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Mitigation of Heat Stress Effects on Chamomile and its Essential Oil Using Melatonin or Gibberellic Acid and some Agricultural Treatments

Shaimaa Ibrahim Mohammad Elsayed[1]; Ahmed E.El-Gohary[2]; Saber Fayez Hendawy[2]; Saied Kamel Mohamed Abd El-Naby[1]

[1]Horticultural Crops Technology Dept. National Research Centre, Dokki, Giza, Egypt.

[2]Medicinal & Aromatic Plants Dept. National Research Centre, Dokki, Giza, Egypt

Abstract

Temperature change risk in horticultural crops includes modified phonology (timing) of leafing, flowering, and harvest. The yield of essential oil of chamomile depends on some factors such as the plant region's environmental conditions. Therefore, this study was carried out during two successive growing seasons on chamomile plants grown under either region; northern Egypt and East of Cairo to reduce the impact of heat stress on growth, oil yield and its components which, the seedlings had been cultivated at half of October and the half of November and sprayed with either gibberellin or melatonin. We observed that spraying melatonin or gibberellins improved growth characteristics compared to control. There was a clear superiority, there was a clear superiority of melatonin on chamomile plants grown under Nubaria which improved most of the previous characteristics. Chamomile seedling can be grown from mid-October to mid-November, so it can be harvested in March before the heat rises and maintains the resulting oil quality maintains the resulting oil quality. The main component of Chamomile oil is (Bisabolol oxide A) and was higher under the conditions of Nubaria when seedling was treated with gibberellin followed in the lowest order by treatment with melatonin, but it was the opposite under the conditions of Sekam region. The second component was (Bisabolol oxide B).

Keywords: chamomile, melatonin, gibberellin, sowing date and locations, oil yield and components.

Introduction

Temperature is one of the major climatic factors that limit geographical distribution of plant species. Crops are sensitive to the magnitude of change in temperature, extreme temperatures (minimums and maximums) and the timing of temperature changes (night vs. day, spring vs. summer), this change will affect not only agricultural production but may alter the geographical distribution and growing season of crops. Temperature change risk in horticultural crops include modified phonology (timing) of leafing, flowering, and harvest[1].Chamomile :(*Matricaria recutita*,L. , *Matricaria chamomilla*,L. *Or Chamomilla recutita* (L.), (Asteraceae) is a well-known medicinal plant species often referred to as the star among medicinal species in the world. Due to its various pharmacological and pharmaceutical properties, the plant possesses great economic value.Pharmacological properties include anti-inflammatory, antiseptic, carminative, healing, sedative, and spasmolytic activity[2]. In addition to pharmaceutical uses, the oil is extensively used the perfumery, cosmetics, and aromatherapy, and food industry[3]. The Egyptian chamomile was categorized under the α bisabololoxide A group[4,5,6].

Over 120 constituents have been identified in chamomile[7]. Chemical constituents identified as secondary metabolites, including 28 terpenoids, 36 flavonoids and 52 additional compounds with potential pharmacological activity[8].The yield of essential oil of chamomile depends on the plant genotype as well as the environmental conditions under which the plants are grown[9,10,4,6,11,12]. Alter planting and established heat-tolerant or low chill tolerant variety and treat the seedlings with natural

*Corresponding author e-mail: shaimaa_elsayed83@yahoo.com .; (Shaimaa M. Elsayed).
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material such as melatonin and gibberellic acid with medicinal& aromatic plants are emergency.

Melatonin is an indolic compound (biogenic indoleamine) structurally related to other important substances, such as tryptophan, serotonin, indole-3-acetic acid (IAA). It can act as a potential modulator of plant growth and development in a dose-dependent manner[13].The dual role of melatonin in plants have recognized as a protector against abiotic and biotic stresses[14]. Melatonin functions in plants can be recognized into three categories: firstly; growth promoters as auxins[15];secondly; antioxidants for free radicals and serve as a first-line defense against oxidative stress[16]; thirdly ;In plants, MEL is considered to be involved in many physiological processes, *e.g.* root and shoot development[17], flowering, flower development, or delaying leaf senescence[18]and other functions (signal molecules for regulation of flower development,[19].Arnao and Hernandez-Ruiz[20] hypothesized that exogenous melatonin might cause changes in the concentration of endogenous free IAA. The chemical structure of melatonin (indoleamine) is like auxin IAA hormone.

Gibberellins are natural growth hormones playing a primary role in stimulating the auxin reaction, that helps in growth and development of many plants as well as its direct effect on internode elongation, flowering, fruiting, quality, and yield. In addition, it has numerous physiological effects on germination, stem elongation, leaf expansion, growth, flowering, and cell expansion[21]. Exogenous application of GA_3 to plants causes the increment in the activities of many key enzymes and photosynthesis [22].

This work aimed to evaluate growth, yield and some chemical constituents of chamomile plants under different locations and different sowing date and investigate the growth promoting activity of some compounds (Melatonin or Gibberellic acid) on the growth of chamomile plants and its reflection on oil yield and its components.

Materials and Methods

This investigation was carried out during two seasons (2018 and 2019) aiming to evaluate growth, yield and essential oil quality of chamomile plants *Matricaria recutita*, L. Grew under different locations, different sowing dates and treatments of growth promoting activity of some compounds. (Chamomile herbarium preserved in the National Research Centre herbarium under No.M143)

Locations were: Sharkia Governorate; Adlya Farm belongs to Sekem Co. (its location is latitude 30° 22' 50.2" N and longitude 31°.39'.1" E.), 80 Km to East of Cairo and National Research Centre Farm for Research and Production at Al-Nubaria region, Al Bahira Governorate its location is latitude 30º 30' 1.4" N, and longitude 30º 19' 10.9" E, Egypt. The climatic data of both locations were presented in Table (1,a and b).

Table 1 (a, b) climate data* for Sekam and Nubaria locations were collected from October to March in 2018 and 2019

a) Sekam location

Date	Soil temperature [°C]	Solar radiation Dgt [MJ/m2]	Wind direction dig [deg]	Precipitation [mm]	Wind speed [m/sec]	Leaf Wetness [min]	HC Air temperature [°C]	HC Relative humidity [%]	Dew Point [°C]
October	22.57	10.14	137.94	0.00	0.66	200.97	21.67	65.58	13.62
Nov.	21.59	7.33	123.03	0.00	0.54	426.67	16.47	75.77	11.19
Dec.	18.55	6.16	159.65	0.00	0.59	611.45	14.89	80.29	10.81
Jan.	16.04	7.63	142.74	0.00	0.87	664.84	12.57	76.81	7.77
Feb.	16.68	10.86	186.64	0.00	0.62	725.36	15.24	76.89	9.99
Mar.	20.15	15.19	186.77	0.00	0.75	130.97	17.98	66.52	9.35

b) Nubaria location

Date	Soil temperature [°C]	Solar radiation Dgt [MJ/m2]	Wind direction dig [deg]	Precipitation [mm]	Wind speed [m/sec]	Leaf Wetness [min]	HC Air temperature [°C]	HC Relative humidity [%]	Dew Point [°C]
October	26.47	5.87	107.77	15.15	0.00	206.14	23.41	70.50	16.70
Nov.	19.87	5.48	112.51	4.19	0.00	418.50	18.77	82.20	14.88
Dec.	17.34	5.47	107.80	0.23	0.00	299.35	15.00	78.71	10.71
Jan.	15.02	9.22	210.43	0.10	0.46	215.16	12.23	60.97	3.58
Feb.	16.29	10.28	214.35	0.06	0.49	202.32	13.49	71.25	7.24
Mar.	19.34	14.36	190.95	0.31	0.55	236.77	15.55	71.90	9.33

*Central Laboratory for Agricultural Climate, Agricultural Research Centre, Egypt.

Two different sowing date (15 of October 15 of November) were carried out under both locations.

The effect of GA_3 and Melatonin (MEL) as a foliar spray on chamomile plants under both locations was studied. These substances were prepared as an aqueous solution at 25 mg/L

The uniform healthy seedlings (10-15 cm length) were obtained from Somasta center nursery and were planting directly in the field on both planting dates. So, the seedlings were transplanted into plots 20 m^2 on rows with 60 cm apart and 25 cm between the seedling, on two sowing date (half of October and half of November) and after about one month of every time, the seedling had been sprayed with either gibberellin or melatonin at 25 mg/L. comparing with another seedling without treated, which sprayed with water. Split plot design was adopted in this study, since locations were put in the main plots, the sowing date was applied to subplots and spray treatments were applied to subplot with three replicates for each treatment.

GA_3 product from Science Lab. com, Inc. Chemical Laboratory Equipment, 14025, Smith Road, Houston, Texas 77396, USA

MEL product from ScienceLab.com, Inc. Chemical Laboratory Equipment,14025, Smith Road, Houston, Texas77396, USA.

All spray solutions contained 0.1% triton B as a wetting agent to avoid the surface tension and sprayed in the morning hours of the day in mist form. The experiment contained twelve treatments as combinations between two planting dates; half of October and half of November at Sekam farm and Nubaria farm and treated by GA_3 and melatonin applications, compared with control.

Other horticultural practices were similar for all plants and as recommended by Agriculture Ministry in Egypt. The drip irrigation system was used to irrigate all the plants.

Soil sample: The physical and chemical properties of the soil samples were analyzed at the beginning of the work (for the Sekam & Nubaria locations) for texture, pH, and electrical conductivity (EC) using the water extract (1:2.5) method.

The soil characteristics of Nubaria location were as follows: sand 57.9%, silt 35.6%, clay 6.5%, pH 7.8, and EC 1.4 ds/m

The soil characteristics of Sekam location were as follows: sand 76.62%, silt 7.96%, clay 15.42%, pH 7.7, and EC 0.83 ds/m.

Data recorded:

Two harvests were obtained during harvests (Feb. & March) for the two times, according to[23].Plant height was recorded. Fresh flower heads were collected from each treatment through flowering stages in both seasons, all of which were air dried. Yield fresh weight and dry weight of flower heads (g/ plant and Kg/ fed.) were obtained.

Samples of the air-dried flower heads from the second harvest were used for the essential oil extraction with hydro-distillation (water distillation) at the Laboratory of Horticultural Crops Technology Dep., National Research Centre, Giza, Egypt. Dry flower heads (100 g) from each treatment with three replicates for the same treatment was hydro-distilled for 3 h using a Clevenger apparatus as described by[24]. The essential oil content was calculated as a percentage. Also, essential oil yield (ml/ plant) was calculated according to the dry weight of flower heads/ plant while essential oil yield per (L/fed) was calculated by multiplying the oil yield per plant by a number of plants per feddan (16800 plants). The extracted oil was dehydrated over anhydrous sodium sulfate and stored in glass vials at deep freezer in the absence of light until used.

Gas chromatography–mass spectrometry analysis (GC-MS)

Most of the compounds were identified using The GC-MS system (Agilent Technologies) was equipped with a gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. Samples were diluted with hexane (1:19, v/v). The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 μm film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.0 ml/min at a splitless mode, injection volume of 1 µl and the following temperature program: 40 °C for 1 min; rising at 4 °C /min to 150°C and held for 6 min; rising at 4 °C/min to 210 °C and held for 1 min. The injector and detector were held at 280 °C and 220 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 50-550 and solvent delay 3 min. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data

Statistical analysis: The data obtained in each respective season were analyzed separately then the combined experiments (Bartlett's test) analysis was used according to[25]. Means were separated by[26] and multiple range test using a significance level of $P<0.05$.

Results and Discussion

1. Growth characters:

Data in Tables (2-6) represented studying the effect of spraying melatonin and gibberellin on chamomile plants grown under two different sown dates in two different locations of cultivation (Sekam and Nubaria) act different climate conditions.

a. Plant height

Results of Table (2) showed clearly that there were significant differences between plants sprayed with gibberellin or melatonin and control plants which had the shortest plants (56.58cm) in the two different locations of cultivation (Nubaria and Sekam) and two sowing dates, whichever gibberellin or melatonin were similar almost and gave highest plants (63.75 and 64.92 cm).

Concerning the effect of sowing date, data in Table (2) show the second sowing date (half of November) in two different locations (Nubaria and Sekam) showed the higher plants (63.94cm) compared with the first sowing date (half of October) which gave the shorter plants (59.56 cm). So, plant height was the tallest under Nubaria location (70.44 cm) compared with Sekam location (53.06 cm). There was a significant effect for the interaction between two different planting locations (Sekam and Nubaria) and spraying melatonin or gibberellin under the first and the second sowing dates. The obtained highest chamomile plants were from Nubaria farm which cultivated at both sowing dates and sprayed with melatonin or gibberellin, so plants sprayed with melatonin at the second sowing date had the sovereign effect. While the shortest plants were recorded with chamomile plants in control treatment which cultivated in Sekam farm (43.00 cm) at first sowing date.

b. Fresh weight of flower heads (g/plant) and (ton/4200m^2.)

Mean of comparisons in Tables (3 and 4) showed that there were significant differences between spray treatments; spraying gibberellin gave the highest values of fresh flower heads weight and fresh yield (167.9 g/plant and 2.821 ton/4200m^2.) followed by melatonin compared with control which gave the lowest value of fresh flower heads weight and fresh yield (108 g/plant and 1.828 ton/4200m^2.) in the two different locations of cultivation (Nubaria and Sekam) and the two sowing dates.

Data in the same Tables there were highly significant differences between two sowing dates; the second sown date showed the higher fresh flower heads weight and fresh yield (152.4 g/plant and 2.651 ton/4200m^2) than the first sowing date in two different planting locations (Nubaria and Sekam).

There was a significant effect for the interaction between two different climate places (Sekam and Nubaria) and spraying treatments at the first and the second sowing date.

The heaviest fresh flower weight and fresh flower yield were obtained with chamomile plants sprayed with melatonin treatment which cultivated in Nubaria farm (206.7 g/plant and 3.472 ton/4200m^2 at the second sowing date, followed by chamomile plants spraying with gibberellin treatment which cultivated in the same location and sowing date (188.7 g and 3.169 4200m^2), respectively. While the lowest fresh flower weight and fresh flower yield were recorded with chamomile plants in control treatment which cultivated in Sekam farm (70.00 g/plant and 1.176 ton/4200m^2) at the first sowing date.

Table 2: The combined effect of Melatonin or Gibberellin spray on plant height (cm/plant) of chamomile plants grown under two different sowing dates at two different planting locations for two seasons

Planting locations	**(Sekam)**		**(Nubaria)**		
sowing Date	**First sowing date***	**Second sowing date****	**First sowing date***	**Second sowing date****	**Mean of Treatments**
Spray Material					
Control	43.00 G	51.00 F	67.33 BC	65.00 BC	**56.58 B**
Melatonin	49.33 FG	59.00 DE	72.33 AB	74.33 A	**63.75 A**
Gibberellin	54.33 EF	61.67 CD	71.00 AB	72.67 AB	**64.92 A**
Mean of planting locations	**53.06 B**		**70.44 A**		
Mean of sowing date	**59.56 B**		**63.94 A**		

Values within each column followed the same letter are not statically significant of 5% level
*Half of October **Half of November

Table 3: The combined effect of Melatonin or Gibberellin spray on flower fresh weight (g/plant) of chamomile plants grown under two different sowing dates at two different planting locations for two seasons

Planting locations	(Sekam)		(Nubaria)		
sowing Date	First sowing date*	Second sowing date**	First sowing date*	Second sowing date**	Mean of Treatments
Spray Material					
Control	70.00 ^{E}	100.0 ^{D}	121.0 ^{CD}	144.3 ^{BC}	**108.8 ^{C}**
Melatonin	130.0 ^{BC}	150.0 ^{B}	185.0 ^{A}	206.7 ^{A}	**167.9 ^{A}**
Gibberellin	100.0 ^{D}	125.0 ^{C}	142.0 ^{BC}	188.7 ^{A}	**138.9 ^{B}**
Mean of planting locations	**112.5 ^{B}**		**164.6 ^{A}**		
Mean of sowing date	**152.4 ^{A}**		**124.7 ^{B}**		

Values within each column followed the same letter are not statically significant of 5% level
*Half of October **Half of November

Table 4: The combined effect of Melatonin or Gibberellin spray on flower fresh yield (ton/4200m^2) of chamomile plants grown under two different sowing dates at two different planting locations for two seasons

Planting locations	(Sekam)		(Nubaria)		
sowing Date	First sowing date*	Second sowing date**	First sowing date*	Second sowing date**	Mean of Treatments
Spray Material					
Control	1.176 ^{E}	1.680 ^{D}	2.033 ^{CD}	2.425 ^{BC}	**1.828 ^{C}**
Melatonin	2.184 ^{BC}	2.520 ^{B}	3.108 ^{A}	3.472 ^{A}	**2.821 ^{A}**
Gibberellin	1.680 ^{D}	2.100 ^{C}	2.385 ^{BC}	3.169 ^{A}	**2.334 ^{B}**
Mean of planting locations	**1.890 ^{B}**		**2.765 ^{A}**		
Mean of sown date	**2.094 ^{B}**		**2.561 ^{A}**		

Values within each column followed the same letter are not statically significant of 5% level
*Half of October **Half of November

c. Dry flower heads weight (g/plant) and (Kg/4200 m^2.)

Data in Tables (5 and 6) indicated that there were significant differences between spray treatments, where spraying melatonin gave the heaviest weight of dry flower heads (64.08 g/plant) and (774.2 Kg/4200m^2). Compared with control which gave the lightest dry flower weight heads (33.17 g/plant) and (557.2 Kg/4200m^2) in the two different locations of cultivation (Nubaria and Sekam) and the two sowing dates. Mean of comparisons in Tables (5 and 6) presented that there was a significant difference between the two sowing dates and two locations recorded the highest dry flower weight g/plant and dry flower yield Kg/4200m^2 were recorded under the second sowing date (44.49 g/plant and 718.7 Kg/4200m^2) at Nubaria location, respectively.

There was a significant effect for the interaction between two different planting locations (Sekam and Nubaria) and spraying melatonin or gibberellin at the first and the second sowing dates. The highest value of dry flower heads weight and dry flower yield was obtained with chamomile plants sprayed with melatonin which cultivated in Nubaria farm (55.00 g/plant and 924.0 Kg/4200m^2) at the second sowing date, followed by chamomile plants sprayed with gibberellin which cultivated in Nubaria farm (50.67 g/plant and 851.2 Kg/4200m^2) at the second sowing date, respectively. In contrast the lowest value of dry flower weight and dry flower yield were recorded with chamomile plants in control treatment which cultivated in Sekam farm (25.00 g/plant and 420.00 Kg/4200m^2) at first sowing date.

Table 5: The combined effect of Melatonin or Gibberellin spray on flower dry weight (g/plant) of chamomile plants grown under two different sowing dates at two different planting locations for two seasons

Planting locations	(Sekam)		(Nubaria)		
Sown Date	First sowing date*	Second sowing date**	First sowing date*	Second sowing date**	Mean of Treatments
Spray Material					
Control	25.00 ^{G}	32.67 ^{F}	35.00 ^{DEF}	40.00 ^{CD}	**33.17 ^{C}**
Melatonin	38.33 ^{CDE}	42.00 ^{C}	49.00 ^{B}	55.00 ^{A}	**46.08 ^{A}**
Gibberellin	33.33 ^{EF}	36.33 ^{DEF}	40.00 ^{CD}	50.67 ^{AB}	**40.08 ^{B}**
Mean of planting locations	**36.78 ^{B}**		**42.78 ^{A}**		
Mean of sowing date	**34.61 ^{B}**		**44.94 ^{A}**		

Values within each column followed the same letter are not statically significant of 5% level
*Half of October **Half of November

Table 6: The combined effect of Melatonin or Gibberellin spray on head dry flower yield (Kg/4200m²) of chamomile plants grown under two different sowing dates at two different planting locations for two seasons

Planting locations	(Sekam)		(Nubaria)		
Sown Date	First sowing date*	Second sowing date**	First sowing date*	Second sowing date**	Mean of Treatments
Spray Material					
Control	420.0 G	548.8 F	588.0 DEF	672.0 CD	**557.2 C**
Melatonin	644.0 CDE	705.6 C	823.2 B	924.0 A	**774.2 A**
Gibberellin	560.0 EF	610.4 DEF	672.0 CD	851.2 AB	**673.4 B**
Mean of planting locations	**581.5 B**		**755.1 A**		
Mean of sowing date	**617.9 B**		**718.7 A**		

Values within each column followed the same letter are not statically significant of 5% level
*Half of October **Half of November

2. Essential oil content and its constituents

a. Essential oil percentage

Results of Table (7) illustrated that spraying melatonin or gibberellin gave the highest essential oil percentage (0.342 % and 0.343%) with insignificant differences between them. while control treatment gave the lowest essential oil percentage (0.295%). This was true in the two different locations of cultivation (Nubaria and Sekam) and the two sowing dates. Results of Table (7) demonstrated that there were significant differences between the two sowing dates and two different locations which, the first sowing date of Sekam location gave the highest essential oil percentage (0.338%, 0.333%) respectively.

There was a significant effect for the interaction between two different cultivation locations (Sekam and Nubaria) and two spraying treatments (melatonin and gibberellin) in the first and the second sowing date. The highest essential oil percentage were obtained with chamomile plants are grown in Sekam farm at the first sowing date which spraying with melatonin (0.366%), followed by chamomile plants spraying with gibberellin which cultivated in Nubaria location at the first sowing date (0.360%). While the lowest essential oil percentage was recorded in chamomile plants in control treatment which cultivated in Nubaria location (0.280%) at second sowing date.

b. Essential oil yield (ml/plant and liter/4200m²)

Mean of comparisons in Tables (8 and 9) explanted that in Sekam and Nubaria farms, the plants spraying with melatonin gave the highest essential oil yield per plant (0.157 ml) and per 4200m² (26.35 liters). While plants in control gave the lowest value of essential oil yield (0.098 ml) and essential oil yield per 4200m² (16.36 liters) at the first and the second sowing date. Also, data showed that there were significant differences between the two sowing dates and between two planting locations, so, the second sowing date showed the highest values of essential oil yield per plant and per 4200m² (0.136 ml and 22.82 liters).

Also, Nubaria farm produced plants contained the highest oil yield per plant and per 4200m². There was a significant effect for the interaction between two different planting locations (Sekam and Nubaria) and two spraying treatments at the first and the second sowing date.

The highest essential oil yield per plant and per 4200m² were obtained with chamomile plants spraying with melatonin which cultivated in Nubaria location at the second sowing date (0.176 ml/plant and 29.64 l/4200m²) and, those which cultivated at the first sowing date (0.169 ml/plant and 28.33 l/4200m².) followed by chamomile plants spraying with gibberellin which cultivated in Nubaria location (0.168 ml and 28.16 liters) at the second sowing date, respectively. In contrast the lowest essential oil yield per plant and per 4200m² were recorded with chamomile plants in control treatment which cultivated in Sekam location (0.080 ml and 13.36 liters) at the first sowing date, respectively.

Our current investigation reported that both foliar application treatments of GA_3 and melatonin enhanced the vegetative growth and increased the flower yield as well as improved flower quality and oil chemical contents compared with untreated control during the two cultivated seasons. It is well known that GA_3 is used as a plant regulator to stimulate both cell division and cell elongation that positively affect the vegetative growth characters. It affects many mechanisms of plant growth including stem elongation by stimulating cell division and elongation, flowering, fruit development, and breaking dormancy[27]. The beneficial effect of GA_3 on plant vegetative growth resulted in more accumulation of quality which is possibly the main reason for flower yield increases and improvement of quality.

Melatonin application as a bio-stimulator could be a good, feasible, and cost-effective method useful in agriculture. Among many functions that melatonin performs in plants, its role as an antioxidant and a growth promoter is most supported by experimental evidence. This compound is an independent PGR, and it may mediate the activities of other PGRs. Due to its antioxidant properties, melatonin can stabilize the cell redox status and protect tissues against reactive oxygen, which accumulated in stressful environments. It is believed that melatonin can increase the food quality (the aspect of functional food) and may improve human health. This compound has the similar chemical structure as auxin; thus, it seems that melatonin may play a similar role in plants as this hormone. Also, the application of melatonin can reduce the negative effect of climate change on plant productivity [20,14,28].

Exogenously applied melatonin affects the developmental processes during both vegetative and reproductive growth. It has been shown that the use of melatonin as an effective biostimulator; antioxidants and growth promoters in agriculture was beneficial and positive in reducing the effect of heat stress on the yield and quality of some crops (Apricot, orange" Navel, Valencia" and Artichoke). Results revealed that between all growth regulators, the foliar application of melatonin on ‘Canino’ apricot trees was the highest vegetative growth following by low second order GA_3 as compared to other treatments, while GA_3 gave the highest yield following by melatonin as compared to other treatments[28]; the low concentration of each of GA_3 and melatonin show a positive effect on most the vegetative growth characteristics and also, fruit set percentage of Washington navel orange was improved by using all spraying materials especially GA_3 and melatonin and this resulted in improving fruit properties and tree yield[29];that spraying with gibberellic acid showed the best vegetative growth of Valencia orange trees grown under sandy soil and led to the highest crop followed in descending order by spraying with melatonin. Most characteristics of the resulting fruits did not differ significantly between all GA_3 and melatonin treatments[30] and so, high productivity of artichoke cultivars could be achieved by using suitable cultivar and cultivation management such as foliar application treatments of GA_3 and melatonin[31].

The yield of essential oil of *Matricaria recutita* depends on the environmental conditions of plant sites as well as the plant genotype[9,10,2,6,12]. The essential oil yields differ with some research which were highly or low and this may be due to many factors e.g., different locations, growing conditions (soil and climate)[32,33]. Some authors have also reported variability of the oil content under the influence of different factors as soil type[33], day light and irradiance[34], and time of flower harvest[6,35]. Thus, in our research cutting was performed two times (February and March). These observations agreed with Bettray and Vomel[23], who report that the essential oil yields increased with the increasing of temperature.

Table 7: The combined effect of Melatonin or Gibberellin spray oil percentage of chamomile plants grown under two different sowing dates at two different planting locations for two seasons

Planting locations	(Sekam)		(Nubaria)		
Sown Date	First sowing date*	Second sowing date**	First sowing date*	Second sowing date**	Mean of Treatments
Spray Material					
Control	0.317 F	0.297 G	0.287 G	0.280 G	**0.295 B**
Melatonin	0.366 A	0.337 CDE	0.343 BCD	0.320 EF	**0.342 A**
Gibberellin	0.353ABC	0.330 DEF	0.360 AB	0.330 DEF	**0.343 A**
Mean of planting locations	**0.333 A**		**0.320 B**		
Mean of sowing date	**0.338 A**		**0.316 B**		

Values within each column followed the same letter are not statically significant of 5% level
*Half of October **Half of November

Table 8: The combined effect of Melatonin or Gibberellin spray oil yield (ml/plant) of chamomile plants grown under two different sowing dates at two different planting locations for two seasons

Planting locations	(Sekam)		(Nubaria)		
Sown Date	First sowing date*	Second sowing date**	First sowing date*	Second sowing date**	Mean of Treatments
Spray Material					
Control	0.080 E	0.097 D	0.101 D	0.112 CD	**0.098 C**
Melatonin	0.141 B	0.142 B	0.169 A	0.176 A	**0.157 A**
Gibberellin	0.118 C	0.120 C	0.144 B	0.168 A	**0.138 B**
Mean of planting locations	**0.116 B**		**0.145 A**		
Mean of sowing date	**0.125 B**		**0.136 A**		

Values within each column followed the same letter are not statically significant of 5% level
*Half of October **Half of November

Table 9: The combined effect of Melatonin or Gibberellin spray on oil yield (l/ 4200m^2.) of chamomile plants grown under two different sowing dates at two different planting locations for two seasons

Planting locations	(Sekam)		(Nubaria)		
Sown Date	First sowing date*	Second sowing date**	First sowing date*	Second sowing date**	Mean of Treatments
Spray Material					
Control	13.36 ^{F}	16.33 ^{EF}	16.90 ^{DE}	18.84 ^{CDE}	**16.36 ^{C}**
Melatonin	23.63 ^{B}	23.81 ^{B}	28.33 ^{A}	29.64 ^{A}	**26.35 ^{A}**
Gibberellin	19.86 ^{CD}	20.12 ^{C}	24.23 ^{B}	28.16 ^{A}	**23.09 ^{B}**
Mean of planting locations	**19.52 ^{B}**		**24.35 ^{A}**		
Mean of sowing date	**21.05 ^{B}**		**22.82 ^{A}**		

Values within each column followed the same letter are not statically significant of 5% level
**Half of October **Half of November

c. The main constituents of essential oil:

Results in Tables (10 and 11) represented the effect of spraying melatonin and gibberellin on the main constituents of the essential oil of chamomile plants cultivated in two different locations (Sekam and Nubaria).

Thirty-seven to forty components were identified in the essential oil of chamomile flower in the two locations (Nubaria and Sekam) by GC-MS at the second cut. The total identified compounds ranged from 96.90 to 99.50% from the separated compounds.

The main constituents of the essential oil were identified by GC-MS in the flower heads of chamomile plants and are shown in Tables (9 and 10) .The major compound was found to be Bisabolol oxide A and accounted for 47.00 and 55.82% for control, 37.18 and 60.76% for melatonin treatment and 41.11 and 57.15% for gibberellin treatment in the essential oil of flowers chamomile plants cultivated in Nubaria farm and Sekam farm, respectively.

The second major compound was identified as Bisabolol oxide B in the essential oil of flower heads of chamomile plants and accounted for 17.74 and 21.88% for control, 23.11 and 8.03, for melatonin spray and 33.34 and 14.08% for gibberellins spray in the essential oil of flowers chamomile plants cultivated in Nubaria farm and Sekam farm, respectively.

Monoterpene Cis beta farnesene in the essential oil of flower chamomile plants cultivated in Nubaria farm was 6.33, 6.64 and 6.95% for control, melatonin, and gibberellin treatments, respectively. On the other hand, in Sekam location monoterpene Cis beta farnesene decreased to 2.59% in the essential oil of flower chamomile control plants.

Chamazulene increased from 1.43% in the essential oil of flower heads chamomile plants sprayed with melatonin to 6.32% in the essential oil of flower chamomile plants sprayed with gibberellin and cultivated in Nubaria farm.

Generally, the total amount of monoterpene increased from 3.08 % in the essential oil of dry flower heads of plants spraying with gibberellin cultivated in Nubaria to 9.26 % in the essential oil of dry flower heads of plants spraying with melatonin cultivated in the same location, while the total amount of monoterpene in dry flower heads of plants cultivated in Sekam location does not affect with spraying treatments.

The sesquiterpene increased from 76.71 in the essential oil of dry flower heads of plants spraying with melatonin cultivated in Nubaria to 94.39 % in the essential oil of dry flower heads of plants spraying with gibberellin in the location. Moreover, the oxygenated compounds increased from 78.86% in the essential oil of dry flower heads of plants spraying with melatonin cultivated in Nubaria to 87.19% in plants spraying with gibberellin and cultivated in Sekam. But non - oxygenated compounds increased from 10.86% in control plants cultivated in Sekam farm to 18.57% in plants sprayed with melatonin cultivated in Nubaria farm.

Chamomile oil is produced conventionally by steam distillation as endorsed in many pharmacopeias. It incorporates several chemical class entities including sesquiterpenes α-bisabolol is known as levomenol, and bisabolol oxides A & B (≤ 78%), farnesene (12-28%) and chamazulene (1-15%); and polyacetylene derivatives, e.g.,spiroethers (cis/trans-en-ynedicycloethers (8–20%)[7]. The qualitative and quantitative chemical characteristics of chamomile oil have revealed the existence of four different chamomile chemotypes, in terms of their essential oil composition[36,37]. Salamon and Abou Zeid[5]compared the oils of chamomile grown in different locations in Egypt, and they reported a chamazulene content of 1.7- 2.6 %, bisabolol-oxide B (1.6-4.9 %), with the lowest content in Giza location, and the highest in Fayoum. Bisabolol-oxide A followed the opposite trend reaching the maximum (68.2%) in Giza and the minimum in Fayoum. Chamazulene content ranged between 1.7 and 2.6 %, while bisabolol between 2.4 and 11.2 % in the different locations. They reported that the Egyptian chamomile belongs to the bisabolol-oxide chemotype A. Weglarz and Roslon, [38] reported that, Bisabolol oxide B and chamazulene was the main component in 9 lines of *Matricaria recutita* (30.42%) in one. Also, Baghalian*et al*.[39] on *Matricaria recutita*, observed that, the main essential oil constituents (α-bisabololoxide B, α-bisabolonoxide A, chamazulene, α-bisabolol oxide A, α-bisabolol, trans-β farnesene). Mayra *et al*.[4]compared five commercial samples of chamomile grown in Brazil and one from Egypt as control. The major compound in Brazilian chamomile samples were α-bisabolol oxide B (25.31 - 32.99 %) while it constituted (9.87 %) of the Egyptian sample. On the contrary, the Egyptian sample contained (46.55%) of αBisabolol oxide A, while it ranged between 11.61 and 16.57 % in the Brazilian samples. Hendawy *et al*.[40] reported that the Egyptian chamomile belongs to the bisabolol-oxide chemotype A.

Table 10 : The main constituents of the essential oil of dry flower heads of chamomile in Nubaria as affected by melatonin or gibberellin spray in the second harvest

RT	Component Name	Formula	Mass/charge ratio (m/z)	Treatments		
				Control	Melatonin	Gibberellin
8.677	alpha.-Pinene	$C_{10}H_{16}$	77, 79, 91, 92, 93	Trac	0.17	Trac
11.125	Sabinene	$C_{10}H_{16}$	41, 43,77, 91, 93	0.10	Trac	0.59
11.854	Benzene, 1-methyl-3-(1-methylethyl)	$C_{10}H_{14}$	91,117,119, 120,134	0.36	0.49	0.12
11.988	D-Limonene	$C_{10}H_{16}$	43, 67, 68, 93, 119	0.89	0.21	trac
12.780	cis-Ocimene	$C_{10}H_{16}$	41, 77, 91, 92.93	-	0.28	-
12.996	beta.-Ocimene	$C_{10}H_{16}$	77, 79, 80, 91,93	0.63	2.14	0.28
13.048	gamma.-Terpinene	$C_{10}H_{16}$	43, 77, 79, 91,93	0.22	2.14	-
13.152	1,5-Heptadien-4-one, 3,3,6-trimethyl	$C_{10}H_{16}$	41, 55, 83	1.47	0.26	-
13.975	Artemisia alcohol	$C_{10}H_{18}O$	41, 43, 55, 57, 85	0.66	2.66	1.59
16.476	Citronellal	$C_{10}H_{18}O$	41, 43, 55, 56, 69	1.52	-	-
16.837	Endo-Borneol	$C_{10}H_{18}O$	41, 55, 67, 69, 95	0.13	0.59	0.50
17.321	Terpinen-4-ol	$C_{10}H_{18}O$	41.43, 55, 69, 71	-	0.58	-
19.303	Cis-3-Hexenyl isovalerate	$C_{11}H_{20}O_2$	41,55,57, 67, 82	-	0.16	-
19.390	3-Methyl-2-butenoic acid, 3-methylbut-2-enyl ester	$C_{10}H_{16}O_2$	55, 69, 83	-	0.14	-
19.489	Butanoic acid, 3-methyl-, hexyl ester	$C_{11}H_{22}O_2$	41, 43, 55, 57,70	-	0.07	-
22.602	gamma.-Elemene	$C_{15}H_{24}$	41, 73, 79, 93, 121	0.31	0.83	-
23.021	Alpha.-Longipinene	$C_{15}H_{24}$	41, 91, 93, 105, 119	-	0.14	0.41
24.385	Beta.-elemene	$C_{15}H_{24}$	41, 57, 69, 81, 91	0.40	0.16	-
24.607	Butanoic acid, 3-methyl-, phenylmethyl ester	$C_{12}H_{16}O_2$	41, 57, 65, 85, 91	0.15	0.32	-
25.213	Beta.-ylangene	$C_{15}H_{24}$	41, 69, 79, 91, 93,	0.39	0.52	0.18
25.522	β-Cubebene	$C_{15}H_{24}$	41, 91, 93, 105, 161	0.15	0.27	-
26.396	Cis-.beta.-Farnesene	$C_{15}H_{24}$	41, 67, 69	6.33	6.64	6.95
27.113	Germacrene D	$C_{15}H_{24}$	93, 105, 119, 120, 161	0.74	1.56	-
27.702	Heptadecane, 2,6,10,15-tetramethyl	$C_{21}H_{44}$	41, 43, 55, 57, 71	-	-	0.16
27.952	Farnesol	$C_{15}H_{26}O$	55, 69, 73, 79, 93	0.44	1.80	0.71
28.180	Benzene, (2-ethyl-4-methyl-1,3-pentadienyl)-, (E)	$C_{14}H_{18}$	115, 128, 141, 142, 157	-	0.42	-
29.678	Caryophyllene oxide	$C_{15}H_{24}O$	43, 69, 79, 81, 93	0.65	0.66	0.46
30.121	(-)-Spathulenol	$C_{15}H_{24}O$	43, 91, 93, 119, 205	2.10	2.19	0.22
30.604	Cedren-13-ol, 8	$C_{15}H_{24}O$	41, 91, 93, 105, 119	0.09	-	0.89
31.764	Lanceol, cis	$C_{15}H_{24}O$	79, 93, 119, 121, 134	0.09	0.83	0.28
33.216	Bisabolol oxide B	$C_{15}H_{26}O_2$	43, 81, 85, 105, 143	17.74	23.11	33.34
34.533	Bisabolone oxide	$C_{15}H_{26}O_2$	43, 67, 93	9.51	0.22	1.23
34.609	Alpha.-Bisabolol	$C_{15}H_{26}O$	43, 69, 109, 119	Trac	Trac	2.29
36.649	Chamazulene	$C_{14}H_{16}$	128, 152, 153, 155, 169	2.70	1.43	6.32
37.692	Bisabolol oxide A	$C_{15}H_{26}O_2$	93, 107, 121, 125, 143	47.00	37.18	41.11
41.895	1,2-Benzenedicarboxylic acid, bis (2-methyl propyl) ester	$C_{16}H_{22}O_4$	41, 43, 57, 104,149	0.85	-	0.27
42.320	1,6-Dioxaspiro[4.4]non-3-ene, 2-(2,4-hexadiynylidene)	$C_{13}H_{12}O_2$	76, 115, 128, 157, 200	3.91	8.96	1.03
Monoterpenes				4.51	9.26	3.08
Sesquiterpenes				88.64	76.71	94.39
Others				6.38	10.33	1.46
Oxygenated Compounds				84.84	79.47	83.92
Non Oxygenated Compounds				14.69	17.43	15.01
Total of identified compounds				99.53	96.90	98.93

Table 11: The main constituents of the essential oil of dry flower heads of chamomile in Sekam as affected by melatonin or gibberellin spray in the second harvest

RT	Component Name	Formula	mass/charge ratio (m/z)	Treatments		
				Control	**Melatonin**	**Gibberellin**
8.677	Alpha.-Pinene	$C_{10}H_{16}$	**77, 79, 91, 92, 93**	0.28	-	0.14
9.143	Camphene	$C_{10}H_{16}$	**41, 57, 67, 79, 91, 93**	0.24	-	0.13
11.125	Sabinene	$C_{10}H_{16}$	**41, 43,77, 91, 93**	0.04	0.08	0.07
11.854	Benzene, 1-methyl-3-(1-methylethyl)	$C_{10}H_{14}$	**91,117,119, 120,134**	-	0.17	0.13
11.988	D-Limonene	$C_{10}H_{16}$	**43, 67, 68, 93, 119**	1.00	1.11	1.53
12.996	Beta.-Ocimene	$C_{10}H_{16}$	**41, 77, 91, 92.93**	-	0.25	0.12
13.048	Gamma.-Terpinene	$C_{10}H_{16}$	**43, 77, 79, 91,93**	0.08	-	0.10
13.152	1,5-Heptadien-4-one, 3,3,6-trimethyl	$C_{10}H_{16}$	**41, 55, 83**	-	0.64	0.24
13.975	Artemisia alcohol	$C_{10}H_{18}O$	**41, 43, 55, 57, 85**	0.90	0.26	0.18
14.581	Linalool	$C_{10}H_{18}O$	**43, 55, 57, 69, 71**	-	0.22	0.06
16.050	Camphor	$C_{10}H_{16}O$	**79, 81, 95**	0.50	-	0.23
16.476	Citronellal	$C_{10}H_{18}O$	**41, 43, 55, 56, 69**	-	1.18	0.53
16.837	Endo-Borneol	$C_{10}H_{18}O$	**41, 55, 67, 69, 95**	0.22	-	0.13
17.321	Terpinen-4-ol	$C_{10}H_{18}O$	**41.43, 55, 69, 71**	-	-	0.05
17.974	Isopulegol	$C_{10}H_{18}O$	**41, 43, 55, 57**	-	-	0.14
20.923	Alpha.-Fenchyl acetate	$C_{12}H_{20}O_2$	**41, 43, 69, 83, 93, 95**	0.77	-	0.58
22.602	Gamma.-Elemene	$C_{15}H_{24}$	**41, 73, 79, 93, 121**	-	0.17	0.10
24.385	Beta.-elemene	$C_{15}H_{24}$	**41, 57, 69, 81, 91**	0.15	0.25	0.26
25.213	β-ylangene	$C_{15}H_{24}$	**41, 69, 79, 91, 93,**	0.56	0.32	0.53
25.522	β-Cubebene	$C_{15}H_{24}$	**41, 91, 93, 105, 161**	0.07	0.15	0.09
26.396	Cis-.beta.-Farnesene	$C_{15}H_{24}$	**41, 67, 69**	2.59	5.82	4.78
27.113	Germacrene-D	$C_{15}H_{24}$	**93, 105, 119, 120, 161**	0.29	0.79	0.53
28.407	Beta.-Cedrene	$C_{15}H_{24}$	**41, 79, 81, 91, 93**	-	0.14	0.64
27.952	Farnesol	$C_{15}H_{26}O$	**55, 69, 73, 79, 93**	-	0.19	0.31
28.506	Myristicine	$C_{15}H_{26}O$	**91, 131, 161, 165,192**	-	-	0.15
29.206	Elemol	$C_{15}H_{26}O$	**41, 43, 59**	0.41		
29.678	Caryophyllene oxide	$C_{15}H_{24}O$	**43, 69, 79, 81, 93**	-	0.43	0.22
30.121	(-)-Spathulenol	$C_{15}H_{24}O$	**43, 91, 93, 119, 205**	1.89	0.73	0.88
30.616	Veridiflorol	$C_{15}H_{26}O$	**41, 43, 69, 81,105, 109**	0.86		
31.566	Bicyclo[4.4.0]dec-2-ene-4ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)	$C_{15}H_{24}O_2$	**41, 43, 55, 57, 71**	-	0.23	0.23
32.586	Tau.-Cadinol	$C_{15}H_{26}O$	**41, 81, 91, 93, 105, 161**	-	0.10	0.26
32.702	Longiborneol	$C_{15}H_{26}O$	**41, 43, 44, 55, 57**	0.21	-	-
33.216	Bisabolol oxide B	$C_{15}H_{26}O_2$	**43, 81, 85, 105, 143**	21.88	8.03	14.81
34.533	Bisabolone oxide	$C_{15}H_{24}O_2$	**43, 67, 93**	5.17	5.51	5.77
34.609	alpha.-Bisabolol	$C_{15}H_{26}O$	**43, 69, 109, 119**	1.99	4.64	2.30
36.649	Chamazulene	$C_{14}H_{16}$	**128, 152, 153, 155, 169**	1.57	3.55	2.53
37.692	Bisabolol oxide A	$C_{15}H_{26}O_2$	**93, 107, 121, 125, 143**	55.82	60.76	57.15
41.895	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	$C_{16}H_{22}O_4$	**41, 43, 57, 104,149**	-	1.04	0.75
42.320	1,6-Dioxaspiro[4.4]non-3-ene, 2- (2,4-hexadiynylidene)	$C_{13}H_{12}O_2$	**76, 115, 128, 157, 200**	-	2.63	2.55
Monoterpenes				**3.26**	**3.27**	**3.54**
Sesquiterpenes				**93.46**	**91.58**	**91.31**
Other				**0.88**	**4.74**	**4.35**
Oxygenated Compounds				**90.73**	**86.06**	**87.28**
Non Oxygenated Compounds				**6.87**	**13.53**	**11.92**
Total of identified compounds				**97.60**	**99.59**	**99.20**

Conclusion

Chamomile seedling can be grown from mid-October to mid-November, so it can be harvested in March before the heat rises and maintaining with the quality of the resulting oil.

Chamomile plants which cultivated at both sowing dates and sprayed with melatonin or gibberellin at Nubaria location gave the tallest plants. The highest value of fresh and dry flower heads weight and fresh and dry flower yield were obtained with chamomile plants sprayed with melatonin which cultivated in Nubaria location at second sowing date. The highest essential oil percentage was obtained with chamomile plants grown in Sekam farm at first sowing date which spraying with melatonin. The main component of Chamomile oil is (Bisabolol oxide A) and was higher under the conditions of Nubaria when seedling was treated with gibberellin followed in the lowest order by treatment with melatonin, but it was the opposite under the conditions of Sekem. The second component was (Bisabolol oxide B).

Author contributions

1- Saber Fayez Hendawy and Saied Kamel Mohamed Abd El-Naby designed this study.
2- Shaimaa Ibrahim Mohammad Elsayed, and Ahmed E.El-Gohary carried out the experimental work.
3- Shaimaa Ibrahim Mohammad Elsayed analyzed the oil samples and the data.

4- Saied Kamel Mohamed Abd El-Naby and Shaimaa Ibrahim Mohammad Elsayed wrote the paper and reviewed the manuscript critically.

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