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Growth and quality of lemon balm (*Melissa officinalis*) as influenced by various plant extracts

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Abstract

Lemon balm (*Melissa officinals*) is one of the most valuable medicinal plants in herbal medicine. Bio-stimulants are likely to gain popularity as an alternative strategy for providing plants with nutrients, vitamins, and hormones that increase yield and quality. Field trials were conducted during two seasons, 2017/2018 and 2018/2019, to assess lemon balm's growth and essential oil response to natural plant extracts with various concentrations. Lemon balm plants were exposed to moringa leaf extract (1, 2, 3%), pomegranate peel extract (0.5, 1, 1.5 %) and banana peel extract (1, 2, 3 %) as a foliar spray. Growth characters (plant height, fresh and dry weights /plant) and chemical contents (NPK and total carbohydrates) were studied. Moringa extract at 3% surpassed, in most cases, other plant extracts in improving the vegetative growth traits of lemon balm. Banana peel extract was more effective in increasing oil percent than moringa or pomegranate extracts. Besides, all extracts caused increases in NPK and carbohydrates over control while they yielded volatile oil without significantly altering the quality.

Key words: Lemon balm, *Melissa officinals,* plant extracts, growth, essential oil

Introduction

The world is becoming more conscious of the risks of intensive chemical fertilizers, which have a negative impacts on agricultural sustainable development as well as human health, the environment, and soil degradation. Therefore, bio-stimulants are likely to receive a great deal of attention as an alternative strategy to chemical fertilizers in whole or in part that provides plants with nutrients, vitamins and hormones and boosts the yield and quality of many crops. At the same time, these eco-friendly natural bio-stimulants produced from plant wastes are freely available in many countries and can be obtained after extraction (Povero *et al.,* 2016). For example, the peels of pomegranate and banana account for about 50% and 47-50% of the total fruit weight, respectively. Thus, large peel waste is produced, representing a valuable fertiliser source (Fatemeh *et al.,* 2012; Belgacem *et al.,* 2021). Plant bio-stimulants are plant extracts with a wide range of unknown bioactive compounds that can improve plant nutrient use efficiency and tolerance to biotic and abiotic stresses (Bulgari *et al.,* 2015).

In this regard, extracts from moringa leaves, pomegranate, and banana peels have emerged as a source of very promising natural plant growth enhancers enriched in biologically active compounds. Moringa leaves are rich in zeatin and other growth-enhancing compounds like vitamin E, phenolics and minerals. Zeatin is one of the phytohormones, a significant component of oil and protein structure. It can be used as a source of cytokinin (Marcu, 2005). Banana peel is rich in dietary fibre, proteins, essential amino acids, polyunsaturated fatty acids and potassium. Banana peel also demonstrated the presence of various phenolic compounds, antioxidants as vitamins and K element (Emaga *et al.,* 2007 and Lee *et al.,* 2010). Pomegranate fruits are usually consumed after the separation of seeds from the peels, and the seeds are used in juice manufacturing. The pomegranate peel is a rich source of bioactive compounds such as phenols, tannins, proanthocyanidins components, flavonoids and complex polysaccharides (Li *et al.,* 2006). In particular, punicalagins and gallic acids are the main active components in the peel and have been correlated with the antimicrobial activity of the extract (Glazer *et al.,* 2012).

Lemon balm (*Melissa officinals*), is one of the most valuable medicinal plants in herbal medicine belonging to the Lamiaceae family. Lemon balm leaves are widely used in Europe as herbal tea for their aromatic, digestive and antispasmodic properties in functional gastrointestinal disorders. It is used worldwide in perfumes, cosmetics, tea, food products and pharmaceutical industries (Peter, 2012). Several studies have investigated the pharmacological effects of lemon balm leaves and essential oils. It has been cited as an efficient hypoglycaemic agent, a mild sedative, a spasmolytic and an antibacterial agent (Chung *et al.,* 2010). It has powerful antioxidant effects that are up to ten times stronger than the effects of vitamins B and C, and it acts as an antiviral agent to heal herpes simplex cold sores. In addition, *Melissa officinalis* can moderate the neurotoxic effects of chemical drugs (Popova *et al.,* 2016). Its pharmacological properties have been attributed to the principal constituents such as rosmarinic acid, which is antiviral and antioxidant, while the essential oil is spasmolytic and antimicrobial (Shakeri *et al.,* 2016). The chemical composition of the essential oil of the lemon balm leaf (0.02-0.3% of dry weight) showed that the major compounds were citronellal and citral (neral and geranial), accompanied by β-caryophyllene, germacrene D, ocimene and citronellol (Moradkhani *et al.,* 2010).

Until now, researchers have published very few reports regarding the impact of plant extracts as stimulators and their application for enhancing plant growth in medicinal plants. Hence, this study examined the growth and chemical composition of lemon balm in response to different plant extracts (moringa leaf, pomegranate, and banana peels) when administered as foliar sprays.

Material and methods

Experimental: Two field trials were carried out during two successive seasons of 2017/2018 and 2018/2019 at the Experimental farm of the Faculty of Agriculture, Cairo University, Egypt (30 °28 05′ N, 31°22′ E).

Table 1. Physical and chemical analysis of the soil

Parameter	Value
Clay (%)	33
Silt (%)	30
Sand (%)	37
OM (%)	1.4
$CaCO_3$ (%)	2.5
EC (dSm^{-1})	1.9
pH	7.7
Available nutrients (ppm)	
N	60
P	15
K	90
Fe	20
Soluble cations mmolc L^{-1}	
Ca	6
Mg	2
Na	6.5
Soluble anions mmolc L^{-1}	
HCO_3	3.9
Cl	4.4
SO_4	6.5

Lemon balm (*Melissa officinalis*) seeds from (Enza Zaden, Assem Doss Co. Egypt) were sown in October in both seasons, and they germinated after two weeks. In the last week of November, uniform, healthy 15-cm seedlings were transplanted to the field in rows 50 cm apart and 30 cm between plants. The plants were sprayed with moringa extract (1, 2, 3 %), pomegranate extract (0.5, 1, 1.5 %) and banana extract (1, 2, 3%) while control plants were sprayed with water. All extracts were dissolved in water and sprayed by Gamma 10 (Italy) hand sprayer four times each season, starting from six weeks after transplanting and every month after that until the leaves were completely wet. Treatments were distributed in a randomized complete block design with three replications. The fertilizer rates used at planting and during the growing season were equivalent to half of the recommended dose for lemon balm plants.

Preparation of the extracts: Fresh leaves of moringa (*Moringa oleifera*), fruit peels of pomegranate and banana were cut into small pieces, dried and ground with a blender. For extraction, ethyl alcohol 80% was added to the powder and occasionally stirred by a rotary shaker at room temperature for 48 hours. Extracts were purified by filtering twice through Whatman filter paper No.1. The crude ethanolic extracts were concentrated using a rotary evaporator under reduced pressure at 45ºC. Each extract was diluted to the required dose.

Harvesting: Lemon balm plants were harvested twice manually 10 cm above the soil surface after 105 and 200 days from transplanting. The following parameters were estimated at harvest: plant height, fresh and dry weights of the plant (dry weight was carried out by drying at 40 ºC for 72 hours in an electric oven), essential oil percentage and composition. Some chemical parameters were measured in the dried leaves, such as NPK % (Skubij *et al.,* 2020) and total carbohydrates % (Deore Sonali and Kadam Vasant, 2016**).**

Essential oil extraction: The oil content of the herb (a sample of 100 g from each treatment) was determined by hydro-distillation for three hours using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia (2016).

GC analysis of the essential: The essential oil samples obtained in the two harvests of the second season were analyzed using gas chromatography (GC) to determine their main constituents. The use of GC in the quantitative determinations was performed using a GC-2010 plus capillary gas chromatography (Shimadzu Corp., Japan) instrument stands at the Laboratory of Medicinal and Aromatic Plants, National Research Centre, equipped with a Shimadzu FID 2010 Plus detector (Flame Ionization Detector).

Statistical analysis: All the data were subjected to Analysis of Variance (ANOVA) using MSTAT-C V.2.1 software package (Freed *et al.,*1989) and treatment means were separated according to the Duncan's Multiple Range test at a probability level of 5% (Duncan, 1955).

Results and discussion

Results presented in Tables 2 and 3 revealed noteworthy differences among plant extracts across all studied traits in both harvests, except for plant height in the second harvest (Table 2). Notably, foliar applications of the three extracts yielded positive effects on all studied traits, with significant increases in plant height and fresh and dry weights compared to the control (Table 3). Among these extracts, moringa extract (ME) exhibited a more pronounced influence on growth parameters compared to pomegranate extract (PE) and banana extract (BE), even though all three extracts led to substantial enhancements over the control.

Plants treated with 3% ME demonstrated the tallest stature, followed by those treated with 1.5% PE, showcasing the highest plant fresh weight (Table 3). The trend continued for plant dry weight, where 3% ME treatment yielded the highest dry weight, followed by 1.5% PE treatment. It's worth noting that the increase in plant dry weight was primarily attributed to the elevated fresh plant weight due to ME's influence.

This trend aligns with previous research that highlights the effectiveness of moringa extract in enhancing growth and yield across various crops (Culver *et al.,* 2012; Matthew, 2016; Iqbal *et al.,* 2014; Abd El–Hamied and El-Amary, 2015; Azra, 2011). The positive effects of moringa extract are linked to its abundant

Table 2. Effect of foliar sprays of moringa, pomegranate and banana extracts on plant growth characters of lemon balm in two harvests of the first season

Treatment	Plant height (cm)		Plant fresh weight (g)		Plant dry weight (g)		Oil percent (%)	
	1st HAR	2nd HAR	1st HAR	2nd HAR	1st HAR	2nd HAR	1st HAR	2nd HAR
ME 1%	48.00 bc	65.70 a	220.00 ef	335.03 bc	45.83 gh	72.03 cd	0.01 d	0.14 ab
ME 2%	50.67 bc	65.00 a	314.00 bc	326.20 bc	70.73 cd	74.45 cd	0.01 d	0.11 cd
ME 3%	61.33 a	65.30 a	477.67 a	542.58 a	98.74 a	132.00 a	0.01 d	0.11 cd
PE 0.5%	43.33 bcd	62.70 a	223.13 ef	361.92 b	50.96 fg	84.53 bc	0.03 bc	0.09 de
PE 1%	48.67 bc	62.30 a	284.67 cd	343.85 bc	64.04 de	76.61 bcd	0.02 cd	0.09 de
PE 1.5%	52.67 ab	68.00 a	356.92 b	419.23 b	83.53 b	98.38 b	0.02 ab	0.08 e
BE 1%	42.33 cd	59.70 a	211.00 ef	243.57 cd	45.50 gh	55.35 de	0.04 ab	0.16 a
BE 2%	41.67 cd	65.00 a	253.67 de	365.83 b	74.98 bc	84.72 bc	0.03 bc	0.14 ab
BE 3%	45.00 bcd	66.00 a	295.33 cd	334.84 bc	59.48 ef	79.69 bc	0.05 a	0.14 ab
Control	37.00 d	59.30 a	184.00 f	188.08 d	38.56 h	44.46 e	0.02 cd	0.12 bc

ME= Moringa leaf extract PE= Pomegranate peel extract BE= Banana peel extract. Values within each column followed by the same letter were not statistically significant at Duncan's test ($P \leq 0.05$).

Table 3. Effect of foliar sprays of moringa, pomegranate and banana extracts on growth characters of lemon balm in two harvests of the second season

Treatment	Plant height (cm)		Plant fresh weight (g)		Plant dry weight (g)		Oil percent (%)	
	1st HAR	2nd HAR	1st HAR	2nd HAR	1st HAR	2nd HAR	1st HAR	2nd HAR
ME 1%	51.00 abc	62.67 a	233.11 ef	323.01 bc	48.95 d	69.92 cd	0.01 d	0.12 c
ME 2%	54.67 ab	63.67 a	300.22 bc	339.20 bc	63.99 bc	75.33 bc	0.01 d	0.08 e
ME 3%	57.00 a	63.00 a	434.33 a	493.41 a	90.69 a	117.20 a	0.02 d	0.12 c
PE 0.5%	45.00 cdef	62.00 a	223.13 f	311.47 cd	48.55 d	72.36 cd	0.02 cd	0.08 de
PE 1%	50.67 bc	58.00 a	271.33 cde	345.18 bc	57.27 bcd	75.66 bc	0.03 bcd	0.08 e
PE 1.5%	50.00 bcd	62.00 a	324.58 b	384.66 b	71.33 b	92.50 b	0.04 b	0.10 d
BE 1%	42.33 f	58.33 a	236.00 ef	249.80 de	51.02 cd	53.49 de	0.03 bc	0.13 bc
BE 2%	43.33 ef	65.67 a	253.67 def	291.69 cd	65.69 b	66.74 cd	0.04 bc	0.14 b
BE 3%	49.00 bcde	63.67 a	291.00 bcd	331.44 bc	60.23 bcd	74.92 bc	0.06 a	0.16 a
Control	44.33 def	55.67 a	168.67 g	203.37 ef	33.31 e	44.75 e	0.02 d	0.09 de

ME= Moringa leaf extract PE= Pomegranate peel extract BE= Banana peel extract Values within each column followed by the same letter were not statistically significant at Duncan's test ($P \leq 0.05$).

Table 4. Effect of foliar sprays of moringa, pomegranate and banana extracts on oil composition of lemon balm

Compound	ME 1%	ME 2%	ME 3%	PE 0.5%	PE 1%	PE 2%	BE 1%	BE 2%	BE 3%	Control
					1st harvest					
Trans-chrysanthemal	5.63	5.13	5.67	10.28	11.4	3.8	4.98	4.45	2.98	3.22
Cis-verbenol	4.99	6.79	11.88	6.77	5.4	4.58	4.8	7.36	5.08	5.41
Nerol	0.89	-	1.14	-	-	1.18	1.44	1.01	1.00	1.25
Neral	27.82	30.83	27.1	28.59	25.18	26.64	26.88	27.62	30.28	22.85
Geraniol	1.05	-	1.23	-	1.04	0.77	1.19	1.23	1.22	1.53
Geranial	44.96	49.45	43.48	48.13	44.51	42.14	41.66	43.38	43.13	43.43
Geranyl acetate	4.3	4.11	3.68	3.87	4.01	3.31	3.22	4.35	3.03	5.67
Caryophyllene	1.15	-	0.765	-	1.42	1.3	1.76	1.29	1.86	1.44
Caryophyllene oxide	3.19	3.69	1.15	-	3.17	3.18	2.33	1.42	0.88	5.76
					2nd harvest					
Trans-chrysanthemal	t	t	t	t	t	t	t	t	t	t
Cis-verbenol	4.83	3.48	1.21	4.43	4.15	4.09	2.46	3.78	1.28	3.26
Nerol	1.61	1.68	1.33	2.7	1.66	1.02	1.39	3.15	0.59	1.10
Neral	30.52	31.04	32.8	31.31	30.3	29.32	32.81	31.75	37.19	32.26
Geraniol	0.98	0.82	0.94	0.78	0.77	1.32	-	1.02	0.64	-
Geranial	48.3	49.03	42.63	46.96	48.89	47.22	47.77	47.12	48.88	50.53
Geranyl acetate	1.7	1.68	1.61	1.78	1.6	1.86	1.99	1.51	1.8	1.69
Caryophyllene	2.23	1.18	1.47	1.89	1.63	1.25	1.77	1.99	4.00	2.4
Caryophyllene oxide	0.76	1.35	2.76	1.47	1.81	3.95	1.84	1.23	4.27	1.74

ME= Moringa leaf extract PE= Pomegranate peel extract BE= Banana peel extract. t= traces

content of endogenous cytokinins such as zeatin and indoleacetic acids (Emongor, 2015; Yap *et al.,* 2021).

Comparable to the findings with moringa extract, pomegranate extract exhibited promising outcomes in enhancing plant growth and yield. Pomegranate peel powder extract has been documented to increase plant growth and yield due to its rich composition of compounds, including potash, iron, zinc, flavonoids, anthocyanins, tannins, proanthocyanidins, phenols, and ascorbic acid (Li *et al.,* 2006; El-falleh *et al.,* 2011).

The second-season data (Table 3) show the trends observed in the first season, with plants treated with 3% ME showcasing the highest values for plant height and fresh and dry weights. Interestingly, banana extract (BE) treatments consistently exhibited higher essential oil percentages than moringa and pomegranate extracts. The positive impact of BE on oil percentage can be attributed to its essential amino acid content, particularly tryptophan, which plays a role in volatile oil biosynthesis.

Furthermore, banana peel extract is rich in vitamins A and C, proteins, carbohydrates, macro and micronutrients, phenolic compounds, fats, and fibres (Nguyen *et al.,* 2003). This nutritional profile, along with its potassium, calcium, sodium, phosphorus, iron, manganese, bromine, rubidium, strontium, zirconium, niobium, organic matter, crude lipid, carbohydrate, phytate, and saponin content, likely contributes to its growth-promoting effects (Chen *et al.,* 2007).

These findings align with prior research demonstrating the efficacy of banana and pomegranate peel extracts in enhancing plant growth and yield (Sadak *et al.,* 2015; Mercy *et al.,* 2014). Furthermore, exogenous application of banana extract has positively impacted parameters such as IAA levels, photosynthetic pigments, phenols, free amino acids, antioxidant enzyme activities, and seed nutritional values (Bakry *et al.,* 2016).

Moreover, applying moringa, pomegranate, and banana extracts through foliar spraying significantly increased N, P, K, and carbohydrate percentages in lemon balm leaves over two harvests (Figs. 1-4). Notably, plants treated with moringa extract consistently displayed the highest averages for these variables, supporting previous findings on other plant species (Mona, 2013).

Regarding essential oil composition, geranial and neral were identified as major constituents in lemon balm essential oil, and their percentages varied slightly among different treatments and the control. This aligns with previous research that suggests essential oil composition is more influenced by plant varieties and environmental factors than foliar application with plant extracts (Sitthithaworn, 2011; Yaseen and Hajos, 2021).

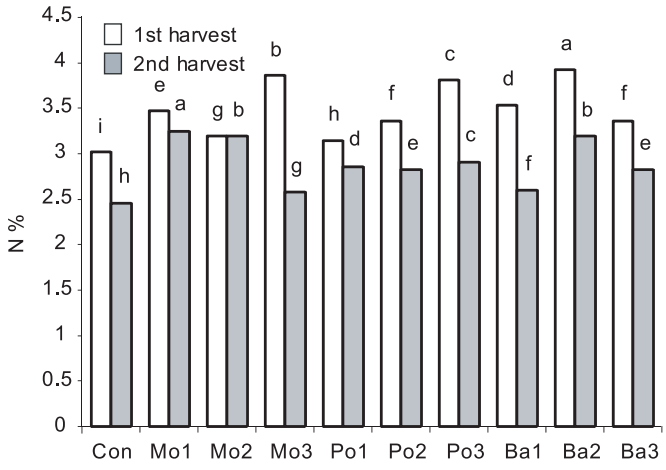

Fig. 1. Effect of foliar spray of moringa, pomegranate and banana extracts on N % of lemon balm

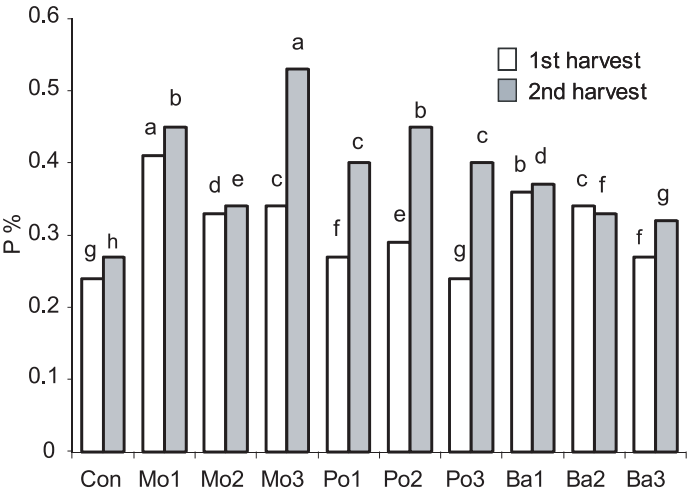

Fig. 2. Effect of foliar spray of moringa, pomegranate and banana extracts on P % of lemon balm

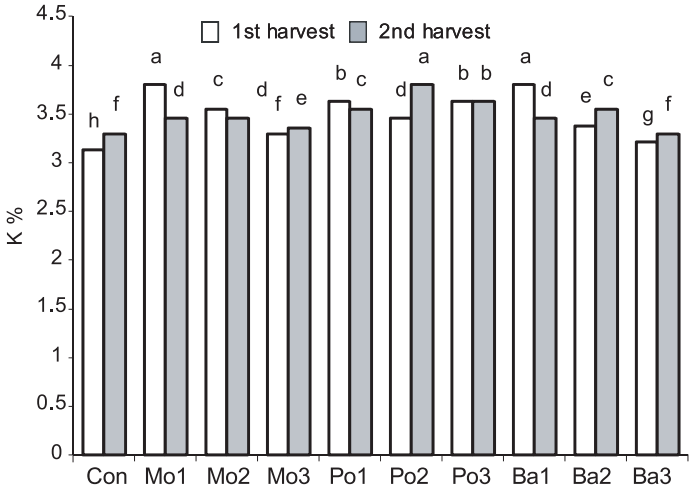

Fig. 3. Effect of foliar spray of moringa, pomegranate and banana extracts on K % of lemon balm

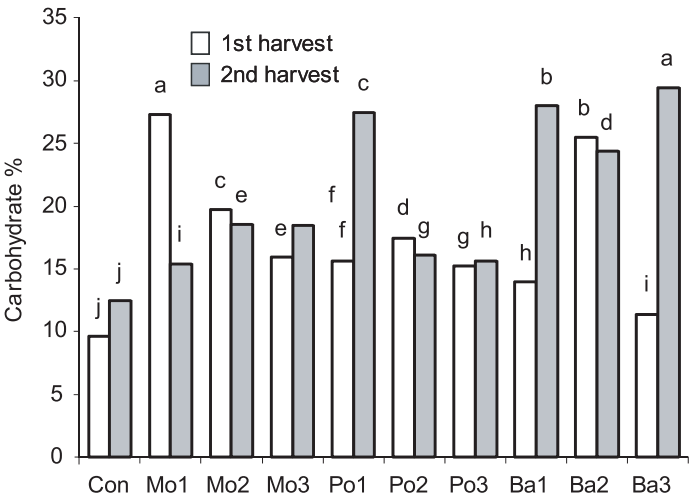

Fig. 4. Effect of foliar spray of moringa, pomegranate and banana extracts on carbohydrate % of lemon balm

In conclusion, the study demonstrates that foliar applications of moringa, pomegranate, and banana extracts significantly positively affect lemon balm's growth, nutrient content, and essential oil composition. These findings are consistent with prior research showcasing the growth-promoting effects of these extracts on various crops. Moringa extract, in particular, stands out due to its rich cytokinin content, while pomegranate and banana extracts contribute through their nutrient and compound profiles.

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