### GROWTH AND ESSENTIAL OIL QUANTITY OF POT MARIGOLD CALENDULA OFFICINALIS IN RESPONSE TO FOLIAR APPLICATION OF MORINGA EXTRACT AND PINK-PIGMENTED FACULTATIVE METHYLOTROPHIC BACTERIA METHYLOBACTERIUM POPULI

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#### ABSTRACT

The use of bio stimulants for promoting plant growth like bacteria and plant extracts has great potential to provide economical and sustainable solutions to current agricultural practices and challenges. Two consecutive field trials in 2019 and 2020 were conducted to investigate the effect of foliar spraying with moringa extract and pink-pigmented methylotrophic bacteria on growth and the essential oil content of Calendula officinalis plant. Four concentrations of moringa extract (1, 2, 4 and 6 %) and four concentrations of pink-pigmented methylotrophic bacteria (0.5, 1, 2. and 3 %) were used. All moringa and methylotrophic bacteria treatments positively pink-pigmented impacted growth characteristics, i.e., plant height, number of leaves and essential oil content in both studied two seasons. Plants sprayed with 2% moringa extract produced the tallest plant and number of leaves meanwhile, plants treated with 4% moringa extract exhibited a significant increase in essential oil contents (ml/plant). A similar trend was observed when plants were treated with 2% pink-pigmented methylotrophic bacteria, and it is suggested that these concentrations be used.

Key words: Moringa extract, Growth, Essential oil, Pink-Pigmented Methylotrophic Bacteria, *Calendula officinalis*, pot marigold.

#### **INTRODUCTION**

Pot marigold *Calendula officinalis* L. is an annual ornamental plant besides being a medicinal plant that belongs to the *Asteraceae* family (Sedghi *et al.*, 2011). It originated from Western Asia, Central and Southern Europe in addition to the US. Marigold has been

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grown throughout the world for ornamental use as cut flowers and as flower-pot plants. Nowadays, its leaves have been valued for pharmaceutical, biological, medicinal, herbal, and cosmetic purposes (Clark et al., 2010, Szopa et al., 2020). Lately, the calendula has received special attention for the commercial production of its seed oil (Gesch, 2013). Marigold could serve as a valuable source of natural colors, since flavonoids and carotenoids are two major classes of pigments (yellow and orange) found in abundance in their inflorescences. In addition, the flavonoids have biological activities and carotenoids can protect against cancer and functions as a precursor for vitamin A. Research studies confirmed antipyretic, anti-tumor, hepato-regenerative, cardioprotective, neuroprotective, anticancer, and cicatrizing effects of the dried flower heads (Piccaglia et al., 1997). Tincture of marigold is employed in homoeopathy for curing mental tension and insomnia disorders (Kumar et al., 2013). It is also used to treat skin rashes and infections of the skin and mucous membranes (Arora et al., 2013). In recent years there has been great interest in sustainable agriculture for improving crop yield and quality in environmentally or ecologically friendly ways. Affordable and effective natural bio stimulants have been explored in the agricultural sector as an alternative to chemical fertilizers which is harmful on human and the environment (Bilalis et al., 2009).

Among the various bio stimulants are bacteria such as pink-pigmented methylotrophic bacteria and plant-derived extracts such as moringa leaf extract which both can play an important role in this regard. They are perceived as natural environmentally friendly products that enhance plant growth, reduce the reliance on synthetic fertilizers and protect plants from potential pathogens (Zhang *et al.*, 2021). Furthermore, the application of bio stimulants particularly in the production of medicinal plants leads to ensure higher quality of the final products according to the global quality standards without affecting yield. (Drobek *et al.* 2019).

Pink-Pigmented Facultative Methylotrophs (PPFMs) are Gram-negative symbiont bacteria with plants. They belong to the genus *Methylobacterium* that can be grown on plant surfaces and isolated from the phyllosphere of several plant species. These bacteria form pink to red colonizers on selective isolation media due to carotenoid synthesis and utilize single carbon such as methanol and methylamine when grown on plant surface or use formaldehyde, and multi-carbon substrates (Savitha *et al.*, 2013). After consuming waste products provided by the plants, these bacteria are known to secrete a variety of metabolites useful for the plants including phytohormones such as auxins, cytokinins and vitamin B12 (Omer *et al.*, 2004). These phytohormones have many desirable effects on plant growth by improving nutrient usage efficiency and alleviating various biotic or abiotic stresses (Hussain *et al.*, 2013).

Foliar application of PPFMs as shown previously in different research studies tend to participate in plant metabolism causing changes in vital and structural processes by consuming plant waste products and secreting a variety of metabolites (Welbaum *et al.*, 2004). There are reports that confirm the potential benefits of these bacteria when applied correctly to the plants like nitrogen fixation, phytohormone production, improving tolerance to abiotic stresses (Balachandar *et al.*, 2008). It is well documented that PPFM can enhance plant growth and productivity optimization in an environmentally friendly manner. PPFM strains enhanced seed germination of same wheat (*Triticum aestivum*) as PPFM inoculation induced number of stomata, chlorophyll concentration and malic acid content. They led to improved plant growth and yield (Verma *et al.*, 2014). In addition, PPFM showed a marked improvement in pod quality of snap bean by enhancing their content of amino acids, protein, total sugars, and ascorbic acid (Abd El-Gawad *et al.*, 2015).

Previous studies have reported that applying moringa extract (ME) can be considered a sustainable tool and meaningful approach to enhance plant growth and yield. Patchouli explants treated with moringa leaf extract (MLE) had higher growth than control plants in this regard the use of MLE at 40 g/L was found as potential substitute for 1.5 ppm zeatin hormone (Nihayati and Najah 2021). Significant increases in *Allium cepa* characteristics

were observed with ME application at a concentration ratio of 1:2 (50%) and 3,6 weeks intervals after transplanting (Mohamed *et al.*, 2013). Similarly, in exogenous application of 30% ME has been shown to improve number of leaves plant-1, stem diameter, plant height, number of florets spike-1, flowering duration (44 days), and vase life of *Antirrhinum majus* (Jan *et al.*, 2022). Whereas, when lettuce plants were exposed to 6% ME the vegetative quality parameters (head structure, head, and root weights) were improved with an obvious decrease in the nitrate content in leaves.

Owing to the significance of both PPFM and ME as biostimulants for growth, the aim of this study was to evaluate their impact as foliar applications on the vegetative traits, chemical compounds, and essential oil content of pot marigold.

### MATERIALS AND METHODS

- Location and Duration: The experiments of this study were carried out at Zouhria Botanic Garden, Horticulture Research Institute, Agricultural Research Center, Giza, for two successive seasons 2019 and 2020.
- 2. Plant Material: Pot Marigold *Calendula officinalis* L. seedlings used in this study were kindly provided from SEKEM Group Companies, Cairo Belbes Desert Road, El Salam City, Egypt.
- **3. Preparation of Moringa Leaf Extract:** Fresh leaves of *Moringa oleifera* were dried and grinded with a blender. Chemical analysis of moringa powder per 100 g dry weight was extracted by 70% methanol using a Soxhelt apparatus for 6 hours. The extracts were concentrated using a rotary evaporator under reduced pressure at 40 °C. The crude extract was then diluted to the required dose. This protocol was guided by the method of Farhan *et al.* (2021). The dried powdered Moringa leaves were subjected to the chemical analysis (crude protein, crude lipids, crude fiber according to the standard methods of A.O.A.C (2010). The phenol-sulfuric acid method was used to determine the total carbohydrates according to Dubois *et al.* (1956). The absorbance read was at 490 nm using a spectrophotometer. The minerals contents of calcium, sodium and potassium were

determined using flame photometry. Magnesium, iron, copper, manganese, zinc, Boron and selenium contents were determined by atomic absorption spectroscopy using the Association of Official Agricultural Chemists (AOAC, 2010) procedures. A standard colorimetric method was applied for phosphorus as mentioned by Borah *et al.* (2009). Sulfur was measured using a gravimetric method according to Hanlon (1998). The ascorbic acid (vitamin c) concentration was analyzed using the method described by Mukherjee and Choudhuri (1983). The tocopherol content (vitamin E) was measured at wavelength 520 nm using a 2.2-dipyridyl reagent according to the method of Philip *et al.* (1954). Vitamin B contents were determined using the method of chemical analysis of food described by Ekinci and Kadakal (2005).  $\beta$ -Carotene content was determined by using a method described by Biswas et al. (2011).

4. Preparation of PPFM Bacteria The PPFM Bacteria solution was collected and graciously supplied by the Department of Horticulture based on a partnership between the Department of Microbiology and the Department of Horticulture, Faculty of Agriculture, Ain Shams University. Pink-Pigmented Facultative Methylotrophic Bacteria Methylobacterium populi were isolated and collected from a variety of plants, including Gossypium herbaceum (cotton), Phaseoulus vulgaris (snap bean), Vcia faba (broad bean), and Datura inoxia. All samples were collected from Qalyubia, Beheira and Giza governorates. Agar medium of mineral ammonium salts (AMS) was used to isolate and purification of PPFM Filtered methanol was added to the sterile medium at a concentration of 0.5%. The minimum mineral medium is M72. This medium was used to test the use of methanol. Methanol was added to sterile medium at different levels concentrations. 0.5, 1, 1.5 or 2%. K medium was used for determination of cytokinins, methanol was filtered. They were added to a sterile medium at a concentration of 0.5% the DSM125 medium was used to determine IAA (Abd El-Gawad et al., 2015).

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- 5. Experimental Treatments: Nine experimental treatments were initiated as follows:
- Moringa extract (ME) at 1, 2, 4 and 6%
- The solution of PPFM (10<sup>9</sup> CFU/ml.) bacteria were sprayed 4 times in both studied seasons, one spray every 30 days, where 4 treatments of PPFM bacteria were applied each for each season. The concentration of PPFM was 10<sup>9</sup> CFU /ml. (colonies forming unit). The following four treatments were prepared:
  - 1-0.5% PPFM concentration (5 ml/liter)
  - 2-1% PPFM concentration (10 ml/liter)
  - 3-2% PPFM concentration (20 ml/liter)
  - 4-3% PPFM concentration (30 ml/liter)
  - 5- Irrigation with water only represented the control group.

### 6. Data Recorded

**a) Vegetative parameters:** The following parameters have been recorded: plant height, number of leaves/ plants, dry weight/plant and number of flowers/ plant.

b) Chemical analysis

Essential oil content (ml/ plant), the extraction and analysis of essential oil content was carried out according to the method described by **Farhan** *et al.*, (2021).

- **7. Experimental Design:** Pot experiments in the open field were arranged in a randomized complete block design (RCBD).
- 8. Statistical Analysis: The collected data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1989). The differences among means were compared by Duncan Multiple Range Test at a probability level s 5% (Duncan 1955). All statistical analyses were carried out using MSTAT-C software package (Freed *et al.*, 1989).

#### **RESULTS AND DISCUSSION**

The positive effect of moringa is usually attributed to the extract which contains many components (phenolics, amino acids, ascorbic acid, tocopherols, selenium, phytohormones such as auxins, gibberellins, zeatin-type cytokinin) and not to a specific compound based (Table 1).

COMPONENT	CONCENTRATION	COMPONENT	CONCENT -RATION	COMPONENT	CONCENT -RATION
Protein (%)	28.5	Zn (mg)	3.0	Iron (mg)	37.5
Fat (%)	4.5	Manganese (mg)	9.0	Sodium (mg)	160
Carbohydrate (%)	35.4	Selenium (mg)	20	Vitamin E (mg)	89
Fiber (%)	15.0	Boron (mg)	3.0	Sulfur (g)	0.74
Calcium (g)	3.0	Vitamin A (mg)	14	Zn (mg)	3.0
Magnesium (g)	0.46	Vitamin B1 (mg)	3	Vitamin C (mg)	13.2
Phosphorus (g)	0.33	Vitamin B2 (mg)	17.7	Copper (mg)	0.75
Potassium (g)	1.86	Vitamin B3 (mg)	6		
Iron (mg)	37.5	Vitamin E (mg)	89		

**Table 1.:** Chemical analysis of moringa powder per 100 g dry weight.

### Effect of moringa extract (ME) and pink-pigmented Facultative Methylotrophic (PPFM) bacteria on plant height

Data presented in Table (2) showed that plant height (cm) progressively increased with increasing ME levels to reach its highest values at 2.0% concentration as compared to other treatments and the control group for the two seasons reaching  $37.27 \pm 0.59$  and  $35.75 \pm 0.35$  cm, respectively.

These results are in accordance with those reported by Ebido *et al.*, (2020) on moringa extract as a good organic foliar fertilizer for enhancing yield of cocoyam. This is attributed to the presence of growth hormones and nutrients which are present in sufficient quantities and suitable proportions, capable of increasing growth and yield of various crops.

 Table (2): Effect of foliar spraying with moringa extract (ME) and pink-pigmented facultative methylotrophic (PPFM) bacteria on plant height of *Calendula officinalis* L.

TREATMENTS				A EXTRAC TRATION		PPFM BACTERIA CONCENTRATIONS				
SEASONS	Control	1%	2%	4%	6%	0.5%	1.0%	2.0%	3.0%	
Firstseason	25.66	33.11	37.27	$26.94 \\ {\scriptstyle \pm 0.82}^{\rm b}$	27.33	12.72	17.44	14.89	15.03	
(2019)	±0.83 <sup>b</sup>	±0.38 <sup>a</sup>	±0.59 <sup>a</sup>		±4.32	±1.09 <sup>c</sup>	±0.48 <sup>c</sup>	±1.73 <sup>c</sup>	±4.19 <sup>c</sup>	
Second season	26.50	29.50	35.75	31.33	30.67	13.25	18.02	6.77 <sub>±1.63</sub>	16.87	
(2020)	±0.71 <sup>d</sup>	±0/72 cd	±0.35 <sup>a</sup>	±1.05 <sup>bc</sup>	±2.87 <sup>b-d</sup>	±2.01 <sup>f</sup>	±2.32 <sup>e</sup>		±2.55 ef	

Values are means  $\pm$ SD. Different letters next to the mean values in each column indicate significant difference at 5 level according to Duncan test.

### Effect of moringa extract (ME) and pink-pigmented Facultative Methylotrophic (PPFM) bacteria on number of leaves

Data presented in Table (3) indicated that number of leaves/plant gradually increased in response to the gradual increasing in ME level to be the maximum at the 2% concentration compared to the remaining treatments and controls in both seasons (164.2  $\pm$ 4.40 and 184 $\pm$ 1.70, respectively). The obtained results are in harmony with those found by Abou El-Nour and Ewais (2017) on *Moringa oleifera* extract who achieved vegetative growth good expressed as plant height, a greater number of leaves, branches, larger leaf area and maximum leaf.

Table (3): Effect of foliar spraying with moringa extract (ME) and pink-pigmented facultative methylotrophic (PPFM) bacteria on number of leaves/plant of *Calendula officinalis* L.

TREATMENTS				EXTRA		PPFM BACTERIA CONCENTRATIONS				
SEASONS	Control	1%	2%	4%	6%	0.5%	1.0%	2.0%	3.0%	
First season (2019)	136.20 ±7.91	125.80 ±3.15 <sup>b</sup>	164.20 ±4.40 <sup>a</sup>	104.00 ±4.25 <sup>c</sup>	110.70 ±1.44 <sup>c</sup>	74.88 ±2.60 <sup>d</sup>	67.89 ±2.79 <sup>d</sup>	68.24 ±1.50 <sup>d</sup>	68.66 ±8.75 <sup>d</sup>	
Second season (2020)	109.30 ±4.78 e	134.00 ±2.39 <sup>cd</sup>	$184.0_{\pm 1.70}$ <sup>a</sup>	129.50 ±5.35 <sup>d</sup>	145.70 ±13.02 <sup>c</sup>	$68.00 \\ {}_{\pm 1.63} ^{\rm f}$	69.17 ±5.31 f	$68.83 \\ {}_{\pm 2.86} ^{f}$	$78.83 \\ {\scriptstyle \pm 3.40}^{\rm f}$	

Values are means  $\pm$ SD. Different letters next to the mean values in each column indicate significant difference at 5 level according to Duncan test

# Effect of moringa extract (ME) and pink-pigmented Facultative Methylotrophic (PPFM) bacteria on dry weight

Table (4) showed that (PPFM) bacteria at its lowest concentration (0.5%) was the best concentration for dry weight/plant. Whilst the remaining treatment were either equal to or less than the control. This was confirmed by the previous findings on other plants. For example, Kheyri *et al.*, (2020) noted that biological fertilizer (mycorrhizal fungi) was applied to enhance the absorption rate of nutrient elements from the soil leading to increase the plant growth. Mycorrhizal fungi improved plant growth by enhancing the nutritional and water conditions of plant with changing the root morphology and water relationship enhancement by that fungus. The treatments had the highest effect on improving the plant height, branchlet number, inflorescence number and flower dry weight.

 Table (4): Effect of foliar spraying with moringa extract (ME) pink-pigmented facultative methylotrophic (PPFM) bacteria on dry weight /plant of *Calendula officinalis* L.

TREATMENTS				EXTRAC		PPFM BACTERIA CONCENTRATIONS				
SEASONS	Control	1%	2%	4%	6%	0.5%	1.0%	2.0%	3.0%	
First season	22.44	17.77	42.55	34.88	43.77	$51.0 \\ 0_{\pm 2.38} a$	19.00	24.78	15.89	
(2019)	±4.40 <sup>d</sup>	±3.00 <sup>ef</sup>	±2.23 <sup>b</sup>	±2.13 c	±0.96 <sup>b</sup>		<sub>±4.63</sub> d- <sup>f</sup>	±2.11 <sup>d</sup>	±5.23 <sup>f</sup>	
Second season	32.17	24.33	64.33	59.83	46.17	71.67	28.83	34.33	$21.83 \\ \pm 3.19^{\ f}$	
(2020)	±0.85 <sup>d</sup>	±0.94 ef	±0.85 <sup>b</sup>	±2.49 <sup>b</sup>	±0.62 <sup>c</sup>	<sub>±</sub> 2.95 <sup>a</sup>	±3.88 <sup>de</sup>	±6.60		

Values are means  $\pm$ SD. Different letters next to the mean values in each column indicate significant difference at 5 level according to Duncan test.

### Effect of moringa extract (ME) and pink-pigmented Facultative Methylotrophic (PPFM) bacteria on number of flowers

Results in Table (5) indicated that PPFM bacteria increased the number of flowers /plant at 3% when compared to the remaining other treatments and the controls in both investigated seasons (57  $\pm$ 0.85 and 56  $\pm$ 4.33, in descending order). Previous studies reported that co-inoculation of two plant growth promoting rhizobacteria (PGPR) can be increased flower yield and Flower essential oil (Sandilya *et al.*, 2022).

 Table (5): Effect of foliar spraying with moringa extract (ME) and pink pigmented facultative methylotrophic (PPFM) bacteria on number of flowers/plant of Calendula officinalis L.

Tractor		Mori	inga extract	concentrat	ions	PPFM bacteria concentrations			
Treatments Seasons	Control	1%	2%	4%	6%	0.5%	1.0%	2.0%	3.0%
First season (2019)	44.00 ±3.89 c-e	41.33 c-e ±2.72	42.33 c-e ±5.33	37.50 ±4.13 <sup>e</sup>	52.83 ab ±0.85	43.67 c-e ±1.22	40.17 de ±1.43	46.33 b-d ±4.29	57.00 ±0.85
Second season (2020)	38.00 bc ±1.65	29.17 ±7.58 <sup>°</sup>	32.00 ±2.94 <sup>c</sup>	29.00 ±8.04 <sup>c</sup>	51.00 ±3.56 <sup>a</sup>	50.00 ±2.04 <sup>a</sup>	38.00 ±3.19 <sup>bc</sup>	46.17 <sub>±</sub> 2.78 <sup>ab</sup>	56.17 ±4.33 <sup>a</sup>

Values are means ±SD. Different letters next to the mean values in each column indicate significant difference at 5 level according to Duncan test.

# Effect of moringa extract (ME) and pink-pigmented Facultative Methylotrophic (PPFM) bacteria on essential oil content

As shown in Table (6), pot marigold *Calendula officinalis* plants produced essential oil ranging from 0.18 to 0.35 % between different treatments. Essential oil content percentage (%) and content (ml/plant) were insignificantly affected by both ME and PPFM bacteria in both seasons. Among ME concentrations, 6% ME gave the highest oil percent was recorded 33,14 % and 27,9 % compared to the control in seasons 1 and 2, respectively. Notably, differences in oil percentage between control and 1% ME were insignificant in both seasons.

Meanwhile, 0.5% and 1% PPFM bacteria had similar equal effects on estimated oil content (ml/ plant) control in both seasons and equal to the treated plants with 2% and 3% PPFM bacteria recorded increases in plant oil content exceeding the control by 214 and 121 % in season 1 and exhibited increases of 152 and 96 % in the season 2, respectively.

Noticeably, all PPFM bacteria were more effective in enhancing oil content (ml/plant) than ME.

Similar outcomes were obtained by Meena *et al.*, (2012) who mentioned that foliar application of PPFM appreciably advanced root and shoot length and the number of leaves in tomato vegetation. Moreover, Madhaiyan *et al.*, (2012) stated that utility of PPFM as foliar spray notably expanded plant top and plant dry weight in cotton and sugarcane. They attributed extraordinary results for the growth to overall cytokinins and PPFM population which act as a bio-stimulant component. Clearly, PPFM bacteria were tremendously diversified institution of microorganisms and enhanced the plant growth with the aid of producing wide types of phytohormones (Meena *et al.*, 2012; Mizuno *et al.*, 2013). The promoting impact of PPFM bacteria on vegetative boom was also mentioned in many flowers (Gashti *et al.*, 2014). The mode of action for the promoting effect might be explained by way of the truth that PPFM microorganism have the potential to provide some growth regulators which included auxins and cytokinins (Lee *et al.*, 2006 and Nadali *et al.*, 2010).

**Table (6):** Effect of spraying with moringa extract (ME) and pink-pigmented facultative methylotrophic (PPFM) bacteria on essential oil content of *Calendula officinalis* L.

Treatments Seasons		Mo	ringa extract	concentrat	ions	PPFM bacteria concentrations				
	Control	1%	2%	4%	6%	0.5%	1.0%	2.0%	3.0%	
First season (2019) Essential oil (%)	0.18 <sup>g</sup>	0.18 <sup>g</sup>	0.22 <sup>cd</sup>	0.23 <sup>bc</sup>	0.24 <sup>b</sup>	0.19 <sup>fg</sup>	0.20 <mark>ef</mark>	0.35 <sup>a</sup>	0.24 <sup>b</sup>	
Second season (2020) Essential oil (%)	0.19 <sup>f</sup>	0.19 f	<sub>0.23</sub> cde	0.24 bcd	0.25 b	0.20 f	0.22 <sup>e</sup>	0.34 <sup>a</sup>	0.25 <sup>b</sup>	
First season (2019) Essential oil content (ml/plant)	0.03 <sup>e</sup>	0.03 de	0.05 bcd	0.08 <sup>a</sup>	0.05 <sup>bc</sup>	<sub>0.03</sub> de	<sub>0.04</sub> cde	0.08 <sup>a</sup>	0.06 <sup>b</sup>	
Second season (2020) Essential oil content (ml/plant)	<sub>0.03</sub> d	<sub>0.03</sub> d	<sub>0.04</sub> cd	0.08 <sup>a</sup>	<sub>0.04</sub> d	<sub>0.03</sub> d	<sub>0.04</sub> d	<sub>0.04</sub> d	0.06 <sup>bc</sup>	

Different letters next to the mean values in each column indicate significant difference at 5 level according to Duncan test.

# Effect of Moringa extract (ME) and pink-pigmented Facultative Methylotrophic (PPFM) bacteria on essential oil constituents

Table 7 showed the aromatic profiles of Calendula officinalis flowers essential oil as affected by different levels of moringa and pink pigmented methylotrophic bacteria extracts. GC/MS and GC analysis of Calendula officinalis flowers essential oil constituents revealed the id entification of 17 compounds accounting for 92.73 to 99.98% of the volatile constituents.

The presence of  $\alpha$  -Cadinol as the main component and the prevalence of  $\Delta$  -Cadinene, Tmuurolol, cadina-3, 9-diene,  $\beta$  -calacorene and pentacosane as majors were found in all samples from the various treatments. Considerable amounts of  $\alpha$ -Pinene (1.1–1.5 %), Sabinene (2.4–3.1 %),  $\alpha$ -Terpineol (2.8–5.2 %) Caryophyllene oxide (1.9–2.7 %) and tau-Cadinol (1.8–2.8 %) were also detected.

Foliar treatments of moringa and pink-pigmented bacterium extracts increased  $\alpha$  - Cadinol percent as the primary component, which increased from 32.78% (control) to 43.71 and 41% as the maximum when plants were sprayed with 4% ME and 3% PPFM bacteria, respectively, compared to the lowest percent with control. The rise in  $\alpha$  -Cadinol concentration was followed by a decrease in  $\Delta$  -Cadinene.

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**Table (7):** Effect of spraying with moringa extract (ME) and pink-pigmented facultative methylotrophic (PPFM) bacteria on essential oil constituents extracted from *Calendula officinalis* L.

COMPOUND	CONTROL	MO	RINGA	EXTRA	АСТ	PPFM				
		1%	2%	4%	6%	0.5%	1%	2%	3%	
cis-muurola- 4(14)-5-diene	2.3	2.3	1.66	-	1.69	1.5	1.52	1.77	-	
γ-Cadinene	2.4	-	1.67	-	2.23	2.18	2.11	1.65	1.66	
$\Delta$ -Cadinene	13.69	15.18	9.56	5.77	14.44	12.88	13.9	11.67	6.12	
α-cadinene	2.1	-	1.77	-	1.95	1.9	1.8	2	2.42	
ß-Patchouli	1.77	1.4	2.13	2.57	1.5	2.45	2.22	1.57	2.28	
$\alpha$ -calcorene	2.2	2.27	2.23	2.75	3	2.66	2.45	1.8	2.46	
Muurol-5-enen- 4-B-oL (cis)	2.12	2.66	2	1.9	1.96	1.83	1.84	1.96	2.17	
ß-calacorene	5.43	7.58	5.56	5.08	4.73	4.87	4.54	5.1	5.48	
Nerolidol	2.33	1.3	2.27	1.5	2.05	2	1.93	1.86	1.88	
Guaiol	2.22	2.43	2.54	-	2.3	2.28	2.05	2.08	2.17	
Acorenol	2.9	3.3	2.74	2.79	2.46	2.23	2.35	2.34	2.83	
cadina-3,9-diene	6.34	6.35	6.7	6.7	6.54	6.06	6.19	5.9	6.57	
T-muurolol	10.5	9.3	11.05	13.15	10.16	8.9	9.67	9.76	11.37	
β -Eudesmol	2.39	3.29	3.66	5.42	3.28	2.41	2.5	2.98	4.19	
Bulnesol	2.98	2.1	-	3.42	1.2	3	1.1	1.16	-	
α -Cadinol	31.2	34.09	41.81	43.71	37.7	34.08	38.14	39.14	41	
Pentacosane	5.29	5.84	2.47	5.1	2.28	1.5	4.06	4.17	7.38	
Total	98.16	99.39	99.82	99.86	99.47	92.73	98.37	96.91	99.98	

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### الذمو وكمية الزينة العطري لنبائة الأقدوان استجابة للتسميد الورقي بمستخلص المورينجا والبكتيريا الميثيلوتروفيك الاختيارية خابت اللون الوردي

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### المستخلص

تم استخدام المنشطات الحيوية لتعزيز نمو النبات وهما البكتيريا القرمزية مثلية التعذية والمستخلص النباتي لأوراق المورينجا لتوفير حلول اقتصادية ومستدامة للممارسات والتحديات الزراعية الحالية. تم إجراء تجربتين حقليتين متتاليتين عامي 2019 و 2020 لدراسة تأثير الرش الورقي لمستخلص أوراق نبات المورينجا والبكتريا القرمزية مثلية التعذية على النمو ومحتوى الزيت العطري في نبات الأقحوان. تم استخدام أربعة تركيزات من مستخلص المورينجا (٤،،،٥،) وأربعة تراكيز من بكتيريا القرمزية مثلية التعذية (٥.5، 1، 2، 3 ٪). وكان للكل من البكتريا القرمزية مثلية التعذية والمورينجا تأثيرا إيجابياً على خصائص ونمو نبات الأقحوان، بالنسبة إلى طول النبات، عدد الأوراق والأفرع ومحتويات الزيوت العطرية في الموسمين. النباتات التي تم رشها بنسبة 2٪ من مستخلص المورينجا أعطت أعلى نسبة ارتفاع الزيوت العطرية في الموسمين. النباتات التي تم رشها بنسبة 2٪ من مستخلص المورينجا أعطت أعلى نسبة ارتفاع النبات وعدد أوراق، وعند رشها بمستخلص المورينجا 3.0% زاد محتوى الزيت العطري (ملجم / نبات)، كما زاد أيضاً محتوى الزيات العطري في نبات الأقحوان عند رش النباتات بالبكتيريا القرمزية مثلية التعذية محتوى الزيات وعدد أوراق، وعند رشها بنسبة 2٪ من مستخلص المورينجا أعطت أعلى نسبة 2٪ النبات وعدد أوراق والأذي ع