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**ORIGINAL RESEARCH ARTICLE**

# Effect of foliar application of Moringa Leaf Extract (MLE) on crop growth, herb yield and oil quality in *Mentha arvensis*

DIPENDER KUMAR[1] • PRIYANKA SURYAVANSHI[2] • PRAWAL PS VERMA[3] • RAJENDRA C PADALIA[1] • AMIT CHAUHAN[1] • VENKATESHA KT[1] • RAKESH K UPADHYAY[1] • AMIT K TIWARI[1]

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**ABSTRACT**

*Enhancing growth and productivity of medicinal and aromatic plants using natural plant growth promoters such as moringa (Moringa oleifera, Lam.) has recently gained wide attention. However, there are no elaborate reports on the impact of Moringa Leaf Extract (MLE) as growth promoter in mint crop. This study was conducted to investigate the effect of Moringa Leaf Extract (MLE) on growth, herb yield and essential oil content in Mentha arvensis L. var. Kosi. Foliar application with MLE at 1%, 2% and 3% concentration on plants were compared with the control (water spray). Results revealed that all the crop-growth parameters were significantly improved as compared to the control. Interestingly, not only essential oil content but also the marker compounds especially menthol and isomenthone, were also increased. Among applied treatments, foliar application of 2% MLE was the most effective in improving the crop performance. This treatment enhanced the menthol content in the oil up to 6.3% in comparison to control. Conclusively, the current study suggests that MLE may be used as a promising natural growth enhancer to improve productivity of Mentha arvensis.*



## INTRODUCTION

Mint (*Mentha arvensis* L.) is one of the most important aromatic plant which is well known for its medicinal and pharmacological properties. Essential oil of mint is extensively used in many pharmaceutical as well as confectionary industries. Globally, India is the largest producer of mint oil, having share of 80-85% (Bose et al., 2013). Due to presence of high menthol content in its essential oil, *M. arvensis* has unique importance in mint family. Essential oil is stored in leaves in specialized secretory structure called as glandular trichomes (Shanker et al., 1999; Sharma et al., 2003). At maturity, the aerial part is cut down and the essential oil is extracted through hydro-distillation process. Vegetative growth, age of plant and foliation patterns become major factors for oil production and its quality. There are several other factors which are

*Corresponding Author; Email: priyanka@cimap.res.in
[1]CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre, Pantnagar, Uttarakhand-263149, India
[2]CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, Uttar Pradesh-226015, India
[3]CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre, Purara (Bageshwar), Uttarakhand-263641, India
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responsible for the quality and yield of essential oil, like season, temperature, moisture, solar radiation and other climatic and rhizospheric situations.

For making farming sustainable and profitable, focus must be on low-cost inputs in agriculture. This includes novel agricultural practices such as foliar sprays on plants with natural, environment-friendly, substances. *Moringa oleifera* (miracle tree) is a well-known plant due to its high nutrition value. Moringa plant is rich in nutrients such as antioxidants, vitamins, minerals, phytonutrients and protein. Researchers have reported the role of Moringa leaves extract of foliar application in enhancing yield of different crop. Some authors found significant enhancement in growth of plants on foliar application of Moringa leaves extract in rocket pea and onion (Abdalla, 2013; Mishra et al., 2013; Mohammed et al., 2013), respectively. Moringa leaf extract is considered one of the most important plant bio-stimulants which can be used as a natural and alternative source of mineral nutrition (Phiri and Mbewe, 2010). Presently, Moringa leaf extract has gained massive importance and attention for its useful application because of presence of cytokinin's like zeatin, antioxidants such as ascorbic acid, amino acids, flavonoids, carotenoids, vitamins A and C, phenolics and macro as well as micro-nutrients (Latif and Mohamed, 2016; Rady et al., 2013). The main hormone found in MLE is Zeatin and its concentration is higher than other studied plants so far (Rady et al., 2013). MLE as a natural and eco-friendly bio stimulant can be applied to improve growth and productivity attributes of various crops grown in normal conditions (Ashraf et al., 2016; Nasir et al., 2016).

Therefore, this study was conducted to investigate the effect of foliar application of Moringa leaves extract at different concentrations on growth, herb yield, essential oil yield and oil quality in *Mentha arvensis L.* (mint).

## MATERIALS AND METHODS

Experiment was carried out at the experimental farm of CSIR-Central Institute of Medicinal & Aromatic Plants, Research Centre, Pantnagar during summer 2020. The experimental site is located between latitude 29° N and longitude 79.38° E, and at an altitude of 243 m above mean sea level. Soil can be characterized as mollisol, with neutral pH of 7.8. The crop, *Mentha arvensis* L. Variety 'Kosi' was raised to study the effect of foliar application of Moringa leaves extract at different concentrations (1%, 2% and 3%) on growth, yield and quality of essential oil. The experiment was laid out in Completely Randomized Design (CRD) with five replications of each treatment along with control (water as foliar spray) in pots. Single plant was planted per pot and foliar spray was started one month after transplanting and applied four times during the whole experiment in morning time at 8 A.M. The foliar spray was applied at 15 days interval. For preparation of Moringa Leaves Extract, young leaves of moringa were harvested from young full-grown trees located at CSIR-Central Institute of Medicinal & Aromatic Plants, Research Centre, Pantnagar (India). For preparation of MLE, 100g young leaves were ground with a pinch of water (10 ml/ 100 g fresh material) using mortar and a pestle. The juice was extracted by hand pressure and was filtered through cheese cloth. The solution was re-filtered using No.2 Whatman filter paper. Following the method developed by Fuglie (2000), the extract was diluted with distilled water at a ratio of 1:32 (v/v) and sprayed directly onto the mint plants. The MLE was used within five hours from cutting and extracting. The prepared extract was stored at 0ºC temperature in a refrigerator and used as stock solution. Further, concentrations were made as per treatments using this stock solution and were taken out only when needed for use.

All the observations related to growth, essential oil yield and quality were recorded at maturity of the crop. For growth measurement, plant height, canopy, leaf: stem ratio, leaf length, leaf width, primary branches, secondary branches per plant were recorded. For yield, herb yield and oil yield were recorded. For quality, the composition of essential oil was studied. To extract the essential oil, 100g leaves with stem from each treatment (in triplicate) were hydro-distilled on the same day. For

maximum recovery of essential oil, fresh leaves were hydro-distilled for 3h. All the samples were hydro-distilled using glass Clevenger type apparatus by recommended method. Oil volume was measured directly in the extraction burette. Yield percentage was calculated as volume (mL) of essential oil per 100g of plant material. Collected oil was dried over anhydrous sodium sulphate in sealed vial at low temperature. Essential oil in samples were analysed using GC-FID and GC-MS techniques. GC-FID analysis of essential oil was carried out on Thermo Fisher Trace GC-1300 equipped with TG-5 (30 m ´ 0.25 mm; 0.25 µm film thickness) fused silica capillary column and with flame ionization detector (FID). Nitrogen was used as carrier gas at 1.0 mL $min^{-1}$. Oven temperature programming was done from 60-230°C at 3°C $min^{-1}$. Injector and detector temperatures were 230°C and 250°C, respectively. Injection volume was 0.02 µL neat with a split ratio of 1:40. GC-MS performed on Clarus 680 GC coupled with Clarus SQ 8C mass spectrometer of PerkinElmer fitted with Elite-5 fused silica capillary column (30 m × 0.25 mm; film thickness 0.25 µm). Oven column temperature program was from 60 to 240°C at 3°C $min^{-1}$ with initial and final hold time 2 min. Helium was used as carrier gas at 1.0 mL $min^{-1}$. Injection volume was 0.02 µL neat with split ratio 1:30. The injector and ion source temperatures were maintained constant at 250°C. Ionization energy was 70 eV (EI). Essential oil constituents were identified by comparing retention indices (RI), mass spectra library search and by comparing their mass spectra (MS) data with literature data (Adams, 2007). Recorded data were statistically analyzed by using OPSTAT statistical software package (Sheoran, 2010) at 5% level of significance.

## RESULTS AND DISCUSSION

### *Growth attributes, herb and essential oil yield*

Data pertaining to growth attributes influenced by foliar spray of MLE at varied concentrations are presented in Table 1. Data revealed that plant height was not significantly influenced by foliar application of MLE at varied concentrations, although, the plant height is slightly higher as compared to the control treatment. (Water spray). Plant canopy was increased significantly as the concentration increased up to 3% MLE (2831.40 $cm^2$) as compared to control (2302.80 $cm^2$). Canopy growth was statistically at par with each other in foliar spray of MLE at 1% (2409.00 $cm^2$) and at (2425.20 $cm^2$) concentration. Canopy formation in treatment of foliar spray of MLE at 3% concentration was significantly higher as compared to rest of the treatments. Leaf-stem ratio and leaf length recorded in $T_2$: 2% MLE as foliar spray was significantly higher as compared to control (Table 1). In case of leaf width (cm) no significant effect of MLE foliar spray at various concentrations were observed. (Table 1). Number of secondary branches were increased significantly. Maximum number of secondary branches were recorded in treatment of 2% MLE as foliar spray (38.00) as compared to control (33.80), while it decreased as the concentration further increased to more than 2%. Yasmeen et al., (2013) and Elzaawely et al., (2016) confirmed the findings that foliar application of MLE have positive affect resulting the plant growth. There are several phytohormones present in MLE such as auxin, which improve cell elongation and promotes stem growth, gibberellins, which enhances plant height and cytokinin having

**Table 1: Effect of foliar spray of Moringa Leaf Extract (MLE) on crop growth parameters of *Mentha arvensis***

| Treatments | Plant height (cm) | Canopy ($cm^2$) | L:S ratio | Leaf length (cm) | Leaf width (cm) | No. of Secondary branches $plant^{-1}$ |
|---|---|---|---|---|---|---|
| **$T_0$: water spray** | 61.0 | 2302.80 | 1.376 | 4.58 | 2.65 | 33.80 |
| **$T_1$: 1% MLE** | 67.8 | 2409.00 | 1.460 | 5.02 | 2.73 | 34.80 |
| **$T_2$: 2% MLE** | 66.4 | 2425.20 | 1.516 | 5.23 | 2.78 | 38.00 |
| **$T_3$: 3% MLE** | 64.4 | 2831.40 | 1.450 | 5.00 | 2.73 | 32.80 |
| **CD at 5%** | **NS** | **174.49** | **0.057** | **0.34** | **NS** | **3.74** |

role in cell division and apical dominance modification (Taiz and Zeiger, 2010). Significant increase in herb yield per plant was observed for MLE treated plants as compared to control (Table 2). Maximum herb yield per plant was recorded in MLE at 2% concentration as compared to control, resulting in 12.5% increase. Similarly, treatment of MLE @ 1% recorded 6.3 per cent increase in herb yield and treatment of 3% MLE recorded 10.37% increase in herb yield when compared to control.

Essential oil yield was significantly enhanced due to MLE application as compared to the control. Highest per cent increase in essential oil yield was found in treatment $T_2$ which was 12.5% more than the control. Similarly, treatment $T_1$ and $T_3$ recorded 6.37% and 10.36% increase over control respectively (Table 2). Moringa Leaf Extract (MLE) has been found as an excellent source of bio stimulant not only for the plant growth but also for the yield in different crops (Ashraf et al., 2016). Yasmeen et al. (2013) and Rady and Mohamed (2015) had reported that due to presence of antioxidants, phenols, essential nutrients and phytohormones in MLE, it is considered as a valuable plant stimulant. Ali et al., (2018) confirmed the findings in Geranium and reported that MLE treatment improved the biomass yield in geranium. Prabhu et al., (2011) found that the herb weight of *Ocimum sanctum* was significantly increased due to MLE application. Our results are in line with the above findings. Because of presence of various chemical components in MLE, it can be used as a bio stimulant in order to enhance the mint growth and productivity.

***Essential oil composition***

The foliar spray of MLE at different concentrations influenced the limonene, menthone, isomenthone, menthol and menthyl acetate in essential oil of *Mentha arvensis* (Table 3). Foliar applied MLE showed stimulatory effect as compared to control. Foliar spray of MLE at 2% concentration showed the highest value of essential oil content in leaves of mentha. 2% MLE as foliar application enhanced menthol content in oil up to 81.30% in comparison to control (76.25%) which showed 6.21% increase over control. 1% MLE application showed 2.42% increase of menthol content over control. In case of isomenthone, the highest percentage was found at 1% MLE application (5.95) followed by 2% MLE application (5.52), 3% MLE application (5.50) and control (5.21). Further, increase in concentration of MLE beyond 2%, resulted in a decline in the value of essential oil content and the percentage of menthone and isomenthone compounds in the essential oil (Table 3). Percentage of limonene, menthone and menthyl acetate were found maximum in control as compared to MLE application. Interestingly, Moringa leaf extract (MLE) application not only increased the herb yield and oil yield but also improved the content of marker compounds in essential oil of *Mentha arvensis* and this might be due to the presence of plant growth promoter in MLE that improved the content of marker compounds in essential oil. Increase in the essential oil composition and content might be due to several components which are present in MLE such as amino acids, nutrient elements and phytohormones. These results are in line with the

**Table 2: Effect of foliar spray of Moringa Leaf Extract (MLE) on herb and essential oil yield of *Mentha arvensis***

| Treatments | Herb yield (gm plant$^{-1}$) | Herb yield (q ha$^{-1}$) | Essential oil (ml per 100gm) | Essential oil yield (Kg ha$^{-1}$) |
|---|---|---|---|---|
| **$T_0$: water spray** | 280.00 | 207.40 | 0.96 | 207.40 |
| **$T_1$: 1% MLE** | 299.00 | 221.50 | 0.96 | 221.50 |
| **$T_2$: 2% MLE** | 320.00 | 237.04 | 1.00 | 237.04 |
| **$T_3$: 3% MLE** | 312.40 | 231.38 | 1.06 | 231.38 |
| **CD at 5%** | **29.06** | **21.54** | **0.064** | **20.27** |

**Table 3: Effect of foliar spray of Moringa Leaf Extract (MLE) on essential oil quality of *Mentha arvensis***

| Compounds | Treatments | | | |
|---|---|---|---|---|
| | $T_0$:water spray | $T_1$:1% MLE | $T_2$:2% MLE | $T_3$:3%MLE |
| Limonene (%) | 2.82 | 2.10 | 1.14 | 2.52 |
| Menthone (%) | 4.63 | 4.50 | 3.30 | 4.22 |
| Isomenthone (%) | 5.21 | 5.95 | 5.52 | 5.50 |
| Menthol (%) | 76.25 | 78.14 | 81.30 | 76.96 |
| Menthyl acetate (%) | 5.41 | 3.87 | 3.58 | 4.02 |
| **Total** | **94.32** | **94.56** | **94.84** | **93.21** |

findings reported for coriander by (Mazrou, 2019), and also for geranium by Ali et. al. (2018) that MLE treatment is a promising growth promoter that positively affects the volatile oil and MLE application improved the volatile oil composition as well (Table 3). Bano et al., (2016) revealed that plant growth regulators improved the physiological and biochemical attributes that directly affect the terpenoid pathway. So, the quantity and quality of volatile essential oil was enhanced.

Therefore, the present study clearly suggests that foliar application of Moringa Leaf Extract (MLE) which is an outstanding source of nutrients, amino acids, phytohormones, phenolics and antioxidants could be used as plant growth regulator in Mentha crop having significant positive effect on growth parameters and also improved the yield component such as herb yield and essential oil yield as well as volatile essential oil content. Moringa leaf extract application showed a potential to improve quantity and quality of this plant. This study suggests that application of MLE through foliar spray at 2% concentration at regular intervals can result in better crop growth and yield and oil quality Mentha in agro-climatic conditions of Pantnagar, Uttarakhand region. However, more research is needed at field level to recommend the novel effect of MLE for better production of essential oils of other aromatic crops.

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