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Impact of wastewater on lemongrass (*Cymbopogon flexuosus*) essential oil yield and quality

Diana Dhayal*and Khajanchi Lal
ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India
**Email: dianadhayal@gmail.com*

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ABSTRACT

Lemongrass (Cymbopogon flexuosus) essential oil yield and quality were evaluated in a field experiment conducted at ICAR-Indian Agricultural Research Institute (IARI) to evaluate the effects of groundwater (GW), untreated wastewater (WW), constructed wetland treated wastewater (TWW) and groundwater in conjunction with untreated wastewater in cyclic mode (CW) in the main plots and three levels of fertilizers in subplots viz. control (no NPK fertilizer application)(N_0), the recommended dose of NPK fertilizers – the amount of NPK added through irrigation water (N_1) and recommended dose of NPK fertilizers (N_2) during 2018-2019.The trial was arranged in a split-plot design with three replications.The total oil yield from three harvests was the maximum (546 L ha^{-1}) in the case of wastewater irrigation and the minimum in the case of groundwater (476 L ha^{-1}). Irrigation with untreated wastewater resulted in significant increase of 11 and 16% in essential oil yield compared to treated wastewater and groundwater irrigation, respectively. The total oil yield from alternative irrigation with wastewater and groundwater was equal to that obtained in the case of irrigation with treated or untreated wastewater. The neral (neral is an isomer of citral i.e. the major compound in the lemongrass oil) and geranial contents obtained from groundwater and treated wastewater irrigation were 11-14% higher compared to alternative irrigations with untreated sewage and groundwater. Overall results indicated that the wastewater can be used for growing lemongrass aromatic crop to produce essential oils without causing any reduction in quantity and quality.

Keywords: Aromatic, citral, essential oil yield, sewage, wastewater

INTRODUCTION

With increasing population, irrigation water demand is expected to increase from 688 BCM in 2010 to 1072 by 2050. It will result in a reduction of the fresh water availability for agricultural use predicted from 83% in 1998 to 67% by 2050, (Gupta and Deshpande, 2004). Therefore, alternate water sources for agriculture is the need of hour. It is expected that by the year 2051, urban wastewater generation might cross 120,000 MLD and that rural India would also generate 50,000 MLD in view of water supply designs for community supplies in rural areas (Bhardwaj, 2005). The concentration of nutrients in wastewater irrigation by 1,000 m^3 per hectare was found to vary considerably: 4-24 kg phosphorus, 16-62 kg total nitrogen, 2-69 kg potassium, 27-182 kg sodium, 9-110 kg magnesium and 18-208 kg calcium (Qadir, 2011).

The use of wastewater for irrigation is relatively safe and considered to be a low-cost wastewater disposal strategy. This technology involves the conservation of water, water supply supplementation for irrigation and the use of nutrients present in the wastewater for productive purposes (Lopez *et al*., 2006). Cultivation of crops with non-edible economic parts like aromatic grasses, cut flowers, etc. has been proposed as a remunerative and a viable option for preventing pollutants entry in food chain (Lal *et al*., 2008a, b). Lemongrass (*Cymbopogon flexuosus*), being a perennial aromatic sedge and leading to huge biomass production, is widely cultivated for its essential oil (Zheljazkov *et al*., 2011). The essential oil is distilled from the foliar leaves of the lemongrass. The herbage on an average contains 0.2- 0.4% oil and the oil yield is 100-120 kg/ha/ year. Oil of lemongrass is a viscous liquid, yellow to dark yellow or dark amber in colour turning red with age. Lemongrass oil is widely being used in cosmetics, perfumes, soaps, detergents and insect repellents. India being the largest producer (300–350 tonnes $annum^{-1}$) of lemongrass oil, exports 80%

of it (National Horticulture Board, Govt. of India, 2005). The lemongrass plant is hardy and flourishes in a wide variety of soil ranging from rich loam type of soils to poor laterite. There has been a growing gap in the global production and demand of lemongrass oil (3900 metric tonnes; Barbosa *et al.*, 2008). Hence, to meet the demand of this industrial crop, expansion of its production to wastewater irrigated lands seems to be a sustainable option. The yield of lemongrass may also be affected by nutrients, salts, pathogens, heavy metals and other pollutants present in wastewater. The magnitude of variation in lemongrass essential oil quality and yield may also be affected by the extent of wastewater treatment or when wastewater is used in conjunction with good quality groundwater. The information on the relative essential oil yield and quality of lemongrass produced with treated and untreated wastewater is not adequate. Therefore, a study was formulated to know the effects of wastewater both treated and untreated on essential oil yield and quality of lemongrass.

MATERIALS AND METHODS

Study site

The study was carried out in the experimental field having latitude of 28°38’21.3" N and longitude of 77°08’56.5" E at an elevation of 230 m above mean sea level located near to a sewage drain covering an area of 150 m^2 inside the ICAR-Indian Agricultural Research Institute (IARI) (Fig. 1).The climate of the study area is semi-arid monsoonal subtropical type. The mean annual temperature (°C) of the study area is 24°C and the mean annual rainfall is 769 mm.

Experimental wastewater treatment framework

The treatment system consisted of wetland in form of 18-treatment cells (or mesocosms) using 500 litre syntax tanks with vertical sub-surface flow (VSSF). Each mesocosm had a 0.20 m base layer which was made of fine gravel with 38% porosity and having a diameter of around 1.5cm overlain by a center layer 0.30 m high consisting of coarse sand. All the mesocosms were painted white to avoid excessive heating. The mesocosms were connected to the main (sewage water) influent discharge line maintaining the maximum hydraulic water head of 16.3 cm. The untreated wastewater was made to pass through a screen and was then collected in a sump (2.36 x 0.68 x 0.762 m^3) before pumping it into the individual mesocosms.

Only 12-mesocosms were then used for planting four replicates of three emergent macrophytes *viz. Typha latifolia* (Cattail), *Phragmites karka* (Reed), *Acorus calamus* (Vachh). The remaining 6-mesocosms were left un-vegetated. Then before the commencement of the experiment, plants in the vegetated mesocosms were allowed to grow and multiply with periodic application of wastewater as a source of nutrients in order to form a dense stand.

Intermittent flooding was done in all mesocosms up to a maximum depth of 16.3 cm with the wastewater during the experimental period (August 2018 to April 2019), thrice in a month. The treated effluent from each mesocosm was collected and stored treatment wise in 500 litre capacity tanks.

Micro-plot experimental field

The experimental field consisted of 36 micro-plots, each of size 1.8 × 1.5m. Root slips of lemon grass (*Cymbopogon flexuosus*)- var. Krishna were transplanted (15 slips per plot) at a spacing of 60 x 30cm on 6th September 2017. The crop was established for first year with uniform inputs using groundwater for irrigation. From September 2018 onwards, the crop was fertilized and irrigated as per treatment. Split plot design was used for experiment with three replications. A total number of 12 treatments were given which consisted of combinations of; (A) Different types of irrigation water in main-plots, *viz.* (i) groundwater (GW), (ii) untreated wastewater (WW), (iii) constructed wetland treated wastewater (TWW) and (iv) groundwater in conjunction with untreated wastewater in cyclic mode (CW) (B) three levels of fertilizers in subplots *viz.* (i) N_0: control (no NPK fertilizer application) (ii) N_1: It is the amount of N, P and K fertilizers added after subtracting the present amount of N, P and K in wastewater used for irrigation from the recommended doses of N, P and K (iii) N_2: It is the amount of N, P and K fertilizers added as per the recommended doses. Groundwater, treated wastewater and untreated wastewater used for irrigation were marginally saline and neutral in reaction. The N, P, K contents in untreated wastewater were significantly higher

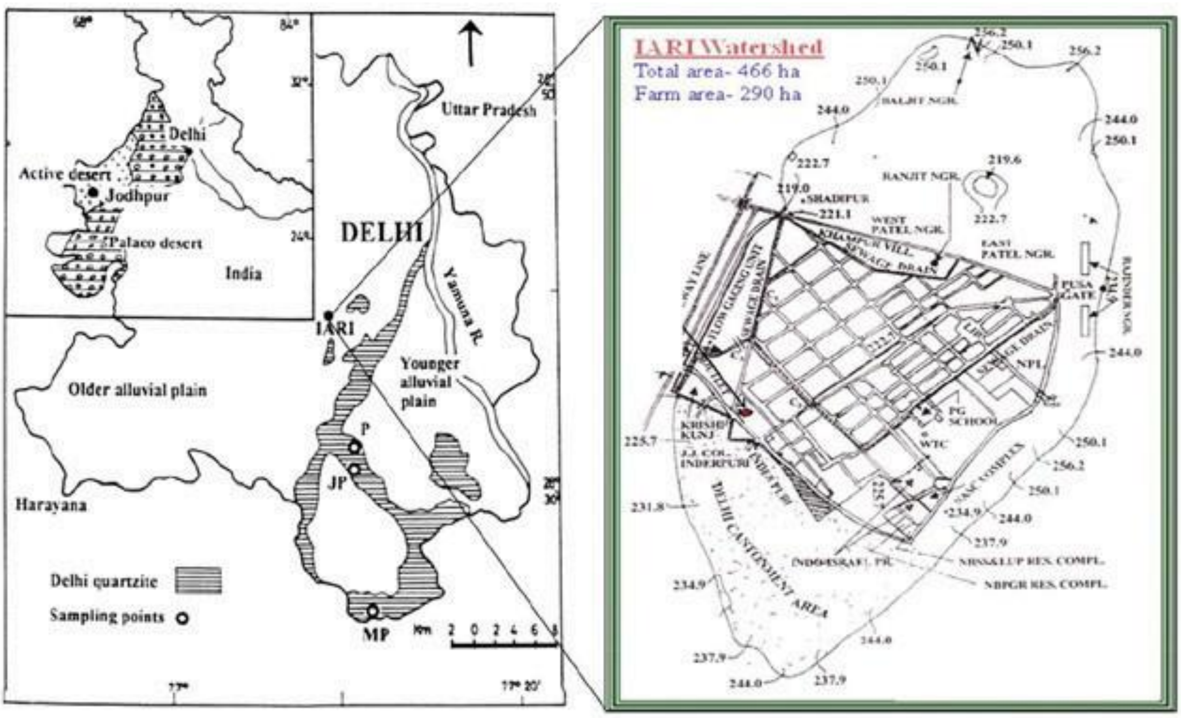

Fig.1 : Project site located within IARI micro-watershed

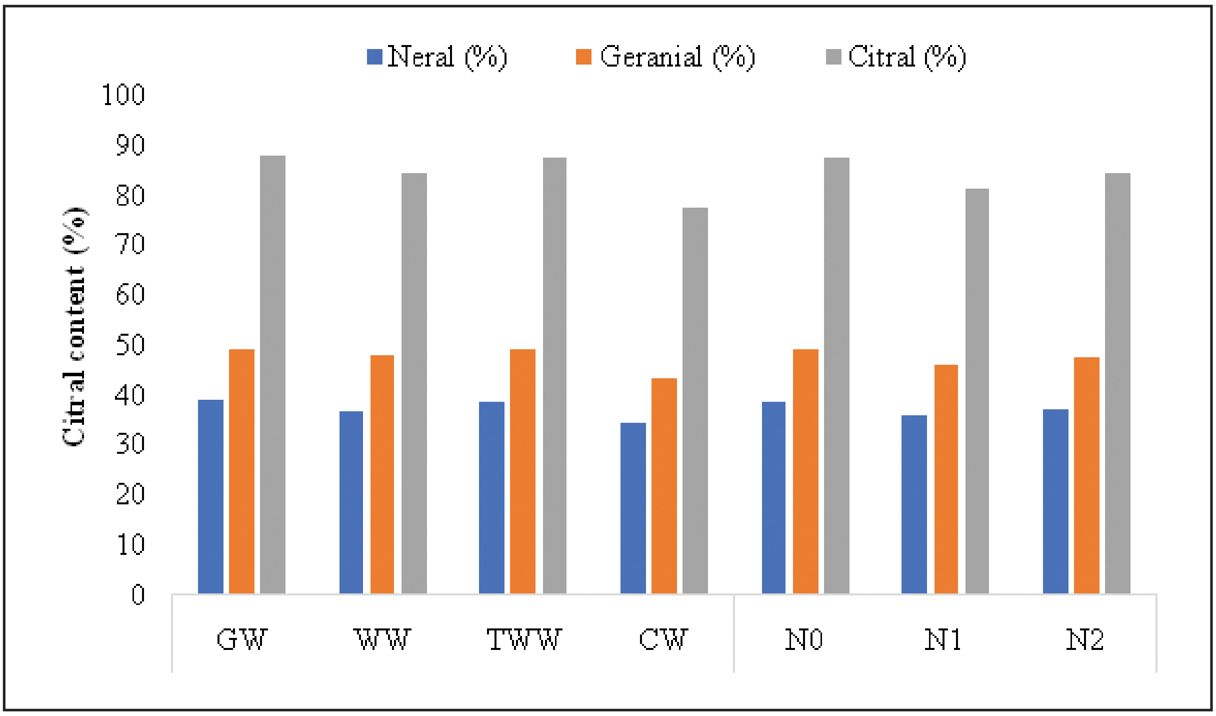

Fig. 2: Citral content (%) in lemongrass essential oil

GW: Groundwater, WW: Untreated wastewater, TWW: Treated wastewater, CW: Conjunctive water, N_0: Control, N_1: Adjusted doses of NPK fertilizers, N_2: Recommended dose of NPK fertilizers and 2.3, 3.7 and 2.7 times more than the constructed wetland treated water (7.5, 1.06 and 5.4 mg L^{-1}), respectively. The fertilizers N, P_2O_5 and K_2O had recommended doses of 150, 60 and 60 kg ha^{-1}. One fourth of N and total P and K were applied as basal dose while the rest of N was top-dressed in three equal splits at 30 and 60 and 90 days after imposition of the treatments. Considering the irrigation frequency of once in 20 days, total 10 irrigations were applied from September to April.

The depth of irrigation was kept 5 cm which required 135 litres of water per irrigation in each subplot. For application of treated wastewater (TWW), 34 litres of treated wastewater obtained separately from mesocosms planted with *Typha latifolia* (Cattail), *Phragmites karka* (Reed), *Acorus calamus* (Vachh) and without vegetation were collected, mixed and applied with the help of 20 litre capacity buckets. In case of conjunctive wastewater irrigation, groundwater and untreated wastewater were applied alternately in a cyclic mode beginning with groundwater irrigation. Hand weeding was also carried out intermittently during the research duration.

Herbage and essential oil yield

During the study period, from each plot the crop was harvested about 10 cm above the ground three times on 15th November 2018, 26th February 2019 and 29th April 2019. The plot wise harvested biomass was weighed and converted into herbage yield per hectare.

To determine essential oil content, a known weight of fresh herbs W_1 (about 250 g) was taken and subjected to steam distillation using Clevenger apparatus and recorded the volume of oil (ml) from the Clevenger burette. The essential oil content (%) on fresh herbage basis was found as given below (equation 1):

$$Oil\ content\left(\%\right)=\frac{Volume\ of\ oil\,(ml)}{W_1(g)}\times 100 \quad ..\ (1)$$

The cut wise essential oil yield was calculated as follows (equation 2):

$$Oil\ \ yield\left(litre\ ha^{-1}\right)=\frac{Volume\ of\ oil\ \left(ml\right)\times 1000\times herbage\ yield\,(Mg\ ha^{-1})}{W_1(g)} \quad ....(2)$$

The cut wise essential oil yields obtained were summed to find essential oil yields per hectare from all the three cuts.

Composition of essential oil

Atomic absorption spectrophotometer was used for the analysis of Zn, Cu, Fe, Mn, Cr, Ni, Pb and Cd contents in the lemongrass essential oil samples. The essential oil collected in the vial from the Clevenger burette was added with a pinch of sodium sulphate to remove water from the oil if any. Further the oil was filtered and the samples were diluted by hexane (5 micro Litres oil in 2 mL hexane) for composition analysis by using Gas Chromatography - Mass Spectrometry (GC-MS). A HP-5MS column (30 m × 0.25 mm;/0.25 m, Thermo Co., USA) was used for GC-MS analysis, which was directly connected to a triple axis mass spectrometer (Thermo Fisher, USA). The injection volume was 1 micro litre with flow mode in split control. Helium (High purity, New Delhi, India) was used as carrier gas at a head pressure of 10 psi and gas flow was set at 1 ml min^{-1}. Below describes the GC-MS condition: Initially, the oven temperature was held at 40°C for 1 minute. Thereafter, the temperature was allowed to rise at a rate of 3°C min^{-1} until it reached to 120°C and was held for 2 minutes. The temperature was again increased at a rate of 5°C min^{-1} up to 220°C and was held for 1 minute. Then finally, the temperature was raised to 280°C at a rate of 4°C min^{-1}. Total time taken was 65 minutes. The MS acquisition parameters were as follows: ion source 180°C, electron ionization 70 eV, full scan mode (50–550 mass units), transfer line temperature 280°C, solvent delay 3 min, and E.M voltage 1376. The ionization energy was 70 eV with a scan time of 1 s and mass range of 50–550 AMU. Compounds were identified by matching their mass spectra. NIST (National Institute of Standards and Technologies) Mass Spectra Library was used as a reference for identifying the essential components.

Statistical analysis

Each parameter data was subjected to a two-way ANOVA analysis (Gomez and Gomez, 1984) with separation of means. All tests of significance were performed at the level of 5% probability.

RESULTS AND DISCUSSION

Herbage yield

The herbage yield of lemongrass was found to be varying from 20.61 to 24.75 Mg ha^{-1} in first cut, 18.27-21.96 Mg ha^{-1} in second cut and 15.48-17.84 Mg ha^{-1} in third cut (Table 1). The total herbage yield achieved from all the three cuts was the maximum (63.45 Mg ha^{-1}) when irrigated with untreated wastewater followed by conjunctive use of wastewater (59.85 Mg ha^{-1}), treated wastewater (58.95 Mg ha^{-1}) and the minimum in case of groundwater irrigation (55.26 Mg ha^{-1}). Compared to groundwater, irrigation with untreated wastewater resulted in a significant increase of 15% in the crop biomass. Soil irrigated with treated wastewater or alternatively with sewage and groundwater yielded statistically at par with untreated wastewater or groundwater irrigation.

Similarly, use of recommended or adjusted doses of N, P and K produced significantly higher total and cut wise crop biomass compared to control (no fertilizer application). The difference in crop biomass obtained with the application of recommended fertilizer doses and doses adjusted due to supply of nutrient from irrigation was not significant.

Content, yield and quality of lemongrass essential oil

The essential oil content and yield obtained from the fresh biomass of lemongrass in three harvesting are presented in Table 2 and 3. In case of first cut, oil content obtained by the herbage harvested from the differentially irrigated plots ranged from 0.75 to 0.81 % with the mean values varying from 0.77 to 0.78% only. Similar values of essential oil content were also recorded in case of second and third harvests. No significant differences were found in the oil content obtained from the differentially irrigated plots in all three cuts. Also, there were no significant differences noted in the oil content of all three cuttings with different nutrient dose treatments.

Mean values of essential oil yield obtained in the first cut varied from 184 to 212 L ha^{-1}. The maximum oil yield was obtained from crop growing in wastewater irrigated plots and the minimum in case of groundwater usage. Oil yield obtained from the crop irrigated with wastewater was 15% higher than that obtained with ground water irrigation. In case of second cut, essential oil yield ranged from 157 to 179 L ha^{-1}. Similar to the first cut, in second cut also untreated wastewater irrigation produced the maximum essential oil yield whereas the minimum (156.84 L ha^{-1}) in case of groundwater irrigation. In second cut, essential oil yield obtained with untreated wastewater irrigation was found significantly higher compared to groundwater and treated wastewater irrigation but at par with conjunctive wastewater use. Similar trend was observed in third cut also.

The total oil yield obtained from three harvests was the maximum (546 L ha^{-1}) in case of wastewater irrigation and the minimum in case of groundwater (476 L ha^{-1}). Irrigation with untreated wastewater resulted in significant increases of 11 and 16% in essential oil yield compared to treated wastewater and groundwater irrigation, respectively. Compared to no fertilizer application, application of recommended or adjusted dose of fertilizers resulted in significantly higher production of cut wise and total essential oil yield. No interaction effects in the herbage and oil yield were observed amongst all the treatments of the experimental study.

For testing the quality of lemongrass essential oil, the diluted samples were aspirated in atomic absorption spectrophotometer for the estimation of Zn, Cu, Mn, Fe, Cd, Cr, Ni and Pb. The results showed absence of heavy metals in the essential oil of lemongrass. Component analysis of the lemongrass oil by GC-MS resulted in a number of chemical compounds (Premathilake *et al*., 2018 and Matasyoh *et al*., 2007). Citral or 3, 7-dimethyl-2,6-octadienal (both E- and Z-isomer) was the major compound in the lemongrass oil.

Citral has two isomers- the E-isomer which is also known as geranial or citral A and the Z-isomer, which is also known as neral or citral B. It was observed that the maximum neral content was present in the oil extracted from the plants irrigated with groundwater and the minimum was found with conjunctive use of water (Fig. 2). The neral content obtained from groundwater usage and alternative irrigations with untreated sewage and groundwater was 14% higher than that of groundwater usage. The neral content obtained from groundwater and treated wastewater were almost the same. Geranial content in the essential oil obtained from groundwater, treated wastewater and untreated

Table 1: Herbage yield (Mg ha^{-1}) obtained from three cuts and their total from the plots irrigated with different types of irrigation water and nutrient levels.

Water quality	Herbage yield (Mg ha^{-1}))			
	First cut	Second cut	Third cut	Total
GW	20.6	18.3	15.5	55.2
WW	24.7	21.9	17.8	63.4
TWW	22.1	19.7	15.8	58.9
CW	22.8	20.2	17.2	59.8
CD (5%)	2.7	2.2	1.6	5.1
Nutrient Doses				
N_0	20.4	18.4	14.9	54.8
N_1	23.0	20.2	17.2	60.3
N_2	24.3	21.4	17.5	63.8
CD (5%)	1.98	1.35	1.35	2.79

GW: Groundwater, WW: Untreated wastewater, TWW: Treated wastewater, CW: Conjunctive water, N_0: Control, N_1: Adjusted doses of NPK fertilizers, N_2: Recommended dose of NPK fertilizers. Mg ha^{-1}: It is Megagram per hectare. Megagram is equal to 10^3 kg.

Table 2: Oil content (%) in above ground biomass of lemongrass

Water quality	Oil content (%)		
	First cut	Second cut	Third cut
GW	0.78	0.76	0.78
WW	0.78	0.75	0.78
TWW	0.78	0.72	0.77
CW	0.77	0.76	0.77
CD (5%)	NS	NS	NS
Nutrient Doses			
N_0	0.78	0.75	0.78
N_1	0.77	0.74	0.78
N_2	0.78	0.75	0.78
CD (5%)	NS	NS	NS

GW: Groundwater, WW: Untreated wastewater, TWW: Treated wastewater, CW: Conjunctive water, N_0: Control, N_1: Adjusted doses of NPK fertilizers, N_2: Recommended dose of NPK fertilizers

wastewater usage were more or less the same but 11 to 13% higher than in the cyclic mode of groundwater and untreated wastewater usage. Similarly, in case of nutrient treatments, maximum neral and geranial content was obtained with control followed by recommended doses and then N_1.

Citral (neral + geranial) content also followed the similar trend and was found the maximum in the oil extracted from plants irrigated with groundwater and from N_0 in case of nutrient dose treatments.

The changes in essential oil contents with changing quality of irrigation water were non-significant. Significant improvement in essential oil yield were mainly ascribed to the increase in herbage yield produced with untreated wastewater irrigation either alone or in conjunction with ground water. Increased soil organic carbon and improved soil fertility with the addition of substantial amount of essential plant nutrients supplied through sole or conjunctive use of untreated wastewater irrigation could have produced higher crop yields compared to ground water irrigation (Lopez *et al.*, 2006; Lal *et al.*, 2013). Compared to the groundwater irrigation an increase of 14-15% in total essential oil yield of lemongrass with sole use

Table 3: Oil yield (L ha^{-1}) in above ground biomass of lemongrass

Water quality	Oil yield (L ha^{-1})			
	First cut	**Second cut**	**Third cut**	**Total**
GW	184	157	135	476
WW	212	179	155	546
TWW	194	158	142	494
CW	193	171	145	508
CD (5%)	18	17	13	47
Nutrient Doses				
N_0	183	153	131	468
N_1	196	169	147	511
N_2	208	176	155	539
CD (5%)	14	11	12	29

GW: Groundwater, WW: Untreated wastewater, TWW: Treated wastewater, CW: Conjunctive water, N_0: Control, N_1: Adjusted doses of NPK fertilizers, N_2: Recommended dose of NPK fertilizers

of wastewater or in conjunctive mode with groundwater was also noticed by Lal *et al*. (2013). Higher yields of lemongrass essential oil with wastewater were also recorded by other workers (Singh, 1998 and Darvishi *et al*., 2010). Compared to sole use of untreated wastewater, half amount of essential plant nutrients was supplied through wastewater irrigation in case of conjunctive use of wastewater. Non-significant difference in herbage yield of lemongrass irrigated solely with wastewater or in combination with groundwater indicated that amount of essential plant nutrients added were sufficient enough for meeting crop requirement (Anwar *et al*., 2010). Nutrients contained in wastewater get recycled when used for irrigation, thus not only saving fertilizers but also improving soil fertility. Sum of amount of nutrients supplied through irrigation and adjusted amount of nutrient added through fertilizer equals to the recommended nutrient doses, which would have been sufficient enough to meet the crop nutrient requirement.Therefore, the differences in yield obtained with adjusted and recommended nutrient doses were not found significant. Kumar *et al*. (2017) also observed that application of 100 % recommended dose of nitrogen along with groundwater irrigation produced herb and oil yield of mentha statistically at par with that obtained by applying 50% recommended dose of nitrogen under wastewater irrigation. Nutrients supplied through irrigation along with application of recommended nutrient doses through fertilizer would have resulted in overdoses of nutrients. Overdoses of nutrients added did not result in significant increase in plant nutrient content or not utilized for photosynthate formation. Simultaneous accumulations of pollutants in soil can lead to environmental issues when overdosing of nutrients along with its low use efficiency takes place and later may lead to leaching in groundwater.

CONCLUSIONS

The overall experimental evidences show the potential of cultivating the aromatic lemongrass with untreated wastewater which will protect the fresh water reserves. The total oil yield from three harvests was the maximum in case of untreated wastewater irrigation and the minimum in case of groundwater. Irrigation with untreated wastewater resulted in significant increases of 11 and 16% in essential oil compared to treated wastewater and groundwater irrigation, respectively. Compared to no fertilizer application, application of recommended or adjusted dose of nutrients (recommended nutrient doses - doses supplied by wastewater) resulted in significantly higher total herbage and essential oil yield. Lemongrass essential oil mainly contained citral in which neral accounted for 34-39% and geranial 43-49%. The neral and geranial contents obtained from groundwater and treated wastewater irrigation were 11-14% higher compared to alternative irrigations with untreated sewage and groundwater.

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