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Vetiver (*Vetiveria zizanioides* L.) Yield and its water use efficiency affected by different plant populations under reclaimed soil conditions

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Abstract
Vetiver (*Vetiveria zizanioides*) is one of the promising plants as raw materials in perfumery industry but it has not been widely introduced in Egypt. Where irrigation and plant population density are important factors in plant growth, a field experiment was conducted at the Experimental Farm of South El-Tahrir, El- Behira governorate, Horticulture Research Institute during the two successive seasons of 2017 and 2018 to establish optimal irrigation rate and plant population densities for vetiver in sandy soils under drip irrigation system using split plot design with three replications. The treatments of three irrigation rates were I1= 1.2, I2= 1.0, and I3= 0.80 of evapotranspiration (ETo), and three plant population densities (D1= 25,000, D2= 38,000 and D3= 50,000 plants /fed), where irrigation treatments were assigned in the main plot and densities were assigned in the split plot. Results indicated that vetiver yield, yield components, and water use efficiency (WUE) increased with I2 and increasing plant population densities. Significant interaction effects between irrigation rate and plant population density were detected in both seasons for yield components, and WUE. The highest growth vegetative and yield parameters, plant height, number of tillers/plant, root length, fresh and dry weight/ plant and /fed. and WUE were obtained from plants irrigated with I1, while the highest values from root length and oil percentage were found in I2. The maximum proline value was obtained under I3. On the other hand, plant density at D2 resulted the highest value from number of tillers/plant, root length, fresh and dry weights per plant and oil percentage. High density at D3 gave the highest root length. While the highest value of proline came from D1 in both seasons.

Keywords: Vetiver, water consumptive use, water use efficiency, planting density, essential oil

Introduction
Vetiver (*Vetiveria zizanioides* L.) Nash Syn. *Chrysopogon zizanioides*) is an eco-friendly plant, and it is the fastest growing perennial tussock grass of the Poaceae (Gramineae) family (Maffei, 2002) [42]. It can prevent soil erosion and it is also helpful in the rehabilitation of metal-polluted soils (Pripdeevech *et al.,* 2006) [49]. In addition, it is an evergreen, gramineous, and perennial herb, and its appearance is similar to that of lemongrass. Under normal circumstances, the height of vetiver can reach up to 1.0 m or more. Furthermore, vetiver grass has short rhizomes and a massive, finely structured root system that grows very quick. It has been reported to grow to a depth as much as ˜4 m (Truong, 2002) [61]. This deep root system makes the vetiver plant extremely drought tolerant and very difficult to dislodge when exposed to a strong water flow (Hengchaovanich, 1998; *Truong et al*., 1995) [30, 62]. Also, the vetiver plants has been cultivated for many industrial applications, including the production of a commercially and medically valued essential oil called vetiver oil, which can be distilled from the roots. The essential oil has potent antioxidant, anti-inflammatory, antimicrobial, and antifungal activities (Chou *et al*., 2012; Kim *et al*., 2005; Luqman *et al*., 2005) [18, 37, 41]. Moreover, the oil has commonly been used as a functional ingredient and fragrance in foods, aromatic products, and cosmetics.
One of the most important factors that increases and exploits the productive potential of plants is the arrangement of plants, especially when combined with irrigation treatments. Plant density is an agronomic factor that manipulates micro environment of the field and affects growth, development and yield formation of crops as well as water use efficiency (Debaeke and Aboudrare, 2004) [20]. Planting density, which can be maintained by adjusting row spacing and plant spacing, is a strong determinant of yield in various crops, since there is no option for filling the space between plants by branching and tillering (Pereira *et al*., 2019) [47]; thus, a

suitable plant density may help in making full use of the entire natural resource and promoting harmonious development of crop populations and individuals. Researchers have indicated that differences in distance between rows and plants influenced the spatial distribution of roots and soil water contents (Cucci *et al*., 2017) [19]. Additionally, plant density can affect plant response to light by affecting the leaf area index. The higher the plant density, the greater is the received solar radiation for photosynthesis. However, with plant density increases, the competition for light between plant increases, which result in a decrease in vegetative and reproductive plasticity of plants (Soleymani., 2017) [57], while a lower plant density, results in a decreased absorption of light by the plants, as well as decrease in crop yield per unit area (Kemanian *et al*., 2004; Ali *et al*., 2013) [35, 5]. Theoretical research and practical experience have shown that higher number of tillers/plant in sole vetiver (30x60 cm) resulted in higher root yield/plant and there was no marked difference in essential oil content of vetiver root due to treatments (Mohd and Dash 2014) [44]. While increasing of the distance within rows from 50 to 75 cm significantly increased plant height, fresh and dry weights of herb per plan and oil yield/plant and feddan of lemongrass, maximum oil percentage was detected by the lowest planting density (Emad *et al*., 2017) [25].

On the other hand, the efficient use of irrigation water is flattering increasingly important in arid and semi-arid regions with limited water resources. Water is vital for plant growth and development; nevertheless, excessively little or too much water from rainfall or irrigation can limit yield potential. Water use by the plant is evaluated based on evapotranspiration rate, which is water removal by soil evaporation (E) and by plant transpiration (Allen *et al*., 1998) [6]. Determination of the correct irrigation level as affected by planting density can result in an efficient use of labor, land, and water resources. Moreover, Shabih *et al*., (2000) [55] indicated that, plant growth was reduced considerably while the level of essential oil was enhanced at both the densities 45x60 and 45x45 under water stress. Oil yield decreased at density 45 × 45 cm2 under water stress. The major oil constituent citronellal decreased significantly at density 45 × 60 cm2 while citronellol content, abscisic acid and IAA increased at both the densities under water stress. To improve water use efficiency, adopting a rational planting density is an effective agronomic measure (Debaeke and Aboudrare, 2004) [20]. Although vetiver is widely cultivated in tropical and sub-tropical regions, it is not commonly grown in arid and semi-arid regions, such as the Middle East and especially in Egypt. Limited studies have been conducted to determine the suitability of vetiver in the semi-arid regions. Therefore, the objective of this study was to assess the effect of plant population density on water use efficiency of vetiver plants grown using three irrigation levels to develop management practices under sandy soil conditions. The hypothesis tested was that increasing plant population density improves water use efficiency by reducing soil evaporation.

Materials and Methods

Experimental zone description

This study was conducted at the Experimental Farm of South El- Tahrir, El- Behira governorate, Horticulture Research Institute, during the two successive seasons of 2017 and 2018. It is situated at an altitude of 6.7 m above sea level and is intersected by 31°02′N and 30° 28′E. Detailed climatic parameters for the experimental site are given in Table 1. The soil of the experimental site is sandy. Different samples of soil at 30 cm depth were taken randomly for physical and chemical analyses and it were done at Soil & Water and Environmental Institute Lab. (A.R.C.) as described by Donald (1996) [22] (Table 2).

Table 1: Monthly climatic data at El- Behira governorate during the growing periods of vetiver in 2017 and 2018

Months	R		WS		RH		TMAX		TMIN		SRAD	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
March	0.3	1.1	3.0	2.6	60.0	50.9	23.2	26.8	11.0	11.9	19.8	20.3
April	0.0	5.5	2.9	2.8	54.9	48.6	26.9	29.3	12.5	14.2	23.7	23.9
May	0.0	0.0	3.1	3.2	49.7	47.4	31.5	33.5	16.9	18.7	27.2	25.8
June	12.7	0.0	3.1	3.2	47.3	45.7	35.3	35.4	20.2	21.0	29.6	29.3
July	14.7	4.0	3.2	3.4	50.0	51.6	37.2	36.4	22.6	22.5	29.1	29.1
August	0.0	0.0	2.9	3.1	53.0	54.4	35.9	35.9	23.1	23.1	26.9	26.9
September	0.0	0.0	2.9	3.0	54.6	55.1	34.2	34.4	20.6	21.9	23.6	23.1
October	30.5	3.3	2.9	2.9	58.7	57.0	29.0	30.6	17.7	19.3	18.8	18.8
November	71.4	14.9	2.9	2.3	56.1	61.1	29.0	25.6	16.0	15.4	22.6	10.4

Where: R: Rain (mm/month); WS: wind speed (m/sec); RH: Relative Humudity %; TMAX, TMIN: maximum and minimum temperatures °C; SRAD: solar radiation (cal/ cm2/ day). [Data were obtained from the agro meteorological Unit at SWERI, ARC].

Table 2: Physical and chemical analysis of the experimental soil

Soil characteristics	Values
Particle size distribution	
Coarse sand (%)	14.6
Fine sand (%)	74.1
Silt (%)	6.1
Clay	5.2
Soil texture	**Sandy soil**
Bulk density (Mg m^{-3})	1.65
Hydraulic conductivity (cm h^{-1})	34.3
$CaCO_3$ ($g.kg^{-1}$)	1.86
Organic matter ($g.kg^{-1}$)	0.21
EC (dSm^{-1})	2.24

Experimental design and treatments

The experiment was designed as a split plot in randomized complete blocks with three replications. It comprised six treatments: three water irrigation levels in the main plot and three vetiver populations in sub-plot. Irrigation treatments were applied with amount of water equals to 120% ETo (I1), 100% (I2) ETo, 80% ETo(I3); Planting density were: D1: planting in rows 40 cm apart (40x40 cm spacing =25000 plants/feddan or 6 plant/m^2), D2: planting in rows 30 cm apart (40x 30 cm spacing = 38000 plants/feddan or 9 plant/m^2) and D3 planting in rows 20 cm apart (40x20 cm spacing = 50000 plants/feddan or 12 plant/m^2).

Agronomic practices

Vetiver offsets (about 20 cm long) were planted on 22th March, 2017 and on 25th March, 2018 in the first and second growing seasons, respectively. Plants was fertilized with the recommended rate of chemical fertilizers, i.e. 450 kg of ammonium sulphate (20.5%N) /fed., 350 kg of calcium super phosphate (15.5% P_2O_5) /fed and 75 kg of potassium sulphate (48% K_2O) /fed. Calcium super sulphate was added before planting in only one dose, while ammonium sulphate and potassium sulphate were added in two equal doses after 45 and 60 days from planting.

Irrigation water was applied every three days using drip irrigation system. It was a surface drip including an irrigation pump (50 hp) connected to sand and screen filters he conveying pipeline system consists of a 63 mm PVC main line connected to 50.8 mm PVC sub-main line. The drip lateral lines of 16 mm diameter are connected to the sub-main line. Each lateral line is 20 m long and spaced at 0.4 m on the sub-main and is equipped with build-in emitters of 2 L/h discharge rate spaced at 0.3 m on the lateral dripper. Soil moisture content was gravimetrically determined in soil samples taken from consecutive depths of 15 cm down to 60 cm. Soil samples were collected before each irrigation, 48 hours after irrigation and at harvest time.

Parameter assessments

Hand harvesting was performed on 28th and 25th September in the first and second seasons, respectively. Vetiver height and the number of tillers were determined. Shoots of 10 plants from each plot were clipped at 10 cm with random above the soil surface, roots were excavated as completely as possible from each experimental unit and were washed gently in deionized water and measured for following data: Root length (cm) and diameter (mm), Root fresh weight (g)/ plant, root fresh weight (ton)/fed., Root dry weight (g)/ plant, Root dry weight (ton)/fed. Essential oil percentage was determined in fresh roots according to the method described by the British Pharmacopoeia (1963) [15]. Oil samples taken from the second season were analyzed using GC/MS as described by Amer *et al*., (2019a) [7]. Proline content (ppm) was analyzed as described by Bates *et al*. (1973) [13].

Water relations

Water consumptive use (WCU)

Crop water use was estimated by the method of soil moisture depletion according to Majumdar (2002) [43] as follows:

$$WCU = \sum_{i=1}^{i=4} \frac{\theta 2 - \theta 1}{100} \times Bd \; x \; d$$

Where: WCU = water consumptive use or crop evapotranspiration, ETc (mm), I = number of soil layer, θ2 = soil moisture content after irrigation, (%, by mass), θ1 = soil moisture content just before irrigation, (%, by mass), Bd = soil bulk density, (g/cm^3), d = depth of soil layer, (mm).

Applied irrigation water

The amounts of applied irrigation water were calculated according to the equation given by Vermeiren and Jopling (1984) [64] as follows:

$$AIW = \frac{ETo \times I}{Ea\,(1 - LR)}$$

Where: AIW = depth of applied irrigation water (mm), ETo = reference evapotranspiration (mm/day) calculated using BISm model, I= irrigation intervals (days), Ea = irrigation application efficiency of the drip irrigation system, LR= Leaching requirements

Water use efficiency (WUE)

Water use efficiency is calculated according to Stanhill (1986) [59] as: WUE=Yield (kg/fed.)/ET (m^3 water consumed/fed.). Where: Y = yield (kg/fed), WCU =Water consumed by the crop during the growing season (m^3/fed).

Statistical analysis

The data were subjected to analysis of variance split plot design (ANOVA) as published by Gomez and Gomez, (1984) [26], with irrigation rate treatments assigned as the main plot, plant population densities as sub plots and replicates as blocks. Means of the treatment were compared by the least significant difference (LSD) at 5% level of significance.

Results and Discussion

Effect of irrigation rate and plant population density on yield components

In order to determine the response of vetiver to plant density, the effects of different plant population density (PPD) under different levels of irrigation water on growth of the plants were determined. The yield components, namely plant height, number of tillers per plant, root length, root diameter, and weight of fresh and dry root per plant as well as per fed. Are presented in Tables 3 and 4. Both the irrigation rates and plant population density significantly affected all growth parameters. Data clearly indicated that low irrigation rate at I3 caused gradual decrease in most of the growth characters except root diameter.

Analysis of variance of the data for growth characters presented in Table 3 showed that in both seasons 2017 and 2018, the mean differences in plant height under different plant density were significant, the same were observed between the irrigation rates. High irrigation rate (I1) increased the plant height (up to 259.14 and 261.38 cm in both 2017 and 2018 seasons, respectively. Moreover, the lowest PPD (D1) showed the same trend of plant height increasing (255.72 and 256.83 cm) during both seasons, respectively.

In addition, in both seasons, the interaction between I3 and D3 demonstrated lowest values for vetiver plant height which gave 227.36 and 228.39 cm, respectively. While the interaction between I1 and D1 showed the highest values in 2017 and 2018 which stated 265.21 and 267.25 cm respectively. On the other hand, in first season, it was observed that the irrigated plant with I1 presented 63.95 tellers/ plant followed by 57.02 and 49.36 for I2 and I3, respectively. While, the plant density (D2) displayed the highest tillers number of 61.38 followed by D3 by 56.07 then

D1 by 52.88. The second season noticed the same trend. Furthermore, there was a significant ($P < 0.05$) difference in tillers number per plant irrigated by I1 interaction with D2 of density and those other treatments, which remarked the highest numbers of 68.40 and 69.42 cm for 2017 and 2018, respectively.

Meanwhile, there were no significant differences between root length (cm) under the treatments of D2 and D3. While the high rate of water irrigation at I1 revealed the maximum values of root length in both seasons. In addition, it was observed that the highest value of root length 58.14 cm obtained at first season under the treatment of I1xD2.

The data presented in the same table also evident that root diameter (mm) boosted under the irrigated of I3 up to 2.37 and 2.40 mm during 2017 and 2018, respectively. Additionally, increasing PPD to D3 exposed growing of root diameter up to 2.07 and 2.09 mm for 2017 and 2018, respectively. Likewise, the interaction between the plant density and irrigation rates treatment showed that, I3 with D3 gave the highest values of root diameter of 2.52 and 2.60 mm for both seasons.

Roots of vetiver fresh and dry weights (g/plant) were also influenced by irrigation rate (Table 4). In both seasons, 0.80 and 1.0 ETo consistently resulted in lower yields than the 1.2 ETo treatment. Average yield decreases for 0.80 and 1.0 ETo relative to 1.2 ET were 20.62 and 9.53% in 2017 and 17.35 and 12.15% in 2018, respectively. Fresh and dry weights (gm/plant) were also influenced by plant population density (Table 4). Root fresh weights were optimum of 458.20 and 458.62 g/plant under densities of D2 in both seasons 2017 and 2018, respectively. While densities of D3 recorded the optimum fresh weight of vetiver root (ton/fed) which of 21.87and 19.83ton/fed. for both seasons, respectively. Dry weight show the same trend of fresh weight for both seasons.

The interaction between irrigation rate and plant population density had a significant effect on fresh weight of vetiver root yield (ton/fed) with quite similar results in both seasons (Table 4). The combination of an irrigation rate of I1 with a density of D3 indicated as the treatment that maximizes fresh yield of vetiver root which of 24.28 or 23.96 ton/fed for 2017 and 2018 respectively using the drip irrigation system.

In this regard, the increments in vetiver yield and its components due to increasing irrigation water might be attributed to the beneficial effect of irrigation on growth and photosynthetic capacity. Consequently, more dry matter accumulated in yield components, which reflected on yield/fed, Moreover, increasing irrigation water amount enhances the ability of plants to effectively utilize the environmental resources. This in turns increases the amount of metabolites synthesized (by plant). Water is generally considered as one of the limiting factors, which affects the physiological and biochemical processes influencing crop productivity. Water is one of the important environmental factors in crops production, and development and effective materials of herbal plants. These results are in harmony with those of Abdel-Motagally (2010) [1], Beheshti and Fard (2010**)** [14]**,** Jahanzad *et al*., (2013) [33] and Afshar *et al*., (2014) [2]. Furthermore, the results indicated that plants subjected to water stress tend to yield components characters less than plants supplied with adequate water requirements. The significant difference in yield between the full irrigated plants and those stressed indicate that the imposed water stress caused a reduction in the physiological activities of the plant as such the plant could not achieve full growth potential which was exhibited in the significantly lower yield recorded. This is in tandem with results obtained by Warrick and Gardner, (1983) [65], Karlen and Camp, (1985) [34] and Averbeke and Marais (1992) [10], who all reported increasing yield with increase in irrigation water supplied. Moreover, planting 38000 plant/fed caused increments in all studied traits, except for root diameter per plant, plant height and fresh weight of root (ton)/fed. In both seasons. Plant density effects on yield components characters can attributable to soil type, fertility level, variety and other environmental conditions and that fertile soils require higher populations than poor soils, Widdicombe and Thelen, (2002) [67] showed increasing yield by increasing the plant population up to a certain limit usually above 70,000 plants/ha.

Table 3: Effect of irrigation rate and plant population density on yield components of vetiver (*Vetiveria zizanioides* L) in 2017 and 2018

Plant population density (plant fed^{-1})	2017				2018			
	I1	I2	I3	Mean	I1	I2	I3	Mean
1. Plant height (cm)								
D1	265.21	256.47	245.47	255.72	267.25	258.56	244.69	256.83
D2	259.98	249.71	236.45	248.71	262.01	251.55	235.87	249.81
D3	252.24	241.41	227.36	240.34	254.87	239.78	228.39	241.01
Mean	259.14	249.20	236.43		261.38	249.96	236.32	
LSD (0.05)	I 3.51	D 3.07	I x D 5.32		I 2.01	D 1.29	I x D 3.23	
2. Number of tillers/plant								
D1	59.33	53.50	45.81	52.88	64.00	52.91	47.97	54.96
D2	68.40	61.21	54.53	61.38	69.42	60.85	56.09	62.12
D3	64.11	56.35	47.75	56.07	65.32	56.62	46.00	55.98
Mean	63.95	57.02	49.36		66.25	56.79	50.02	
LSD (0.05)	I 0.53	D 0.82	I x D 1.43		I 0.79	D 1.37	I x D 2.37	
3. Root length (cm)								
D1	49.11	45.87	38.22	44.40	50.00	46.28	41.57	45.95
D2	58.14	56.28	51.99	55.47	57.25	56.84	48.36	54.15
D3	54.50	54.79	53.47	54.25	55.80	53.11	52.55	53.82
Mean	53.92	52.31	47.89		54.35	52.08	47.49	
LSD (0.05)	I 1.15	D 1.59	I x D 2.02		I 1.62	D 3.39	I x D 5.87	
4. Root diameter (mm)								
D1	1.35	1.75	2.20	1.77	1.37	1.76	2.19	1.77
D2	1.50	1.96	2.39	1.95	1.49	1.95	2.41	1.95
D3	1.70	2.00	2.52	2.07	1.69	1.98	2.60	2.09
Mean	1.52	1.90	2.37		1.52	1.90	2.40	
LSD (0.05)	I 0.13	D 0.12	I x D 0.21		I 0.10	D 0.08	I x D 1.17	

Table 4: Effect of irrigation rate and plant population density on yield components of vetiver (*Vetiveria zizanioides* L) in 2017 and 2018

Plant population density (Plant fed^{-1})	2017				2018			
	I1	I2	I3	Mean	I1	I2	I3	Mean
5. Root fresh weight (g)/plant								
D1	475.15	425.53	371.23	423.97	421.91	429.33	368.57	406.60
D2	506.34	460.74	407.52	458.20	498.37	467.54	409.94	458.62
D3	485.57	440.87	385.74	437.39	479.22	332.58	378.08	396.63
Mean	489.02	442.38	388.16	---	466.50	409.82	385.53	---
LSD (0.05)	I 1.92	D 2.83	I x D 4.90		I 2.63	D 3.91	I x D 5.20	
6. Root fresh weight (Ton)/fad.								
D1	11.88	10.64	9.28	10.60	10.55	10.73	9.21	10.16
D2	19.24	17.51	15.49	17.41	18.94	17.77	15.58	17.43
D3	24.28	22.04	19.29	21.87	23.96	16.63	18.90	19.83
Mean	18.47	16.73	14.68	---	17.82	15.04	14.57	---
LSD (0.05)	I 0.70	D 1.11	I x D 2.20		I 1.50	D 1.35	I x D 2.33	
7. Root dry weight (g)/ plant								
D1	80.41	71.85	59.88	70.71	78.35	69.85	62.45	70.22
D2	88.57	76.63	65.27	76.82	86.38	74.95	67.11	76.15
D3	82.81	73.52	62.98	73.10	83.87	69.87	62.17	71.97
Mean	83.93	74.00	62.71	---	82.87	71.56	63.91	---
LSD (0.05)	I 1.54	D 1.27	I x D 2.20		I 2.07	D 2.72	I x D 3.58	
8. Root dry weight (Ton)/fad.								
D1	2.01	1.80	1.50	1.77	1.96	1.75	1.56	1.76
D2	3.37	2.91	2.48	2.92	3.28	2.85	2.55	2.89
D3	4.14	3.68	3.15	3.66	4.19	3.49	3.11	3.60
Mean	3.17	2.79	2.38	---	3.14	2.70	2.41	---
LSD (0.05)	I 0.05	D 0.08	I x D 0.10		I 0.06	D 0.07	I x D 0.13	

Effect of irrigation rate and plant population density on chemical composition

Essential oil percentage

Results indicated that essential oil percentage was affected significantly by different densities of planting (Table 5). The maximum essential oil percentage 1.75 and 1.73 % were produced under plant density of D2 in the first and second seasons, respectively. The results also show that the D1 and D3 treatment had little significant difference affected which produced (1.51 and 1.59 %) and (1.49 and 1.57 %) for both treatments in two seasons, respectively. Also, essential oil percentage was significantly affected by irrigation rate in both season, I3 and I1 consistently resulted in lower essential oil content than I2 treatment. The optimum value was achieved under I2 being (1.77 and 1.75 %) for 2017 and 2018 respectively. Average essential oil content for I3 and I1 were 1.65 and 1.43% in 2017 and 1.64 and 1.40 % in 2018, respectively. Interaction between plant density and irrigation regime on essential oil content was significant (Table 5). The D2 produced significantly higher yield at the I2 treatment when compared with the rest of treatments which recorded1.95% for 2017 and 1.89% for 2018.

For essential oil production, multiple factors are effective including: plant ontogeny, site of oil production, photosynthesis, photoperiodic modulation, light quality, seasonal and climatic variations, nutritional relationships, plant growth regulators, plant density, moisture, salinity, temperature. In general, each factor that is influenced on the photosynthesis can be affected on the production of essential oil. Plant density by affecting the absorption of nutrients and exposure of the plant to the light can be affected on the photosynthesis and production of essential oil Khorshidi, *et al*., (2009) [36]. For the irrigation level effects, the increased oil percentage under 100% ETo similar results were observed by (Amer *et al*., 2019b) [8]. A previous study on *Artemisia annua* demonstrated that water stress decreased the density of glandular trichomes on both surfaces of leaves and, as a result, decreased the essential oil content **(**Yadav *et al*., 2014) [68]. On the other hand, the observations on other aromatic plants such as German chamomile (Pirzad *et al*., 2006) [48], rosemary, spearmint and lemongrass under water stress, either maintained or enhanced the level of essential oil content, depending on the species and magnitude of the stress condition (Delfine *et al*., 2005) [21].

Proline content

The results for proline content (Table 5) indicate that the highest content (5.79%) which was recorded by I3 of the irrigation treatment. This value was greater than that recorded with I2 by about 4.48 %. The I1 of irrigation treatment yielded about 41.10 % less proline content than the I3 which stated 3.41%. On the other hand, the difference in proline content was however not statistically significant ($P < 0.05$) between the treatments of PPD. Moreover, the proline content significantly affected by the interaction between PPD and irrigation rates. The D1 of plant density produced significantly higher value (6.01%) at I3 treatment when compared with the I1 and I2 of irrigation regime. These results were achieved at the first season of study and the second one had the same trend of results.

Research has indicated a correlation between decreasing proteins and increasing amino acids with water stress (Gorbanli *et al*., 1998) [27]. The distinctive effects of deficit soil moisture on leaf proline accumulation have also been reported researchers (Yoshiba *et al*., 1997; Ain-Lhout *et al*., 2001) [69, 4]. An increase in proline accumulation in vetiver root due to water deficit and consequent water stress is reported to play a significant role in adaptation to ambient conditions (Heuer and Nadler, 1998; Sarker *et al*., 2005) [31, 54]. Proline is highly active in the osmotic regulation of available nitrogen accumulation (Ashraf, 1994) [9]. Results of the regression analysis between the amount of applied irrigation water and proline accumulation in vetiver root are plotted in table 5. Increasing proline accumulation was observed with water decrease. This increase has mostly been reported as a defense mechanism of the plant against water stress (Bandurska, 2004; Ahire *et al*., 2005; Sankar *et al*., 2007; Hamidou *et al*., 2007) [11, 3, 53, 29].

Table 5: Effect of irrigation rate and plant population density on essential oil percentage and proline contents of vetiver (*Vetiveria zizanioides* L in 2017 and 2018

Plant population density (plant fed^{-1})	2017				2018			
	I1	I2	I3	Mean	I1	I2	I3	Mean
1. Essential oil percentage								
D1	1.35	1.63	1.54	1.51	1.29	1.65	1.52	1.49
D2	1.50	1.95	1.80	1.75	1.48	1.89	1.81	1.73
D3	1.45	1.72	1.61	1.59	1.43	1.70	1.59	1.57
Mean	1.43	1.77	1.65		1.40	1.75	1.64	
LSD (0.05)	I 0.20	D 0.23	I x D 0.40		I 0.11	D 0.08	I x D 0.14	
2. Proline content								
D1	3.51	5.00	6.01	4.84	3.55	4.79	5.88	4.74
D2	3.40	4.32	5.75	4.49	3.39	4.33	5.70	4.47
D3	3.31	4.11	5.60	4.34	3.28	4.12	5.61	4.34
Mean	3.41	4.48	5.79		3.41	4.41	5.73	
LSD (0.05)	I 00.75	D 0.39	I x D 0.80		I 0.03	D 0.06	I x D 0.07	

GC analysis of essential oil

Analysis and identification of fresh root oil chemical composition using gas chromatography (GC) are summarized in Table 6 during 2018. From the results, fifteen compounds were identified and can be seen that sesquiterpenes constitute is the predominant class of compounds. Alcohols and acids were the most important components. Among the alcohols, preziza-7(15)-en-12-ol, cedren-5-en-15-ol, preziza-7(15)-en-3α-ol, ziza-6(13)-en-12-ol (khusirnol), ziza-5-en-12- ol, 12-nor-ziza-6(13)-en-2 β-ol, khusian-2- ol (helifolan- ol) were the major compounds. These results are in agreement with Hammam and Hassan (2014) [32] on vetiver. The main component was prezizanoic acid, Hexadecanoic acid and zizanoic acid. The sesquiterpene hydrocarbons were mainly represented by compound γ-vetivenene, while sesquiterpene aldehydes signified by prezizaan- l5- al. Prezizanoic acid were identified as a chief component. Data of the effect of interaction between treatments (table 6) indicated that, the highest prezizanoic acid ratio (36%) was recorded at I3D3 treatment, whereas it was decreased to (33.9 %) at I1D1 treatment. The preziza-7(15)-en-12-ol content tended to increase at I3D2 treatments by (9.9%) in compare with 8.5 % for I1D1. cedren-8-en-15-ol generally increased at I3D1 and I3D2 treatments by 9.2 % while stated 7.0 % under I1D1 treatments. In general, previous data show that, the irrigation treatment had a significant effect on the composition values, which tented to increase under the low level of irrigation (I3) compared to the high level (I1). On the other hand, slight changes were spotted in composition percentage under the plant population densities. In this concern, Water availability is a central resource affecting plant fitness. Predicted increases in the frequency of extreme precipitation events under ongoing global climate change (Bates *et al*., 2008; Donat *et al*., 2016) [12, 23] threaten reliable sources of water for terrestrial ecosystems (Easterling *et al*., 2000; Weltzin *et al*., 2003) [24, 66]. Plants experiencing drought or flooding can adjust their morphology to optimize water uptake by the roots while decreasing the rate of photosynthesis by the leaves, thereby changing the production of growth and defense metabolites (Koricheva *et al*., 1998; Grant *et al*., 2005; Nicotra *et al*., 2007; Kleine and Mueller, 2014) [39, 28, 45, 38].

Water deficit in plants may lead to physiological disorders, such as reduction in photosynthesis and transpiration (Sarker *et al*., 2005) [54] and in the case of aromatic crops may cause significant changes in the yield and composition of essential oils. In aromatic plants, growth and essential oil production are influenced by various environmental factors, such as water stress (Burbott and Loomis 1969) [16]. (Solinas and Deiana 1996) [58] reported that secondary metabolites of plants can be altered by environmental factors and water stress is a major factor affecting the synthesis of natural products. Changes in essential oil extracted from aromatic plants and their composition were observed with water stress (Sabihet *et al*., 1999). According to (Turtola *et al.*, 2003) [63], plants produce high terpene concentrations under environmental stress conditions because of a low allocation of carbon to the growth, suggesting a trade-off between growth and defence. Earlier studies with *O. vulgare* and *A. annua* have demonstrated significant changes in essential oil content and composition under water stress conditions (Said-Al Ahl and Hussein, 2010; Chalchat *et al*., 1994) [51, 17]. Such an increase in essential oil content in *O. vulgare* subsp. gracile and sesquiterpenoids in *O. vulgare* subsp. virens under water stress implicate the important role of monoterpenoids and sesquiterpenoids in the protection of oregano against water stress conditions (Tholl, 2015) [60].

Table 6: Percentage component of the essential oil of vetiver (*Vetiveria zizanioides* L) under effects of irrigation rate and plant population density during 2018

No.	Components %	Treatments					
		I1 D1	I1 D2	I1 D3	I3 D1	I3 D2	I3 D3
1	prezizanoic acid	33.9	34.7	34.0	35.4	35.6	36.0
2	preziza-7(15)-en-12-ol	8.5	8.9	8.6	9.7	9.9	9.8
3	cedren-8-en-15-ol	7.0	7.4	7.1	9.2	9.2	9.1
4	myristicin	0.3	0.3	0.4	0.3	0.5	0.4
5	preziza-7(15)-en-3α-ol	7.0	7.6	7.3	7.1	7.4	7.3
6	vetiselinenol	0.6	0.8	0.7	0.9	0.9	0.9
7	Hexadecanoic acid	2.8	3.0	3.0	3.5	3.7	3.6
8	ziza-6(13)-en-12-ol (khusirnol)	2.5	2.2	2.6	2.6	2.8	3.0

9	2-epi-ziza-6(13)-en-3α-ol	0.2	0.2	0.2	0.4	0.4	0.5
10	ziza-5-en-12- ol	3.1	3.4	3.3	3.6	3.8	3.7
11	zizanoic acid	5.4	5.6	5.6	6.0	6.4	6.2
12	prezizaan- l5- al	2.0	2.4	2.3	2.6	3.0	2.8
13	12-nor-ziza-6(13)-en-2 β-ol	0.9	1.1	1.1	1.4	1.6	1.6
14	khusian-2- ol (helifolan- ol)	1.9	2.0	2.0	2.5	2.5	2.7
15	γ-vetivenene	0.3	0.2	0.2	0.7	1.0	0.9
	Total	76.4	79.8	78.4	94	96.8	96.6

Water relations

1. Applied irrigation water and water consumptive use

The values of the applied irrigation water are shown in Table (7). It indicated that irrigating with full irrigation under high population densities consumed more water than other treatments. The interaction between irrigation rate and plant population density on the applied water and water consumptive use were measured in both season (Table 7). The highest amount of the applied water and water consumed were 6899 and 6479 m3/fed respectively in 2017 and it was 6940 and 6520 m3/fed respectively in 2018 under 120% ETo and high population densities of 50000 plant/fed. This could be due to higher density of roots which enabled the plants to extract large amounts of water. By contrast, the minimum values were observed under 80% ETo and low population densities of 25000 plant /fed being (5030 and 5058 m3/fed) and (4610 and 4638 m3/ fed) for applied water and water consumed in both 2017 and 2018, respectively. Increasing of water use for this behavior may be owing to the abundance of soil moisture in the soil and the plants tend to grow luxuriantly and hereafter use more water. Sani *et al*., (2008) [52] reported that irrigation levels may not have much impact on soil water content beyond the surface soil layers. Moreover, it has been mentioned by Kowal (1970) [40] that in the lower depths of the soils, the amount of available water decreases and the proportion of strongly held water increases so that the extraction of water by plants becomes increasingly difficult with time.

2. Water use efficiency

Additionally, the combined effects of irrigation rate and plant population density on irrigation water use efficiency (WUE) were significant in both seasons (Table 7). The maximum values of WUE (3.75 and 3.67 kg/m3) were observed under the high population density in 2017 and 2018, respectively. The lowest value of WUE (1.97 and 1.82 kg/m3) occurred with the low plant density in 2017 and 2018, respectively. This increase in WUE with increasing plant density maybe due to high density planting accommodating a higher density of roots, which enabled the plants to extract larger amounts of water than occurs under low density. The minimum WUE at low population densities may be due to increased levels of evaporation. Pandey *et al* (1988) [46] observed that higher plant density gave higher consumptive use, rate of moisture use and water use efficiency as compared to the lower plant density. Also, Singh *et al*., (2003) [56] reported that water use efficiency of wheat was higher at higher population density than low population density.

Table 7: Effects of irrigation rate and plant population density on crop water relation parameters of vetiver (*Vetiveria zizanioides* L) in 2017 and 2018

Plant population density (Plant fed^{-1})	2017			2018		
	I1	I2	I3	I1	I2	I3
Applied Water						
D1	6464	6023	5030	6212	5635	5058
D2	6711	6252	5335	6644	5995	5346
D3	6899	6350	5641	6940	6415	5682
Water consumptive use						
D1	6044	5603	4610	5792	5215	4638
D2	6291	5832	4915	6224	5575	4926
D3	6479	5930	5221	6520	5995	5262
Water use efficiency						
D1	1.97	1.90	2.01	1.82	2.06	1.99
D2	3.06	3.00	3.15	3.04	3.19	3.16
D3	3.75	3.72	3.69	3.67	2.77	3.59

Conclusion

From the results of this study, it can be concluded that for this particular condition, application of 120% ETo is not economical. It is advisable to irrigate with 100% ETo. This gives as similar to yield while saving a lot on water and labor. However, the 38,000 plants per fed treatment should be used as it translates to higher yield and more protection for the soil.

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