing during February 2013.

The drip system was installed during 2016 to irrigate pomegranate plants. The experiment was designed in Factorial RBD with two cultivars of pomegranate (V_1 - Ganesh & V_2 - Bhagwa), four levels of nutrient management (T_1 - Vermicompost 30 kg/pl, T_2 - FYM 30 kg/pl, T_3 - T_1 + T_2, and T_4 - Recommended doses of chemical fertilizers/pl). The experiment was replicated thrice and each treatment was having four plants of each cultivar. A control of pure lemongrass was also maintained separately. The Krishna variety of lemongrass procured from CSIR - Central Institute of Medicinal and Aromatic Plants, Lucknow, Uttar Pradesh, India, was planted during July - August 2016 at spacing 50×40cm in all treatments, except treatment T_4 in a plot size of 56 m^2 excluding the area covered by pomegranate plants. The lemongrass was grown purely in rain - fed conditions and without the application of any fertilizer. During July - August 2018, due to an increase in tussock size, lemongrass spacing was converted into 100×80 cm by removing one tussock in between the two tussocks. The treatments of organic nutrition were applied in pomegranate plants during July of each year. The whole experiment is being maintained by adopting the necessary cultural practices. In lemongrass, two cuts (May and October) were taken and fresh yield (t/ha) was recorded. Simultaneously, the samples from each treatment were collected for both the cuts and estimated oil in the laboratory through steam extraction by using a Clevenger apparatus after keeping the samples in shade for 24 hours. For data analysis, fresh yield and estimated oil (kg/ha) of both the cuts were clubbed together and analyzed through OPSTAT (Two Factor Analysis) an online software developed by Sheoran *et al*, (1998) [31]. Since lemongrass was not planted in treatment T_4, only three treatments were used for data analysis, and pure lemongrass (control) data was used for comparison.

2.3 Statistical analysis: Statistical analyses were performed at The computer section, CCS HAU, Hissar using one - way analysis of variance ANOVA, and the significant difference between means was determined by Duncan's multiple range test using SPSS 20 software. Significance was defined at $P<$ 0.05.

3. Results and Discussion

Descriptive and affective field evaluation trials of the lemongrass intercropped with pomegranate crops play an important role to increase the double income of the Indian marginal farmers as well as for the perfumery, cosmetic, essential oil, and fruits/food industries. Several circumstantial factors such as habits, beliefs, attitudes, and aroma values influence consumer choices. Geck *et al*. (2017) [7] reported that the perception of gustatory and olfactory sensations is highly subjective, depending on genetic, physiological, environmental, and especially cultural variables. Cardinal *et al*. (2015) [5] proved that aroma, as well as fruits/food - science - related consumers (FSRC), checked pomegranate fruit - flavored powdered beverages differently to the other two consumer segments, women, and children. In the current study, the lemongrass intercropped with pomegranate analysis revealed the economic importance and differences between oasis and regular orchard with full sun exposure in the aril colour intensity of the pomegranate and quality and yield of the lemongrass essential oil. If we see the different combinations of FYM and vermicompost the combinations with V1 were always high for all traits except the three (lemongrass fresh herb yield/plot and t/ha, and the essential oil on a green basis lit/ha (Table 1).

More or less the same trends were found for V1 treatments for all the traits except the trait essential oil on a green basis kg/ha (Table 2; Figure 1, 2, and 3). The pooled analysis of variance (ANOVA) for varieties and manure combinations indicated the highly significant differences for the varieties, manure combinations, and varieties × manure combinations (Table 3). Therefore, high magnitudes of variation were present in the treatments (Table 3). Arils from pomegranate fruits plus lemongrass cultivated under the full intercropping system, particularly dry areas of Bundelkhand of Uttar Pradesh and Madhya Pradesh states of India, were characterized by high intensity of the red colour pomegranate fruits which made them well appreciated by the consumer panel with high - quality essential oil of the lemongrass. However, these differences in the essential oil of the lemongrass aroma and pomegranate fruit taste may be influenced by the sunlight and plant density, and colour of the flush. Similarly, the overall liking was also higher in the pomegranates grown under intercropped with lemongrass oasis, as compared with those samples grown under individual without utilizing interspaced full sunlight).

In general, microclimate conditions due to intercropping with lemongrass had a positive effect on the aril colour (less red colour intensity was reached), which was linked to a lower overall satisfaction degree and also for the colour, aroma, and taste degrees and the yield and quality of the essential oil of lemongrass. These results suggested that an environmental condition arises by intercropping influenced pomegranate fruit appearance (colour intensity) and the yield and quality of the essential oil of lemongrass. This would directly affect the final perception by consumers (overall, appearance, and flavor) and ultimately on the farmer's economy. It is also authenticated by observations/data well.

3.1 Volatile compounds

The lemongrass and pomegranate fruits produce a range of volatile compounds that make up their characteristic aroma and contribute to their flavour (Lal *et al*., 2014; Lal *et al*., 2020a, b, c, d) [15, 17, 18, 20, 22]. Aromatic compounds identified in lemongrass (citral and some other minor compounds) and in pomegranate fruit juice samples were high - quality taste and concentration. The total concentrations of volatile compounds in the headspace of pomegranate juices obtained from only fleshly arils ranged from 45 μg L−1 to 140 μg L−1. These compounds can be grouped into six chemical families: (a) monoterpenes: limonene, α - pinene, β - pinene, p - cymene, γ - terpinene, sabinene, and α - bergamotene; (b) aldehydes: hexanal, nonanal; (c) alcohols: 1 - hexanol; (d) monoterpenoids: α - terpineol, linalool, dihydrocarveol; (e) esters: linalyl acetate; (f) hydrocarbons: decane. Monoterpenes, aldehydes, and alcohols represented the predominant groups in the pomegranate juices. These results are consistent with those of Melgarejo *et al*. (2011) [3] and Calin - Sanchez *et al*. (2011) [3]. In general, the most abundant compounds in the investigated samples were limonene (sensory descriptors: mild, citrus, sweet, orange, lemon), hexanal (fatty, green, grassy, powerful), 1 - hexanol (mint, grass), and decane and citral in lemongrass essential oil (Tables 4).

Nevertheless, the intercropping environment affected the accumulation of volatile compounds in lemongrass and pomegranate fruits that may contribute to their aroma and flavour variation (Holland *et al*., 2009; Styger *et al*., 2011; Lal, 2012; Lal, 2013; Lal, 2014; Lal *et al*, 2021; Kumar *et al*., 2021) [10, 13 - 15, 19, 32]. The concentration and the relative percentages of volatile components varied greatly following orchard intercropping microclimates. In general, the total concentrations of volatile compounds were greater in lemongrass and pomegranate fruits from comparisons to separate cultivation of both crops (Figure 1 and 2). Similar results were reported by Jia *et al*. (2005) [9] who showed that an increase in temperature caused by sun exposure has contributed to the degradation of aroma compounds. Generally, the full shade conditions of the oasis were favourable to accumulate hexanal in pomegranate fruits. Limonene also showed high concentrations in lemongrass and pomegranate fruits grown under the intercropping system.

The meticulous study of correlations results between different soil and treatment factors indicated the NAG is positive and significantly correlated with P (kg/h); organic carbon % and EC but negatively correlated with ph and FUME, respectively. The Acidic phosphatase is positive and significantly correlated with B - GLU but strangely B - GLU was negatively associated with N (kg/h). The N (kg/h) was also positively and significantly correlated to the most important traits lemongrass fresh (t/ha) and essential oil yield (kg/ha) therefore this is an important achievement. The organic carbon % is also positively and significantly correlated to EC but negatively with FUME. The EC and FUME were negatively correlated. Another important highly significant and positive correlations were also recorded between the lemongrass fresh herb yield (t/ha) and the essential oil yield of the lemongrass. The selection of one factor will certainly affect other dependent factors positively or negatively. The weak positively and negatively correlations were also recorded between all studied factors.

Nevertheless, the correlations between soil and manures factors, and intercropped varieties of pomegranate and lemongrass, irrigated water, elevated moisture in the soil, humidity, temperatures due to intercropping impaired the expression of 1 - deoxy - D - xylulose - 5 - phosphate synthase transcripts required for isopentenyl pyrophosphate (IPP) synthesis, the universal precursor for the biosynthesis of terpenes. Our research findings are also in the agreement of several research workers (Rohdich *et al*., 2002; Potchter *et al*., 2008; Calin *et al*., 2011; Carbonell *et al*., 2012; Lal *et al*., 2018a, b; Lal *et al*., 2020d; Kumar *et al*., 2021) [3, 4, 16, 21, 22, 29, 30]. Belancic *et al*. (1997) [1] also affirmed that intercropping exposure enhances the accumulation of terpenoids in the pomegranate fruits, while too high temperatures can have inverse effects on both crops.

Fig 1: Pomegranate + Lemongrass, pomegranate at flowering stage and harvested lemongrass

Fig 2: Pomegranate at fruiting stage intercropped with lemongrass

Fig 3: Lemongrass high essential oil yielding variety CIMAP - Suwarna

Table 1: Initial growth of pomegranate and fresh and oil yield of lemongrass (Lg) on green basis in I[st] cut after four months of planting

Treat	Ht (m)	CD (cm)	EW (m)	NS (m)	Ft. No.	Lg fresh (kg/56 m^2)	Lg fresh (t/ha)	Oil on green basis (ml/100g)	Oil on green basis lit/ha
T1V1	2.39*	6.24*	1.52*	1.55*	21.42*	2.27	0.405	1.1	4.455
T1V2	2.28	4.36	1.46	1.45	15.65	2.82*	0.504*	1.1	5.544*
T2V1	2.52*	5.80*	1.65*	1.68*	21.33*	0.93	0.166	1.0	1.660
T2V2	2.08	3.98	1.42	1.45	14.25	1.42*	0.254*	1.0	2.540*
T3V1	2.19	4.61*	1.46*	1.44*	24.17*	2.25*	0.402*	1.07	4.301*
T3V2	2.23*	4.59	1.44	1.40	20.75	2.20	0.393	1.07	4.205
T4V1	2.24*	5.12*	1.55*	1.52*	21.17*	-	-	-	-
T4V2	2.08	4.29	1.40	1.47	17.17	-	-	-	-
T5 (Lg pure)	-	-	-	-	-	2.05	0.366	1.20	4.392

*High value; T_1V_1 - cv. Ganesh with 30 kg vermicompost, T_1V_2 - cv. Bhagwa with 30 kg vermicompost, T_2V_1 - cv. Ganesh with 30 kg FYM, T_2V_2 - cv. Bhagwa with 30 kg FYM, T_3V_1 - T_1 + T_2, T_3V_2 - T_1 + T_2, T_4V_1 - cv. Ganesh with RDCF, T_4V_2 - cv. Bhagwa with RDCF and T_5 - Pure lemongrass (control).

Table 2: Effect of treatments on growth and yield of pomegranate and green and oil yield of lemongrass (Lg) on green basis during 2017

Treat	Ht (m)	CD (cm)	EW (m)	NS (m)	Ft. yield (kg/pl)	Lg fresh (t/ha)	Oil on green basis kg/ha
T1V1	2.95*	7.33*	1.82*	1.87*	4.64*	10.15*	49.15*
T1V2	2.60	5.28	1.79	1.68	3.00	5.14	26.54
T2V1	3.25*	7.14*	2.05*	1.99*	5.62*	7.15*	33.54
T2V2	2.09	4.69	1.36	1.43	2.85	6.73	35.71*
T3V1	2.74*	5.47*	1.78*	1.67*	5.34*	6.73*	32.32
T3V2	2.45	5.24	1.53	1.52	4.05	9.48	44.71*
T4V1	3.10*	6.79*	1.82*	1.80*	4.54*	-	-
T4V2	2.19	5.46	1.52	1.67	3.82	-	-
T5 (Lg pure)	-	-	-	-	-	11.68	131.18

T_1V_1 - cv. Ganesh with 30 kg vermicompost, T_1V_2 - cv. Bhagwa with 30 kg vermicompost, T_2V_1 - cv. Ganesh with 30 kg FYM, T_2V_2 - cv. Bhagwa with 30 kg FYM, T_3V_1 - T_1 + T_2, T_3V_2 - T_1 + T_2, T_4V_1 - cv. Ganesh with RDCF, T_4V_2 - cv. Bhagwa with RDCF and T_5 - Pure lemongrass (control).

Table 3: Pooled analysis of variance (ANOVA) for varieties and manures combinations

Source of variation	d.f.	Mean sum of squares		
		Fresh herb yield (t/ha)	Essential oil yield/ha	Significance
Replications	2	0.82	40.54	
Varieties (A)	1	17.40	4633.66	**
Manures combinations (B)	2	19.60	822.79	**
A×B	2	11.16	709.11	**
Error	10	2.46	94.65	
Total	17			

**-p<0.01

Table 4: The correlations among different soil and treatments factors

	De-hydrog-enase	NAG	Acidic phosph atase	B-GLU	N (kg/h)	P (kg/h)	Organic carbon %	PH	EC	FUME	Lg fresh (t/ha)	Oil on green basis lit/ha	Lg Fresh (t/ha)	Oil on green basis kg/ha
Dehydrogenase	-	-0.216	0.081	0.668	-0.312	0.169	-0.288	-0.411	-0.397	0.315	0.252	0.237	-0.575	-0.225
NAG		-	0.383	-0.135	-0.282	0.617*	0.712*	-0.672*	0.863*	-0.709*	-0.004	-0.026	-0.214	-0.361
Acidic phosphatase			-	0.642*	-0.402	-0.211	0.423	-0.480	0.098	-0.230	-0.151	-0.148	-0.154	-0.109
B-GLU				-	-0.654*	-0.219	-0.159	-0.281	-0.498	0.361	-0.270	-0.302	-0.762	-0.294
N (kg/h)					-	-0.133	-0.328	0.233	-0.010	0.198	0.266	0.370	0.914**	0.865*
P (kg/h)						-	0.110	-0.345	0.453	-0.321	0.045	0.042	-0.044	-0.276
Organic carbon %							-	-0.552	0.842*	-0.937**	0.405	0.338	-0.347	-0.472
PH								-	-0.561	0.448	-0.360	-0.344	0.478	0.274
EC									-	-0.874*	0.333	0.300	0.004	-0.286
FUME										-	-0.519	-0.466	0.174	0.387
Lg Fresh (t/ha)											-	0.990**	0.004	0.036
Oil on green basis lit/ha												-	0.104	0.169
Lg Fresh (t/ha)													-	0.809*
Oil on green basis kg/ha														-

* - P<0.05 and ** - P<0.01 level of significance

4. Conclusion

A significant variation in pomegranate fruit composition was noted following the variation of the orchard microclimate. The important highly significant and positive correlations were also recorded between the lemongrass fresh herb yield (t/ha) and the essential oil yield of the lemongrass. The selection of one factor will certainly affect other dependent factors positively or negatively. The weak positively and negatively correlations were also recorded between all studied factors. The intercropping containing different varieties and manures combinations were separated from those with two crops and mono - varietal orchards. The fruits from full shade oases of Ganesh and Bhagwa and intercropped lemongrass variety Krishna showed an attractive yield with the red colour of arils and a reduction in total sugar concentrations. Hexanal and limonene were showed to be responsible for a typical aroma profile of fruits grown under a full shade oasis system. Pomegranate arils from such an intercropping system were found to be the most appreciated by consumers in terms of colour, aroma, and taste. However, full sun conditions of trees grown outside the intercropping system harmed titratable acidity and the satisfaction degree of consumers for sensory attributes such as aril colour, aroma, taste, and overall quality, but they improved the synthesis of malic acid. The special oasis ecosystem should be safeguarded as a heritage of global importance and considered to improve sensory traits related to taste perception. These results extended earlier studies suggesting that the environment conditioned pomegranate fruit quality. Further studies will be required to grasp the complex factors underlying quality in pomegranate in or outside the oasis. Typical intercropping with lemongrass, pomegranate properties could be used for its labeling as a geographical indication (GI) or protected designation of origin (PDO). The correlations in the intercropped varieties of pomegranate and lemongrass, irrigated water, soil and manure factors, elevated moisture in the soil, humidity, temperatures due to intercropping impaired the expression of high yield of essential oil of the lemongrass, and also the production of the fruits of the pomegranate.

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8. Credit authors statements

AKK was involved in planning, actual experimentation, statistical analyses, manuscript preparation; MK experimentation; AM, data collection, distillation; samples preparation for chemical analysis; RKL - statistical analyses, manuscript preparation.

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