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Melatonin Dose-Mediated Increment in Growth, Floral Production, Essential Oils Yield and Composition in Marigold

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ABSTRACT

Globally, African marigold (MG) derived essential oils (EOs) have attained an immense economic pertinence in the flavor, fragrance, food, medicinal, and floricultural industries, which necessitate boosting its production on a commercial scale. Therefore, we aimed to assess varying levels (0, 50, 100, 150, and 200 mg L^{-1}) of exogenously applied melatonin (MT) as a growth hormone to trigger growth, flower yield, and EOs of MG. The MT was applied as a foliar spray after thirty days of transplantation of MG plants and repeated thrice at fifteen-day intervals. The results depicted that exogenous MT (150 mg L^{-1}) recorded the maximum plant height and leaves number along with fresh and dry weights of leaves and roots. The same treatment exhibited 66%, 64%, and 18% higher flower fresh and dry weights and flower yield respectively, than the control. Additionally, MT remained effective in reducing days taken to bud emergence and flowering, while flower retention duration increased by 11 days. Following the trend of vegetative growth traits, foliar-applied MT (150 mg L^{-1}) remained unmatched in terms of physiological attributes (transpiration rate, stomatal conductance, chlorophyll a, and b contents) of MG. Moreover, for EOs extracted from fresh and dry flowers and leaves, this treatment remained effective by producing 77%, 73%, 53%, and 85% higher content, respectively than the control. Finally, the chemical profiling analyses detected eighty-seven chemical constituents (Caryophyllene oxide was the most dominant compound, and cis-Z-alpha-Bisabolene epoxide followed it) in MG flowers. In terms of the retention time of different chemical compounds in MG flowers, Calarene epoxide had the highest retention time of 19.75 minutes among major compounds. Based on these results, 150 mg L^{-1} dose of MT may be recommended to growers for boosting MG growth, floral yield and EOs content sustainably.

Keywords: *Tagetes erecta*, GC-MS analysis, hydro distillation, melatonin, essential oil, floral yield.

INTRODUCTION

African marigold (*Tagetes erecta*) (MG) is an annual flowering plant and belongs to the genus *Tagetes* and family Asteraceae (Guzman et al., 2023). Globally, MG has become a promising cash crop that can be grown in varied pedo-climatic conditions; however, it is typically grown in tropical regions on a wide scale. Recently, it has acquired immense economic importance in terms of aesthetic value and as a source of EOs worldwide (Tudora et al., 2024), which has boosted its commercial scale production in South Asian countries, particularly Pakistan (Zulfiqar et al., 2020). MG finds its uses as a culinary spice in the preparation of a variety of traditional medicines for wound healing and pesticides. Likewise, its flowers contain pigments, particularly zeaxanthin and lutein, that serve as food coloring agents of high quality as a substitute for saffron, and edible dye preparation. Moreover, secondary metabolites such as carotenoids, lutein, triterpenes,

thiophenes, and flavonoids are abundant in *T. erecta* flowers. It also contains complex mixtures of volatile and bioactive compounds called EOs. The *Tagetes* spp. derived EOs are generally yellow to red-amber and have a strong, sweet, and fruity particularly citrus-like aroma. These EOs have medium viscosity, however, these undergo polymerization and resultantly become thick like gel (Mir et al., 2018).

Domestic and international market demand of MG driven EOs has been persistently on the rise owing to their utilization in the production of pharmaceuticals (due to the presence of antioxidants), cosmetics, and food-grade flavors, along with being diuretics, antibacterial, vision protection, and anti-inflammatory (Aslam et al., 2016). The MG-derived EOs have shown a wide range of antinociceptive, insecticidal, antifungal, anticancer, antiepileptic, allelopathic, larvicidal, hepatoprotective, anti-diabetic, antidepressant, and mosquitocidal actions (Safar et al., 2020). Although, several studies have reported EOs presence in MG, however, the composition of EOs tends to vary significantly depending on biological factors like the genetic potential of varieties and abiotic factors (temperature, precipitation, soil salinity, etc.) along with agronomic management practices (Zhnag et al., 2020). This implies that there could be pronounced variations in the chemical compositions of EOs derived from MG which necessitate conducting fresh studies to ascertain the composition of EOs extracted from indigenous potent varieties of MG.

The exogenous application of melatonin (MT: N-acetyl-5-methoxytryptamine), which is a low molecular weight substance, might be developed as a pro-environment, farmers-friendly, economically viable, and potent strategy to boost MG growth (Moustakas et al., 2023). The MT was initially identified in the cow's pineal glands and was found to have antioxidant activity and critical roles in modulating numerous physiological processes in animals. Thereafter, MT was also isolated as an indoleamine hormone from different vascular plants in 1995. Previously, it has been reported that MT exerted an auxin-like function which triggered plant growth and development (Talaat et al., 2023). Li et al. (2021) opined that MT prevented oxidative harm to lipids, proteins, and nucleic acids by directly scavenging ROS (reactive oxygen species) or by controlling the activity and biosynthesis of enzymes and non-enzymes antioxidants. Additionally, it functioned as a crucial signaling molecule that triggered plant physiological functions and shielded plants from various abiotic stressors. Likewise, Mukherjee and Bhatla (2020) noted that MT effectively lowered the biosynthesis of reactive oxidative species (ROS) and restricted cell damage and electrolyte leakage under unfavorable conditions. They also reported MT's role as a promoter of plant development, plant stress reliever, and a blooming and fruit maturation regulator. Moreover, Chen et al. (2021) reported a pronounced influence of MT on morphological characteristics, mineral nutrition, nitrogen, and ROS metabolism in alfalfa, while it also delayed senescence in rice. Furthermore, Okunlola et al. (2023) inferred that exogenously applied MT (50 μM) increased the accumulation of osmolyte, biosynthesis of pigments, and inorganic ions concentration in capsicum species. Sardar et al. (2023) reported MT (50 and 100 μM) doses also remained effective in boosting plants vegetative and physiological growth of broccoli plants.

Although the role of MT in the biosynthesis of EOs in flowering plants like MG is still poorly understood, the underlying mechanisms of MT need to be studied from the perspective of linkage between plant growth attributes and the chemical composition of EOs. Moreover, research gaps regarding the optimum dose of exogenous MT necessitate conducting fresh studies to boost MG cultivation as a cash crop on a commercial scale. Therefore, we hypothesized that MG plants might respond differently to atypical doses of MT in terms of yield attributes, yield, and EOs composition owing to the varying impact of MT concentrations on vital physico-chemical processes of MG. Thus, a study was designed with the prime aim of improving the growth and yield of marigolds through foliar-applied melatonin, quantification, and chemical composition analysis of essential oils extracted from the leaves and flowers of marigolds grown in a South Asian environment.

MATERIALS AND METHODS

Plant materials and experimental treatments

Marigold (*Tagetes erecta*) healthy and superior quality seeds were used as planting material in this study and sown to raise nursery at the Horticulture Research Sub-Station for Floriculture and Landscaping, Agriculture Complex, Multan, Punjab, Pakistan (Latitude 30.19679,

longitude 71.47824 and altitude of 131 m from the sea level) having semi-arid conditions during 2022–2023. One-month-old seedlings of MG were transplanted manually in the open field by maintaining P×P and R×R spacing of 45 cm and 90 cm, respectively. The trial was comprised of different concentrations of MT (0, 50, 100, 150, and 200 mg L^{-1}) that were applied as a foliar spray on MG plants after one month of transplantation. The treatments were repeated thrice at fifteen days' intervals. The trial was conducted using the randomized complete block design (RCBD) in regular arrangement with 5 replications (no separate replication was maintained for recording response variables). For conducting the EOs analysis, the fresh aerial parts (flowers and leaves) of the MG plants were collected from each treatment early in the morning to avoid solar radiation impact and subsequently, samples were stored in pre-tagged Kraft paper bags for further analysis.

Recording of response variables

For recording response variables, five randomly selected plants were used and then their average was computed. Plant height and root length were estimated using a tailor's measuring tape (Iqbal et al., 2021), whereas roots and shoot's fresh weights were determined using a digital balance immediately after plant harvesting (Tarikuzzaman et al., 2024). The dry weights were determined by putting shoots and root samples in an oven (70 °C) until constant weights were obtained (Iqbal et al., 2024).

For estimating the pigment content of MG, fresh leaves were selected, and a clean mortar and pestle was used for grinding purposes. To maintain a low temperature during the extraction process, a small amount of liquid nitrogen and pre-chilled acetone were added to the mortar to prevent the degradation of chlorophyll pigments. Thereafter, the paste was shifted to a clean centrifuge tube containing acetone (usually 80% acetone). Following the extraction period, the leaf extract was gently swirled to ensure thorough mixing. A spectrophotometer was used to measure the chlorophyll content in the extract. The spectrophotometer was set to the appropriate wavelengths for chlorophyll measurement, typically around 645 nm and 663 nm. Absorbance readings at the specified wavelengths were recorded. Using the obtained absorbance values, the chlorophyll content was calculated using appropriate equations or formulas. Common equations include the Arnon (1948) equation.

$$\text{Chlorophyll a (mg g-1) in original tissue sample} = \text{Chlorophyll a (mg/ml)} \times \text{final volume (ml)} \quad (1)$$

$$\text{Chl a} = [12.7(OD663) - 2.69(OD645)] \times \times V/1000 \times W \quad (2)$$

$$\text{Chlorophyll b (mg g}^{-1}\text{) in original tissue sample} = \text{Chlorophyll a (mg/ml)} \times \text{final volume (ml)} \quad (3)$$

$$\text{Chl b} = [22.9(OD645) - 4.68(OD663)] \times \times V/1000 \times W \quad (4)$$

An infrared gas analyzer (CI-340 portable, Hoddesdon, England) was used for transpiration rate estimation. The water vapor pressure in the chamber was kept between 6.0 and 8.9 m bar, the molar flow of air per unit leaf area was kept at 403.3 mmol m^2 s^{-1}, the ambient temperature of 22.4–27.9 °C, ambient CO_2 concentration (352 mol mol^{-1}), leaf temperature of 28.4–32.4 °C, and atmospheric pressure 99.9 KPa (Abbas et al., 2023).

Extraction of essential oil

The EOs were extracted from fresh flowers and leaves by using a hydro-distillation technique (Ahmad et al., 2024). Firstly, fresh and healthy flowers and leaves were collected and air dried for 5 days at room temperature. Subsequently, the flower's petals were separated and placed (300 g) in the flask of hydro-distillation apparatus containing the distilled water (1 L). After that, the flask was kept on a heating mantle and connected to the Clevenger apparatus. When boiling started the vapors moved from the flask to the Clevenger apparatus where vapors were condensed back to water due to the continuous circulation of water in the condenser and then started to settle in the Clevenger column. Heating continued for 3 hours, and EOs settled on the top of the water in the column due to being light in weight. Water was drained out and EOs were collected in Eppendorf for further gas chromatography-mass spectrometry (GCMS) analysis.

The EOs yield was calculated by following Equation 5.

$$\text{EOs (\%)} = (\text{EOs extracted(ml)})/ /(\text{Total weight of petals (g)}) \times 100 \quad (5)$$

GC-MS analysis

To analyze the chemical composition of EOs, a gas chromatography-mass spectrometer (GC-MS) (model GCMSQP2010 SE, Japan) with a Rxi-624silMS capillary column (length 30 m,

diameter 0.32 mm, and thickness 1.80 µm) and a turbo molecular pump (58 L/sec for He) were used. Additionally, a rotary pump operating at 30 L/min (60Hz) was also utilized. The GC-MS analysis was conducted under the following conditions: 1.0 µl of injection volume, (250 °C) of injection temperature, oven temperature (40–300 °C in 27 min), 1.40 ml/min of helium carrier gas, split ratio of 50.0, 1 min of sampling time, injection mode split less, (200 °C) of ionization, raw control mode pressure (9.7 kPa), interface temperature (250 °C), and 6 min of solvent cut time. Finally, the components were identified through comparison with a pre-stored mass spectrum (MS) database. Finally, the GC-MS was used to determine the chemicals' relative concentrations.

Statistical analyses

The data of all response variables under study were recorded, arranged, and analyzed statistically as Fisher's analysis of variance (one-way ANOVA) was employed for estimating overall significance. Thereafter, a comparison for treatment means was used by employing the least significant difference (LSD) test at the level of 5% probability with the help of the SAS statistical package (9.2 Version, SAS Institute, Cary, NC, USA) (Gomez and Gomez, 1984).

RESULTS

Vegetative growth traits

As per recorded findings, exogenous MT had highly significant effects on all vegetative growth parameters of the MG plant (Table 1). The tallest plants (116.90 cm) were recorded by 150 mg L^{-1} which was 33% higher than the plant height (87.85 cm) recorded by the control treatment. Interestingly, the highest dose of MT (200 mg L^{-1}) produced 18% shorter plants than 150 mg L^{-1}, however, it performed better than the rest of the treatments. Likewise, all doses of MT remained effective in increasing leaves and branches number per plant of MG, however, 150 mg L^{-1} dose surpassed the rest of the treatments by exhibiting 56% and 57% greater leaves and branch numbers, respectively than the control, whereas it was followed by 200 mg L^{-1} treatment. Moreover, MT application remained instrumental in triggering the stem diameter and leaf area of MG, especially 150 mg L^{-1} treatment remained unmatched by recording the maximum values (65% and 114% greater than the control treatment, respectively). Besides the leaf area of MG, the foliage applied MT (150 mg L^{-1}) effectively improved leaf fresh and dry weights (33% and 400, respectively) than the control treatment, whereas it remained statistically at par with the 200 mg L^{-1} dose for leaf dry weight. On the similar patterns of leaf fresh and dry weights, foliage applied MT triggered leaf growth as well as evident from improved root fresh and dry weights, especially 150 mg L^{-1} dose increased these root traits by 378 g and 21 g respectively than the control treatment. As far as whole plant weight and plant dry matter were concerned, 150 mg L^{-1} dose of MT remained superior by recording the maximum values (155 g and 3%, respectively greater than the control treatment) of these traits. Overall, the increasing dose of MT remained effective in boosting the vegetative growth of MG up to the dose of 150 mg L^{-1}, whereas 200 mg L^{-1} treatment performed below par to 150 mg L^{-1}, except for leaf dry weight, however, it remained superior to rest of foliage applied MT treatments under investigation.

Reproductive growth traits

The reproductive growth traits of MG were significantly influenced by different doses of MT

Table 1. Effect of different doses of foliar applied melatonin on vegetative growth traits of marigold

Treatments	Plant height (cm)	Leaves number per plant	Branches number per plant	Stem diameter (cm)	Leaf area (cm^2)	Leaf fresh weight (g)	Leaf dry weight (g)	Root fresh weight (mg)	Root dry weight (mg)	Whole plant weight (g)	Dry matter (%)
Control	87.85e	169.01e	21.12e	1.14e	2.13e	1.10e	0.20d	134.33e	13.73e	651e	65.17e
50 mg L^{-1}	90.48d	192.43d	24.05d	1.36d	3.26d	1.18d	0.23c	269.39d	18.24d	752d	66.09d
100 mg L^{-1}	94.12c	220.27c	27.53c	1.50c	3.30c	1.36c	0.27b	356.18c	28.70c	787c	67.08c
150 mg L^{-1}	116.90a	264.93a	33.12a	1.89a	4.56a	1.47a	0.29a	596.44a	39.63a	851a	68.50a
200 mg L^{-1}	98.48b	256.75b	32.09b	1.75b	4.16b	1.38b	0.28ab	512.33b	34.54b	806b	67.41b

Note: *values having atypical letters within the same column vary significantly from each other at $P \leq 0.05$.

applied as a foliar spray (Table 2). The control treatment exhibited the maximum days taken to bud emergence and flowering, whereas MT foliage application (150 mg L^{-1}) remained effective in reducing the number of days for these traits by 3 and 8 days, respectively. It was followed by the MT dose of 150 mg L^{-1} which in turn was followed by 200 mg L^{-1}. Likewise, the MT dose of 150 mg L^{-1} remained superior to other doses in terms of flowering duration which were 11 and 4 days greater than the control and 200 mg L^{-1} treatments, respectively. As far as flower number per plant and flower diameter were concerned, foliage applied MT (150 mg L^{-1}) remained effective in boosting these traits by 37% and 133%, respectively, compared to the control treatment. This treatment also remained instrumental in boosting the flower number per plant and flower diameter by 1% and 84% respectively, compared to the following treatment of 200 mg L^{-1}. As far as flower fresh and dry weights were concerned, 150 mg L^{-1} doses significantly improved these traits (66% and % higher than the control treatment), while it was followed by the greatest dose of MT (200 mg L^{-1}) which in turn was followed by 100 mg L^{-1} dose of foliar applied MT (Table 2). For flower yield, the exogenously applied MT (150 mg L^{-1}) outmatched other treatments by producing 18% greater flower yield than the control treatment, whereas the following treatment 200 mg L^{-1} produced 11% higher flower yield than the control treatment. Overall, following the trend of vegetative growth traits, the increasing dose of MT remained effective in boosting the reproductive growth traits of MG up to the dose of 150 mg L^{-1}, whereas 200 mg L^{-1} treatment performed below par to 150 mg L^{-1}, however, this treatment also remained superior to rest of foliage applied MT doses under investigation.

Physiological growth traits

The recorded findings revealed that foliage applied MT in different doses remained effective in significantly affecting the physiological growth parameters of MG (Table 3). It was noted that MT (150 mg L^{-1}) significantly triggered the transpiration rate of MG that was 3.2 mmol H_2O $m^{-2}s^{-1}$ higher than the control treatment, whereas it was also higher than the transpiration rate recorded by MG plants receiving 200 mg L^{-1} dose of MT. The lowest dose of foliar-applied MT performed below par with the rest of the treatments, however, it recorded a significantly higher transpiration rate than the control treatment. Likewise, the stomatal conductance of MG plants was effectively triggered by the MT dose of 150 mg L^{-1} which was higher than the control treatment by 39 µmol H_2O $m^{-2}s^{-1}$. It was followed by the maximum dose of MT (200 mg L^{-1}) that recorded 24% higher

Table 2. Effect of different doses of foliar applied melatonin on reproductive growth traits of marigold

Treatments	Days to bud emergence	Days to flowering	Flowering duration (days)	Number of flowers per plant	Flower diameter (cm)	Flower fresh weight (g)	Flower dry weight (g)	Flower yield (g)
Control	13.49a	61.53a	111.16e	70.35e	3.20e	7.46e	1.49e	635.96e
50 mg L^{-1}	12.30b	59.30b	113.61d	76.68d	3.74d	8.51d	1.70d	647.33d
100 mg L^{-1}	11.70c	56.73c	115.51c	82.10c	4.45c	9.75c	1.95c	658.55c
150 mg L^{-1}	10.25e	53.51e	122.78a	96.38a	7.14a	12.36a	2.47a	751.74a
200 mg L^{-1}	10.89d	55.24d	118.87b	95.43b	5.24b	10.21b	2.04b	705.01b

Note: *values having atypical letters within the same column vary significantly from each other at $P \leq 0.05$.

Table 3. Effect of different doses of foliar applied melatonin on physiological growth traits of marigold

Treatments	Transpiration rate (mmol H_2O $m^{-2}s^{-1}$)	Stomatal conductance (gs) (µmol H_2O $m^{-2}s^{-1}$)	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)
Control	2.91e	106.0e	0.15d	0.11e
50 mg L^{-1}	3.31d	116.0d	0.16c	0.13d
100 mg L^{-1}	3.62c	123.0c	0.18b	0.14c
150 mg L^{-1}	6.10a	145.0a	0.19a	0.16a
200 mg L^{-1}	4.40b	131.0b	0.18b	0.15b

Note: *values having atypical letters within the same column vary significantly from each other at $P \leq 0.05$.

stomatal conductance compared to control. Interestingly, even the lowest dose of foliage applied MT remained instrumental in boosting the stomatal conductance of MG by 9% than control. Pertaining to the chlorophyll (a and b) content, 27% and 45% increments in chlorophyll-a and chlorophyll-b were recorded for the MT dose of 150 mg L^{-1}, which was followed by 200 mg L^{-1} which in turn remained statistically at par to 100 mg L^{-1} for chlorophyll-a content (Table 3). Following the trend of vegetative and reproductive growth traits of MG, all foliage-applied doses of MT remained effective in triggering the physiological growth traits of MG under investigation, however, 150 mg L^{-1} remained unmatched which was followed by the highest dose (200 mg L^{-1}) which in turn was followed by 100 mg L^{-1}, whereas the lowest dose (50 mg L^{-1}) even performed significantly better than the control treatment.

Essential oils yield

The essential oils were extracted from fresh and dry flowers as well as leaves of MG using the hydro-distillation technique and analyzed by GC-MS (Gas chromatography- mass spectrometry) protocol. The results revealed that foliage-applied MT in different doses remained effective in improving the EOs productivity from flowers and leaves of the MG (Table 4). As per recorded findings, EOs extraction from fresh flowers and leaves was on the higher side than the corresponding values recorded for dry flowers and leaves of MG. Among foliage-applied doses of MT, 150 mg L^{-1} remained superior by producing significantly higher EOs in fresh and dry flowers of MG that were 44% and 74% higher than the control treatment, respectively. The MT dose of 200 mg L^{-1} produced 11% and 18% fewer EOs from fresh and dry flowers of MG, respectively than the most performing treatment of 150 mg L^{-1}. This treatment was followed by 100 mg L^{-1} which in turn was followed by 50 mg L^{-1} for EOs production from fresh and dry flowers of MG. Overall, fresh and dry leaves of MG produced significantly higher EOs than the corresponding values recorded by fresh and dry flowers of MG (Table 4). The results revealed that leaves of MG responded positively to foliage-applied MT especially 150 mg L^{-1} dose remained unmatched by producing 53% and 80% greater EOs in fresh and dry leaves of MG, respectively compared to the control. This treatment was closely followed by the highest dose of MT (150 mg L^{-1}), which in turn was followed by 100 mg L^{-1}. Interestingly, in comparison to the control treatment, the lowest dose of foliar-applied MT produced 17% and 35% higher EOs in fresh and dry leaves of MG, respectively, however, it was significantly lower than the corresponding values recorded by the most performing treatment of 150 mg L^{-1}.

Essential oils profiling

The chemical composition analyses of MG flowers revealed the presence of a total of 87 chemical compounds which entailed 9 major, 14 micro, and 64 trace compounds, whereas the corresponding values in leaves (total 100 chemical compounds identified) were 14, 15, and 71, respectively (Tables 5 and 6). As per characterization analyses, among major compounds in the EOs extracted from flowers of MG, Caryophyllene oxide was the most dominant compound and it was followed by cis-Z-alpha-Bisabolene epoxide, whereas humulene and bicyclo [10.1.0] tridec-1-ene were the most dominant minor and trace compounds, respectively (Table 5). In terms of the retention time of different chemical compounds in the MG flowers, Calarene epoxide had the highest retention time of 19.75 minutes among major compounds. Likewise, 2,2':5',2''-Terthiophene, and Bicyclo [7.2.0] undec-4-ene had the maximum retention time of 26.86 and 29.96 minutes respectively among minor and trace compounds.

Table 4. Effect of different doses of foliar applied melatonin on essential oils (EOs) production of fresh and dry flowers and leaves of marigold

Treatments	EO from fresh flowers (%)	EO from dry flowers (%)	EO from fresh leaves (%)	EO from dry leaves (%)
Control	0.22e	0.19e	0.30e	0.20e
50 mgL^{-1}	0.29d	0.24d	0.35d	0.27d
100 mgL^{-1}	0.31c	0.27c	0.39c	0.33c
150 mgL^{-1}	0.39a	0.33a	0.46a	0.37a
200 mgL^{-1}	0.35b	0.28b	0.43b	0.35b

Note: *values having atypical letters within the same column vary significantly from each other at $P \leq 0.05$.

Table 5. The chemical composition of essential oils extracted from the marigold flowers using the GC-MS analysis

Major components			
Sr. No.	Chemical Compound	Retention time (minutes)	Percentage
1	Caryophyllene oxide	15.58	10.86
2	cis-Z-.alpha.-Bisabolene epoxide	18.96	10.10
3	Benzenemethanol,	16.26	7.29
4	Calarene epoxide	19.75	7.07
5	(-)-Spathulenol	15.51	5.75
6	Naphthalene,	16.18	4.02
7	Globulol	16.55	4.68
8	Naphthalene	16.51	2.46
9	Spiro [4.4]nonan-2-one	15.90	2.15
Minor components			
10	Humulene	13.75	1.98
11	Silane, trimethylphenyl	9.62	1.97
12	2-Cyclohexen-1	10.72	1.95
13	Benzene, 1-ethoxy-4-ethyl	12.02	1.28
14	Caryophyllene	13.25	1.31
15	1-(2-Hydroxycyclohexyloxy)-1H-pyridin-2-one	13.94	1.06
16	Benzene	15.85	1.32
17	Globulol	15.99	1.04
18	alpha. –Guaiene	16.08	1.75
19	Cyclopentanecarboxaldehyde	18.51	1.05
20	3-Cyclohexene-1-carboxaldehyde	18.81	1.37
21	Longifolenaldehyde	19.01	1.20
22	n-Hexadecanoic acid	21.49	1.25
23	2,2':5',2''-Terthiophene	26.86	1.10
Trace components			
24	beta.-Pinene	6.17	0.11
25	.gamma.-Terpinene	6.88	0.54
26	p-Cresol	7.65	0.11
27	1,6-Octadien-3-ol, 3,7-dimethyl	7.93	0.58
28	1,6-Octadien-3-ol, 3,7-dimethyl	8.11	0.73
29	5-Octen-2-one, 3,6-dimethyl	8.39	0.14
30	2H-Pyran, 3,4-dihydro-6-methyl	8.62	0.54
31	Cyclohexene, 1,2-dimethyl	8.89	0.15
32	Benzenemethanol, .alpha.,.alpha	9.39	0.41
33	.gamma.-Terpinene	9.51	0.18
34	Ethanone, 1-(3-methylphenyl)	9.57	0.29
35	L-.alpha.-Terpineol	9.75	0.47
36	1H-Benzimidazole, 5-methoxy	10.07	0.22
37	2-Cyclohexen-1	10.46	0.42
38	2,6-Octadien-1-ol	10.88	0.34
39	4-Hexen-1-ol	11.05	0.14
40	p-Cymen-7-ol	11.26	0.31
41	Quinuclidine	11.37	0.41
42	2,4-Pentadien-1-ol	11.46	0.18
43	Acetic acid	11.75	0.25
44	2,6-Octadienal, 3,7-dimethyl	12.12	0.30

45	o-Methyl o-butyl isopropylphosph	12.49	0.26
46	Isoborneol	12.70	0.37
47	2-Hexanoylfuran	13.07	0.22
48	2-Pentene	13.36	0.14
49	2-Methylene cyclopentanol	13.59	0.51
50	Furan	13.67	0.13
51	5,5-Dimethyl-1,3-hexadiene	14.11	0.34
52	Naphthalene	14.22	0.49
53	2-Isopropenyl-4a	14.31	0.23
54	Santolina epoxide	14.40	0.15
55	2-Isopropylidene-3-methylhexa-3	14.99	0.63
56	Bicyclo [10.1.0] tridec-1-ene	15.09	0.99
57	alpha.-Farnesene	15.15	0.93
58	Caryophyllene	15.25	0.76
59	Cyclohexene	15.42	0.22
60	Guaiol	15.70	0.18
61	Alloaromadendrene	15.78	0.59
62	Caryophyllene oxide	16.68	0.72
63	Caryophyllene oxide	16.73	0.92
64	Spiro[5.6]dodecane	16.81	0.43
65	Alloaromadendrene	17.10	0.53
66	4-Decyne	17.30	0.89
67	Camphene	17.45	0.75
68	Solavetivone	17.58	0.89
69	Epianastrephin	17.86	0.59
70	Isobornyl propionate	18.58	0.72
71	Caryophyllene oxide	19.17	0.48
72	Isoaromadendrene epoxide	19.31	0.20
73	cis-p-mentha-1(7)	19.46	0.16
74	Cycloheptane	19.55	0.16
75	2-Propenoic acid	19.97	0.23
76	Thianthrene	20.11	0.23
77	Isoaromadendrene epoxide	20.98	0.38
78	Isoaromadendrene epoxide	22.01	0.13
79	1(2H)-Phenanthrenone	22.46	0.49
80	Phytol	22.70	0.30
81	Octadecanoic acid	23.37	0.29
82	11H-Indeno[1,2-b]quinoxaline	25.72	0.96
83	Heptadecane	28.91	0.63
84	2(1H)-Naphthalenone,	28.33	0.20
85	Triacontyl acetate,	29.48	0.26
86	Bicyclo[7.2.0]undec-4-ene	29.78	0.16
87	Bicyclo[7.2.0]undec-4-ene	29.96	0.22

DISCUSSION

In this study, foliar application of melatonin had significant effects on vegetative, reproductive, and physiological growth traits along with essential oils production of marigold flowers and leaves. The recorded findings revealed that growth traits, flower yield, and EOs production were significantly improved by increasing the concentration of foliage-applied MT, however,

the highest dose of MT could not perform at par with its lower dose. Particularly, the 150 mg L^{-1} dose surpassed the rest of the treatments by recording the maximum vegetative growth (plant height, leaves and branches number per plant, stem diameter, fresh and dry weights of leaf and roots, leaf area, and whole plant weight). These findings corroborate with those of Moustakas et al. (2023), who inferred that MT application (10 and 100 μM) imparted significant influence on chlorophyll content and leaf area as in our study that triggered the photosystem II (PSII) function which promoted robust source-sink relationship and ultimately vegetative growth was triggered in mint plants. It was also inferred that both doses of MT played a strategic role in preventing the biosynthesis of reactive oxygen species (ROS) which improved plant growth under the un-noticed incidences of abiotic stresses (particularly heat and drought stress) (Ramírez-Estrada et al., 2023). Moreover, robust vegetative growth recorded by foliage-applied MT was attributed to the non-photochemical quenching (NPQ) mechanism which improved chlorophyll content and PSII functionality causing significant improvement in plant height, stem diameter, and leaf area. These improvements caused more biomass accumulation of roots and ultimately plants fresh and dry weights were also increased. Overall, foliage application of MT (100 μM) was recommended as a photosynthetic bio-stimulant for improving the vegetative growth traits especially root and shoot of crop plants under both favorable and stressed conditions. Moreover, the sub-optimal performance of the highest dose (200 mg L^{-1}) in this study might be attributed to suppression of PSII and biosynthesis of ROS (Islam et al., 2023; Sagar et al., 2023), however future research needs to explore the underlying mechanisms. Similarly, MT's roles in boosting morphological characteristics, energy status, and nitrogen metabolism in alfalfa have also been reported previously by Chen et al. (2021), and similar findings were reported by Cui et al. (2017) in wheat. Moreover, Yang et al. inferred that exogenously applied MT boosted endogenous MT levels and stimulated the organic acids biosynthesis in the root system, and ultimately, root fresh and dry weights were improved along with whole plant weight. Dai et al. (2020) also reported a significant influence of foliage-applied MT on the root growth of rapeseed. Furthermore, it was concluded that exogenous application of MT remained instrumental in boosting the biosynthesis of endogenous MT along with phosphoglycerate kinase, fructose-1,6-bisphosphate esterase, and fructose-1,6-bisphosphate aldolase which improved all vegetative growth traits of tomato seedlings (Yang et al., 2023).

As far as reproductive growth traits (time taken to bud emergence and flowering, number of flowers, flower diameter, flower fresh and dry weights, flowering duration, and flower yield) of MG were concerned, MT (150 mg L^{-1}) foliar application remained effective in boosting these traits on the similar pattern of vegetative growth parameters under investigation. It might be attributed to improved water and nutrient use efficiencies achieved through greater photosynthetic efficiency caused by MT-triggered stomatal conductance and decreased canopy temperature (Sharif et al., 2018). Our findings report a similar trend as recorded by Talaat (2023), who opined that barley plants responded positively in terms of reproductive growth traits to MT (70 μM) under field capacity levels of 30%, 70%, and 100%. It was inferred that Calvin cycle enzyme activity was triggered by MT that boosted gas exchange capacity and strengthened the chlorophyll fluorescence system, which led to greater diversion of assimilated toward reproductive sink-sites, and ultimately, flower diameter and their fresh and dry weights were enhanced. Furthermore, exogenously applied MT increased the concentration of endogenous MT along with indole-3-acetic acid, gibberellins, and cytokinins which played key roles in enhancing the floral retention duration and flower yield. Previously, similar findings have also been reported by Yang et al. (2023), who inferred that foliage-applied MT (100 μmol L^{-1}) improved the endogenous MT levels and chlorophyll fluorescence induction kinetics along with neutralizing the deleterious impacts of alkaline (60 mmol L^{-1} $NaHCO_3$) environment which significantly promoted flowering and fruit development in tomatoes. Moreover, Fu et al. (2023) administered four concentrations of MT (50, 100, 150, and 200 Mm) to cotton seedlings and noted that all doses of MT remained effective in boosting the reproductive growth (flowering and boll formation) by modulating the biosynthesis of superoxide dismutase (SOD), and peroxidase (POD) which improved growth of crop plants even when there was frequent incidence of chilling stress coupled with saline environment. Furthermore, Meftahizadeh et al. (2023) observed that MT triggered the modulation of redox

reactions and improved the phytochemical status which improved the growth of guar.

Besides vegetative and reproductive growth traits, the physiological parameters (transpiration rate, stomatal conductance, chlorophyll a, and chlorophyll b content) of MG plants were pronouncedly improved by 150 mg L^{-1} level of MT. It might be inferred that the same dose of MT resulted in the maximum leaf area and leaf diameter which led to the highest chlorophyll content. Similar findings have been reported by previous studies wherein the NPQ mechanism triggered by exogenous application of MT was attributed to increased chlorophyll (a and b) content (Imran et al., 2021; Kaya and Doganlar, 2018). Moreover, Talaat (2023) concluded that exogenously applied MT effectively improved endogenous MT concentration, which led to enhanced transpiration rate and stomatal conductance, and ultimately, chlorophyll synthesis (owning to strengthening of chlorophyll fluorescence system) and photosynthesis rate were also improved. Moreover, foliage application of MT (50–150 Mm) triggered the photosynthesis rate, and it was attributed to improved chlorophyll content in the leaves of crop plants. Furthermore, Ramírez-Estrada et al. (2023) administered different doses of MT (1, 10, and 100 μM) and results revealed that MT doses especially 10 μM treatment remained unmatched in terms of SPAD values and photosynthetic efficiency, while it was inferred that MT remained effective to promote photoinhibition and improved chlorophyll contents and ultimately photosynthesis rate was increased in green bean plants. Okunlola et al. (2023) also reported similar research findings whereby exogenously applied MT (50 μM) significantly influenced several physiological functions in capsicum species including accumulation of osmolyte, biosynthesis of pigments (chlorophyll a and b), and inorganic ions concentration. Moreover, Imran et al. (2021) also reported MT-induced regulation of physiological functions in soybean plants via the biosynthesis of endogenous nitric oxide. Contrastingly, Jiang et al. (2016) record`ed MT-associated physiological function modulation through antioxidant capacity improvement and ion homeostasis in maize seedlings and root development of sweet cherry (Sarropoulou et al., 2012). Furthermore, Sharif et al. (2018) attributed MT associated physiological growth to the regulation of abscisic acid metabolism and the strengthening of the antioxidant system by MT that maintained stomatal opening and increased pigment content. However, Campos et al. (2019) and Mohammadi et al. (2021) observed a significant reduction in oxidative stress by MT application and concluded that it could impact several other physiological functions in young coffee plants and Anise hyssop.

In this study, EOs were extracted from fresh leaves and flowers of MG that contained monoterpene hydrocarbons (e.g., ocimene, limonene, terpinene, myrcene, etc.) and acyclic monoterpene ketones (e.g., tagetone, dihydrotagetone, tagetenone). Similar findings were previously reported wherein smaller amounts of sesquiterpene hydrocarbons and oxygenated compounds were also present in MG flowers (Sharma et al., 2020; Arnao and Hernández-Ruiz, 2019). In this study, essential oil yield increased with the increase in MT application, which could be attributed to the increasing division rate of the meristematic cells which produced more enzymes that played a vital role in triggering the biosynthesis of EOs. Likewise, the IAA and MT were reported to entail similar chemical structures and performed auxin-like functions to increase the biosynthesis of EOs in tea, cotton, and pistachio plants (Kamiab, 2020). Our results indicated 87 compounds in flowers. The EOs contents were increased by the effect of MT, which improved plant growth and development. There were three types of compounds based on their percentage in oil, which were identified as significant (more than 2%), minor (less than 2% and more than 1%), and trace compounds (less than 0%). Among these compounds, Caryophyllene oxide (10.86%), cis-Z-alpha-Bisabolene epoxide (10.10%), Benzenemethanol (7.29%) were major compounds, Humulene (1.98%), Silane, trimethylphenyl (1.97%), 2-Cyclohexen-1(1.95%) were minor compounds and 2-Isopropylidene-3-methylhexa-3 (0.99%), Bicyclo [10.1.0] tridec-1-ene (0.93%), Caryophyllene oxide (0.92%) were trace compounds were analyzed in flowers of MG.

Our results were different from earlier studies which reported 18 compounds that might be attributed to different varieties, agro-environmental conditions, agronomic management practices etc. whereas the major chemical constituents were piperitone (19.2%), βcaryophyllene (15.2%), limonene (11.7%), methyl eugenol (12.3%), (E) – ocimene (13.7%), piperitenone (8.1%) and terpinolene (11.9%) (Irshad et al., 2021). The study reported a substantial variation in the relative quantities of monoterpenoids, sesquiterpenoids,

and some of the unreported higher molecular weight hydrocarbons in the EOs of aerial parts. Comparing the composition of *T. erecta* flower's essential oil showed some similarities and differences with previous studies (Tudora et al., 2024; Safar et al., 2020). While the major constituent in the flower oil was β-caryophyllene, cis-ocimene, and l-limonene were identified as the major constituent in aerial parts. Unlike, high levels of piperitone and low levels of limonene and piperitenone were reported in *T. patula* EOs that could be attributed to the differential roles of MT in free-radical scavenging and stomatal behavior of plants under abiotic stresses (Shen et al., 2021; Li et al., 2019). This difference between previous studies and these results could be probably due to different species of MG and the MT application mode (seed priming) and time of foliar spray. Parsa Motlagh et al. (2024) also recorded a significant influence of foliage-applied MT (0.5 and 1 mM) on fatty acid composition in sesame.

CONCLUSIONS

Here in this study, we have explored the production potential of marigold by optimizing the foliar applied doses of melatonin. The recorded findings were completely in line with the research hypothesis which demonstrated that exogenous foliar application of MT significantly affected the agro-botanical, physiological, and biochemical characteristics of MG. Overall, the MT dose of 150 mg L^{-1} remained unmatched by triggering the vegetative and reproductive growth of MG by boosting the physiological growth processes (transpiration rate, stomatal conductance, Chlorophyll a, and b contents). The highest dose of MT (200 mg L^{-1}) could not perform at par with 150 mg L^{-1}, however, it remained superior to the rest of the MT doses as well as control treatment. The chemical profiling of EOs extracted from flowers identified 87 chemical constituents. As per characterization analyses, among major compounds in the EOs extracted from flowers of MG, Caryophyllene oxide was the most dominant compound, and it was followed by cis-Z-alpha-Bisabolene epoxide, whereas humulene and bicyclo [10.1.0] tridec-1-ene were the most dominant minor and trace compounds, respectively. In terms of the retention time of different chemical compounds in MG flowers, Calarene epoxide had the highest retention time of 19.75 minutes among major compounds. Likewise, 2,2':5', 2''-Terthiophene, and Bicyclo [7.2.0] undec-4-ene had the maximum retention time of 26.86 and 29.96 minutes, respectively, among minor and trace compounds. Thus, these findings might serve as a baseline to develop MT exogenous application as a biologically viable and potent strategy to boost MG growth and flower yield sustainably. Future studies need to perform the economic analysis of employed MT treatments concerning the added advantages offered in the form of flower yield increment and essential oil yield. Moreover, various underlying mechanisms responsible for inducing MT-related boosts in plant physiological processes still need to be explored for increasing MG yield and essential oil production to meet the persistently increasing demand in food, medicinal, and pesticide industries.

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