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RESEARCH ARTICLE

EVALUATION OF THE CHEMICAL CONSTITUENTS OF WILD AND CULTIVATED MARIGOLD PLANTS.

Heba Ibrahim Abd El-Moaty[1] and Hanan Ali El-Sayed[2].

1. Assistant professor of Phytochemistry,Medicinal and Aromatic Plants Department, Desert Research CenterEl-Mataria, Cairo, Egypt.
2. Researcher of cultivation of Medicinal and Aromatic Plants Medicinal and Aromatic Plants Department, Desert Research CenterEl-Mataria, Cairo, Egypt.

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Abstract

For evaluation of different sowing dates and foliar fertilization effects on cultivated marigold, a factorial experiment with split plot design and three replications was carried out. Marigold (*Calendula officinalis*) was cultivated at Baloza Research Station, Desert Research Center, North Sinai Governorate, Egypt,during cropping seasons (2014/2015 and 2015/2016). Three sowing dates (15 September, 1 October and 15 October) and three foliar fertilization (Control, Ever Full Grow and Biomagic product) and their interaction were studied. Results showed that, sowing dates and foliar fertilization have significant effects on plant height, flower dry weight, primary metabolite constituents in marigold flowers such as (carbohydrates, nitrogen, protein and lipids) and flavonoids as secondary metabolites when comparing with the estimated component of wild *C. officinalis*. However, the best interaction treatment was the sowing date at 1 October and biomagic, which gave a highly significant effect on evaluated traits compared to other ones. So, this treatment was chosen to investigate the separated primary and flavonoid compounds compared with wild plants. The obtained data declared that the highest concentrations of the separated free and combined sugars was inulin. Meanwhile the lysine was the highest percentages of the separated protein amino acids. On the other hand, the highest percentage of fatty acids was palmitic acid. Investigation of flavonoids using HPLC analysis revealed that the plant contained 22 flavonoids compounds in the best interaction treatment and wild *C. officinalis*. It was noticed that, the obtained major compounds for the chosen cultivated plants were Apigenin-6- arbinose -8-glactose (169.790mg/100g), Hespiridin (102.330mg/100g) and Luteolin-6- arbinose-8- glucose (85.565mg/100g). While the obtained major flavonoids compounds for the wild *C. officinalis* were Hespirtin(117.340 mg/100g), Luteolin-6- arbinose-8- glucose (21.587 mg/100g) and Apignin-6- glucose -8-rhamnose (11.778 mg/100g).

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Corresponding Author:-Heba Ibrahim Abd El-Moaty.
Address:-Assistant professor of Phytochemistry, Medicinal and Aromatic Plants Department, Desert Research CenterEl-Mataria, Cairo, Egypt.

Introduction:-

Calendula Officinalis L. is an annual. It´s origin is the west of Asia and Mediterranean and cultivated as an ornamental plant before its medicine properties known as an herbal medicine. The plant was first cultivated as an herbal medicine in Europe in 17th century and now there is in Germany, Czech Republic, Slovakia, Austria and Switzer Land, Hungary and recently in Egypt and Syria (Penelope, 1993 and Pala Paul *et al* 2002).

Calendula officinalis use in medical (treating gastric and intestinal disease coetaneous wounds and an anti-inflammation medicine), cosmeticin various creams and nutritional in coloring the foods like cheese and butter. Also, the oil extracted from the seeds has industrial and pharmaceutical application (Bernath, 2000; Dinda and Craker, 1998). Recently, some evidences have been discovered the positive effects of its essence on HIV (Kalvatchev, 1997)

Biomagic product is a biological promoter of microbial origin and contains many of the biological products, which affect the plant growth and productivity and increase the plant immunity to microbial diseases. Biomagic product consists of amino acids, vitamins, macro elements and micro elements. Biomagic product does not contain any of the synthetic phytohormones, (El-Sibaie, 1995).

Foliar nutrition is widely used in order to correct specific nutrient deficiency or to prove nutrient, what is preferable especially in newly reclaimed soil. Plants response to foliar nutrition varies according to several factors such as plant species and environmental conditions. Several researches reported the beneficial effect of foliar fertilization on growth and yield of different medicinal and aromatic plants (Khalil *et al* 2001, Khalil and El-Sherbeny, 2005).

Sowing date play an important role in plant growing and effect on active substance in medicinal plants, significantly (Ghani, 2011).Applying various sowing dates results in facing to different temperature, solar radiations and day length by plant growing processes, so that impact on plants growth and yield (Dadashi and Khajepour, 2004).

Materials and methods:-

The present work was carried out at the Experimental Station of Desert Research Center (D.R.C.) at Baloza, North Sinai Governorate during the two successive seasons of 2014/2015 and 2015/2016 to study the influence of sowing date and Complete fertilizer or biomagic product treatments on vegetative characters and chemical constituents of cultivated marigold and compare it with the wild *(CalendulaOfficinalis.* L.) plantswhich collected from MersaMattruh, Egypt during April season (2015).

Seeds of Marigold (*Calendula Officinalis* L.) plants were kindly provided from SEKEM Company of Medicinal and Aromatic Plants. The seeds were sown in the nursery bed. Meanwhile, seedlings were transplanted in the experimental area after 45 days from swing dates for the two seasons in sandy soil. The mechanical and chemical properties of the used soil are shown in Table (A) according to Chapman and Pratt (1971).

Table A:-Physical and chemical properties of the experimental soil.

Particle size distribution (%)			Texturesoil	$Ecdsm^{-1}$	pH	Available nutrients (Cations)					Available nutrients (Anions)			
Sand	Silt	Clay				Na %	P %	K %	Ca mg/l	Mg mg/l	CO_3	HCO_3 mg/l	Cl^-	So^-_4
90	5	5	sandy	1.37	8.20	4.78	0.42	0.54	3.65	4.40	-	3.85	3.3	6.5

The irrigation system of the experiment was drip irrigation with the drippers of four liters/h for one hour twice every week by using plastic tanks on the first of lateral side. The lateral sides were pipe lines from plastic material diameter 16 mm and with 30 m tall. The spaces between them (pipe) were 50 cm, 50 cm between the plants on the row and 2 m between the treatments. The lateral side of every replicate was 8.6 m and contained 17 plants. Every treatment had three replicates and contained 51 plants. The chemical analysis of the used water is shown in Table (B).

Table B:- Chemical analysis of irrigation water.

Samples	pH	E.C. (ppm)	S.A.R	Soluble cations (me/l)				Soluble anions (me/l)			
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO_3^{=}$	HCO_3^{-}	$SO_4^{=}$	Cl^{-}
1 st. season	7.45	1456	3.80	2.90	3.20	8.60	0.60	0.10	5.60	2.10	7.50
2 nd. season	7.10	1512	3.52	3.25	3.05	9.50	0.40	0.50	3.81	3.69	8.20

pH: Acidity, E.C.: Electrical conductivity, dSm^{-1}: Dec Siemen per meter, S.A.R: Sodium adsorption ratio, me/l: mille equivalent per liter

To evaluate the chemical constituents of each of wild Marigold and cultivated one, we need to choose the appropriate agricultural treatments to get the best productivity of the crop to compare the chemical components of wild plant and cultivated one under the appropriate agricultural treatments. Hence we sowed Marigold under the following treatments.

SowingDate:-

The seeds were sown in the nursery bed on 15th September, 1st and 15th October in the two seasons (2014-2015). Meanwhile, seedlings were transplanted in the experimental area on 1st, 15th November and 1st December (2014-2015) for the two seasons.

Complete fertilizer or biomagic product treatments:-

- The used complete fertilizer with commercial name of Ever Full Grow contained macro and micronutrients. Ever Full Grow fertilizer was obtained from Ever Grow for Specialty Fertilizers Co. Sadat Factories, Industrial zone No 7043, Sadat City, Egypt. The chemical composition of Ever Full Grow fertilizer was as follows: Macro elements: nitrogen (N) 19 %, phosphorus (P_2O_5) 19 %, Potassium (K_2O) 19% and magnesium (Mg) 0.5%. Amended with chelated & mineral elements (Fe, Zn, Mn, Cu, B and Mo). The concentration used of Ever Full Grow as foliar spray in both seasons was 2.5g/L as recommended (Ever Grow for Specialty Fertilizers Co.). The amount of Ever Full Grow was dissolved in aqueous solution.
- The used biomagic product which is a biological promoter of microbial origin (El-Sibaie, 1995). Biomagic was provided from Microbiology Department, D. R. C. The biomagic does not contain any of the synthetic phytohormones but it contains many of the biological products, which affect the plant growth and productivity and increase the plant immunity to microbial diseasses. Biomagic has pH of 5.5 and consists of the following:
  - Amino acids (1.907%) i.e. arginine, cystine, glycine, histidine, isoleucine, leucine, lysine, phenyl alanine, theronine, tryptophane, tyrosine and valine.
  - -Vitamins (0.38%) i.e. thiamin, biotene, choline, folic acid, niacin, potothinic, pyrodxine and rhiboflavin.
  - Macro elements (in mg/L) i.e. N(1125), P_2O_5 (550)and K_2O(625)
  - Micro elements (in mg/L) i.e. Fe (160), Zn(124), Mn (100), Mg(45), Cu(45), B(14), Mo(12), Cd (7) and Ni(4).
  - The concentration used of biomagic as foliar spray in both seasons was 7.5 g/L as recommended (El-Sibaie 1995). The amount of biomagic was dissolved in aqueous solution.
  - The plants were sprayed using hand-held sprayer and the used volume of the solution was maintained just to cover the whole plant foliages completely every 21 days from the first spray date after 15 days from transplanting date till the last harvesting (1st May).
- The control plants were sprayed with tap water.

Interaction treatments between sowing date and complete fertilizer or biomagic product:-

Each date of sowing (three dates) was combined with each treatment of complete fertilizer or biomagic product (three treatments including control) to form 9 interaction treatments. All the plants received normal agricultural practices when they need

Harvesting:-

Harvesting of flowers was carried out every 7 days from 1 January until 15 May in the first and the second seasons.

Recorded data were as follows:-

Vegetative characters:-

A random sample of five plants from each replicate was taken and the following data were recorded

Plant height (cm) recorded on 10 April of the two seasons.

Flowers dry weight per plant (g)

Investigation of primary metabolites in flowers:-

- **Determination of total carbohydrates percentageand content** according to (Chaplin and Kennedy, 1994).
- **Identification of free sugars and combined sugars** for cultivated plant under the best interaction treatment in this study and wild plant by HPLC according to (Zielinski *et al.*, 2014).
- **Determination of total nitrogen percentageand content** by using Kjeldahl method according to James (1995).
- **Determination of total protein percentageand content** according to James (1995).
- **Investigation of total amino acids** for cultivated plants under the best interaction treatment in this study and wild plantsaccording to Csomos and Simon-Sarkadi (2002), using Amino Acid Analyzer.
- **Determination of total lipids percentageand content** according to Farag*et al*. (1986).
- **Determination of saponifiable matter (fatty acids)** for cultivated plants under the best interaction treatment in this study and wild plants.They were determined using GLC (Farag*et al*., 1986).

Investigation of flavonoids as secondary metabolitesin flowers:-
Estimation of Flavonoid percentageand content according to Karawya and Aboutable (1982).

Qualitative and quantitative of flavonoids for cultivated plants under the best interaction treatment in this study and wild plantsby HPLC

The ethanol extracts of *Calendula Officinalis* flowers were analyzed usingHPLC. The employed HPLC system consisted of HP 1090M Series II high performance liquid chromatography equipped with an HP 1090M Series II diode array and an eight-channel electrochemical coulometric array detector ((EC); Esa Inc., USA). The EC was operated using 100-800 mV potentials (100mV intervals). The detector array was housed in a temperature-regulated compartment at 35ºC.

Flavonoid separation was done by ODS-3 (4.0 × 150 nm, 3µm) column with a C-18 guard column, with temperature set at 35ºC. The flow rate of the mobile phase was 0.7mL/min, and the injection volumes were 10µL of the standards and sample extracts. All flavonoids were quantified using the external standard method. Quantification was based on peak area (DAD) or beak hight (EC). (Mattila*et al*., 2000).

Design and Statistical Analysis:-
The experimental design was factorial experiment between sowing date and complete fertilizer or biomagic product treatments in Split blot design (Main plots were consisted of sowing date and sub plots included the complete fertilizer or biomagic product) with three replicates. Data of the present study were statistically analyzed and the differences between the means of the treatments were considered significant when they were more than least significant differences (L.S.D.) at the 5% or 1% levels according to Steel and Torrie(1980).

Results and discussion:-

Growth parameters:-

Plant height:-

Data presented at Table (1), revealed that sowing dates (15th September, 1st October and 15th October) gave insignificant increase in plant height in the first season, but in the second season the first and second date gave a highly significant increase compared to the third date in this regard. Moreover, the tallest plants were obtained from the second sowing date (1 October) in the first season, meanwhile it obtained from the first sowing date (15 September) in the second season. These results are similar to those found by Mehdi *et al*., (2016) on *Calendula officinalis* plants. The significant decrease in plant height with delaying in sowing date could be attributed to shorter period of vegetative growth of plants. Imholte and Carte (1987) reported that delay of sowing caused a decline in plant height.

Moreover, spraying marigold plants with complete fertilizer (Ever Full Grow) or biomagic product gave a highly significant increase in plant height compared to control. Moreover, the tallest plants were obtained from biomagic treatment, then decreased gradually with Ever Full Grow followed by control treatments. These results are in agreement with those found by Khalid and Shedeed(2015) on *Nigella sativa* L. plants by using foliar nutrition**,** (Hafez, 2013) on Jerusalem Artichoke by using biomagic and Hashem (2007) on thyme plants by using complete fertilizer and biomagic. These results may be due to the Biomagic contents of proteins, amino acids, vitamins and hormones, as well as some micro nutrients (Hafez, 2013).

Meanwhile, the interaction between the second sowing date (1 October) and biomagic treatment recorded the tallest plants and gave a highly significant increase in this regard compared to the other ones. Furthermore, the interaction treatment between second sowing date and control recorded the shortest plants in comparison with other interaction treatments in the first season, but in the second season the interaction between the third date (15 October) and control showed the shortest plants compared with other treatments.

Flowers dry weight / plant:-

Table (1) indicate that, the best sowing date was (15 September) which gave the highest flowers dry weight per plant and it was significantly increased compared to other dates under the study. Since, flowers dry weight per plant was decrease gradually by delaying in sowing 1 October followed by 15 October. These results are in harmony with those reported by (Hossein *et al.*, 2014) and (Seghatoleslami and Mousavi, 2009) on marigold plants.

most studies about sowing date indicate that delaying in sowing date leads to decrease qualitative and quantitative yield. In an investigation on *Calendulaofficinalis* L. medicinal plant and *Menthapiperita*indicated that sowing date effect on dry weight of *Calendula officinalis* L. and *Menthapiperit*a wassignificant and delaying in sowing decreased growth parameters and chemical constituents (TahmasbpourandMohamadin, 2006). Also, the overall response for planting marigold in September was better due to the availability of favorable temperature and day length (duration of light) before the onset of flower bud initiate and flowering (Singh, 2015) on *Tageteserecta*Linn plant .

Data recorded in Table (1) reveal that, the treatment of biomagic was the best one and gave a highly significant increase in flowers dry weight per plant compared to Ever Full Grow and control treatments.

Table 1:-Effect of planting date and fertilization treatments as well as their interaction on plant height (cm), flowers dry weight / plant (g) and totalcarbohydrates % and content / plant flowers of *Calendula officinalis* during the two seasons (2015 - 2016).

Treatments	Plant height (cm)								Flowers dry weight / plant (g)							
	F1	F2	F3	Means (D)	F1	F2	F3	Means (D)	F1	F2	F3	Means (D)	F1	F2	F3	Means (D)
	First season				Second season				First season				Second season			
D1	33.33	42.00	46.00	40.44	33.69	42.33	44.61	40.21	24.88	38.28	44.11	35.76	25.71	45.15	47.75	39.54
D2	31.67	40.00	54.17	40.94	30.42	40.25	48.69	39.79	13.19	23.21	53.77	30.06	14.81	22.99	53.85	30.55
D3	33.43	42.00	45.33	40.26	27.75	37.08	44.42	36.42	16.34	21.68	28.17	22.06	15.66	25.95	36.77	26.12
Means (F)	32.81	41.33	47.50		30.62	39.89	45.91		18.14	27.73	42.02		18.72	31.36	46.12	
LSDat 5%	For(D)= N.S.	For(F)= 1.42	For(D*F)=3.04		For(D)=1.12	For(F)= 1.60	For(D*F)=2.52		For(D)=1.81	For(F)=1.16	For(D*F)= 2.42		For(D)=1.70	For(F)=0.55	For(D*F)=1.85	
LSDat 1%	For(D)= N.S.	For(F)= 1.99	For(D*F)=4.65		For(D)=1.85	For(F)= 2.24	For(D*F)=3.63		For(D)=2.99	For(F)=1.63	For(D*F)= 3.70		For(D)=2.81	For(F)=0.77	For(D*F)=2.97	
	Total carbohydrates %								Total carbohydrates content							
D1	20.44	24.33	28.37	24.38	16.45	15.71	17.67	16.61	5.086	10.731	10.861	8.893	4.229	7.094	8.437	6.587
D2	16.17	18.72	20.87	18.59	16.22	18.62	18.52	17.79	2.133	4.345	11.223	5.900	2.491	4.280	9.974	5.581
D3	19.19	25.70	17.87	20.92	14.73	18.40	19.82	17.65	2.880	7.240	3.815	4.645	2.307	4.774	7.294	4.792
Means (F)	18.60	22.92	22.37		15.80	17.58	18.67		3.366	7.439	8.633		3.009	5.383	8.568	
LSDat 5%									For(D)=0.647	For(F)=0.268	For(D*F)= 0.745		For(D)=0.293	For(F)=0.098	For(D*F)=0.322	
LSDat 1%									For(D)=1.073	For(F)=0.375	For(D*F)= 1.177		For(D)=0.485	For(F)=0.138	For(D*F)=0.516	
Wild plant	16.95															

= Sowing date D1= 15th September D2= 1st October D3= 15th October

F= Foliar spray F1= control F2= Ever Full Grow F3= Biomagic

Meanwhile, spraying marigold plants with Ever Full Grow or biomagic gave a highly significant increase in flowers dry weight per plant compared to control.

These results may be due to the Biomagic contents of proteins, amino acids, vitamins and hormones, as well as some micro nutrients (Hafez, 2013). The increase of flowers dry weight per plant might be due to the application of complete fertilizer which consist of macro and microelements such as nitrogen for its importance to consist the amino acids to form the protein which participate in cell enlargement and cell division. While, phosphorus have an important role in producing energy for the physiological processes as synthesis proteins by formation the coenzyme adenine triphosphate (ATP). Furthermore, potassium plays a direct or indirect role in plant metabolism, as explained by (Devlin, 1979).

In addition, such promoting effect on all parameters might be due to that Zn had an important role in indole acetic acid synthesis in plant tissues as it is an activator of enzymetryptophane synthesis (the precursor of auxin), (Krishnamoorthy, 1981). Also, manganese had a regulatory role in biosynthesis of proteins of photosystem II, as reported by (Khmara, 1984). At the same time, Fe might play an important role in the prophyrin structure of chlorophyll. In green plants, there is often a good correlation between the level of supply and the chlorophyll content, (Dekock*et al.*, 1960). Generally, the used fertilizer might play an active role (direct or indirect) in cell division and / or cell enlargement (elongation) in stem tissue leading to more growth parameters (flowers dry weight).

Moreover, the interaction between biomagic and second sowing time (1 October) treatment recorded the highest flowers dry weight per plant and gave a highly significant increase in this respect compared to other interaction treatments. Under each sowing date flowers dry weight per plant was increased gradually by using complete fertilizer (Ever Full Grow) flowed by biomagic. These results were similar in both seasons.

Investigation of primary metabolites:-

Total carbohydrates percentage and content:-

Table (1), indicate that the best sowing date was (15 September) which gave the best value of total carbohydrates percentage and content compared to other planting times and wild plant. Moreover, total carbohydrates content decrease gradually by delaying the sowing from 1 October and followed by 15 October. Also, the first sowing date (15 September) showed a highly significant increase in total carbohydrates content in comparison with second and third dates. These results are in similar with those stated by (Al-Doghachi*et al.*, 2016) on*Withaniasomnifera*L. plant.

On the other hand, total carbohydrates percentage and content increased by using complete fertilizer (Ever Full Grow) or biomagic product compared to control. Meanwhile, spraying marigold plants with Ever Full Grow gave the highest value in total carbohydrates percentage during the first season, but in the second season biomagic treatment recorded the best value in total carbohydrates percentage and content in comparison with other treatments. The treatment of biomagic gave a highly significant increase in total carbohydrates content compared to Ever Full Grow and control treatments. These results are in harmony with those reported by (Hafez, 2013) on Jerusalem Artichoke and (El-Hifny and El-Sayed, 2011) on sweet pepper plant.

Furthermore, the highest increase in total carbohydrates percentage was obtained from the interaction treatment between the first sowing date and biomagic during the first season but, in the second season it observed by interaction treatment between the third sowing date and biomagic compared to other interaction treatments. Moreover, the best interaction treatment was that between the second sowing date (1 October) and biomagic which recorded a highly significant increase in carbohydrates content per plant flowers compared to other interaction treatments.

Investigation of free sugars:-

The data illustrated in Table (2) show that, the separation of the free sugars contents achieved using High Pressure Liquid Chromatography (HPLC), where twelve of free sugars were detected at cultivated marigold plant, which produced from the best interaction treatment in this study (1 October and biomagic) , while eleven of free sugars were detected at wild plants . It was noticed that, the highest concentrations of the separated free sugars at cultivated and wild plants was inulin (9.71% and 13.82 %, respectively).

Investigation of combined sugars:-

Data recorded in Table (2) reveal that, the separation of the hydrolyzed combined sugars were achieved using HPLC, where eleven of combined sugars were detected at cultivated marigold plant which produced from the best interaction treatment in this study (1 October and biomagic), while ten of combined sugars were detected at wild plants. The highest percentage of the separated sugars at cultivated and wild plants was inulin (2.95% and 1.29 %, respectively).

Table 2:- Relative percentage of free and combined sugars in flowers of *Calendula Officinalis* L. for cultivated and wild plants.

No.	Sugars	Cultivated plant		Wild plant	
		Free sugar (%)	Combined sugar (%)	Free sugar (%)	Combined sugar (%)
1	Inulin	9.71	2.95	13.82	1.29
2	Glucuronic	0.56	0.18	-	-
3	Stachyose	1.21	0.18	0.29	0.27
4	Galacturonic	0.44	0.06	-	-
5	Sucrose	0.25	0.05	0.06	0.11
6	Maltose	-	-	-	0.67
7	Glucose	1.44	0.30	0.13	0.07
8	Xylose	0.18	-	0.10	0.18
9	Galactose	-	0.03	0.05	-
10	L- Rhaminose	0.26	0.05	0.07	0.11
11	Fructose	1.87	-	0.89	-
12	Raffinose	5.83	1.36	-	0.13
13	Manitol	-	-	0.10	-
14	Sorbitol	0.31	0.02	0.01	0.05
15	Ribose	0.48	0.01	0.01	0.02

Total nitrogen and protein percentage and content:-

Data recorded in Table (3), show that the best values in nitrogen and protein percentage and content per plant flowers were obtained during planting in 15 September in the first season but, in the second season were observed during planting in 1 October in comparison with other sowing dates. Also, swing dates (15 September or 1 October) recorded a highly significant increase in total nitrogen content compared with the third sowing date (15 October) in first and second seasons. These results are accordance with those stated by (Al-Doghachi *et al*., 2016) on *Withaniasomnifera*L. plant.

From the same Table (3) it could be noticed that total nitrogen percentage and content increased gradually by using complete fertilizer (Ever Full Grow) followed by biomagic treatment. Moreover, spraying marigold plants with Ever Full Grow or biomagic recorded an increase in total nitrogen percentage and content per plant flowers compared to control. Furthermore, the best treatment was that spraying marigold plants with biomagic which gave a highly significant increase in total nitrogen content per plant flowers compared to other treatments. These results were found in both seasons. These results are in agreement with those stated by (Khalid and Shedeed, 2015) on *Nigella sativa* L. plants by using foliar nutrition and (Hashem, 2007) on thyme plants by using complete fertilizer and biomagic.

Furthermore, the highest values of total nitrogen and protein percentage were observed by interaction treatment between the third planting date (15 October) and biomgic in the two seasons compared to the other interaction treatments and the wild plant. Moreover, the best interaction treatment was that between first sowing date and Ever Full Grow which recorded highly significant increase in total protein content per plant flowers during the fist season but, in the second season was that between the second sowing date and biomagic in this respect compared to other interaction treatments. Also, under each of sowing date total nitrogen and protein content were increased gradually by Ever Full Grow followed by biomagic.

Table 3:-Effect of planting date and fertilization treatments as well as their interaction on Total nitrogen & protein % and content / plant flowers of *Calendula officinalis* during the two seasons (2015- 2016).

Treatments	Total nitrogen %								Total nitrogen content							
	F1	F2	F3	Means (D)	F1	F2	F3	Means (D)	F1	F2	F3	Means (D)	F1	F2	F3	Means (D)
	First season				Second season				First season				Second season			
D1	2.02	2.35	2.35	2.24	2.35	2.02	1.68	2.02	0.503	1.037	0.900	0.813	0.604	0.912	0.802	0.773
D2	2.58	1.68	1.57	1.94	2.47	2.91	2.80	2.73	0.340	0.390	0.844	0.525	0.366	0.669	1.508	0.848
D3	1.34	1.79	2.69	1.94	2.35	2.35	3.25	2.65	0.201	0.504	0.574	0.427	0.246	0.610	1.195	0.684
Means(F)	1.98	1.94	2.20		2.39	2.43	2.58		0.348	0.644	0.773		0.405	0.730	1.168	
LSDat5 %									For(D) =0.060	For(F)=0.025	For(D*F)=0.069		For(D)=0.039	For(F)=0.015	For(D*F)=0.044	
LSDat1 %									For(D) =0.100	For(F)=0.035	For(D*F)=0.110		For(D)=0.065	For(F)=0.020	For(D*F)=0.070	
Wild plant	2.02															
	Total protein %								Total protein content							
D1	12.63	14.69	14.69	14.0	14.69	12.63	10.50	12.61	3.143	6.481	5.498	5.041	3.779	5.703	5.017	4.833
D2	16.13	10.50	9.81	12.13	15.44	18.19	17.50	17.04	2.129	2.437	5.275	3.280	2.256	4.184	9.424	5.288
D3	8.38	11.19	16.81	12.13	14.69	14.69	20.31	16.56	1.262	3.155	3.585	2.667	1.535	3.814	7.467	4.272
Means (F)	12.38	12.13	13.77		14.94	15.19	16.13		2.178	4.024	4.786		2.523	4.567	7.303	
LSDat5 %									For(D) =0.352	For(F)=0.154	For(D*F)=0.411		For(D)=0.244	For(F)=0.095	For(D*F)=0.274	
LSDat1 %									For(D) =0.584	For(F)=0.216	For(D*F)=0.647		For(D)=0.410	For(F)=0.134	For(D*F)=0.435	
Wild plant	12.63															

D= Sowing date D1= 15th September D2= 1st October D3= 15th October
F= Foliar spray F1= control F2= Ever Full Grow F3= Biomagic

Investigation of total amino acids (protein-amino acids):-

From data presented in Table (4), results show that the investigation of hydrolyzed protein-amino acids, achieved using amino acid analyzer, where fifteen amino acids of different types were detected at cultivated marigold plant which produced from the best interaction treatment in this study (1 October and biomagic), while fourteen amino acids of different types were detected at wild plant . The highest percentage of the separated amino acids was that of lysine at biomagic and wild plants (15.86 % and 25.00 %, respectively).

Table 4:- Relative percentage of total amino acids in flowers of cultivated and wild *Calendula Officinalis* L.

No.	Compound name	Cultivated plant	Wild plant
		Total amino acids (%)	Total amino acids (%)
1	Asparagine	10.59	15.06
2	Threonine	2.15	1.73
3	Serine	3.84	4.27
4	Glutamine	11. 16	12.80
5	Proline	0.31	0.51
6	Glycine	10.37	7.68
7	Alanine	9.37	7.24
8	Valine	6.71	5.74
9	Methionene	0.79	-
10	Isoleucine	4.59	3.48
11	Leucine	8.72	6.97
12	Tyrosine	7.24	2.89
13	Phenylalanine	2.87	3.30
14	Histidine	5.41	3.29
15	Lysine	15.86	25.00

Total lipids percentage and content:-

Data recorded in Table (5) revealed that the total lipids percentage and content per plant flowers decreased gradually by delaying sowing date 1 October followed by 15 October. Moreover, the best sowing date in this regard was that 15 September compared to other sowing dates. However, the first sowing date recorded a highly significant increase in total lipids content in comparison with other ones. These results coincided with those found by (El-Saady*et al*. 2013) on *Lallemantiaiberica*plant.

Meanwhile, total lipids percentage and content of marigold flowers increased gradually by using Ever Full Grow followed by biomagic treatments. Since, spraying marigold plants with biomagic gave a highly significant increase in total lipids content compared to Ever Full Grow and control. Also, the treated plants with Ever Full Grow or biomagic led to an increase in this regard compared to control. These results are in agreement with those recorded by (Khalid and Shedeed, 2015) on *Nigella sativa* L. plants by using foliar nutrition and (El-Sherbeny*et al*., 2007) on *Rutagraveolens*L. plant by using foliar fertilizers.

Furthermore, the best interaction treatment was that between the first sowing date (15 September) and complete fertilizer (Ever Full Grow) which recorded the highest value of total lipids percentage compared to other interaction treatments but the wild plant recorded the highest value in this respect in comparison with all interaction treatments during the two seasons. Moreover, the highest content of total lipids was obtained from the interaction treatment between the second sowing date (1 October) and biomagic which gave a highly significant increase in this regard compared to other ones.

Table 5:-Effect of planting date and fertilization treatments as well as their interaction on Total flavonoids & lipid % and content / plant flowers of *Calendula officinalis* during the two seasons (2015-2016).

Treatments	Total lipid %								Total lipid content							
	F1	F2	F3	Means (D)	F1	F2	F3	Means (D)	F1	F2	F3	Means (D)	F1	F2	F3	Means (D)
	First season				Second season				First season				Second season			
D1	5.42	6.98	7.75	6.72	2.23	4.33	10.41	5.66	1.349	3.079	2.964	2.464	0.573	1.955	4.971	2.500
D2	6.45	4.09	6.20	5.58	7.01	2.83	5.42	5.09	0.851	0.947	3.334	1.711	1.038	0.651	2.919	1.536
D3	5.34	5.24	5.33	5.30	2.22	2.24	4.74	3.07	0.801	1.476	1.138	1.139	0.348	0.581	1.743	0.891
Means(F)	5.74	5.44	6.43		3.82	3.13	6.86		1.000	1.834	2.479		0.653	1.062	3.211	
LSDat5%									For(D)=0.177	For(F)=0.073	For(D*F)=0.203		For(D)=0.095	For(F)=0.037	For(D*F)=0.107	
LSDat1%									For(D)=0.293	For(F)=0.102	For(D*F)=0.321		For(D)=0.157	For(F)=0.051	For(D*F)=0.170	
Wild plant	10.99															
	Total flavonoid %								Total flavonoid content							
D1	1.298	1.297	1.252	1.282	1.299	1.298	1.298	1.298	0.323	0.572	0.479	0.458	0.334	0.586	0.620	0.513
D2	1.298	1.297	1.297	1.297	1.298	1.298	1.298	1.298	0.171	0.301	0.697	0.390	0.192	0.298	0.699	0.397
D3	1.298	1.298	1.298	1.298	1.298	1.298	1.248	1.281	0.195	0.366	0.277	0.279	0.203	0.337	0.459	0.333
Means (F)	1.298	1.297	1.282		1.298	1.298	1.281		0.230	0.413	0.485		0.243	0.407	0.593	
LSDat5%									For(D)=0.041	For(F)=0.016	For(D*F)=0.047		For(D)=0.022	For(F)=0.0007	For(D*F)=0.024	
LSDat1%									For(D)=0.068	For(F)=0.023	For(D*F)=0.074		For(D)=0.056	For(F)=0.010	For(D*F)=0.038	
Wild plant	1.299															

D= Sowing date D1= 15th September D2= 1st October D3= 15th October
F= Foliar spray F1= control F2= Ever Full Grow F3= Biomagic

These results are in agreement with those found by (El-Sherbeny*et al*., 2007) on *Rutagraveolens*L. plant.

Investigation of saponifiable matter (fatty acids):-

From Table (6) it could be noticed that the fatty acid contents of the lipids were determined using GLC technique, where they obtained results revealed the presence of six saturated fatty acids and two unsaturated fatty acids with different range of concentrations, where the highest percentage of fatty acids at cultivated marigold plant, which produced from the best interaction treatment in this study (1 October and biomagic) and wild plants was Palmitic acid (39.44 % and 42.62 %, respectively.

Table 6:- Relative percentage of fatty acids in flowers of cultivated and wild *Calendula Officinalis* L.

Compound name	No. of carbon atom	Cultivated plant	Wild plant
		fatty acids (%)	fatty acids (%)
Lauric acid	C12:0	2.93	2.13
Myristic acid	C14:0	27.93	15.80
Pentadecylic acid	C15:0	11.64	22.45
Palmitic acid	C16:0	39.44	42.62
Stearic acid	C18:0	5.35	6.66
Arachidic acid	C20:0	6.65	4.35
Oleic acid	C18:1	2.94	3.97
Linoleic acid	C18:2	3.12	2.02

Investigation of flavonoids as secondary metabolites;-

Total flavonoids percentage and content:-

The data illustrated in Table (5) show that, no differences between all sowing dates treatments and wild plants in total flavonoids percentage during the two seasons. On the other hand, total flavonoids content per plant flowers decreased gradually by delaying sowing date from 15 September, 1 October flowed by 15 October. Furthermore, the first sowing date 15 September gave a highly significant increase in this regard compared to other sowing dates. These results are hold true in both seasons.

Moreover, there were no differences between complete fertilizer (Ever Full Grow), biomagic, control treatments and wild plants in total flavonoids percentage. Since, spraying marigold plants by biomagic was the best treatment which gave a highly significant increase in total flavonoids content per plant flowers in comparison with Ever Full Grow or control treatments. Spraying marigold plants by Ever Full Grow or biomagic led to an increase in this respect compared to control. These results were similar in both seasons.

As regard to total flavonoids content per plant flowers, it could be noticed from Table (5) that, the best interaction treatment was that between the second sowing date (1 October) and biomagic product. Meanwhile, there were no differences between all interaction treatments and wild plant in total flavonoids percentage. On the other hand, the interaction treatment between the second sowing date and biomagic gave a highly significant increase in this regard compared to other ones.

Qualitative and quantitative of flavonoids by HPLC:-

Data at Table (7) indicate that, investigation of flavonoids by HPLC revealed the presence of 22 compounds of the cultivated marigold plant, which produced from the best interaction treatment in this study (1 October and biomagic) and wild *Calendula Officinalis*, where the major compounds at cultivated plant were Apigenin-6- arbinose -8- glactose (169.790mg/100g), Hespiridin (102.330mg/100g) and Luteolin-6- arbinose-8- glucose (85.565mg/100g). While the major compounds at wild plants were Hespirtin (117.340 mg/100g), Luteolin-6- arbinose-8- glucose (21.587 mg/100g) and Apignin-6- glucose -8- rhamnose (11.778 mg/100g).

Table 7:- HPLC analysis of the flavonoids in flowers of cultivated and wild *Calendula Officinalis* plants.

No.	Flavonoids	Cultivated plant (Mg/100g)	Wild plant (Mg/100g)
1	Luteolin-6- arbinose-8- glucose	85.565	21.587
2	Luteolin-6- glucose -8- arbinose	16.384	7.624
3	Apigenin-6- arbinose -8-glactose	169.790	4.271
4	Apignin-6- rhamnose -8- glucose	5.648	2.272
5	Apignin-6- glucose -8- rhamnose	25.279	11.778
6	Luteolin-7- glucose	14.812	2.230
7	Narengin	20.353	3.213

8	Rutin	8.725	1.345
9	Hespiridin	102.330	9.538
10	Quercetin-3-O-glucoside	3.100	1.002
11	Rosmarinic	1.627	0.4863
12	Apigenin-7-O- neohespiroside	0.943	0.543
13	Kampferol-3,7-dirhamoside	1.707	1.633
14	apigenin-7- glucose	0.995	0.212
15	Quercetrin	0.966	0.399
16	Quercetin	0.461	2.911
17	Naringenin	0.406	3.952
18	Hespirtin	30.136	117.340
19	Kampferol	0.953	1.085
20	Rhamnetin	0.387	0.750
21	Apignin	0.857	0.215
22	Acacetin	37.531	22.488

The significant decrease in all parameters with delaying in sowing date could be attributed to shorter period of vegetative growth of plants. Also, the used fertilizer might play an active role (direct or indirect) in cell division and / or cell enlargement (elongation) within stem tissue leading to more growth parameters and chemical constituents (carbohydrates, nitrogen, protein, lipids and flavonoids).

Conclusion:-

It could be concluded that, the sowing date of 1 October and spraying plants by biomagic product is suitable for producing the highest flower yield /plant and chemical constituents content of cultivated marigold at North Sinai conditions. These results demonstrated that we can use the cultivated marigold instead of wild plant to keep out the wild plant from extinction and our autecology.

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