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Allelopathic Effect of the Foliar Spray by the Aqueous Seeds Extract of Moringa (*Moringa oleifera*) and Fenugreek (*Trigonella foenum graecum*) on Cineraria (*Pericallis x hybrida*) Plant.

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ABSTRACT

The chemical fertilizers caused many problems in our planet like soil and underground water pollution, beside the residuals of these fertilizers in the edible fruits or the ornamental plants. Thus, two seeds extract (moringa and fenugreek) were evaluated for their ability as biotic elicitors in the fertilization process of cineraria plants under different concentrations (0, 50, 75 and 100), each alone or in combinations on some vegetative growth, flowering parameters and chemical contents of this pot plant. Results cleared that combination between 50% MSE+ 75% FSE produced the shortest and compact plants. Moreover, the interaction between 75% MSE+ 50% FSE recorded the heaviest fresh and dry weights, reduced number of days for starting flowering and increased the inflorescence crown diameter. In addition, most of the highest concentration extracts between both 100% MSE + 100% FSE, 100% MSE + 75% FSE and 75% MSE + 100% FSE tabulated highest N and K% in cineraria leaves. Finally, using 50% MSE individually or in combination with 50% FSE enhanced the pigments content (total chlorophyll and carotenoids).

Abbreviations; MSE (Moringa Seeds Extract) and FSE (Fenugreek Seeds Extract)

Keywords: fenugreek extracts, moringa extracts, *Cineraria, Pericallis X hybrida*

INTRODUCTION

The positive or negative effects which are obtained as a result from the interaction between plant species in which plants are affected by other plants, is called allelopathy. These effects mainly due to the chemical constitute which found in these plants as, allelochemicals, (Iqbal *et al.,* 2014). These allelochemicals can enhance some vital plant processes such as, cell division, enzyme activation, ion uptake and finally increasing plant growth, (Gniazdowska, 2005), in a trial to minimize use of the chemical fertilizers.

Fenugreek (*Trigonella foenum graecum*) Family Leguminosa (Fabaceae) and Moringa (*Moringa oleifera*) family Moringaceae, seeds extract are rich in many substances which stimulate the plant growth. Fenugreek seeds are rich in amino acids such as, folic acid, niacin, thiamin and vitamins such as A, C and B6 (Sharma *et al.,* 1996). Also, it is considered a rich storehouse of many minerals such as, calcium, copper, iron, potassium, zinc, manganese, selenium and magnesium. Likewise, moringa seeds extract can be used for different purposes, one of these is as a plant growth promoter (Fuglie, 1999). In addition, moringa contains a natural cytokinin (zeatin) in its leaves (Aregheore, 2002), along with other minerals and inorganic salts which increase the plant growth and yield, if applied exogenously, (Akinbode and Ikotun, 2008). Moreover, moringa is a potential source of vitamin A and C, iron, calcium and riboflavin (Nambiar *et al.,* 2005).

Cineraria (*Pericallus x hybrida*) belongs to Family Asteraceae is one of the largest genera of flowering plants with petite daisy like flowers surrounded by large green leaves. A big variation in flowers color could be observed with this plant (purple, white, blue and red etc.). Each flower has an eye in the middle surrounded by a small white ring. One of the most commercial show for this plant is to be compact with a big flowering crown which covers most of the plant leaves. For achieving that, a moderate fertilization and watering must be done.

The aim of this article is to study the allelopathy influence of some plants extract (fenugreek and moringa seed extracts) as natural sources for fertilization on the vegetative, flowering growth characters and some chemical contents of this plant.

MATERIALS AND METHODS

Plant material

This investigation was carried out in the Experimental Farm and Laboratory of the Vegetable and Ornamental Plants Dept., Faculty of Agriculture, Mansoura Univ., Egypt, during the two successive seasons of 2013/2014 and 2014/2015. Winter annual cineraria (*Pericallus x hybrida*) seeds were obtained from France. Seeds were sown in September 10th for each season in 10 cm^2 plastic pots filled with a mixture of sand and clay (1:2 v/v), then transplanted into 25 cm^2 pottery pots filled with 7kg from the previous culture mixture in November 20th during both seasons. The N, P and K (19:19:19 ratio) compound fertilizer was added to the culture mixture soils as a basal dose (2g/pot) for all treatments.

Seeds extract

Allelopathic water extracts of fenugreek and moringa were prepared by following the method of Cheema and Khaliq (2000) with replacing the leaves by the seeds. Seeds with any kind of visible damage were discarded. Dry seeds from both plants (fenugreek and moringa) were crushed into a powder using a grinding machine; 100 gm of the dried ground seeds were taken in a glass jar and one liter of hot boiled distilled water (1:10 w/v) were poured on it and was kept at room temperature for 24 hours. The whole mixtures were then stirred for 60 min at room temperature (25 °C) using a magnetic stirrer with occasional shaking. Moreover, these mixtures were filtered through four layers of cheesecloth to remove the fiber debris, then in Whitman No.1 filter paper and the purified extract was adjusted to

pH 6.8 with NaOH 10%. Different concentrations (50, 75 and 100%) were prepared from the stock seeds extract solution, in addition to the control (distilled water).

Experimental design:

The experimental treatments were arranged in a factorial experiment in randomize complete block design with 4 replicates each of which included 3 plants. Experiment design included 16 treatments as moringa seeds extract (MSE) and fenugreek seeds extract (FSE) were used as a foliar spray with concentrations of 0, 50, 75 and 100% individually or in combinations. Both of the seeds extract were conducted to the chemical analysis for determining their contents, as shown in Table (1).Also, the relevant data of the soil analysis are presented in Table (2).

Table 1. Chemical analysis of moringa and fenugreek seeds extract

Extract		Moringa	Fenugreek
(mg/100 ml)	P	1.21	2.76
	K	66.7	48.3
	Ca	234.2	203.8
	Mg	409.5	541.9
	Na	41.7	106.3
	Fe	53.9	133.2
	Mn	0.81	6.05
	Zn	0.36	1.46
	Cu	0.19	0.93
	N	0.08	0.14
(%)	Total phenol	598.7	316.2
	Flavonids	3.66	2.95
	Alkaloids	0.72	0.47

Table 2. Chemical and physical analysis of the soil used in the experiment.

Mechanical analysis		Chemical analysis		Soluble cations and anions	
Coarse sand (%)	1.96	Available N (ppm)	42	Cations (meq/100 g soil)	
Fine sand (%)	29.33	Available P (ppm)	6.3	Ca^{++}	1.83
Silt (%)	37.03	Available K (ppm)	330	Mg^{++}	1.27
Clay (%)	31.68	Organic matter (%)	2.13	Na^{+}	0.97
Texture	Clay loamy	E.C.*	0.26	K^{+}	0.08
		pH**	8.14	Anions (meq/100 g soil)	
		$CaCO_3$	1.95	CO_3^{-}	0.00
				HCO_3^{-}	2.53

* 1: 5 soil: water extraction
** 1:2.5 soil suspension

Data recorded

1. **Vegetative growth parameters;** Plant height (cm), leaf number, leaf fresh and dry weights (g) were measured.
2. **Flowering parameters;** Days number for starting flowering (emergence of the inflorescence), inflorescence crown diameter (cm) and number of florets/inflorescence.
3. **Chemical determinations;** Total chlorophyll and total carotenoids were determined in leaf samples (mg/g fresh matter) according to Mackinney (1941), N% was determined by modified micro Kjeldahle method as described by Pregl (1945), P% was determined according to Jackson (1967) and K% was estimated according to Black (1965**).**

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using Genstat v 11.1, 2008. Mean comparisons were performed using the Duncan multiple method, according to Gomez and Gomez (1984). A significant level of 0.05 was adapted for all statistical analysis for both seasons.

RESULTS

1. Vegetative growth parameters

Data presented in Table (3) showed that cineraria plants which were sprayed with 50% moringa seeds extract in both seasons produced the tallest plants of 39 and 38cm. In addition, this treatment also tabulated the maximum leaf number of 16.67 leaves in both seasons, when compared with most of the other treatments. On the other side, the shortest plants of 21.67, 20.33cm were recorded when 50% MSE+75% FSE were used in both seasons, respectively. Cineraria plants sprayed with 50% MSE+100% FSE tabulated the lowest leaf number of 8.67 leaves, but in the second season the lowest leaf number of the same value was recorded due to the combination between 50% MSE+75% FSE.

Also, a relationship between the fresh and dry weight was observed in the same Table (3). Since, the combination of 75% MSE+ 50% FSE gave significantly the superior values for fresh and dry weights of 254.26 and 44.45 g/plant, respectively when compared with all of the other cases in the first season. Similar results were obtained in the second season in this respect. In contrast, the lowest values for the previous characters (fresh and dry weights) were recorded for plants sprayed with 50% MSE+75% FSE, as they were 78.98 and 15.73 g/plant, respectively and a similar trend was observed in the second season.

2. Flowering growth parameters

For the interaction between moringa and fenugreek seeds extract on number of days for starting flowering, inflorescence crown diameter (cm) and florets number in the inflorescence, data illustrated in Fig. (1, 2 and 3) cleared that using the combination of 75% MSE + 50% FSE, 75% MSE + 75% FSE, 75% MSE + 100% FSE or 100% MSE alone significantly reduced the days number for starting flowering, when compared with most of the other cases, since they were 173.67, 174.00, 174.00 and 175.67 days, respectively.

In addition, the highest combination concentrations of 100% from both of the plant seeds extracts or 50% FSE alone delayed flowering to record 185 days for starting flowering. Also, the combination of 75% MSE + 50% FSE or 75% MSE + 75% FSE,

increased the inflorescence crown diameter of cineraria plants to 27.00 and 25.67 cm, respectively (Fig. 2). There were no significant differences in inflorescence crown diameter among the control and the combinations of 50% MSE+75 FSE, 50 MSE+ 100 FSE, 75% MSE alone, 100% FSE alone, 100%MSE+ 50%FSE, 100% MSE+75%FSE and 100% MSE+100%FSE, since they tabulated the lowest diameter values. These results were also in a similar trend in the second season.

Table 3. Effect of moringa and fenugreek seeds extract concentrations solely or in combinations on plant height, leaf number, fresh and dry weight of cineraria plant during the two seasons of 2013/2014 and 2014/2015.

Seeds extract		Vegetative growth parameters of cineraria plants							
MSE	FSE	Plant height (cm)		Leaf number		Fresh weight (g/plant)		Dry weight (g/plant)	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
0	0	26.33 bcd	23.67 cd	16.33 a	15.33 abc	96.51 hij	96.10 j	19.19 cd	18.83 cde
0	50	28.67 abcd	26.00 bcd	12.33 abcd	12.00abcd	105.26 ghi	103.50i	19.54 cd	17.66 de
0	75	36.33 ab	35.67 ab	9.33 bcd	9.00 cd	113.9 fghi	112.90h	21.76 cd	21.41 cde
0	100	30.33 abcd	25.67 bcd	12.67 abcd	11.33abcd	166.28 cd	165.20d	23.73 c	23.51 cd
50	0	39.00 a	38.00 a	16.67 a	16.67a	217.34 b	216.00b	36.16 b	35.49 b
50	50	30.33 abcd	29.33abcd	11.67 abcd	10.67abcd	102.50 ghij	102.00i	20.31 cd	20.36cde
50	75	21.67 d	20.33 d	9.00 cd	8.67 d	78.98 j	76.00 l	15.73 d	15.40e
50	100	23.00 d	22.00 d	8.67 d	9.33 bcd	122.63 fg	121.00g	20.6 cd	20.36cde
75	0	25.67 cd	24.67 cd	12.00 abcd	12.00abcd	185.21 c	182.40c	19.76 cd	19.34cde
75	50	35.67 abc	34.00 abc	15.67 abc	15.00abcd	254.26 a	251.40a	44.45 a	43.78a
75	75	37.33 a	35.33 ab	16.00 ab	15.00abcd	152.39 de	150.40e	26.32 c	25.99c
75	100	22.67 d	20.67 d	12.33 abcd	12.00abcd	135.52 ef	133.80f	23.10 cd	23.11cde
100	0	35.33 abc	30.67abcd	13.33 abcd	12.33abcd	123.26 fg	121.20g	24.41 c	23.74cd
100	50	25.33 cd	24.67 cd	11.33 abcd	10.33abcd	93.08 ij	93.40 jk	20.44 cd	19.79cde
100	75	24.33 d	23.00 d	14.00 abcd	13.00abcd	120.52 fgh	117.90 g	20.55 cd	19.73cde
100	100	24.67 d	23.00 d	16.33 a	15.67ab	90.83 ij	91.70 k	19.71 cd	19.43cde

Means having the same letter (s) in a column are not significant difference at 5% level according to Duncan's multiple range test.
MSE; Moringa Seed Extract, FSE; Fenugreek Seed Extract.

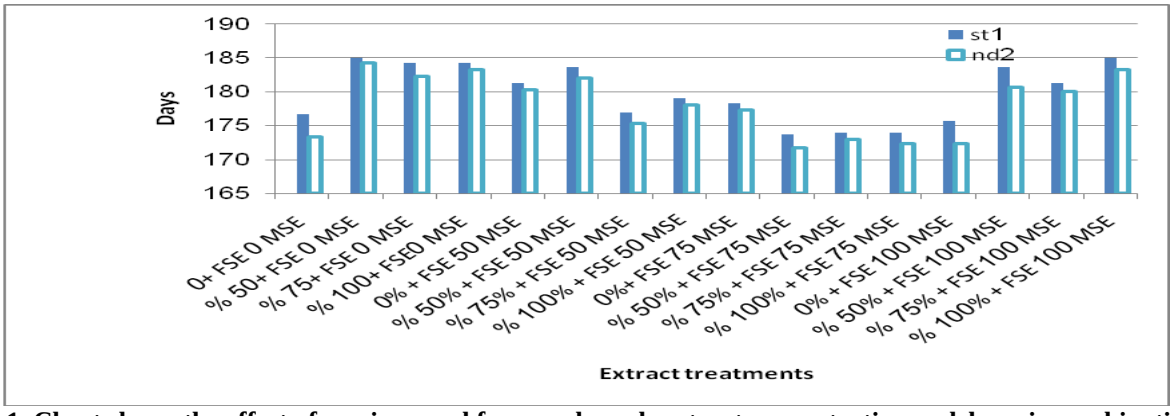

Fig 1. Chart shows the effect of moringa and fenugreek seeds extract concentrations solely or in combinations on number of days from starting flowering during the two seasons of 2013/2014 and 2014/2015.

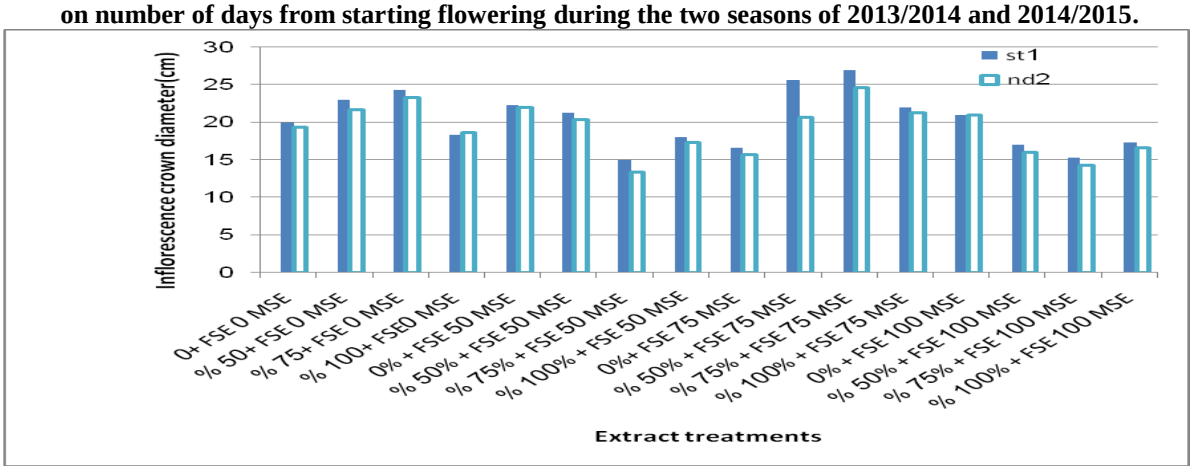

Fig 2. Chart shows the effect of moringa and fenugreek seeds extract concentrations solely or in combinations on inflorescence crown diameter (cm) during the two seasons of 2013/2014 and 2014/2015.
MSE; Moringa Seed Extract, FSE; Fenugreek Seed Extract.

Data illustrated in Fig. (3) showed that fenugreek seeds extract (FSE) had the upper hand in increasing florets number in the inflorescence, as 50 or 100%, recorded the highest number of 38 and 39.33 florets/inflorescence, respectively. However, non-significant differences were found between the superior previous treatments and combinations of 75% MSE + 75% FSE, 75% MSE + 100% FSE and 100% MSE + 50% FSE, since they gave 33.33, 31.33 and 31.00 florets/inflorescence in the first season, respectively. In contrast, in the second season, the fenugreek seeds extract only at concentrations of 50 and 100%, significantly tabulated the highest florets number of 42.33 and 38.67 florets/inflorescence, respectively.

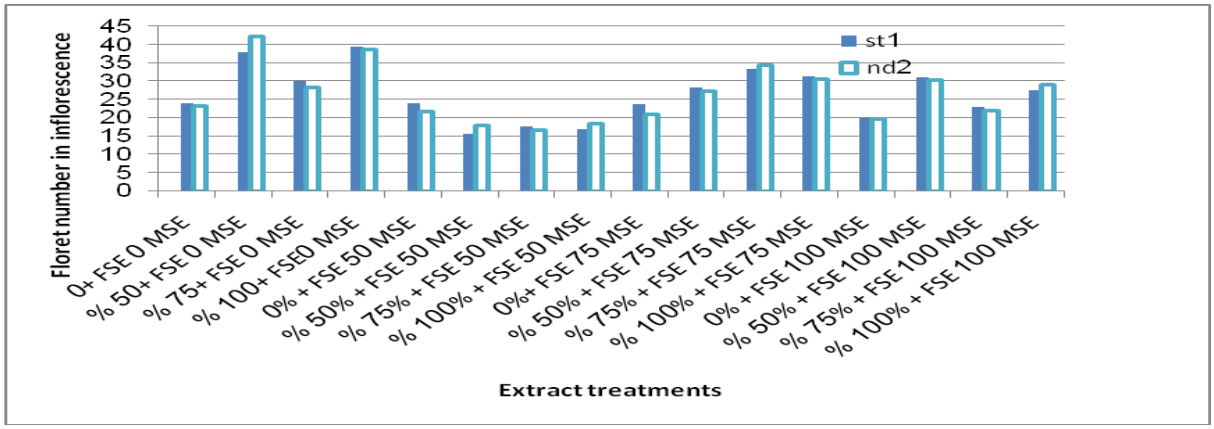

Fig 3. Chart shows the effect of moringa and fenugreek seeds extract concentrations solely or in combinations on florets number in the inflorescence during the two seasons of 2013/2014 and 2014/2015.
MSE; Moringa Seed Extract, FSE; Fenugreek Seed Extract.

3. Chemical determinations

With respect to the effect of MSE and FSE on N, P, K%, total chlorophyll and carotenoids contents, data presented in Table (4) showed that the combinations between MSE and FSE at 100% from both of them, 100% MSE + 75% FSE and 75% MSE + 100% FSE increased the N, P and K% in the leaves. However, it was very clear that no significant differences were conducted among the different combinations on phosphorus% compared to the control in both seasons. In contrary, cineraria plants sprayed with distilled water (control) produced the lowest N, P and K% in both seasons. Highest values for total chlorophyll and carotenoids were obtained when MSE was sprayed at the concentration of 50% alone or in combination with FSE at 50%. While, the lowest values of 11.39 and 6.94 mg/g f.w. were recorded for plants treated with 50% FSE in the first season and similar results were obtained in the second season.

Table 4. Effect of moringa and fenugreek seeds extract concentrations solely or in combination on N, P, K%, total chlorophyll and carotenoids contents (mg/g f.w.) of cineraria plants during the two seasons of 2013/2014 and 2014/2015.

Plant seeds extract		Carotenoids (mg/g f.w)	Total chlorophyll (mg/g f.w.)	Chemical contents of cineraria plants							
				K%		P%		N%		Carotenoids (mg/g f.w)	
MSE	FSE	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st
0	0	1.86 i	1.87k	0.29 a	0.34a	2.18 g	2.23k	22.88 abcd	22.90abcd	10.67 ab	9.67ab
0	50	1.94 hi	1.97j	0.29 a	0.35a	2.25 g	2.29jk	11.39 e	12.07f	6.94 d	6.24c
0	75	1.94 hi	2.12i	0.30 a	0.36a	2.28 fg	2.45i	19.28 abcde	20.28abcdef	10.13 abc	9.43ab
0	100	2.01 gh	2.18hi	0.30 a	0.37a	2.37 efg	2.51i	22.48 abcd	22.15abcde	9.03 abcd	8.32abc
50	0	2.33 cd	2.03j	0.31 a	0.36a	2.44 defg	2.34j	28.39 a	27.05ab	10.30 ab	9.61ab
50	50	2.12 efg	2.34g	0.32 a	0.39a	2.55 def	2.80g	27.07 ab	28.40a	11.37 a	10.30a
50	75	2.17 ef	2.45f	0.32 a	0.39a	2.63 cde	2.80g	18.04 bcde	18.37bcdef	9.07 abcd	7.95abc
50	100	2.24 de	2.51ef	0.33 a	0.41a	2.72 bcd	2.93f	20.01 abcde	21.34abcde	9.41 abcd	8.34abc
75	0	2.05 fgh	2.25h	0.31 a	0.38a	2.46 defg	2.61h	18.93 abcde	19.93abcdef	9.46 abcd	8.73abc
75	50	2.31 cd	2.58e	0.34 a	0.41a	2.88 abc	3.02e	22.91 abcd	22.58abcd	9.72 abcd	8.63abc
75	75	2.42 bc	2.65d	0.37 a	0.42a	3 ab	3.12d	15.31 cde	14.98 cdef	8.31 bcd	7.25bc
75	100	2.58 a	2.81c	0.38 a	0.43a	3.15 a	3.25c	21.96 abcd	22.63abcd	9.68 abcd	9.33ab
100	0	2.24 de	2.34g	0.34 a	0.38a	3.10 a	2.64h	14.21 de	14.88def	8.49 abcd	7.43abc
100	50	2.37 c	2.70d	0.35 a	0.42a	2.95 ab	3.18d	13.98 de	13.65ef	7.38 cd	6.98bc
100	75	2.52 ab	2.91b	0.37 a	0.44a	3.06 a	3.33b	20.36 abcde	21.36abcde	8.08 bcd	7.27bc

Means having the same letter (s) in a column are not significant difference at 5% level according to Duncan's multiple range test.
MSE; Moringa Seed Extract, FSE; Fenugreek Seed Extract.

DISCUSSION

The active reaction of moringa and fenugreek seeds extract on the vegetative, flowering parameters and chemical components of cineraria plants may be due to their nutrients content. As a medicinal plant, the seeds extracts of M. *oleifera* provides a rich and rare combination of zeatin, quercetin, b-sitsterol, caffeoylquinic acid and kaempferol which have antifungal and antibacterial activities (Anjorin *et al.*, 2010). Also, it's antimicrobial property may be due to the 4-(K-L-rhamnosyloxy) benzyl thiocyanate found in the seeds and has antioxidant activities (Rao *et al.*, 1999). In addition, moringa is rich in other growth enhancing compounds like ascorbates, phenolic, phytohormones and inorganic salts like Ca, K, and Fe that are in a naturally balanced concentrations which increase yield of the crops by promoting the growth when applied exogenously (Makkar and Becker, 1996). However, as any growth regulator or chemical fertilizer, has a growth curve, since in the optimal dose, it works as a promoter and in the high doses it retards or inhibits the plant growth. Such findings were obtained by Abou-Zeid and El-Darier (2014), as they proved that there was an inverse proportional relationship between increasing the severity of different percentages of M. *oleifera* extract on one hand, and leaves content from chlorophyll a and b, carotenoids and total chlorophyll content on the other hand.

Besides, Sinha (2012) reported that leaves of M. *oleifera* contained a number of phytochemicals such as alkaloids, glycerides, flavonoids, steroids, terpenoids, saponins, tannins and anthraquinone. These allelochemicals appear to alter a variety of physiological processes, enzyme activation, cell division, more membrane permeability, increased ion uptake and increased plant growth (Gniazdowska, 2005). In addition, zeatin content found in M. *oleifera* seeds could enhance the antioxidant properties of many enzymes and protects the cells from aging effects of reactive oxygen species (Zhang and Ervin, 2004). Also, it is evident from earlier reports that moringa leaf possesses plant nutrients and antioxidants in considerable amounts (Yang *et al.*, 2006) and improve the leaf chlorophyll contents under salinity (Hanaa *et al.*, 2008), by modulating enzymatic and non-enzymatic antioxidants, total soluble proteins, increased leaf K contents with decreased Na and Cl (Azra *et al.*, 2013). Moreover, the foliar application of moringa extracts prevents the premature leaf senescence and resulting in more leaf area with higher photosynthetic pigments (Rehman and Basra, 2010).

Fenugreek seeds endosperm had a high saponin (4.63 g/100 g) and protein (43.8 g/100 g) contents which showed antioxidant activity respectively by free-radical scavenging method (Naidu *et al.*, 2011). Also, fenugreek seeds are rich in some amino acids like leucine, valine, lysine and phenylalanine which play the main role in synthesis of proteins (Hounsome *et al.*, 2008) and bio-stimulating the plant growth, yield and significantly mitigates the injuries caused by abiotic stresses (Kowalczyk and Zielony, 2008), increasing N, P and k content of shoots (Liu *et al.*, 2008), and total sugars (Abo Sedera *et al.*, 2010). Meantime, it contains some inorganic salts as; manganese, magnesium, zinc and copper (Nour and Magboul, 1986). Finally, fenugreek seeds are good sources of protein, fat, dietary fiber and flavonoids such as quercetine, trigonelline, saponins, phytic acid and polyphenols (Sangeetha, 2010).

In conclusion, most of the growth parameters were significantly affected by moringa and fenugreek seeds water extract and gave even higher results when these were applied in combination as compared to their sole application and the control treatments in most of the studied characteristics. In the light of these findings, it could be suggested that foliar application of allelopathic crop water extracts such as moringa and fenugreek can be used as growth promoter and yield enhancer. In future these economically cheap and environment friendly growth promotor can be developed commercially in order to minimize the use of chemical fertilization.

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تأثير التداخل النباتي للرش بمستخلص بذور المورينجا والحلبة على نبات السنانير

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تتسبب الأسمدة الكيماوية فى العديد من المشاكل على كوكبنا مثل تلوث التربة والماء الارضى بجانب الاثار المتبقية لهذه الأسمدة في النباتات الصالحة للأكل أو حتى نباتات الزينة. لذلك تم تقييم اثنين من مستخلصات البذور (المورينجا والحلبة) لقدرتهم كمحفزات حيوية على نبات السنانير تحت تركيزات مختلفة (٠، ٥٠، ٧٥، ١٠٠%) كل على حده أو بالتفاعلات بينهم ودراسة تأثيرهم على بعض صفات النمو الخضري والزهري والمحتوى الكيماوي لذلك النبات. أوضحت النتائج أن التفاعل بين ٥٠% مستخلص بذور المورينجا + ٧٥% مستخلص بذور الحلبة أعطى اقصر النباتات في الارتفاع أو أكثرها اندماجا. علاوة على ذلك فلقد سجلت معاملة التفاعل بين ٧٥% مستخلص بذور المورينجا + ٥٠% مستخلص بذور الحلبة أعلى وزن طازج وجاف وقللت عدد الأيام اللازمة لبداية التزهير معزيادة قطر النورات الزهرية بالمقارنة بمعظم المعاملات الأخرى. بالإضافة لذلك فقد سجلت معظم التفاعلات بين التركيزات المرتفعة من مستخلص المورينجا والحلبة (١٠٠% + ١٠٠% ، ١٠٠% + ٧٥% و ٧٥% + ١٠٠%) على التوالي أعلى نسبة مئوية للنيتروجين والبوتاسيوم بأوراق نبات السنانير. وأخيرا فقد سجلت معاملة ٥٠% مستخلص بذور المورينجا منفرده أو بالتداخل مع ٥٠% من مستخلص بذور الحلبة أعلى محتوى للكلوروفيل الكلى والكاروتينويدات.