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Impact of Radiation and Chemical Mutagens on The Biochemical Profiling of Some Chamomile Cultivars: GC-MS analysis and SEM.

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Abstract

Egyptian chamomile is highly regarded in export markets. This study aimed to improve the essential oil yield and quality of four chamomile cultivars: Bode Gold, Zloty Lan, Local Improved, and Local. Treatments included gamma irradiation (5, 10, and 20 Gy) and foliar applications of colchicine and dimethyl sulfoxide (DMSO) at concentrations of 0.1, 0.2, and 0.4 ppm, conducted over two generations (M1 and M2, 2022–2024). Bode Gold demonstrated the highest essential oil percentage, attributed to superior vegetative and flowering traits, followed by Local Improved and Zloty Lan, with the Local cultivar yielding the least. Higher doses of gamma radiation (20 Gy) and elevated concentrations of colchicine and DMSO (0.4 ppm) significantly increased essential oil production in all cultivars compared to controls. Cross-pollination further enhanced yield, with Bode Gold contributing positively to other cultivars. Additionally, Bode Gold outperformed others in total essential oil components and mineral content. These results highlight the potential of advanced treatments and cross-pollination in enhancing chamomile oil productivity and quality, promoting diversification and competitiveness in global markets. Bode Gold emerges as a promising candidate for optimizing chamomile cultivation in Egypt.

Key words: Chamomile Cultivars, essential oil, gamma, colchicine, DMSO, scanning electron microscope (SEM).

Introduction

Chamomile, *Chamomilla recutita* (L.), (Fam. Asteraceae), is an annual herbaceous plant, native to South and East Europe, North and East Africa and West Asia, Chamomile had good history at Ancient Egyptians, Greek and Roman and still has great importance as a medicinal plant in Egypt and other countries **(Blumenthal, 2001)**, Chamomile flowers and/or its oil are evaluated for their numerous curative effects including anti-inflammatory, antiseptic, carminative, healing, and spasmolytic activities. Accordingly, it has been included in the Pharmacopeias of many countries**.** Recently, it was reported that chamomile can be successfully employed for soil reclamation (**Balak and Misra, 2004**)**.** Till the seventieth of the last century the plant was evaluated by its contents of essential oil and Chamazulene. Due to progress in analytical techniques and pharmacological methods, new constituents were discovered and their biological activities were proven **(Lawrence, 1986).** Countries like Poland, Hungary, Germany, Argentina, and Slovakia, which are the major suppliers of chamomile for the world market, have recently initiated intensive plant improvement programs to produce plants with high level of the biologically active compounds. Some European cultivars were used and compared with the local cultivar to diversify the local market with various sources of chamomile (**Shalaby, *et.al.*, 2010)**. This also aims to meet the global market demand for Egyptian chamomile, ensuring optimal conditions for chamomile production and reflecting on the quality and quantity of output, **(Andrea, 2008),** mentioned that the chamomile plant is one of the most important, oldest, and most famous medicinal plants, as it is used for its multiple properties.

Unluckily, chamomile genotypes are extremely limited, and the majority of chamomile cultivars are either landraces or populations **(Oko, *et.al.*, 2013; Otto *et.al.*, 2015)**. As a result, the drive to discover new sources of diversity is Critical. Increased genetic diversity and new character induction were used to actively conduct mutation breeding for crop breeding **(Kharkwal and Shu 2009; Bhor, *et.al.*, 2014; Lal, *et.al.*, 2019),** The use of physical and chemical mutagens artificially induce mutations, which increases the mutation frequency when compared to spontaneous occurrence. However, excellent production efficiency is required for widespread use of these mutants in plant breeding

(Konzak, 1965; Gaul, *et.al.*, 1972). With the use of induced mutagenesis, it is possible to improve economic traits and quality characters for crop improvement in a short improvement of time **(Manjaya and Nandanwar, 2007; Khursheed, *et.al.*, 2015).**

Scanning Electron Microscope **(SEM)** micrographs showing pollen grains of four chamomile cultivars under study for family Asteraceae and also showing detailed exine ornamentation, these results were in agreement with **(Khan, *et.al.*, 2021)** by studying the Morpho-palynological assessment of some species of the Family Asteraceae using Scanning Electron Microscopy; **(Umber, *et.al.*, 2021)** by examining pollen grains morphology of selected taxa of Family Asteraceae using **(SEM)** and **(Khan, *et.al.*, 2023)** by studying the morphological assessment of Asteraceae taxa using **(SEM).**

This study was conducted with the aim of increasing the quantity and quality of the essential oil of the four chamomile cultivars under study, which is rich in compounds (Chamazulene, α-Bisabolol oxide A and α-Bisabolol oxide B). GC-MS analysis was conducted for the essential oil, as well as the use of electron microscope examination of the mineral components of the seeds of the four cultivars under study.

Therefore, the effect of gamma irradiation at concentrations (5,10 and 20 gray) and spraying with colchicine and DMSO with concentrations (0.1,0.2 and 0.4 ppm) on seedlings of the four chamomile cultivars (Bode Gold, Zloty lan, local improved and local) under study to determine the best of them in quality and quantity of flower heads as well as early Flowering.

Materials and Methods

This field study was conducted at the Agricultural Research and Experiments Farm - Faculty of Agriculture - Moshtohor - Benha University - Qalyubia Governorate – Egypt, in M_1 and M_2 generations during (2022/2023 and 2023/2024).

The soil physical and chemical characteristics of the experimental field were analyzed in compliance with the methods cited by (**Black, *et.al.*, 1982)** and (**Jackson, 1973)** and are shown in **Table (1)**.

Table1. Physical and chemical properties of the experimental soil units at Moshtohor Agric. EXP. Station during each of the two successive seasons (2022/2023 and 2023/2024):

Properties		Seasons	
		2022/2023	**2023/2024**
Physical analysis:			
Coarse sand	**(%)**	2.09	2.03
Fine sand	**(%)**	23.94	24.61
Silt	**(%)**	21.74	21.23
Clay	**(%)**	52.23	52.13
Textural class		Clay	Clay
Chemical analysis:			
$CaCo_3$	**(%)**	1.05	1.08
Organic matter	**(%)**	2.09	2.13
N available	**(mg/kg)**	0.88	0.92
P available	**(mg/kg)**	0.31	0.35
K available	**(mg/kg)**	0.71	0.77
E.C	**(ds. m^{-1})**	0.93	0.98
pH		7.68	7.75

Plant materials:

All seeds of chamomile cultivars were obtained from SEKEM Company and the used cultivars are Bode Gold (introduced from Germany), Zloty lan (introduced from Poland), Local Improved (resulted from selection program for 6 years) and Local (the locally grown).

Field experiment:

The seeds of *Matricaria recutita* cultivars (Bode gold, Zloty lan, local Improved and Local) were sown in the nursery in the first of October and then planted in permanent soil in mid-November. The experiment included 40 treatments which were the combination of four cultivars and ten treatments in three replicates in M_1 and M_2 generations during two successive seasons (2022/2023 and 2023/2024).

The layout of the experiment was a split-plot design with three replicates. The four cultivars were devoted to main plots, whereas treatments were assigned to be sub-plots. The area of the experimental unit was one meter square (M2). It contains two lines, 50 cm apart. In each line, Four furrows were planted per row, each furrow containing two plants. The recommended agricultural practices for growing chamomile regularly were applied.

Treatments:

a- Gamma irradiation

The seedlings of four chamomile cultivars (Bode gold, Zloty lan, Local Improved and Local) have been exposed to three different doses of gamma rays (5, 10, and 20 Gy) in addition to as a control (0.0 Gy). Gamma irradiation was executed at National Center for Radiation Research and Technology (NCRRT), Nasr city, Cairo, Egypt. Irradiation treatments were achieved by Cobalt emitting gamma rays (Co60) with time periods of 23, 45, and 90 seconds for 5, 10, and 20 Gy, respectively and the dose rate was 0,806 Kg/h.

b- Colchicine concentrations:

The study was conducted by using 3 concentrations of colchicine (0.1, 0.2, and 0.4 ppm), where the first spraying took place two weeks after transplanting in the permanent soil, the second two weeks after the first, and the third two weeks after the second.

c- Di Methyl Sulfoxide (DMSO) concentrations:

The study was conducted by using 3 concentrations of DMSO (0.1, 0.2, and 0.4 ppm), where the first spraying took place two weeks after transplanting in the permanent soil, the second two weeks after the first, and the third two weeks after the second.

Plant parameters :

The following parameters were estimated:

1- Essential oil percentage:

Essential oil content (%) in the air dried flower heads were determined. Dried flower heads samples (100 g) were subjected to hydro distillation for 6 hours using the Clevenger apparatus for essential oils (**Clevenger apparatus**).

2- The chemical composition of chamomile essential oil:

The chemical composition of chamomile essential oil was analyzed using a Trace GC-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG—5MS (30 m x 0.25 mm x 0.25 mm film thickness). The initial temperature of the oven was set to 50°C and then increased by 5°C /min to 250°C and held for 2 min. The temperature was increased to the final temperature of 310°C by 30°C /min and held for 2 min. The injector and MS transfer line temperatures were kept at 270, 260°C, respectively. The carrier gas was helium with a constant flow rate of 1 ml/min. The solvent delay was 3 min and diluted samples of 1 ml were injected automatically using an auto sampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50 650 in full scan mode. The ion source temperature was set at 250°C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

3- Scanning seeds of four chamomile cultivars using Electron Microscope (SEM):

The general morphological shape of chamomile seeds were obtained using the electron microscope at magnifications of x30 and x110 (Central lab faculty of science Benha university), SEM images were recorded using BED-C 015.0KV, Joel, equipped with EDX unit for analyzing the surface morphology.

Statistical analysis:

The obtained data in both seasons of the study were subjected to analysis of variance as a factorial experiment in split plot design. L.S.D. method was used to differentiate between means according to **(Snedecor and Cochran, 1989).**

Results and discussion:

1- Essential oil percentage in dry flower heads of four chamomile cultivars (Bode Gold, Zloty lan, local improved and local):

The data in **Table (2) and Fig. (1 and 2),** showed that chamomile cultivars (Bode Gold, Zloty lan, local Improved and local) had significant differences between them, where the cultivar (Bode Gold) scored superiority in the percentage of essential oil in dry flower heads and came in the first place where it scored (1.267% and 1.250 %) in M_1 and M_2 respectively. Then came in the second place (local Improved), which scored (1.192% and 1.214 %), followed by (Zloty lan), which scored (1.157% and 1.178 %), and then came in the fourth and last place, the cultivar (local) where it scored (**1.131**% and 1.157 %) in M_1 and M_2 generations during (2022/2023 and 2023/2024).

The results shown in **Table (2)** and **Fig. (1 and 2),** also showed that all gamma rays treatments (5,10 and 20 gray) gave an increase in the percentage of essential oil in the dry flower heads, where the dose (20 gray) caused the highest increase were recorded (**1.243** % and **1.256 %**) in both seasons respectively. Also treatments with different concentrations of DMSO (0.1, 0.2 and 0.4 ppm) came in second place, where the highest concentration (0.4 ppm) gave an increase in the percentage of essential oil in the dry flower heads, (1.212 % and 1.225 %) in both seasons respectively, while the concentrations of colchicine (0.1, 0.2 and 0.4 ppm) ranked third and last compared to gamma irradiation and DMSO treatments, where the treatment with high concentration of colchicine (0.4 ppm) gave an increase in the percentage of essential oil in the dry flower heads, which recorded (**1.193** % and **1.205 %**) compared to the untreated plants, which recorded (**1.103** % and **1.141 %**) in M_1 and M_2 generations during (2022/2023 and 2023/2024).

The results shown in **Table (2)** and **Fig. (1 and 2),** also showed that the interaction between chamomile cultivars (Bode gold, Zloty lan, local Improved and local) and (gamma rays, colchicine and DMSO) treatments under study increased the percentage of the essential oil for dry flower heads. Where the cultivar Bode gold with the treatment of gamma rays (20 gray), recorded the highest increase (**1.353** % and **1.313 %**), while the cultivar local Improved came in the second place with the same treatment, which scored (**1.250** % and **1.273 %**), then zloty lan cultivar recorded (**1.193** % and **1.223 %**) and the lowest was the (local) cultivar, which scored (**1.177** % and **1.213 %**) compared to the untreated plants in M_1 and M_2 generations during (2022/2023 and 2023/2024).

The results obtained showed the superiority of the Bode Gold chamomile cultivar in terms of essential oil content compared to the other chamomile cultivars under study. This productivity is the result of a cumulative effect of the vegetative and flowering characteristics of each plant. Followed by Local improved cultivar, while Zloty Lan cultivar ranked third compared to Local cultivar. As a result of this study, many chamomile cultivars are available to chamomile producers to meet diverse export specifications.

Table 2. Essential oil percentage in dry flower heads, of four chamomile cultivars (Bode Gold, Zloty lan, local improved and local) in M1 and M2 generations during (2022/2023 and 2023/2024).

Cultivars / Treatments	Essential oil % in dry flower heads, of four chamomile cultivars (Bode Gold, Zloty lan, local improved and local)				
	Bode-gold	**Zloty lan**	**Local I.**	**Local**	**Mean**
	1^{st} season (2022/2023 - M_1)				
Control (D.W.)	**1.200**	**1.100**	**1.143**	**0.967**	**1.103**
Gamma Rays (5)	**1.290****	**1.163****	**1.200****	**1.137****	**1.198****
Gamma Rays (10)	**1.307****	**1.170****	**1.220****	**1.160****	**1.214****
Gamma Rays (20)	**1.353****	**1.193****	**1.250****	**1.177****	**1.243****
Colchicine (0.1)	**1.220****	**1.140****	**1.160****	**1.120****	**1.160****
Colchicine (0.2)	**1.243****	**1.150****	**1.180****	**1.143****	**1.179****
Colchicine (0.4)	**1.270****	**1.160****	**1.190****	**1.153****	**1.193****
DMSO (0.1)	**1.233****	**1.150****	**1.170****	**1.133****	**1.172****
DMSO (0.2)	**1.260****	**1.163****	**1.193****	**1.153****	**1.192****
DMSO (0.4)	**1.293****	**1.183****	**1.210****	**1.163****	**1.212****
Mean	**1.267****	**1.157***	**1.192**	**1.131**	**1.187**
LS D. at 5% for	**cultivars = 0.023 & Treatments = 0.010 & cultivars x Treatments = 0.017**				
LS D. at 1% for	**cultivars = 0.041 & Treatments = 0.015 & cultivars x Treatments = 0.022**				
	2^{nd} season (2023/2024 – M_2)				
Control (D.W.)	**1.187**	**1.113**	**1.157**	**1.107**	**1.141**
Gamma Rays (5)	**1.250****	**1.173****	**1.217****	**1.163****	**1.201****
Gamma Rays (10)	**1.290****	**1.207****	**1.237****	**1.197****	**1.233****
Gamma Rays (20)	**1.313****	**1.223****	**1.273****	**1.213****	**1.256****
Colchicine (0.1)	**1.203****	**1.157****	**1.187****	**1.127****	**1.169****
Colchicine (0.2)	**1.233****	**1.167****	**1.207****	**1.140****	**1.187****
Colchicine (0.4)	**1.253****	**1.187****	**1.217****	**1.163****	**1.205****
DMSO (0.1)	**1.223****	**1.163****	**1.193****	**1.137****	**1.179****
DMSO (0.2)	**1.257****	**1.183****	**1.217****	**1.153****	**1.203****
DMSO (0.4)	**1.287****	**1.207****	**1.233****	**1.173****	**1.225****
Mean	**1.250****	**1.178****	**1.214****	**1.157**	**1.200**
LS D. at 5% for	**cultivars = 0.007 & Treatments = 0.003 & cultivars x Treatments = 0.005**				
LS D. at 1% for	**cultivars = 0.013 & Treatments = 0.005 & cultivars x Treatments = 0.007**				

DMSO = Dimethyl sulfoxide Local I. = Local Improved

Fig. (1): Essential oil percentage in dry flower heads, of four chamomile cultivars (Bode Gold, Zloty lan, local improved and local) in M1 generations during (2022/2023).

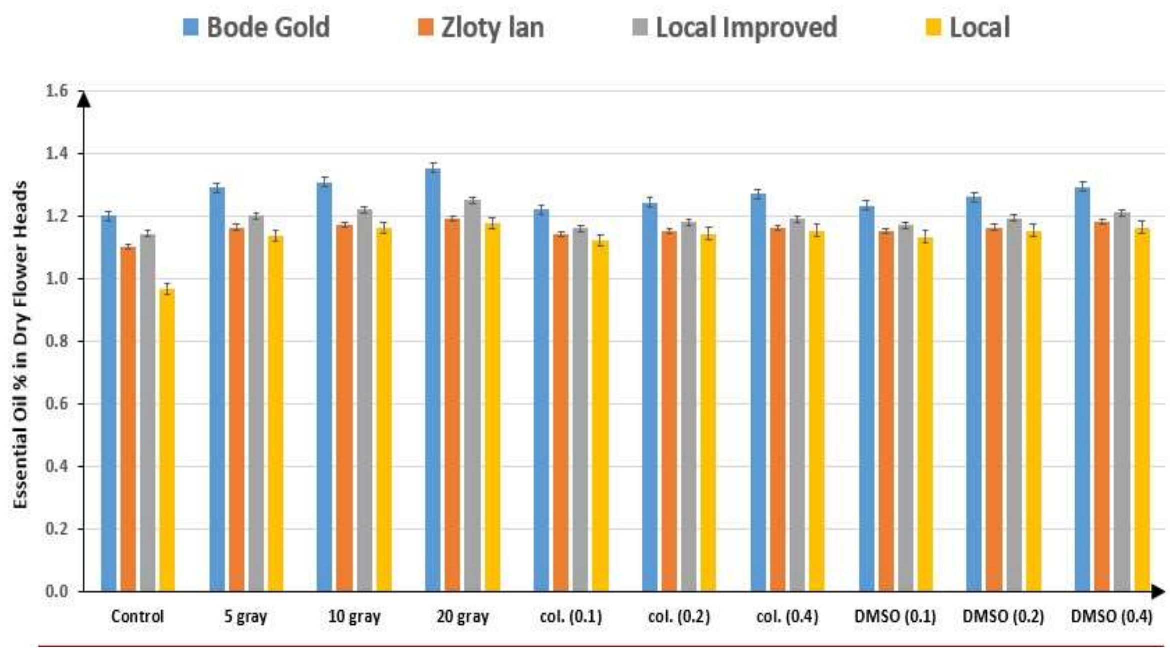
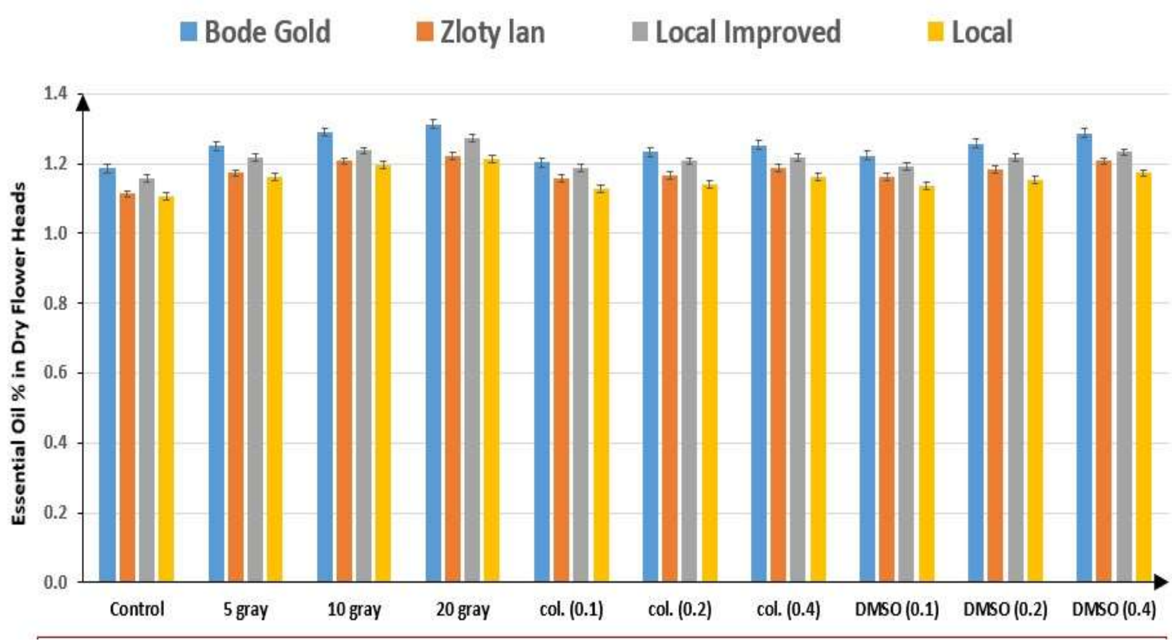


Fig. (2): Essential oil percentage in dry flower heads, of four chamomile cultivars (Bode Gold, Zloty lan, local improved and local) in M2 generations during (2023/2024).



These results were in agreement with **(Svab, *et.al.*, 1967); (Lal, *et.al*., 2000):** found that there is a significant and positive correlation between the yield of fresh flower heads with the number of branches on the plant, the yield of dry flower heads, the area of spread, and the oil yield at both the genetic and phenotypic levels. Oil content and dry flower heads yield were also associated with oil yield. All traits showed a high heritability, **(Andrea, 2002); (Blessing and Salamon, 2006)** found that; There was a noticeable improve in the oil content of the Local improved cultivar. It also led to an increment in the total content of the four major constituents compared to local cultivar. Moreover, **(Salamon and Abou Zeid, 2006); (Sashidharam, *et.al*., 2006)** analyzed the essential components of the essential oil of the L *Matricaria recutita*. plant, where forty-one components were identified, representing 97.5% of the oil, including the main components alpha-Bisabolol A oxide, Farnesene, alpha-Bisabolol, and Chamazulene. It was found that the yield of the main components in chamomile oil Increased by 0.5 to 2 hours of distillation the oil showed a dark blue color and a strong characteristic aroma. **(Repčák and Krausová, 2009); (Shalaby, *et.al*., 2010),** found that Bode Gold chamomile cultivar was superior in terms of essential oil content compared to other chamomile cultivars under study, and this productivity is a net accumulation of the vegetative and flowering growth traits of each plant

2- Chamomile essential oil Composition (GC-MS analysis):

Based on the GC-MS analysis of the essential oil samples of the four chamomile cultivars under study in **Table (3) and Fig. (3),** the components were determined for each cultivar separately, as the Bode Gold cultivar showed the following components (Butanoic acid, 2-methyl-, ethyl ester, (E)-β-Farnesene, 1,9-Decadiyne, α-Bisabolol oxide B, α-Terpineol, Chamazulene, α-Bisabolol oxide A, cis-ene-yne-Dicycloether, (E)-Tibetin spiroether, Decanoic acid, 4-pentadecanol, Undec-10-ynoic acid, 1,11-Dodecadiyne and 13-Tetradece-11-yn-1-ol)

The Zloty lan cultivar also showed the following components (E)-β-Farnesene, α-Bisabolol oxide B, α-Terpineol, Chamazulene, α-Bisabolol oxide A, cis-ene-yne-Dicycloether and Decanoic acid). Also, the Local Improved cultivar showed the following components (Butanoic acid, 2-methyl-, ethyl ester, (E)- β -Farnesene, 1,9-Decadiyne, Germacrene D, α-Bisabolol oxide B, α-Terpineol, Chamazulene, α-Bisabolol oxide A, (cis)-5-hepten-1-yne, cis-ene-yne-Dicycloether, (E)-Tibetin spiroether, Decanoic acid, Undec-10-ynoic acid, 1,11-Dodecadiyne and 13-Tetradece-11-yn-1-ol). The Local cultivar also had the following components (Butanoic acid, 2-methyl-, ethyl ester, (E)- β -Famesene, 1,9-Decadiyne, α-Bisabolol oxide B, α-Terpineol, Chamazulene, α-Bisabolol oxide A, cis-ene-yne-Dicycloether, (E)-Tibetin spiroether, Decanoic acid and 13-Tetradece-11-yn-1-ol). As shown by the GLC examination of essential oil samples in the four chamomile cultivars under study, it showed that the common components between them were (α-Bisabolol oxide A, α-Bisabolol oxide B, Chamazulene, cis-ene-yne-Dicycloether, α-Terpineol, (E)-β-Farnesene and Decanoic acid).

The results of the GC-MS analysis of essential oil samples recorded that Zloty Lan cultivar had the highest percentage of α-Bisabolol oxide A (99.08%), then the Local cultivar, where the rate of α-Bisabolol oxide A (95.59%) was recorded, then the Bode Gold cultivar, where the rate was recorded. α-Bisabolol oxide A compound (93.33%), then the Local Improved cultivar was recorded, where the percentage of α-Bisabolol oxide A compound was recorded (91.58%).

The results of the GC-MS analysis of essential oil samples were recorded as the Local Improved cultivar, where it was found that the percentage of α-Bisabolol oxide B was (2.38%), followed by the Local cultivar, where the percentage of α-Bisabolol oxide B was recorded (1.38%), then the Bode Gold cultivar. Where the percentage of α-Bisabolol oxide B was recorded (1.00%), then Zloty Lan ranked the highest percentage of α-Bisabolol oxide B (0.20%).

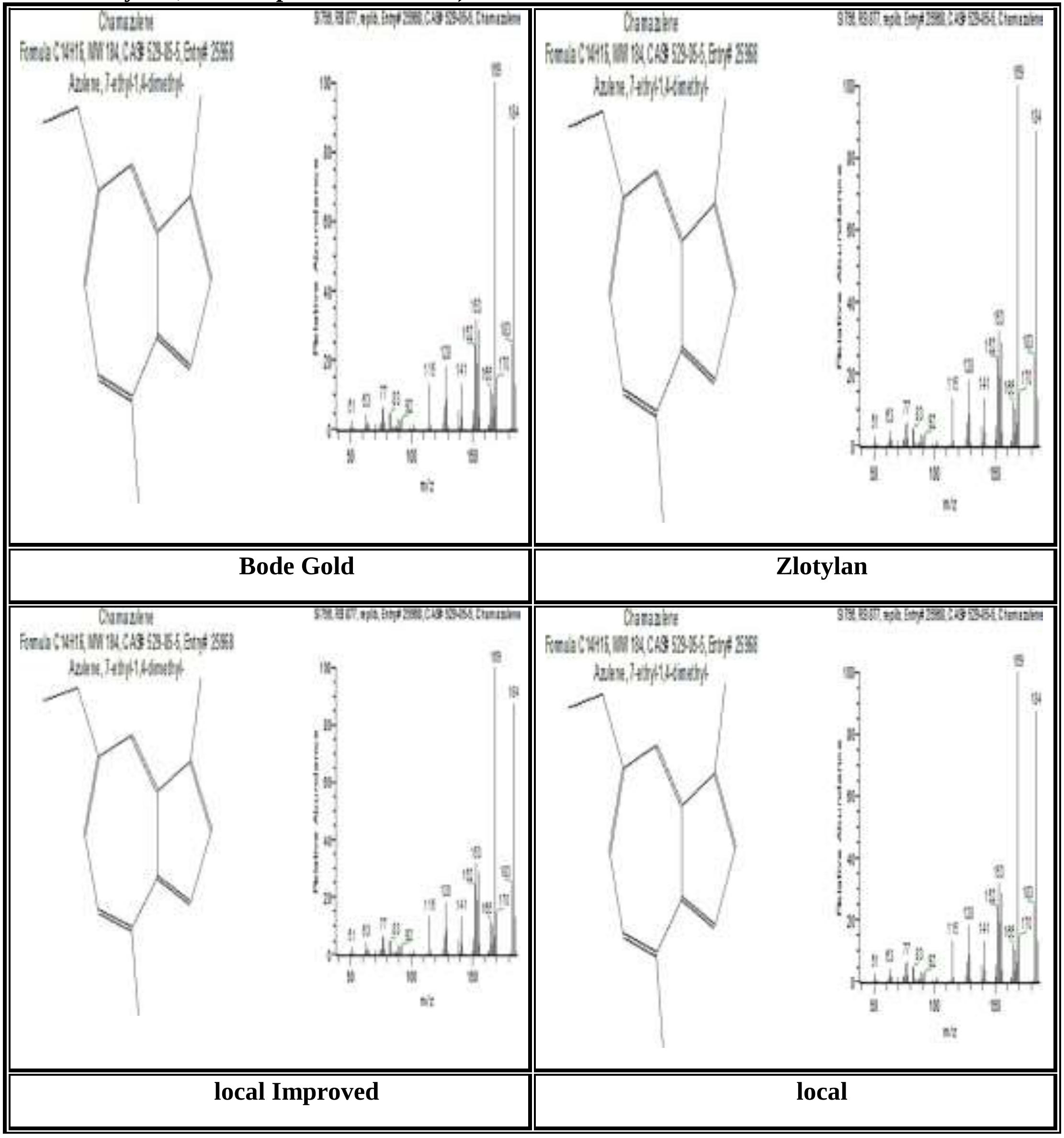
While the results of the GC-MS analysis of essential oil samples were classified as Local Improved, where the percentage of Chamazulene compound was recorded (0.25%), then it was classified as Bode Gold, where the percentage of Chamazulene compound was recorded (0.07%), then it was classified as Local, where the percentage of Chamazulene compound was recorded (0.07%), then Zloty Lan was classified with the highest percentage of Chamazulene (0.03%). These results were in agreement with (**Blessing and Salamon, 2006)** found that; There was a noticeable improve in the oil content of the Local improved cultivar. It also led to an increment in the total content of the four major constituents compared to local cultivar. Moreover, **(Sashidhara *et.al*., 2006)** analyzed the volatile components of the volatile oil of the L *Matricaria recutita*. plant, where forty-one components were identified, representing 97.5% of the oil, including the main components alpha-Bisabolol A oxide, Farnesene, alpha-Bisabolol, and Chamazulene. It was found that the yield of the main components in chamomile oil Increased by 0.5 to 2 hours of distillation the oil showed a dark blue color and a strong characteristic aroma.

Table 3. GC-MS analysis on essential oil content of dry flower heads, on four chamomile cultivars (Bode Gold, Zloty lan, local improved and local).

Bode Gold					
Peak	**RT**	**Name**	**Formula**	**Area**	**Area Sum %**
1	**9.653**	**Butanoic acid, 2-methyl-, ethyl ester**	**C7H14O2**	**47083.76**	**0.05**
2	**27.369**	**(E)-β-Farnesene**	**C15H24**	**47252.12**	**0.05**
3	**30.327**	**1,9-Decadiyne**	**C10H14**	**8611.24**	**0.01**
4	**32.124**	**α-Bisabolol oxide B**	**C15H26O2**	**926844.75**	**1.00**
5	**32.576**	**α-Terpineol**	**C10H18O**	**503041.99**	**0.54**
6	**33.554**	**Chamazulene**	**C14H16**	**67929.15**	**0.07**
7	**34.126**	**α-Bisabolol oxide A**	**C15H26O2**	**86288915.7**	**93.33**
8	**36.215**	**cis-ene-yne-Dicycloether**	**C13H12O2**	**4452638.28**	**4.82**
9	**37.68**	**(E)-Tibetin spiroether**	**C14H14O2**	**15091.66**	**0.02**
10	**38.298**	**Decanoic acid**	**C10H20O2**	**58141.14**	**0.06**
11	**38.441**	**4-pentadecanol**	**C15H32O**	**4394.43**	**0.00**
12	**41.37**	**Undec-10-ynoic acid**	**C11H18O2**	**8773.17**	**0.01**
13	**41.639**	**1,11-Dodecadiyne**	**C12H18**	**4924.84**	**0.01**
14	**41.823**	**13-Tetradece-11-yn-1-ol**	**C14H24O**	**19063.02**	**0.02**
Zloty lan					
Peak	**RT**	**Name**	**Formula**	**Area**	**Area Sum %**
1	**27.351**	**(E)-β-Farnesene**	**C15H24**	**5360.43**	**0.11**
2	**32.089**	**α-Bisabolol oxide B**	**C15H26O2**	**9728.96**	**0.20**
3	**32.541**	**α-Terpineol**	**C10H18O**	**8382.47**	**0.17**
4	**33.537**	**Chamazulene**	**C14H16**	**1537.65**	**0.03**
5	**34.035**	**α-Bisabolol oxide A**	**C15H26O2**	**4891398.7**	**99.08**
6	**36.135**	**cis-ene-yne-Dicycloether**	**C13H12O2**	**18788.75**	**0.38**
7	**38.24**	**Decanoic acid**	**C10H20O2**	**1481.74**	**0.03**
nd	**nd**	**Butanoic acid, 2-methyl-, ethyl ester**	**C7H14O2**	**nd**	**nd**
nd	**nd**	**1,9-Decadiyne**	**C10H14**	**nd**	**nd**
nd	**nd**	**(E)-Tibetin spiroether**	**C14H14O2**	**nd**	**nd**
nd	**nd**	**4-pentadecanol**	**C15H32O**	**nd**	**nd**
nd	**nd**	**Undec-10-ynoic acid**	**C11H18O2**	**nd**	**nd**
nd	**nd**	**1,11-Dodecadiyne**	**C12H18**	**nd**	**nd**
nd	**nd**	**13-Tetradece-11-yn-1-ol**	**C14H24O**	**nd**	**nd**
Local Improved					
Peak	**RT**	**Name**	**Formula**	**Area**	**Area Sum %**
1	**9.659**	**Butanoic acid, 2-methyl-, ethyl ester**	**C7H14O2**	**20669.99**	**0.00**
2	**27.397**	**(E)- β - Farnesene**	**C15H24**	**1868482.1**	**0.42**
3	**30.356**	**1,9-Decadiyne**	**C10H14**	**436460.32**	**0.10**
4	**31.74**	**Germacrene D**	**C15H24**	**66084.15**	**0.01**
5	**32.158**	**α-Bisabolol oxide B**	**C15H26O2**	**10523446**	**2.38**
6	**32.616**	**α-Terpineol**	**C10H18O**	**8102831.5**	**1.83**
7	**33.583**	**Chamazulene**	**C14H16**	**1118113.6**	**0.25**
8	**34.252**	**α-Bisabolol oxide A**	**C15H26O2**	**404871463**	**91.58**
9	**34.504**	**(cis)-5-hepten-1-yne**	**C7H10**	**23558.51**	**0.01**
10	**36.255**	**cis-ene-yne-Dicycloether**	**C13H12O2**	**14456510**	**3.27**
11	**37.703**	**(E)-Tibetin spiroether**	**C14H14O2**	**107871.48**	**0.02**
12	**38.349**	**Decanoic acid**	**C10H20O2**	**245233.06**	**0.06**
13	**41.41**	**Undec-10-ynoic acid**	**C11H18O2**	**90512.86**	**0.02**
14	**41.651**	**1,11-Dodecadiyne**	**C12H18**	**36561.53**	**0.01**
15	**41.834**	**13-Tetradece-11-yn-1-ol**	**C14H24O**	**127387.44**	**0.03**
nd	**nd**	**4-pentadecanol**	**C15H32O**	**nd**	**nd**
Local					
Peak	**RT**	**Name**	**Formula**	**Area**	**Area Sum %**
1	**9.653**	**Butanoic acid, 2-methyl-, ethyl ester**	**C7H14O2**	**54419.87**	**0.04**
2	**27.38**	**(E)- β -Farnesene**	**C15H24**	**336463.11**	**0.24**

3	**30.333**	**1,9-Decadiyne**	**C10H14**	**18097.28**	**0.01**
4	**32.135**	**α-Bisabolol oxide B**	**C15H26O2**	**1942262**	**1.38**
5	**32.576**	**α-Terpineol**	**C10H18O**	**756802.51**	**0.54**
6	**33.554**	**Chamazulene**	**C14H16**	**59980.49**	**0.04**
7	**34.149**	**α-Bisabolol oxide A**	**C15H26O2**	**134939341**	**95.59**
8	**36.198**	**cis-ene-yne-Dicycloether**	**C13H12O2**	**2978224.6**	**2.11**
9	**37.68**	**(E)-Tibetin spiroether**	**C14H14O2**	**12816.9**	**0.01**
10	**38.292**	**Decanoic acid**	**C10H20O2**	**41648.81**	**0.03**
11	**41.822**	**13-Tetradece-11-yn-1-ol**	**C14H24O**	**22416.63**	**0.02**
nd	**nd**	**Germacrene D**	**C15H24**	**nd**	**nd**
nd	**nd**	**(cis)-5-hepten-1-yne**	**C7H10**	**nd**	**nd**
nd	**nd**	**Undec-10-ynoic acid**	**C11H18O2**	**nd**	**nd**
nd	**nd**	**1,11-Dodecadiyne**	**C12H18**	**nd**	**nd**
nd	**nd**	**4-pentadecanol**	**C15H32O**	**nd**	**nd**

Fig. (3): GC-MS analysis on oil content of dry flower heads, on four chamomile cultivars (Bode Gold, Zloty lan, local improved and local).



3- Scanning Electron Microscope (SEM) micrographs showing pollen grains of four chamomile cultivars under study for family Asteraceae and also showing detailed exine ornamentation:

Based on Fig. (4), The general morphological shape of chamomile seeds were obtained using the electron microscope: (a) Scanning of chamomile seeds at magnification x110 and (b) Scanning of chamomile seeds at magnification x30 ; **As shown in Fig. (4),** Morpho-palynological assessment for pollen grains of *chamomilla recutita* (Family Asteraceae): (c) Bode gold cultivar (**3.708 µm**), (d) Zloty lan cultivar (**3.210 µm**), (e) Local Improved cultivar (**3.017 µm**) and (f) Local cultivar (**2.803 µm**) on the basis of scanning electron microscopy.

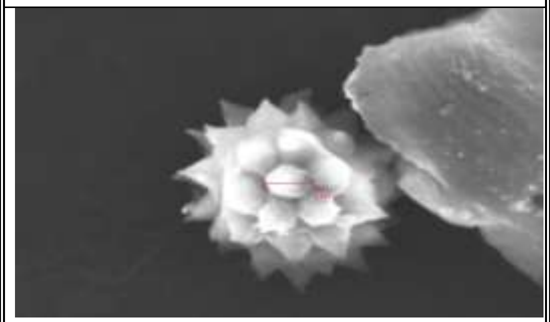
These results in agreement with **(Khan, *et.al.*, 2021)** by studying the Morpho-palynological assessment of some species of the Family Asteraceae using Scanning Electron Microscopy; **(Umber, *et.al.*, 2021)** by studying the effect of **(SEM)** for pollen morphology of selected taxa of Family Asteraceae and **(Khan, *et.al.*, 2023)** by studying the morphological assessment of Asteraceae taxa using SEM.

Fig. (4): Scanning Electron Microscope (SEM) micrographs showing pollen grains of four chamomile cultivars under study for family Asteraceae and also showing detailed exine ornamentation.

(a) Scanning of chamomile seeds at magnification x110

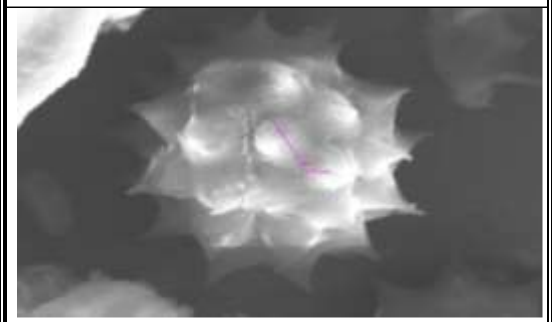
(b) Scanning of chamomile seeds at magnification x30

(c) Bode Gold (3.708 µm)



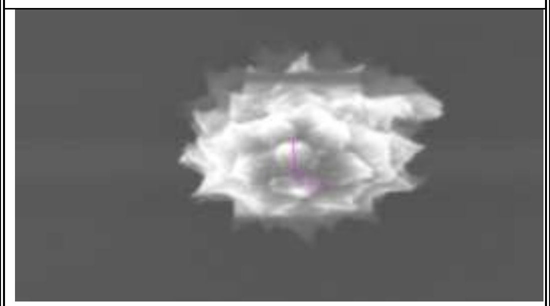
5 µm

(d) Zloty Lan (3.210 µm)



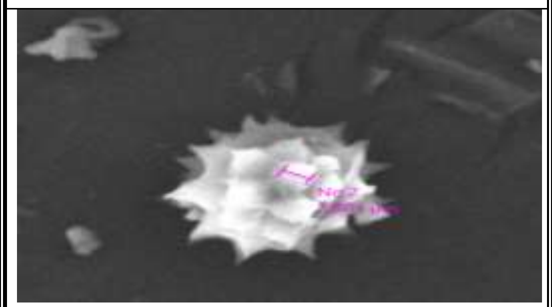
5 µm

(e) Local Improved (3.017 µm)



5 µm

(f) Local (2.803 µm)



5 µm

4- Chemical Composition in seeds of four chamomile cultivars (Bode Gold, Zloty lan, local improved and local) using Electron Microscope (SEM):

The results were mentioned in **Table (4) and Fig. (5)** showed that the seeds of the four cultivars under study using electron microscope contain the following mineral elements (Boron (B), Carbon (C), Nitrogen (N), Oxygen (O), Fluorine (F), Sodium (Na), Magnesium (Mg), Aluminum (Al), Silicon (Si), Phosphorus (P), Sulfur (S), Chlorine (Cl), Potassium (K), Calcium (Ca), Scandium (Sc), Titanium (Ti), Vanadium (V), Chromium (Cr), Manganese (Mn), Iron (Fe), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Selenium (Se), Bromine (Br) and Zirconium (Zr).

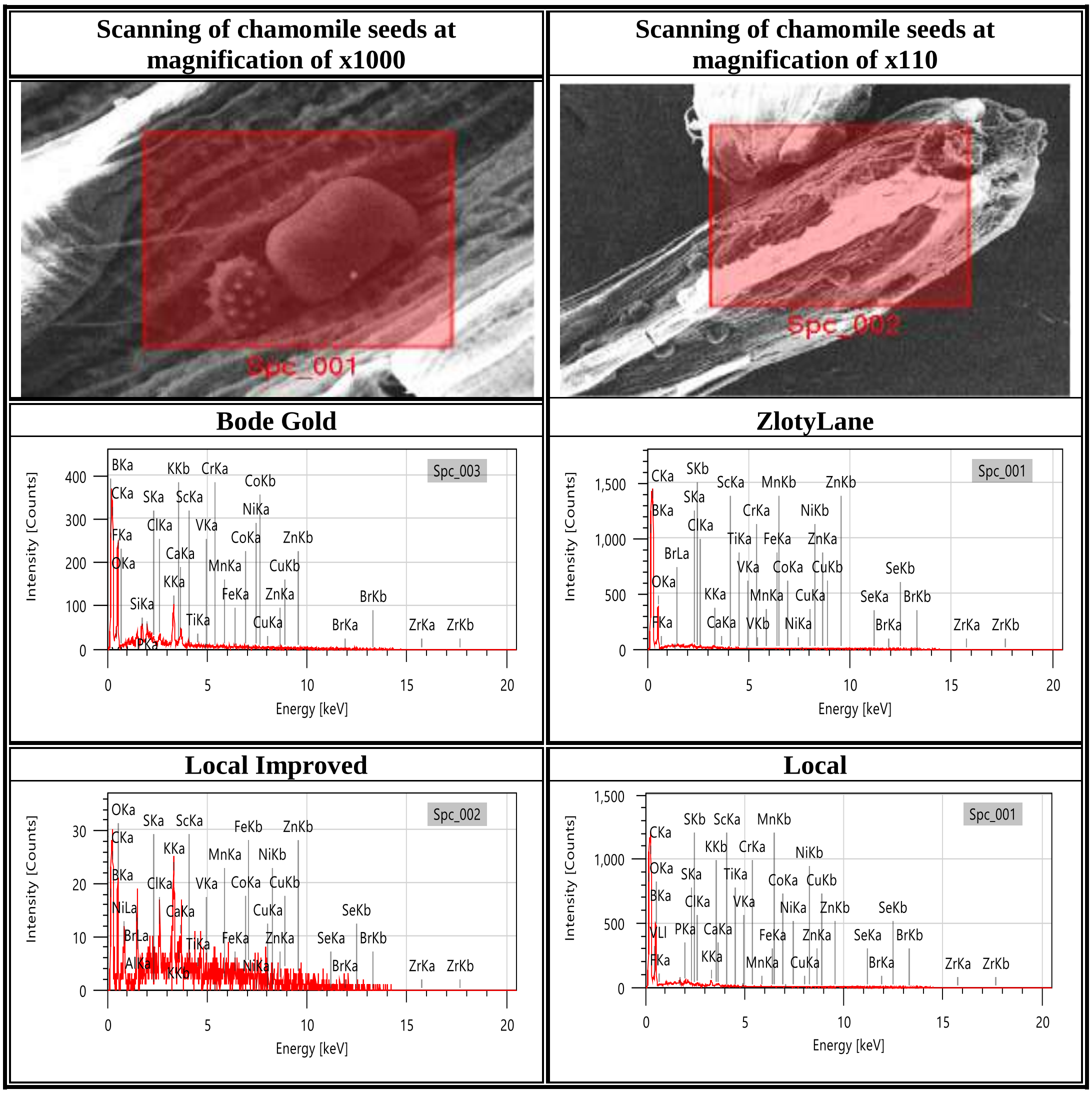
Also, through electron microscope to examine the mineral elements in the seeds of the four chamomile cultivars under study, some mineral elements did not appear such as (Nitrogen (N), Chromium (Cr) and Zirconium (Zr)). In addition to, Local Improved cultivar some mineral elements did not appear in it, as (Fluorine (F), Sodium (Na) and Magnesium (Mg)). It was also shown that some mineral elements did not appear in the seeds of the local cultivar, including (Scandium (Sc), Cobalt (Co), Zinc (Zn) and Bromine (Br)).

Perhaps, The distinguishing feature of the Bode gold cultivar lies in its high content of essential oil compared to other cultivars under study. This increase in seed size and mineral content may promote vegetative growth, thereby enhancing flower heads productivity and quality. Consequently, there is an increase in the percentage of essential oil and the concentration of active components within the essential oil. These results in agreement with **(Ahmed, *et.al.*, 2009)** by studying the chemical taxonomic validation of the herbal drug chamomile, **(Stanojevic, *et.al.*, 2016)** reported the presence of 52 components in chamomile oil, with the highest content of beta-Farnesene, α-Farnesene, α-Bisabolol and Chamazulene, as shown Chamomile oil has the best antioxidant properties. **(Khan, *et.al.*, 2021)** by studying the Morpho-palynological assessment of some species of the Family Asteraceae using Scanning Electron Microscopy; **(Umber, *et.al.*, 2021)** by studying the effect of (SEM) for pollen morphology of selected taxa of Family Asteraceae and **(Khan, *et.al.*, 2023)** by studying the morphological assessment of Asteraceae taxa using SEM.

Table 4. The Chemical Composition in seeds of four chamomile cultivars (Bode Gold, Zloty lan, local improved and local) through Electron Microscope.

Species	Bode gold		Zloty lane		Local improved		Local	
	Quantification method ZAF	**Result Type Metal**	**Quantification method ZAF**	**Result Type Metal**	**Quantification method ZAF**	**Result Type Metal**	**Quantification method ZAF**	**Result Type Metal**
Elements	**Mass%**	**Atom%**	**Mass%**	**Atom%**	**Mass%**	**Atom%**	**Mass%**	**Atom%**
B	**31.50±0.72**	**38.71±0.88**	**32.41±0.33**	**36.31±0.37**	**28.49±2.26**	**39.29±3.12**	**33.08±0.37**	**37.71±0.42**
C	**34.09±1.71**	**37.70±1.89**	**52.09±1.13**	**52.53±1.14**	**28.63±4.86**	**35.53±6.03**	**45.93±1.14**	**47.13±1.17**
N	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
O	**24.28±1.67**	**20.16±1.38**	**14.19±0.78**	**10.74±0.59**	**18.56±4.51**	**17.29±4.20**	**18.68±0.88**	**14.39±0.68**
F	**0.05±0.10**	**0.04±0.07**	**0.17±0.07**	**0.11±0.05**	**nd**	**nd**	**0.12±0.07**	**0.08±0.05**
Na	**0.19±0.09**	**0.11±0.05**	**0.05±0.03**	**0.03±0.01**	**nd**	**nd**	**0.08±0.04**	**0.04±0.02**
Mg	**0.28±0.09**	**0.16±0.05**	**0.01±0.01**	**0.00±0.01**	**nd**	**nd**	**0.03±0.02**	**0.02±0.01**
Al	**0.34±0.18**	**0.17±0.09**	**nd**	**nd**	**1.00±0.87**	**0.55±0.48**	**0.10±0.03**	**0.04±0.01**
Si	**0.61±0.13**	**0.29±0.06**	**0.02±0.02**	**0.01±0.01**	**0.33±0.31**	**0.17±0.16**	**0.15±0.04**	**0.06±0.02**
P	**0.42±0.11**	**0.18±0.05**	**nd**	**nd**	**0.21±0.27**	**0.10±0.13**	**0.21±0.05**	**0.08±0.02**
S	**0.24±0.08**	**0.10±0.03**	**0.03±0.02**	**0.01±0.01**	**0.28±0.29**	**0.13±0.13**	**0.04±0.02**	**0.01±0.01**
Cl	**0.47±0.12**	**0.18±0.04**	**0.11±0.03**	**0.04±0.01**	**2.42±0.80**	**1.02±0.34**	**0.16±0.04**	**0.06±0.01**
K	**3.23±0.35**	**1.10±0.12**	**0.17±0.05**	**0.05±0.01**	**5.84±1.43**	**2.22±0.55**	**0.46±0.08**	**0.15±0.02**
Ca	**1.54±0.27**	**0.51±0.09**	**0.19±0.05**	**0.06±0.02**	**2.44±1.02**	**0.91±0.38**	**0.24±0.06**	**0.07±0.02**
Sc	**0.00±0.06**	**0.00±0.02**	**nd**	**nd**	**0.41±0.54**	**0.14±0.18**	**nd**	**nd**
Ti	**0.04±0.08**	**0.01±0.02**	**nd**	**nd**	**nd**	**nd**	**0.04±0.03**	**0.01±0.01**
V	**nd**	**nd**	**nd**	**nd**	**1.24±1.00**	**0.36±0.29**	**0.05±0.04**	**0.01±0.01**
Cr	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Mn	**0.38±0.22**	**0.09±0.05**	**0.10±0.06**	**0.02±0.01**	**nd**	**nd**	**0.14±0.08**	**0.03±0.02**
Fe	**0.59±0.29**	**0.14±0.07**	**0.01±0.03**	**0.00±0.01**	**2.15±1.69**	**0.57±0.45**	**0.12±0.08**	**0.03±0.02**
Co	**0.29±0.25**	**0.07±0.06**	**0.01±0.04**	**0.00±0.01**	**nd**	**nd**	**nd**	**nd**
Ni	**0.23±0.26**	**0.05±0.06**	**0.20±0.12**	**0.04±0.02**	**2.90±2.51**	**0.73±0.64**	**0.05±0.07**	**0.01±0.01**
Cu	**1.19±0.61**	**0.25±0.13**	**0.03±0.07**	**0.01±0.01**	**0.40±1.66**	**0.09±0.39**	**0.32±0.18**	**0.06±0.04**
Zn	**nd**	**nd**	**0.01±0.08**	**0.00±0.02**	**0.11±1.98**	**0.02±0.45**	**nd**	**nd**
Se	**nd**	**nd**	**0.07±0.05**	**0.01±0.01**	**0.41±0.53**	**0.08±0.10**	**0.02±0.04**	**0.00±0.01**
Br	**0.02±0.39**	**0.00±0.07**	**0.15±0.06**	**0.02±0.01**	**4.18±2.35**	**0.78±0.44**	**nd**	**nd**
Zr	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Fig. (5): Scanning seeds of four chamomile cultivars (Bode Gold, Zloty lan, local improved and local) through Electron Microscope (SEM).



Conclusion

This study recommended the cultivation of (Bode gold) cultivar, (local improved) cultivar then (Zloty lan) cultivar as chamomile cultivars that outperform the local cultivar and suiting the Egyptian climate for their production. Therefore, several cultivars of chamomile are available to chamomile producers to meet the changing specifications for export and (Bode gold) cultivar is considered one of the most important cultivars of chamomile adapted to local climatic conditions and is a good cultivar for high quality and productivity of flower heads and essential oil, as well as its effective components that are involved in many pharmaceutical and cosmetics industries.

This study also confirmed that the use of high concentration of colchicine (0.4 ppm) gave the highest values in the yield of fresh and dry flower heads, as well as the percentage and yield of essential oil for the four cultivars (Bode gold, Zloty lan, Local improved and Local) under the study and it was the best treatment, As for (Bode gold) cultivar, which was treated with colchicine at a concentration of (0.4 ppm), it gave the highest productivity of fresh and dry flower heads and essential oil yield compared with other cultivars with the same treatment under study.

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الملخص العربى

تحت إشراف و متابعة فسم الوراثة – كليه الزراعة بمشتهر – جامعه بنها تمت هذه الدراسة في مزرعة الزينة بقسم البساتين كلية الزراعة بمشتهر جامعة بنها في موسمين ناجحين متتالين 2002/2023 و 2023/2024م تحت عنوان دراسات كيميائية حيوية ووراثية سيتولوجية على التأثير الطفورى للاشعاع والكولشيسين و (DMSO) على نبات البابونج بهدف زراعة بعض الاصناف الاجنبية لنباتات البابونج عالية الجودة والانتاجية في مصر ومنها صنف (Bode gold, Zlotylan, and Improved local) مقارنة بالصنف المحلي، باعتبارها من أهم الأصناف عالية الجودة والإنتاجية للنورات الزهرية والزيت العطري، فضلاً عن مكوناته الفعالة التي تدخل في العديد من الصناعات الصيدلانية حيث تم تعريض شتلات البابونج قبل الزراعة بالأرض المستديمة مباشرة لجرعات أشعة جاما (Gamma rays 5, 10 and 20 gr) كذلك رش النباتات بالكولشيسين (Colchicine و ثنائي الميثيل سلفوكسيد (DMSO)) بعد الزراعة بالأرض المستديمة بثلاث أسابيع (21 يوم) بتركيزات (0.1, 0.2 and 0.4 ppm.) تم رشة ثانية بعد اسبوعين من الرشة الاولي ثم رشة ثالثة بعد اسبوعين من الرشة الثانية بالإضافة معاملات الكنترول بدون رش وقد تم أخذ البيانات التالية :

فحص الميكروسكوب الالكتروني لبذور البابونج للأصناف الأربعة تحت الدراسة و المقارنة بينهم من حيث التركيب المورفولوجي و المحتوي المعدني والتعرف علي محتوي البذور من العناصر المعدنية.

القياسات البستانية ومنها النسبة المئوية للزيت الطيار والتركيب الكيماوي للزيت .

ويمكن تلخيص النتائج المتحصل عليها حيث سجل الصنف (Bode gold) أعلي نسبة زيت طيار في النورات الزهرية الجافة يليه الصنف المحسن ثم الصنف (Zloty lan) بالمقارنة بالصنف المحلي كما سجلت النتائج المتحصل عليها ان من أهم مكونات الزيت الطيار Bisabolol oxide A و α-Bisabolol oxide B و Chamazulene.

التوصية:

أوصت هذه الدراسة بزراعة الصنف (Bode gold) والصنف (المحلي المحسن) ثم الصنف (Zloty lan) كأصناف بابونج تتفوق في احتوائها علي نسبة عالية من لزيت الطيار بالمقارنة بالصنف المحلي كما انها تتناسب مع المناخ المصري لإعطاء أعلي إنتاجية كما يتوفر العديد من اصناف البابونج للمنتجين لتلبية المواصفات المتغيرة للتصدير ويعتبر صنف (Bode gold) من أهم أصناف البابونج الملائمة والمناسبة مع الظروف المناخية المحلية وهو صنف يمتاز بالجودة العالية وإنتاجية عالية للنورات الزهرية والزيت العطري، بالإضافة إلى مكوناته الفعالة التي تدخل في العديد من الصناعات الدوائية ومستحضرات التجميل كذلك الرش بالكولشيسين بتركيز عالي (0.4 ppm) أعطى أعلى القيم من حيث محصول النورات الطازجة والجافة، وكذلك نسبة ومحصول الزيت العطري الطيار للأصناف الأربعة (Bode gold, Zloty lan, and local Improved) تحت الدراسة مقارنة بالصنف المحلي.