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Maximizing Essential Oil Yield and Quality in Menthol Mint (*Mentha arvensis* L.) by Reducing Water Requirement through Deficit Irrigation Practices

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ABSTRACT

Menthol mint (*Mentha arvensis* L.) is an aromatic and medicinal plant worldwide cultivated for high-value essential oil. It comes under angiospermic plant and belongs to family Lamiaceae commonly known as Japanese mint. The high-value essential oil is obtained from the stem and leaf of plant by the process of hydro-distillation through which major ingredient L-menthol is obtained that is used in aroma and pharmaceutical industries. This investigation aimed to reduce water requirements which can enhance its productivity for sustaining menthol mint cultivation in India. The present investigation has been undertaken as treatment combination which includes two varieties *viz.* i) Kosi and CIM-Kranti; three depths of soil moisture *viz.* i) 3 cm, ii) 6 cm and iii) 9 cm along with three moisture regimes, i.e., i) 20±5%, ii) 40±5%, and 60±5% available soil moisture (ASM) were tested in split-split plot design during 2018 and 2019 (from February to June). Results from the present study revealed that the maximum oil yield was recorded 116.78 kg ha^{-1}in cv. Kosi and 107.23 kg ha^{-1} in cv. for CIM-Kranti, whereas menthol yield was recorded 89.23 kg ha^{-1}in cv. Kosi and 85.13 kg ha^{-1}in cv. CIM-Kranti under 6 cm depth of irrigation when applied irrigation at 40±5% available soil moisture (ASM) in menthol mint. However, the lowest water requirement was recorded 118 mm in cv. Kosi and 148 mm in cv. CIM-Kranti. The highest water use efficiency was recorded 0.61 in cv. Kosi and 0.51 kg oil ha^{-1} mm^{-1} in cv. CIM- Kranti under 3 cm depth of irrigation and when irrigations were applied at 20±5 % ASM. The highest net returns of \$ 1140.91 ha^{-1} and \$ 989.70 ha^{-1} have been recorded in Kosi and CIM-Kranti, respectively were computed at 6 cm depth of irrigation and when irrigations were applied at 40±5% available soil moisture as compared with other treatments. The irrigation depth (6 cm) applied at 40±5% ASM was found to be a perfect combination for obtaining maximum oil yield, water use efficiency, net returns, and benefit-cost ratio.

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1. Introduction

Menthol mint (*Mentha arvensis* L.) is also known as Japanese mint, which is an essential oil-bearing plant of the family Lamiaceae. The biosynthesis of essential oil takes place in specialized tissues, i.e., trichomes found as epidermal outgrowth on the aerial part of leaves (Tiwari, 2016). It is a source of l-menthol (70-80%), which is widely used in aroma, flavour and pharmaceutical industries. This crop is commercially cultivated in various countries *viz.* India, China, Brazil, Japan, USA, France, Australia, Thailand, Angola and Argentina. In India, it is cultivated in an area of more than 0.30-0.35 million hectares and produces approximately 0.030-0.035 million metric tonnes of essential oil per annum (Kumar *et al.*, 2020; Kumar *et al.*, 2022). The high-water application during the summer season is becoming a major constraint in its production. The water table in the mint growing area

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follow rapidly decreasing trends due to the cultivation of this crop (Kumar *et al*., 2022). Water deficit stress is an important abiotic factor that affects the productivity and quality of a number of crops where secondary metabolites are the end product (Kostrzewska *et al*., 2017). But it has been reported by number of researchers in many aromatic plants where secondary metabolites are the end product, that water stress enhances its biosynthesis and production per unit area and time (Vilanova *et al*., 2018). The highest essential oil yield in *Lavandula angustifolia* and *Salvia fruticosa* was reported under moderate water deficit as compared with adequate irrigation and severe water deficit (Chrysargyris *et al*., 2016).

The lowest consumptive use of water and maximum water use efficiency were recorded under 0.9 irrigation water/cumulative pan evaporation ratio (IW/CPE ratio) as compared with other treatments. However, the highest gross return, net returns and benefits: cost ratio were recorded under moisture stress conditions (Kumar *et al*., 2020; Kumar *et al*., 2022). The maximum dry weight per plant, root volume, root length, root area and root density were recorded under 100% water requirements as compared with 80% and 60% in *Mentha piperita* L. (Ghamarnia *et al*., 2023). It has also been reported that deficit irrigation practices following strategic water stress can reduce the requirement of water in several crops with enhanced production of secondary metabolites. However, it has not been worked out in the case of menthol mint, where the essential oil is a valuable product and comes under the category of secondary metabolites. There is no study reported to date about the amount of water required for one kg of essential oil production of menthol mint. Moreover, maximizing the essential oil yield and quality characteristics of menthol mint, targeting the least water usage in this crop needs to be worked out. However, studies on how much deficit of water is to be applied and when to be applied have not been studied yet in this crop. Hence, the studies on the different depths and levels of moisture were planned in two commercial cultivars of menthol mint with the following objectives,) to reduce the water requirement with enhanced productivity for getting maximum water use efficiency and ii) to reduce the cost of cultivation and enhance net return, and benefit-cost ratio.

2. Materials and methods

2.1. Site description and climatic features

The field experiment was conducted in year 2018 and 2019, (February to June) at the experimental farm of CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, located at 26.5º N latitude, 80.5º E longitudes with an elevation of about 120 m above mean sea level under the sub-tropical plains of north India. The experimental site is classified as semi-arid subtropical, along with severe hot summers and cold winters. Monsoon sets typically from the second fortnight of June to the last week of September, along with average annual rainfall of 1025 mm. July and August receive about 80% of the monsoon precipitation. Due to the cyclonic disturbance in the Arabian Sea, rainfall occurs during the winter season. The maximum mean temperature varied from 44.11 ℃ to 38.47 ℃ whereas the minimum 18.63 ℃ to 20.63 ℃. The temperature was recorded the lowest during the first fortnight of January, which further showed an increasing trend from the third week of May and it reached to highest at the end of May to the first week of June. After that, it gradually decreased after the onset of the monsoon in the last week of July. The soil profile of the experimental plot was sandy loam in texture, having a filed capacity 20%, and a permanent wilting point is 6%, and 7.7 pH (Table 1). Weather conditions (temperature, relative humidity, rainfall, and sunshine hours) prevailing during experimental periods of both years have been depicted in Fig. 1.

2.2. Treatment and experimental design

In the present study only two cultivars were considered *viz.* a) Kosi and, b) CIM-Kranti, three depths of irrigations, i.e., 3, 6, and 9 cm, and three levels of moisture regimes, i.e., a) 20±5%, b) 40±5%, and c) 60±5% available soil moisture (ASM). The experiment was evaluated under as split-split plot design with an individual plot size of 7.5 m^2 (2.5 × 3 m) in three replications. The flat bed was prepared using the check basin method. The cultivars, depth of irrigations and moisture regimes were kept in the main plot, subplot and sub-subplots. The N:P:K was applied just before transplanting through Urea, DAP, and MOP (1/3rd of N:50 kg ha^{-1}, full dose of P:80 kg ha^{-1} and K: 60 kg ha^{-1}), respectively at the time of plowing during both the year of experimentation. The remaining dose of urea was applied in two splits through top dressing *viz*., 30 and 45 days after transplanting. Thereafter, the experimental field was leveled using computerized laser leveler. After that, transplanting was done in the second week of February under spacing of 40 cm line to the line and 25 cm plant to plant. The irrigations were applied as per treatments after the establishment of transplanted plants. Other agricultural operations were followed, as suggested by Kumar et al. (2021).

Table 1. Physicochemical properties of the soil at experimental field.

Characters / Years	Sand (%)	Silt (%)	Clay (%)	pH	EC (d Sm^{-1})	OC (%)	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
2018	88±7.2	7±1.15	5±0.95	7.7±1.0	3.36±0.50	0.45±0.11	56.40±5.6	41.70±5.8	64.25±9.25
2019	85±7.8	8±1.42	7±1.18	7.5±1.0	3.45±0.2	0.48±0.22	61.25±6.15	45.44±6.15	69.50±10.22

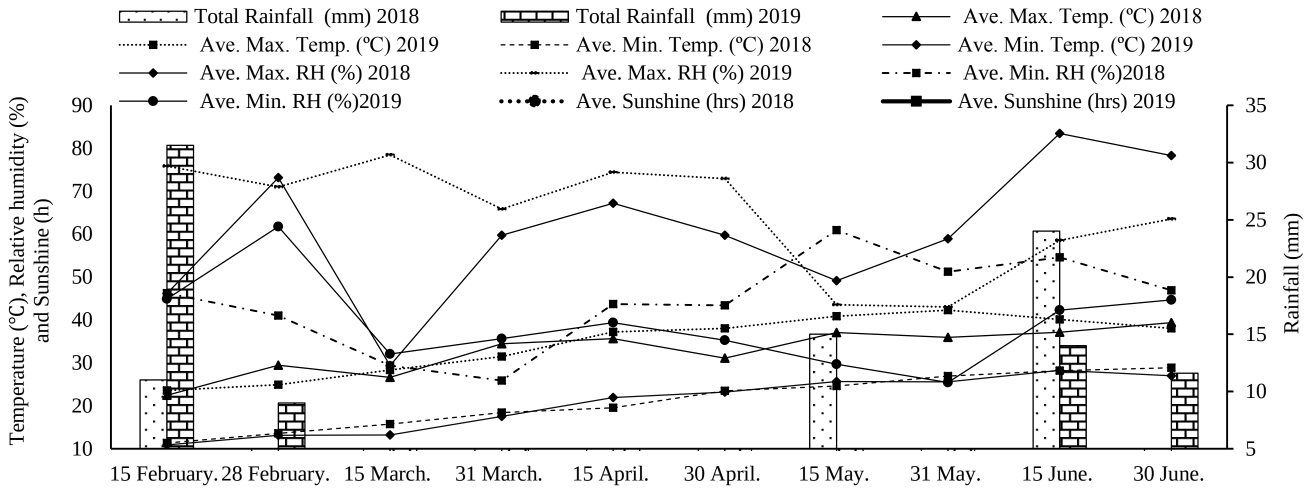

Figure 1. Mean bi-weekly air temperature, relative humidity, sunshine hours, and total rainfall over the cropping season 2018 and 2019.

2.3. Calculation of irrigation depth of water

The moisture content in the soil was tested through the gravimetric method. The depth of irrigation water was worked out by using a technique prescribed by Ekren et al. (2012) and calculated via Equation 1.

$$IR = \frac{FC - Qi}{100} \times \gamma s \times D \quad (1)$$

Whereas, IR=the depth of irrigation water, FC= field capacity (gg^{-1}), Qi= soil moisture at the time of irrigation (g cm^{-3}),γs= Soil bulk density (g cm^{-3}), and D=soil depth (cm). After that, the volume of water required for irrigation in each plot was calculated using the Equation 2 in a formula:

(2) Volume of water $plot^{-1}$ (m^3) = Area of plot (m^2) × depth of irrigation (cm)

Water requirement (cm) was worked out by using Equation 3 prescribed by Kumar et al. (2021).

(3) WR= depth of irrigation applied (cm)× number of irrigations + effective rainfall (cm)

2.3.1. Available soil moisture and water use efficiency

Moisture regimes were designed based on the water-holding capacity of the field soil. Water from a fully saturated soil i.e. (maximum water holding capacity) keeps on draining by free flow and then due to gravitational force. At a certain point, this process gets slower till no more water drains, here the soil is said to be at “field capacity”. However, evaporation or absorption creates a further loss which leads to a stage recognized as the “wilting point” where the plants are unable to fetch water that is required for their vital activities and eventually die in scarcity of moisture. Hence, these three moisture characteristics are crucial for plant growth and agricultural experiments. The soil maximum water holding capacity (WHC_{max}) was determined gravimetrically (Karhu *et al*., 2014) by taking field soil in and wetting it for 2 hours, followed by draining it through filter papers (Whatman 42). The soil was at a maximum saturation point at that time. Then moisture content of the soil was measured after drying a sample at 105ºC for 24h using the formula (Equation 4) used by Alandia et al. (2016).

(4) MC (d.b.) = $W_{f.s} - W_{d.s} / W_{d.s} \times 100$

Whereas, MC= Moisture content, d.b.= dry basis, $W_{f.s}$= Weight of fresh soil, and $W_{d.s}$= Weight of dry soil.

This moisture was WHCmax, 45±5% of that was considered as available soil moisture (ASM). Before the implementation of different moisture regimes, the soil moisture was maintained between field capacity and wilting point (available soil moisture level 45%±5% of WHCmax) for the acclimatization and establishment of the crop. After the crop was established, based on the FC and WHCmax, three soil moisture levels, i.e. higher moisture levels 60%±5% of WHCmax (available soil moisture considered as control), moderate lower moisture levels 40%±5% of WHCmax and lower moisture level 20%±5% of WHCmax. Available soil moisture was measured daily for each treatment using 6050X3K5B Mini Trace Kit (Soil Moisture Equipment Corp., Goleta, CA, USA).

The effective rainfall was calculated by using a method as suggested by Mishra and Ahmed (1987). Water use efficiency (kg oil ha^{-1} mm^{-1}) was calculated by using Equation 5 as prescribed by Singh et al. (1997).

(5) WUE (kg oil ha^{-1} mm^{-1})= Essential oil yield (kg ha^{-1})/ Water applied (mm) + effective rainfall (mm)

2.4. Sampling of plants and biometric observation

For each water stress treatment, six plants were selected randomly in three replications (except border plants to avoid border effect) for recording observations of growth and yield attributes at the stage of harvesting. Plant height was measured from ground level to the upper tip of the youngest leaf, measured by measuring scale and mean values were used for statistical analysis. Leaf area index (LAI) was evaluated before harvest in all treatments using a portable leaf area meter (Model: Li-Cor 3100, USA). Plants were harvested from the soil surface and weighed (fresh weight) using electronic balance to prevent moisture loss immediately and then samples were sent to the lab for distillation. Besides, three samples were dried in shed for converting essential oil content on a dry weight basis to have a valid comparison. For the estimation of leaf: stem ratio, freshly harvested samples were used for separating leaves and stem and these weights were utilized for the calculation of leaf: stem ratio. Finally, after harvesting (kg m^{-2}), the fresh and dry herb yield is converted into (Mg ha^{-1}).

2.5. Estimation of essential oil content and yield

As per the respective treatment of depth of each irrigation and moisture regime, each plant of *Mentha arvensis* L. was placed in 2000 ml water capacity round bottom flask and distillation was done using hydro-distillation through the Clevenger's type apparatus at 70-80°C for 180 minutes (Clevenger, 1928). Then, the essential oil was collected in glass vials and stored at 4°C temperature. To eradicate moisture in essential oil, anhydrous sodium sulphate was applied in sealed glass vials. The obtained essential oil of menthol mint was clear, bright, and yellow. The essential oil yield (EOY) is the primary monetary product for the growers of menthol mint. The formula of essential oil yield (EOY) is given in the Equation 6.

(6) Essential oil yield (kg ha^{-1}) = Dry matter yield (kg ha^{-1}) × Essential oil content (% of d.w. basis) × specific gravity of the oil

2.6. Estimation of menthol content and yield

Menthol is the major aroma constituent and quality parameter in the essential oil of menthol mint. This quality parameter was assessed through a gas chromatograph (Perkin Elmer Auto system XL GC) fitted with Elite wax column (30 m×0. 25 mm×0. 25 µm film thickness). The oven temperature was programmed from 40 ºC (40-120 at the rate of 3ºC min^{-1}) with 9 minutes hold, 120-140ºC for 2 ºC min^{-1} with 2-minute hold and 140-250 ºC at 5ºC min^{-1} with 2 minutes final hold time). Hydrogen gas was used as the carrier gas at 7.5 psi constant column head pressure; the split ratio was 1:40, injection size 0.02 µl neat; injector and detector temperatures were maintained at 250 °C. Characterization of constituents was done based on co-injection with standards (Sigma-Aldrich) along with hydrocarbons (C_7 to C_{30}) (Kumar *et al.*, 2022). The total yield of menthol per unit area is the final economic product in menthol mint, and it was calculated by using the formula as expressed in Equation 7.

(7) Menthol yield (kg ha^{-1}) = Menthol content (%) × Essential oil yield (kg ha^{-1})

2.7. Harvesting

Harvesting of the crop was done at the full maturity stage. It was determined that the youngest leaves seem shorter as compared with earlier stages of the crop and old leaves started yellowing and falling on the ground. It was checked as per regular basis field visits when the crop became around 70-75 days old.

2.8. Estimation of economics

The evaluation of the economical profitability of different treatments was also worked out. The cost of cultivation, gross return, net profit, cost of one-kilogram oil production, and benefit-cost ratio were calculated by using the prevailing market price of inputs and produce. The cost of cultivation is included in fixed as well as variable inputs in menthol mint. The cost of key inputs and outputs depended on the standard market price. Gross returns are the economic return of the essential oil produced from the menthol mint, which was worked out by considering the price of essential oil at the rate of \$ 14.58 kg^{-1} (Kumar *et al.*, 2022). Net returns were calculated by subtracting the cost of cultivation from gross returns and the price of one-kilogram essential oil production is the significant character in menthol mint. The cost of one-kilogram essential oil production was estimated when the yield was divided by the cost of cultivation. Similarly, the calculation of the benefit-cost ratio is worked out by dividing the net profits by the cost of cultivation.

2.9. Statistical analysis

Data on various observations such as biological yield, quality traits, water requirement, and water use efficiency were individually analysed. The statistical analysis was done by using the technique of analysis of variance for the split-split plot design of field experimentation, as suggested by Panse and Sukhatme (1958). A critical difference between different treatment means was calculated at a 5% (P=0.05) level of significance.

3. Results and discussion

3.1. Growth attributes

The result of the present investigation indicated that various depths of irrigation and moisture regimes significantly (P<0.05) affected the growth parameters in menthol mint (Table 2). The highest depth of irrigation (9 cm) recorded maximum plant height (58.79 and 56.25 cm), leaf area index (2.83 and 2.69), and leaf: stem ratio (1.64 and 1.54) in cv. CIM-Kranti and Kosi as compared with 6 cm and 3 cm depth of irrigation (Fig. 2). Plant height (20.77 %), leaf area index (21.20 %), and leaf stem ratio (29.26 %) were significantly recorded higher under 9 cm depth of irrigation as compared with lower depth of irrigation i.e., 3 cm depth of irrigation. Moreover, maximum plant height (59.74 cm), leaf area index (3.24), and leaf stem ratio (1.64) were recorded under 60±5% ASM as compared with other moisture regimes (40±5% and 20±5% ASM) in cv. CIM-Kranti. Moreover, a similar trend was also observed in cv. Kosi during the experiment. Differences in the interaction effect on fresh herb yield, i.e., depth of irrigation × moisture regimes were found significant in the menthol mint. The role of drought stress conditions on plant growth parameters and productivity has been reported previously by several researchers in various medicinal and aromatic crops such as oak species, *Thymus vulgaris*, *Mentha* × *Piperita* L., and *Cassia angustifolia* Vhal. (Pellegrini *et al.*, 2019), (Llorens-Molina and Vacas, 2016), (Abdi *et al.*, 2019), (Nilofer *et al.*, 2018). Moreover, the lower depth of irrigation (3 cm) and increments in moisture stress i.e., 20±5% ASM resulted decrease in plant growth attributes; it might be due to an increase in evapotranspiration resulted in a significant reduction in photosynthesis and cell turgidity (Caser *et al.*, 2019; Rahimi *et al.*, 2017). Correspondingly, the increase in depth of irrigation and decreased moisture stress conditions resulted in a proper reaction of photosynthesis which increased plant height, leaf area index, leaf stem ratio, leaf size, number of leaves, primary and secondary branches of menthol mint (Saeidnejad *et al.*, 2013). Significantly maximum growth attributes were achieved at 9 cm and when irrigations were applied at 60±5% ASM as compared with other treatments in both the cultivars of menthol mint. It might be due to the reduction in cellular elongation in water stress-treated plants (3 cm depth and 20±5% ASM). It is in close agreement with the results of Feng et al. (2016) who have reported that the decrease in water restricted plant cell elongation by reducing cell turgor, depending on moisture and osmotic pressure. It has been noted that in the case of numerous aromatic and medicinal plants such as *M. pulegium*, *M. piperita*, *Rosmarinus officinalis*, and *Salvia* spp (Hassanpour *et al.*, 2014), (Rahimi *et al.*, 2017), (Delfine *et al.*, 2005), and (Caser *et al.*, 2012).

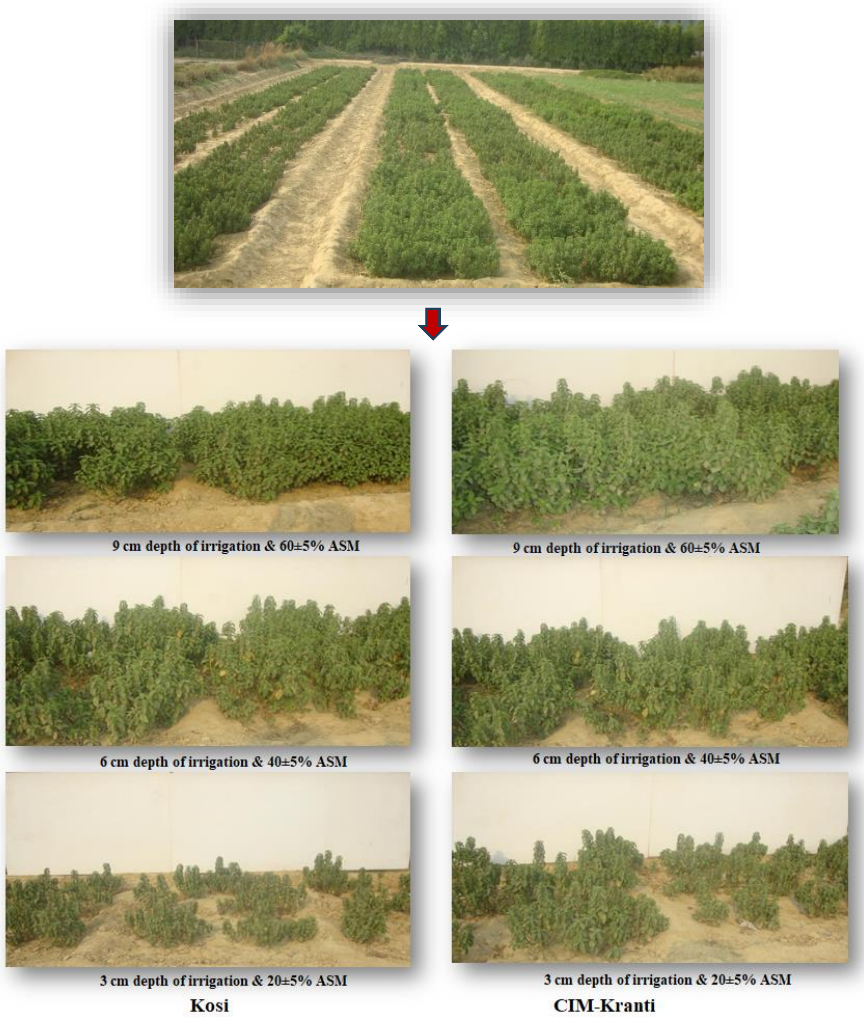

Figure 2. Field view of depth of irrigation and moisture regimes of menthol mint.

Table 2. Plant height (cm), leaf area index, and leaf stem ratio as affected by depth of irrigation and moisture regimes in *Mentha arvensis* L.

Depth of irrigation / Moisture regimes	3 cm	6 cm	9 cm	Mean	3 cm	6 cm	9 cm	Mean	3 cm	6 cm	9 cm	Mean
	Plant height (cm)				Leaf area index				Leaf stem ratio			
Kosi												
60±5% ASM	50.25^{a}	57.52^{a}	63.69^{a}	57.15^{a}	2.77^{a}	2.95^{a}	3.50^{a}	3.07^{a}	1.29^{a}	1.52^{a}	1.81^{a}	1.54^{a}
40±5% ASM	45.62^{b}	51.67^{b}	57.05^{b}	51.45^{b}	2.25^{b}	2.53^{ab}	2.86^{b}	2.55^{b}	1.11^{b}	1.27^{b}	1.55^{b}	1.31^{b}
20±5% ASM	37.81^{c}	43.19^{c}	48.00^{c}	43.00^{c}	1.32^{c}	1.56^{c}	1.70^{c}	1.53^{c}	0.88^{c}	1.12^{c}	1.26^{c}	1.09^{c}
Mean	44.56^{c}	50.79^{b}	56.25^{a}	50.53	2.11^{bc}	2.35^{ab}	2.69^{a}	2.38	1.09^{c}	1.30^{b}	1.54^{a}	1.31
CIM-Kranti												
60±5% ASM	52.52^{a}	60.12^{a}	66.57^{a}	59.74^{a}	2.92^{a}	3.11^{a}	3.69^{a}	3.24^{a}	1.37^{a}	1.62^{a}	1.93^{a}	1.64^{a}
40±5% ASM	47.68^{b}	54.01^{b}	59.63^{b}	53.77^{b}	2.37^{ab}	2.67^{b}	3.02^{b}	2.69^{b}	1.18^{b}	1.35^{b}	1.65^{b}	1.40^{ab}
20±5% ASM	39.52^{c}	45.14^{c}	50.17^{c}	44.94^{c}	1.39^{c}	1.65^{c}	1.79^{c}	1.61^{c}	0.94^{c}	1.19^{c}	1.34^{c}	1.16^{bc}
Mean	46.57^{c}	53.09^{b}	58.79^{a}	52.82	2.23^{bc}	2.48^{ab}	2.83^{a}	2.51	1.16^{c}	1.39^{b}	1.64^{a}	1.40
	SEm±		CD at 5%		SEm±		CD at 5%		SEm±		CD at 5%	
Cultivar (cv.)	0.65		NS		0.04		NS		0.01		NS	
Depth of irrigation (D)	0.79		1.62		0.07		0.25		0.01		0.18	
Moisture regimes (M)	0.79		1.62		0.07		0.15		0.01		0.11	
cv.×M	0.79		NS		0.07		NS		0.01		NS	
cv.×D	1.12		NS		0.10		NS		0.02		NS	
M×D	1.37		NS		0.13		2.12		0.03		1.15	
cv.×M×D	1.94		NS		0.18		NS		0.04		NS	

Letters showing the difference (P>0.05), NS=Non-significant, and ASM= Available Soil Moisture

3.2. Dry herb yield

The present investigation's results revealed that the depth of irrigation and moisture regimes significantly influenced the dry herb yield of menthol mint (Table 3). The cv. CIM-Kranti has shown a higher dry herb yield (3.25 Mg ha^{-1}) as compared with Kosi (3.20 Mg ha^{-1}). The yield enhanced significantly with an increase in the depth of irrigation being the highest (3.67 Mg ha^{-1}) under 9 cm depth of irrigation in cv. CIM-Kranti. However, a lower yield (2.45 Mg ha^{-1}) was recorded under 3 cm depth of irrigation in cv Kosi. Moreover, 60±5% ASM recorded the highest dry herb yield (3.41 and 3.36 Mg ha^{-1} in cv CIM-Kranti and Kosi, respectively). The lowest dry herb yield (2.95 and 3.01 Mg ha^{-1} in cv Kosi, and CIM-Kranti) was recorded under 20±5% ASM. This could be due to an increment in moisture stress resulting in decreases in dry herb yield. Dry herb yield declined significantly when water stress was imposed beyond 40±5% ASM. The amount of irrigation water applied resulted in an increment in the corresponding dry herb yield, which was significantly influenced by the treatment of depth of irrigation and available soil moisture each year. CIM-Kranti cultivar recorded more herb yield, which might be due to a maximum number of leaves, enlargement in size of leaves, proper growth, and development. Dry herb yield increased with an increase in depth of irrigation and moisture level as higher moisture availability encouraged higher growth. The dry herb yield reduced under lower depth of irrigation and where the moisture stress increased, it might be due to the decrease in cell enlargement and leaf senescence caused by decreased turgor pressure. It might be due to altered leaf area index and a reduction in photosynthesis during moisture stress resulting in minimum herb yield (Shao *et al*., 2008). Decreased stomatal conductance leads to reduced photosynthetic rate, and it may decrease plant growth (Leithy and Meseiry, 2006). It might be possible that in the present study moisture stress resulted in reduction of photosynthesis which led to a reduction in herbage yield (Jaleel *et al*., 2009). Leaf area decreased, resulting in lower light utilization and this result might have a lower rate of photosynthesis. However, lower dry herb yield was reported with a minimum depth of irrigation and moisture stress. A shortage of water causes a reduction in turgor pressure, which might have resulted in improved growth and development of plants (Ekren *et al*., 2012).

Table 3. Dry herb yield (Mg h^{-1}), oil content (%), and oil yield (kg ha^{-1}) as influenced by depth of irrigation and moisture regimes in *Mentha arvensis* L.

Depth of irrigation / Moisture regimes	3 ha cm	6 ha cm	9 ha cm	Mean	3 ha cm	6 ha cm	9 ha cm	Mean	3 ha cm	6 ha cm	9 ha cm	Mean
	Dry matter yield (Mg ha^{-1})				Oil content (%)				Oil yield (kg ha^{-1})			
					Kosi							
60±5% ASM	2.58^{a}	3.65^{a}	3.80^{a}	3.36^{a}	3.11^{a}	3.00^{b}	2.85^{b}	2.99^{c}	80.52^{a}	109.61^{b}	108.54^{a}	100.77^{b}
40±5% ASM	2.54^{a}	3.47^{b}	3.54^{b}	3.20^{b}	3.34^{a}	3.36^{a}	3.20^{a}	3.30^{a}	85.18^{a}	116.78^{a}	113.20^{a}	105.78^{a}
20±5% ASM	2.16^{b}	3.27^{c}	3.38^{c}	2.95^{c}	3.31^{a}	3.16^{a}	2.97^{a}	3.15^{b}	71.72^{c}	103.46^{c}	100.36^{b}	93.11^{c}
Mean	2.45^{bc}	3.49^{ab}	3.61^{a}	3.20^{b}	3.26^{a}	3.18^{b}	3.01^{c}	3.15^{a}	80.14^{c}	111.10^{a}	108.58^{ab}	101.01^{a}
					CIM-Kranti							
60±5% ASM	2.62^{a}	3.69^{a}	3.86^{a}	3.41^{a}	2.96^{b}	2.88^{b}	2.67^{b}	2.84^{b}	77.69^{b}	106.50^{a}	103.00^{a}	96.97^{b}
40±5% ASM	2.58^{a}	3.53^{a}	3.60^{a}	3.25^{b}	3.29^{a}	3.04^{a}	2.96^{a}	3.10^{a}	85.04^{a}	107.23^{a}	106.69^{a}	100.89^{a}
20±5% ASM	2.20^{b}	3.33^{b}	3.44^{b}	3.01^{c}	3.39^{a}	3.00^{a}	2.76^{a}	3.06^{c}	74.77^{c}	100.02^{b}	94.95^{b}	91.98^{c}
Mean	2.49^{bc}	3.55^{ab}	3.67^{a}	3.25^{a}	3.22^{a}	2.98^{b}	2.80^{c}	3.00^{a}	80.54^{b}	105.68^{a}	102.68^{a}	97.85^{a}
	SEm±		CD at 5%		SEm±		CD at 5%		SEm±		CD at 5%	
Cultivar (cv.)	0.20		0.61		0.08		0.24		2.15		7.68	
Depth of irrigation (D)	0.07		0.25		0.07		0.10		0.65		2.23	
Moisture regimes (M)	0.08		0.25		0.11		0.10		0.65		2.23	
cv. ×M	0.12		NS		0.08		NS		0.65		2.23	
cv. ×D	0.08		NS		0.14		NS		2.15		NS	
cv. ×D	0.15		0.72		0.20		NS		2.45		NS	
cv.×M×D	0.25		NS		0.22		NS		2.85		NS	

Letters showing the difference (*P*>0.05), NS=Non significant, and ASM= Available Soil Moisture

3.3. Essential oil content

The essential oil content was slightly higher (3.15%) in cv. Kosi as compared with CV. CIM-Kranti (3.0%). Maximum oil content was recorded (3.26% and 3.22%) under 3 cm depth of irrigation. Whereas, minimum essential oil content (3.01% and 2.80%) was observed under 9 cm depth of irrigation in cv. Kosi and CIM-Kranti. Likewise, the oil content was found higher

(3.30% and 3.10% in cv. Kosi and cv. CIM-Kranti) with 40±5% ASM as compared with other treatments of moisture stress. Essential oil content reduces significantly in cv. Kosi with an increase in the depth of irrigation water. There was not any significant reduction in essential oil content with the imposition of the levels of moisture stress. It suggested that the imposition of stress up to 20±5% ASM is equally useful for increment in oil content (Table 3). Minimum depth of irrigation and moisture stress reported maximum oil content. The reason might be that an increase in the numbers of leaf peltate hair during moisture stress caused higher essential oil content (Matraka *et al.,* 2010). Similarly, in the present study, several researchers reported an increment in essential oil content with an increase in water stress. Simon et al. (1992) reported that maximum oil gland density, reduction in the area of leaf and increment in essential oil content was induced by moisture stress. In, *Cuminum cyminum L.,* seed phenolics, leaf phenolic, anthocyanin, and essential oil content were enhanced under water stress conditions (Alinian *et al*., 2016). Similar findings were also reported by several researchers in various aromatic and medicinal plants *viz., Pelargonium graveolens* L. (Khalid *et al*., 2010), *Carum carvi.* (Laribi *et al.,* 2009), black cumin (Bannayan *et al.,* 2008) and *Matricaria chamomilla* (Baghalian *et al.,* 2011).

3.4. Essential oil yield, menthol content and menthol yield

Essential oil yield in cv. Kosi was higher (101.01 kg ha^{-1}) over CIM-Kranti (97.85 kg ha^{-1}); however, the differences were not statistically significant. Maximum oil yield (111.10 kg ha^{-1}) was reported at depth of irrigation up to 6 cm in cv. Kosi, followed by (105.68 kg ha^{-1}) in cv. CIM-Kranti. However, concerning available soil moisture, essential oil yield was recorded as highest (105.78 and 100.89 kg ha^{-1} in cv. Kosi and CIM-Kranti) when irrigations were applied at 40±5% ASM. Essential oil yield enhanced with an increase in depth of irrigation up to 6 cm. There was no significant increase in oil yield with further enhancement in the depth of irrigation. Similarly, essential oil yield was higher when irrigations were applied at 40±5% ASM as compared with 60±5% & 20±5% ASM.

There was no significant difference in menthol content between depth and level of moisture stress. Cultivar Kosi reported higher menthol content (78.05%) as compared with cv CIM-Kranti (75.64%). Maximum menthol content (79.35%) was obtained at 3 cm depth of irrigation as compared with other treatments. Likewise, higher menthol content (79%) was recorded at 20±5% ASM, followed by other treatments. In cultivar CIM-Kranti, the highest menthol content (76.34%) was also recorded with 3 cm depth of irrigation. Similarly, maximum (76.35%) menthol content was recorded under 20±5% ASM over other treatments of moisture regimes. There was a slight decrease in menthol content with an increase in moisture stress (Table 4).

Maximum menthol yield (86.61 kg ha^{-1}) was reported at 6 cm depth of irrigation, whereas lower menthol yield (65.58 kg ha^{-1}) was reported under 3 cm depth of irrigation. Likewise, the highest menthol yield (80.53 kg ha^{-1}) was obtained when irrigations were maintained at 40±5% ASM in cv. Kosi. The highest menthol yield (84.02 kg ha^{-1}) was observed under 6 cm depth of irrigation in cv. CIM-Kranti. Similarly, the maximum menthol yield (80.53 kg ha^{-1} in cv. Kosi was recorded when irrigations were applied at 40±5% ASM. Whereas in cv. CIM-Kranti, it was recorded (79.83 kg ha^{-1}) under 60±5% ASM. Enhancement in menthol yield was recorded with an increase in depth irrigation up to 6 cm, further increasing in the depth of irrigations did not result in any significant increase or decrease in menthol yield. There was no significant reduction in menthol yield with an increase in moisture stress in cv. Kosi. However, menthol yield reduced significantly with the increase in moisture stress beyond 40±5% ASM in cv. CIM-Kranti (Table 4). The present investigation revealed that essential oil yield was found higher under 6 cm depth of irrigation and when irrigations were applied at 40±5% ASM. However, menthol content and menthol yield were recorded significantly higher in this treatment as compared with other treatments. A combination of 6 cm depth of irrigation and 40±5% ASM has proved effective in enhancing oil yield, menthol content, and menthol yield. Cultivar Kosi has reported significantly higher essential oil yield over CIM-Kranti due to higher oil content and genetic variability.

Moreover, the highest oil yield in the optimized depth of irrigation and available soil moisture (6 cm and 40±5%) might be presumably due to the balance in water and oxygen supply to the root which results in enhanced water use efficiency. Whereas the highest depth of irrigation and available soil moisture (9 cm and 60±5%) has led to waterlogging leading to anoxic conditions near the root zone of the plant, which created unfavourable conditions for plant growth and yield. Similarly, oil yield decreased in treatments with higher moisture conditions. Therefore, the optimum moisture condition for essential oil yield was 6 cm depth of irrigation with 40±5% ASM. Similarly, a minimum depth of irrigation and moisture level observed lower yield, due to limited availability of water to plants. Moreover, the growth and development of plants were slightly decreased in the plants of menthol mint. Menthol content was found higher in cv. Kosi over CIM-Kranti. It might be due to the CV. Kosi showed a higher oil yield as compared with CIM-Kranti. Under hot climatic conditions, the growth and development of *Ocimum basilicum* L. were found poor. Whereas the oil quality and content were enriched due to moisture stress (Kalamartzis *et al*., 2020). Likewise, higher menthol yield was recorded in cv. Kosi as compared with CIM-Kranti. Therefore, the optimum depth of irrigation and available soil moisture (6 cm and 40±5%) resulted in higher oil yield with significant enhancement in menthol yield. An increase in water stress resulted in an increment in essential oil as reported by various researchers Yaseen et al. (2003) in *Ocimum basilicum* L., and (Khalid, 2006) in other *Ocimum* species. Under different moisture stress conditions, the essential oil content increased in *Ocimum basilicum* L. (Khalid, 2006). Similarly, Telci et al. (2006) also reported that various constituent and linalool content in *Ocimum basilicum* L. significantly increased under water deficit conditions. Essential oil content and various chemical constituents were also affected by various moisture stress in *Matricaria chamomilla*. The linalool content was higher at I_{50} as compared with I_{125} of field capacity in *Ocimum basilicum* L., (Ekren *et al.*, 2012). Several researchers have reported the positive impact of moisture stress in several aromatic plants such as *Mentha arvensis* L. (Mishra and Srivastava, 2000), *Mentha arvensis* L. (Behera *et al.*, 2014), *Pelargonium graveolens* L.(Eiasu *et al*., 2008), *Mentha piperita* L. (Khorasaninejad *et al.*, 2011) and *Cymbopogon winterianus* (Shabih *et al.*, 2000).

Table 4. Menthol content (%), and menthol yield (kg ha^{-1}) as influenced by depth of irrigation and moisture regimes in *Mentha arvensis* L.

Moisture regimes \ Depth of irrigation	3 ha cm	6 ha cm	9 ha cm	Mean	3 ha cm	6 ha cm	9 ha cm	Mean
	Menthol content (%)				Menthol yield (kg ha^{-1})			
	Kosi							
60±5% ASM	78.99^{a}	76.18^{a}	74.87^{a}	76.68^{c}	66.46^{a}	84.59^{a}	83.99^{a}	78.91^{b}
40±5% ASM	79.03^{a}	78.38^{a}	78.03^{a}	78.48^{ab}	67.63^{a}	89.23^{a}	83.20^{a}	80.53^{a}
20±5% ASM	80.04^{a}	78.79^{a}	78.17^{a}	79.00^{a}	61.34^{b}	84.38^{a}	80.27^{a}	75.89^{c}
Mean	79.35^{a}	77.79^{ab}	77.02^{bc}	78.05	65.58^{c}	86.61^{a}	82.98^{b}	78.94
	CIM-Kranti							
60±5% ASM	75.73^{a}	74.94^{a}	74.54^{a}	75.07^{c}	67.55^{a}	85.50^{a}	84.41^{a}	79.83^{a}
40±5% ASM	76.45^{a}	75.80^{a}	74.27^{a}	75.50^{ab}	65.30^{a}	85.13^{a}	81.67^{a}	77.77^{b}
20±5% ASM	76.82^{a}	76.52^{a}	75.71^{a}	76.35^{a}	59.78^{b}	80.13^{a}	74.41^{b}	72.19^{c}
Mean	76.34^{a}	75.75^{ab}	74.84^{bc}	75.64	64.68^{c}	84.02^{a}	80.68^{b}	77.07
	SEm±		CD at 5%		SEm±		CD at 5%	
Cultivars (cv.)	3.15		NS		3.25		NS	
Depth of irrigation (D)	0.25		0.96		0.45		1.42	
Moisture regimes (M)	0.28		0.96		0.39		1.42	
cv. ×M	0.65		NS		0.65		NS	
cv. ×D	3.15		NS		3.05		2.01	
cv. ×D	3.08		NS		3.58		NS	
cv. ×M×D	3.15		NS		3.65		NS	

Letters showing the difference (*P*>0.05), NS=Non-significant, and ASM=Available Soil Moisture

3.5. Water requirement and water use efficiency

The effects of depth and available soil moisture on irrigation water requirement and water use efficiency are presented in Table 5. The water requirement was comparatively less in cultivar Kosi (488 mm) as compared to CIM-Kranti (508 mm). Maximum water was utilized (658 mm) under 9 cm depth of irrigation in cv. Kosi whereas it was significantly higher in cv. CIM-Kranti, i.e., 748 mm. Likewise, the utilization of water in menthol mint by influenced considerably by available soil moisture. Usage of water was higher (628 mm and 688 mm in cv. Kosi and CIM-Kranti, respectively) when irrigations were applied at 60±5% ASM as compared to 20±5% ASM, i.e., (208 and 268 mm in cv. Kosi and CIM-Kranti, respectively). Water requirement increases with an increase in depth of irrigation irrespective of cultivars, whereas it reduces with the rise of moisture stress.

Water use efficiency was recorded highest (0.23 and 0.19 kg oil ha^{-1} mm^{-1}) in cv Kosi and CIM-Kranti. The highest water use efficiency (0.34 and 0.30 kg oil ha^{-1} mm^{-1}) was observed under 3 cm depth of irrigations as compared with (0.17 and 0.14 kg oil ha^{-1} mm^{-1} in cv. Kosi and CIM-Kranti) under 9 cm depth of irrigations in menthol mint. Likewise, maximum water use efficiency (0.45 and 0.34 kg oil ha^{-1} mm^{-1}) was obtained at 20±5% ASM over (0.16 and 0.14 kg oil ha^{-1} mm^{-1} in both the cultivars) under 60±5% ASM (Table 5). However, it was increased with increased moisture stress. Water requirement was significantly higher in treatment up to 9 cm depth of irrigation and 60±5% ASM as compared to 3 cm depth of irrigation and when irrigations were applied at 20±5% ASM. It might be due to that increased irrigation water might have cause resulted in maximum percolation and utilization of water, which resulted in improved biological yield in both the experimental years. In this treatment, plants have grown properly without any stress conditions leading to an increase in biological yield.

Similarly, irrigation water requirement was reduced at a treatment combination of 3 cm depth of irrigation and 20±5% ASM). The water use efficiency was recorded higher under 3 cm depth of irrigations and irrigations were applied at 20±5% ASM. Severe water stress might have caused a reduction in percolation and evapotranspiration, which adversely affected leaf photosynthesis and tissue water potential. Correspondingly, it resulted in decline growth, development, and yield of plants. Farre and Faci (2009) have also reported that yield and water use efficiency of the crop can be increased by optimization of irrigation regimes that can improve the benefits of irrigation water as compared to cost as well as help in saving water resources. In this way, irrigation schedule optimization is often performed by considering the sensitivity of plants with respect to water stress at various life spans (Kirda, 2002). It was also reported that an increased in number of irrigations resulted in improved water use efficiency in winter wheat (Dong *et al*., 2011).

Table 5. Water requirement (mm) and water use efficiency (kg oil ha^{-1} mm^{-1}), water requirement for one kg oil production (L kg^{-1}) as influenced by depth of irrigation and moisture regimes in *Mentha arvensis* L.

Moisture regimes \ Depth of irrigation	3 cm	6 cm	9 cm	Mean	3 cm	6 cm	9 cm	Mean
	Water requirement (mm)				Water use efficiency (kg oil ha^{-1} mm^{-1})			
Kosi								
60±5% ASM	328^a	628^a	928^a	628^a	0.25^c	0.17^c	0.12^b	0.16^c
40±5% ASM	268^b	508^b	748^b	508^b	0.32^b	0.23^b	0.15^b	0.21^b
20±5% ASM	118^c	208^c	298^c	208^c	0.61^a	0.50^a	0.34^a	0.45^a
Mean	238^c	448^b	658^a	448^b	0.34^a	0.25^b	0.17^c	0.23^a
CIM-Kranti								
60±5% ASM	358^a	688^a	1018^a	688^a	0.22^c	0.15^c	0.10^{bc}	0.14^c
40±5% ASM	298^b	568^b	838^b	568^b	0.29^b	0.19^b	0.13^b	0.18^b
20±5% ASM	148^c	268^c	388^c	268^c	0.51^a	0.37^a	0.24^a	0.34^a
Mean	268^c	508^b	748^a	508^a	0.30^a	0.21^b	0.14^c	0.19^b
	SEm±		CD at 5%		SEm±		LSD at 5%	
Cultivars (cv.)	6.18		23.97		0.01		0.02	
Depth of irrigation (D)	3.54		12.65		0.01		0.01	
Moisture regimes (M)	3.98		12.65		0.01		0.01	
cv.×M	4.67		12.65		0.01		NS	
cv.×D	6.15		NS		0.02		0.01	
M×D	5.08		2.26		0.01		2.26	
cv.×M×D	4.15		NS		0.02		NS	

Letters showing the difference (P>0.05), NS=Non significant, and ASM=Available Soil Moisture

3.6. Economics

The lowest cost of cultivation was computed in cv. Kosi as compared with CIM-Kranti. The minimum cost of oil production was recorded under 3 cm depth of irrigation over other treatments (Fig. 3). Likewise, in the case of moisture regimes, the minimum value of cost oil production was computed when irrigations were applied at 20±5% ASM over 60±5% ASM and 40±5% ASM. The cost of cultivation was increased with an increase in the depth of irrigation. The cost of oil production was reduced with an increase in moisture stress. As shown in Fig. 2, the highest gross and net return were computed under 6 cm depth of irrigation as compared with 3 and 9 cm depth of irrigation in both the cultivars of menthol mint Similarly, maximum gross returns were computed under 40±5% ASM over 60±5% ASM and 40±5% ASM.

Net returns were computed higher under 6 cm depth of irrigations and when irrigations were applied at 40±5% ASM irrespective of cultivars in menthol mint. The cost of one-kilogram essential oil production was always lower in CV. Kosi over CIM-Kranti. It might be due to higher production of essential oil in cv. Kosi as compared with CIM-Kranti. The depth of irrigation and moisture regimes (6 cm and 40±5% ASM) was also computed to lower the cost of one-kilogram essential oil production, irrespective of cultivars. Correspondingly, the benefit: costs ratio was also computed higher under 6 cm depth of irrigation and when irrigations were applied at 40±5% ASM in both the cultivars of menthol mint, respectively, but cv. Kosi obtained a slightly higher benefit-cost ratio as compared to CIM-Kranti. The cost of one kilogram of essential oil production was lower and the benefit: cost ratio was higher under the treatment combination of 6 cm depth of irrigations and 40±5% ASM, it might be presumably that the essential oil yield, gross and net return was computed higher in both the cultivars of menthol mint (Fig. 3 and 4).

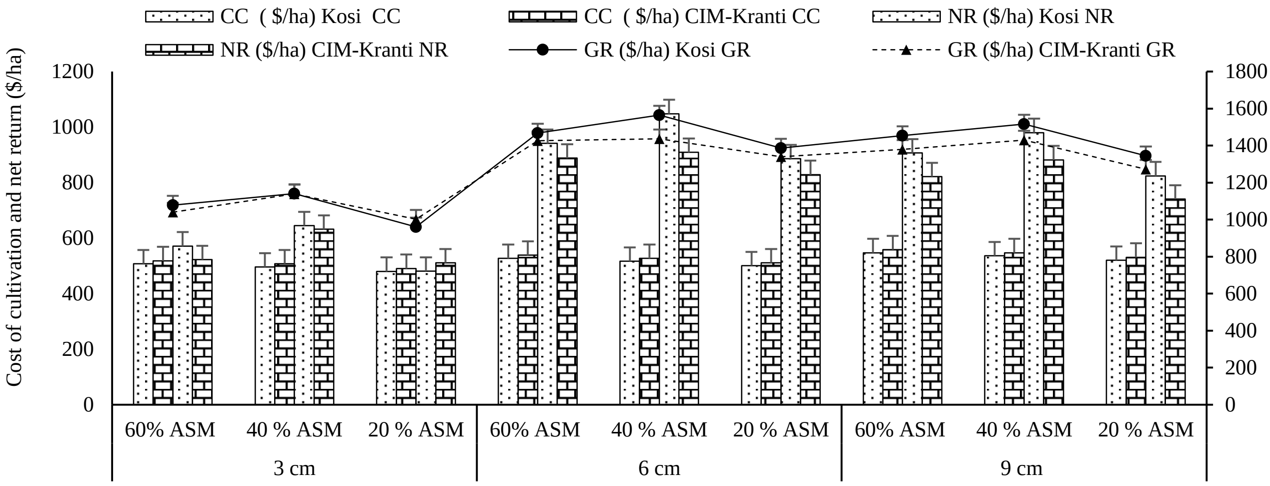

Figure 3. Cost of cultivation, gross, and net return influenced by the depth of irrigation and moisture regimes in *Mentha arvensis* L. CC=Cost of cultivation; GR=Gross Return; NR=Net return; 1 USD= 74.68 INR; USD=United States Dollar; INR=India Rupees; cm= centimetre; ASM= Available Soil Moisture, and Error bars= Standard deviation (SD)

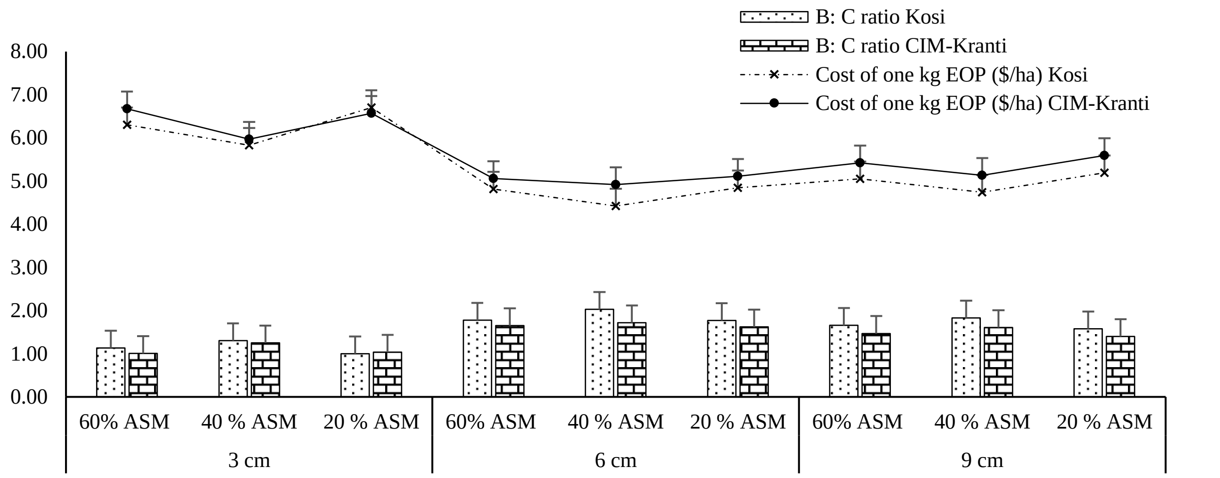

Figure 4. Cost of one-kilogram essential oil production and benefits cost ratio influenced by the depth of irrigation and moisture regimes in *Mentha arvensis* L. B:C ratio= Benefits cost ratio; EOP= Essential oil production; cm= centimetre; ASM= Available Soil Moisture, and Error bars= Standard deviation (SD)

4. Conclusion

The treatment combination of 9 cm depth of irrigation and 60±5% ASM will potentially increase herb yield. The essential oil yield and menthol content will highly improve under the optimized treatment combination of 6 cm depth of irrigation and when irrigations were applied at 40±5% ASM in both the cultivars of menthol mint. It might be due to the physiological and genetic makeup that fevers have higher essential oil content and yield under this treatment. Among the treatments, minimum water requirement and maximum water use efficiency were recorded under 3 cm depth of irrigation and when irrigation was applied at 20±5% ASM. It is concluded from the present investigation that deficit irrigation practices gave the highest essential oil, menthol yield, and water use efficiency with net economic returns. Overall maximum yield was recorded under 6 cm depth of irrigation and when irrigations were applied at 40±5%ASMin cv. Kosi and CIM-Kranti of menthol mint.

Hence, a combination of 6 cm depth of irrigation and when irrigation was applied at 40±5% ASM was found to be the most beneficial combination in cv. Kosi and CIM-Kranti. This combination can be most productive and profitable to the farmers as well as industries. It was also economically the most valuable option due to better resource use efficiency and reduced input cost, especially for water consumption.

Conflict of interests

The authors declare that they have no recognized competing financial interests that could have seemed to impact the work reported in this paper. There are no conflicts of interest that appear for the manuscript submission.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

Actual experimentation, writing a review, and writing an original draft, were done by Devendra Kumar; Help in recording observation by Anuj Kumar: Help in writing and recording the observation in the manuscript by Nilofer: Statistical and data analysis by Anil Kumar Singh; Help in editing the manuscript by Archana Choudhary; Help in recording observation by Kushal Pal Singh: Tabulation and data analysis by Rakesh Kumar; Help in field study during irrigation by Santosh C. Kedar; Chemical analysis done by C.S. Chanotiya: Help in writing and editing the manuscript by Puja Khare: Conceptualization and Supervision by Saudan Singh. All the authors agree to the submission of the manuscript.

Informed consent

The authors declare not to use any patients in this research.

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