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Physiological response of *Moringa oleifera* to stigmasterol and chelated zinc

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Abstract. *El-Moursi A, Talaat IM, Bekheta MA, Gamal El-Din K. 2012. Physiological response of* Moringa oleifera *to stigmasterol and chelated zinc. Nusantara Bioscience 4: 118-123.* Two pot experiments were carried out in the screen of the National Research Centre, Dokki, Giza, Egypt, during two successive seasons (2009/2010 and 2010/2011), respectively to study the effect of foliar spray with chelated zinc (100, 200 and 300 mg/L) and stigmasterol (50, 100 and 150 mg/L) on growth and chemical constituents of moringa plants (*Moringa oleifera*). The results indicated that treatment of plants with 300 mg/L chelated zinc or 150 mg/L stigmasterol significantly influenced the vegetative growth of moringa plants. The same treatments also significantly increased total sugars%, total protein%, total phosphorous and microelements contents in the leaves. The changes in the pattern of protein electrophoresis (SDS-PAGE) extracted from the newly formed leaves of moringa plants treated with different concentrations of chelated Zinc (Zn) or stigmasterol showed beneficial influences for improving plant growth, leaves quality and quantity.

Key words: *Moringa oleifera*, stigmasterol, chelated zinc

Abstrak. *El-Moursi A, Talaat IM, Bekheta MA, Gamal El-Din K. 2012. Tanggapan fisiologis* Moringa oleifera *terhadap stigmasterol dan kelat seng. Nusantara Bioscience 4: 118-123.* Dua pot percobaan dibuat di kebun percobaan Pusat Riset Nasional, Dokki, Giza, Mesir, selama dua musim secara berturut-turut (2009/2010 dan 2010/2011), masing-masing untuk mempelajari pengaruh penyemprotan daun dengan kelat seng (100, 200, 300 mg/L) dan stigmasterol (50, 100 , 150 mg/L) terhadap pertumbuhan dan kandungan kimia tanaman kelor (*Moringa oleifera*). Hasil penelitian menunjukkan bahwa perlakuan tanaman kelor dengan 300 mg/L kelat seng atau 150 mg/L stigmasterol berpengaruh signifikan terhadap pertumbuhan vegetatif tanaman. Perlakuan yang sama juga secara signifikan meningkat persentase gula total, protein total, fosfor total dan kandungan unsur mikro dalam daun. Perubahan pola elektroforesis protein (SDS-PAGE) yang diekstrak dari daun yang baru terbentuk dari tanaman kelor yang diperlakukan dengan konsentrasi kelat seng (Zn) atau stigmasterol yang berbeda menunjukkan pengaruh yang menguntungkan untuk meningkatkan pertumbuhan tanaman, kualitas dan kuantitas daun.

Kata kunci: *Moringa oleifera*, stigmasterol, kelat seng

INTRODUCTION

The search for new drugs of plant origin has yielded fruitful result in the past. Today it is possible to use molecular biology techniques for detection of genetic variability and tagged desired traits as well as culling out duplicates in accessions. The availability of high through out screen has made the possibility of covering 'hits' into lead compounds in comparatively short time. Drug development from plant sources using gene/molecular techniques are becoming increasingly important. Although few people have ever heard of Moringa oleifera tree today, Moringa could soon become one of the world's most valuable plants, at least in humanitarian terms. Perhaps the fastest-growing of all trees, it commonly reaches three meters in height just 10 months after the seed is planted. Furthermore, it has more than a dozen important uses, yielding, among other things, several types of food as well as oil, wood, paper, shade, beautification and liquid fuel (Morton 1991).

Moringa oleifera Lam. leaves on ethanolic extraction yielded a number of amino acids viz, aspartic acid, glutamic acid, serine, glycine, threonine, β-alanine, valine, leucine, isoleucine, histidine, lysine, arginine, phenylalanine, tryptophan, cystine, and methionine. The ether extract of leaves yielded α-and β-carotene. Also, 9 amino acids in the flowers, 8 in the fruits and 7 each in the protein hydrolysate of flowers and fruits of *M. oleifera* were identified. Alanine, arginine, glutamic acid, glycine, serine, threonine, and valine were common in all the parts tested, whereas aspartic acid was present in the flowers as well as the fruits, and lycine occurred only in the flowers. The flowers contained both sucrose and d-glucose, whereas the fruits showed the presence of sucrose only (Ram 1994).

The juice from the leaves and stem bark of *M. oleifera* inhibited *Staphylococcus aureus* but not *Escherichia* coli. The 50% ethanolic extract of root bark of *M. oleifera* showed antiviral activity against vaccinia virus, but was inactive against Ranikhet disease virus. *M. oleifera* root extract (50% ethanolic) at a dose of 200 mg/kg led to fetal

resorption in 60% of female pregnant rats. Ethanolic extract (50%) of *M. oleifera* (whole plant excluding roots) showed anticancer activity against human epidermoid carcinoma of nasopharynx in tissue culture and P388 lymphocytic leukemia in mice (Jayavardhanan et al. 1994).

The roots are carminative, stomachic, abortifacient, cardiac tonic and also used in paralytic conditions and intermittent fever; also useful as rubefacient in rheumatism, in spasmodic affections of the bowels, hysteria, and flatulence as well as in epilepsy. Root bark is used as fermentation to relieve spasm. Bark is considered to be an abortifacient. The fruit is recommended in diseases of liver and spleen, in tetanus and paralysis. Flowers are stimulant and aphrodisiac. Seed oil is applied externally in rheumatism. Leaves are emetic and their juice with black pepper is used in headache. The poultice of leaves is used in reducing glandular swellings. The gum is given in dental caries with sesamum oil and also for relief of otalgia and it is applied with milk on the temples in headache. Seeds are used in veneral affections and to relieve the pain of gout and acute rheumatism (Eilert et al. 1981; Pal et al.1995).

Stigmasterol is a structural component of the lipid core of cell membranes and is the precursor of numerous secondary metabolites, including plant steroid hormones, or as carriers in acyl, sugar and protein transport (Genus 1978). Brassinosteroids (BR) is known as a group of naturally occurring polyhydroxy steroids. All brassinosteroids isolated from plants are characterized as 5-∝-cholestane derivatives that classified as C27, C28, C29 steroids as revealed by (Yokota et al. 1982). BR has the same biological action as gibberellins and auxins. The pollen grains of plant flowers contained the highest values of BR compared with the other plant organs (Horgan et al. 1984). Brassinosteroids have been found to evoke both cell elongation and cell division resulting in elongation, swelling, curvature, and splitting of the internode (Mandava 1988). Physiological functions proposed for Brassinosteroids included cell elongation, cell division, leaf bending, vascular differentiation, proton pump-mediated membrane polarization, sink/source regulation responses (Sasse 1999). In addition, brassinosteroids caused changes in enzymatic activities, membrane potential, DNA, RNA, protein synthesis, photosynthetic activity and changes in the balance of endogenous phytohormones (Steven and Jeneth 1998). Particular interest in sterols was elicited by enhanced growth characters and yield of chamomile plant (Abdel-Wahed and Gamal El-Din 2004). Recently, these studies provided strong evidence that sterols could be essential for normal plant growth and development (Ozdemir et al. 2004).

In recent years, zinc is one of the most important elements for the growth and flowering of some plants as reported by Chandler (1982). Abou-Leila et al. (1994) found that foliar application of Ocimum basilicum L with Zn at 75 mg/l gave the highest values of herbal yield, carbohydrate and oil contents. The effect of Zn on enzyme system responsible for biosynthesis of carbohydrates was reported by Sandmann and Boger (1983). The necessity of Zn for most crops was emphasized by Singh and Ganguar (1973). They mentioned that Zn participates in the production of IAA which resulted in an increase in growth and sugar production in sugar beet. Adding Zn fertilizers as foliar application was suggested in Egyptian alkaline soils, where the availability of Zn and other microelements for plant roots becomes relatively low (El-Sayed 1971).

MATERIAL AND METHODS

Plant materials. *Moringa oleifera* L. seeds secured from the Institute of Horticulture Research, Ministry of agriculture, Giza, Egypt. Two pot experiments were carried out in the Screen of the National Research Centre, Dokki, Giza, Egypt, during two successive seasons (2009/2010 and 2010/2011), respectively to study the effect of foliar spray with chelated zinc (100, 200 and 300 mg/L) and stigmasterol (50, 100 and 150 mg/L) on growth and chemical constituents of moringa plants. On 15th October 2009 and on 19th October 2010, the seeds were sown in pots (35 cm in diameter), each pot contained 12 kg clay loamy soil. Treatments were distributed in complete randomized block design with five replications, five pots each. Fifteen days after sowing, the seedlings were thinned to the most three uniform plants in each pot. Each pot received equal and adequate amounts of water and fertilizers. Phosphorous as calcium superphosphate was mixed with the soil before sowing at the rate of 4.0 g/pot. Three g of nitrogen as ammonium sulfate were added in three applications (one g each) with intervals of two weeks started 30 days after sowing. Also, two g of potassium sulfate was added as soil application. Other agricultural processes were performed according to normal practice. *M. oleifera* plants were sprayed with stigmasterol and chelated zinc solutions 45 days after sowing. The volume of the spraying solution was maintained just to cover completely the plant foliage until dripping. Distilled water was sprayed in the same previous manner on untreated plants (control plants). The first sample (vegetative stage) was taken 10 days after treatment. The second sample (full vegetative stage) was taken at 15th May 2010 and 19th May 2011, respectively. Measurement of growth parameters; plant height (cm), number of branches per plant, fresh and dry weights of leaves and stems (g/plant) were determined.

Chemical analysis. Represented samples of the leaves of each treatment were subjected to the following different chemical analyses. Determination of total sugars was carried out according to Dubois et al. (1956). Total nitrogen (modified micro-Kjeldahl) was determined as described by Jackson (1973) and from which protein was calculated. Potassium, calcium and phosphorous were determined according to the procedure described by Brown and Lilliand (1946) and Troug and Meyer (1939), respectively. Iron was determined by atomic absorption spectrophotometer (Chapman and Pratt 1961).

Data analysis. Data obtained (means of the two growing seasons) were subjected to standard analysis of variance procedure. The values of LSD were calculated, whenever F values were significant at 5% level as reported by (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

Effect on vegetative growth

Table 1 clearly revealed that foliar spray of stigmasterol and chelated zinc significantly increased plant height at all treatments. The most effective treatments in this respect was that of 300 mg/L Zn and 150 mg/L stigmasterol which recorded the highest values of plant height (35 cm and 37.33 cm) compared to 27.25 cm of untreated control. Number of leaves/plant showed the same trend of plant height which recorded 44.17 and 40.50 at treatments 300 mg/L Zn and 150 mg/L stigmasterol, respectively compared to (28.75) of untreated plants. The same treatments resulted in the highest values of fresh and dry weights of leaves which recorded 7.34 and 9.17 g/plant compared to 3.75 g/plant of untreated plants as fresh weights and 1.58 and1.88 g/plant compared 0.93 g/plant of untreated plants as dry weights. The least increases were obtained at treatments 100 mg/L Zn and 50 mg/L stigmasterol related to slight increases in total sugars% and total protein% in the same treatments.

Data presented in Table 2 indicated that foliar application of stigmasterol and chelated zinc significantly increased plant height, number of leaves, fresh and dry weights of branches/plant, fresh and dry weights of leaves/plant. The most effective treatments in this concern was that of 300 mg/L Zn and 150 mg/L stigmasterol. On the other hand, number of branches/plant was not significantly affected.

The positive effect of stigmasterol and chelated zinc on plant growth was previously reported on wheat plant (Abdel-Wahed et al. 2000), sugar beet plant (Abdel-Wahed and Ali 2001), and *Tagetes erecta* plant (Balbaa et al. 2008), geranium plant (Ayad et al. 2009), flax plant (El-Lethy et al. 2010; Hashem et al. 2011), *Matthiola incana* plant (Mahgoub et al. 2011), basil plant (Youssef et al. 2004) and fenugreek plant (Gamal El-Din 2005).

The pronounced increases of vegetative growth of moringa plants, when treated with stigmasterol, could be attributed to its role in cell elongation and division (Mandava 1988; Clouse and Sasse 1998). The favorable action of chelated zinc might be attributed to its role in the synthesis of tryptophan (the precursor of IAA) which in turn affected several plant phenomena as reported by Valke and Wecker (1970).

Effect on chemical constituents

Data presented in Table 3 also indicate that foliar application of chelated zinc and stigmasterol to moringa plants significantly increased potassium (K) content in the leaves at treatments 100 mg/L and 150 mg/L stigmasterol, but treatments 100, 200, 300 mg/L chelated zinc and 50 mg/L stigmasterol were not significant. Treatment 150 mg/L stigmasterol recorded the highest value of potassium content (51.00 mg/ 100 g dry weight) compared to (41.00 mg/100 g dry weight) of untreated control.

Data also indicated that all treatments of chelated zinc and stigmasterol resulted in significant increases in calcium content and the highest values were recorded in plants treated with 300 mg/L chelated zinc and 150 mg/L stigmasterol which recorded 133.35 and 130.38 mg/100 g dry matter compared to 87.10 mg/100 g dry matter of untreated control.

Data presented in Table 3 reveal that foliar spray of chelated zinc and stigmasterol significantly increased iron content except in plants treated with 100 mg/L chelated zinc which was not significantly affected. Application of 300 mg/L chelated zinc recorded the highest value of iron content (83.67 mg/100 g dry matter) followed by treatment with 150 mg/L stigmasterol which recorded 82.00 mg/ 100 g dry matter, while untreated control recorded (63.50 mg/g dry matter).

Data also indicated that spraying plants with 200 or 300 mg/L chelated zinc and 150 mg/L stigmasterol resulted in significant increases in phosphorous content of moringa leaves. Meanwhile, treatment of plants with 50 or 100 mg/L stigmasterol was non-significant (Table 3).

Treatment of moringa plant with chelated zinc and

Table 1. Physiological effect of chelated zinc and stigmasterol on vegetative growth of moringa plants.

Treatments (mg/L)	Plant height (cm)	Number of leaves	Fresh wt of leaves (g/plant)	Dry wt of leaves (g/plant)
Zn 100	30.67	32.00	5.54	1.44
Zn 200	31.33	35.00	6.20	1.48
Zn 300	35.00	44.17	7.34	1.58
Stigma 50	31.00	31.00	6.12	1.30
Stigma100	33.00	38.00	6.15	1.32
Stigma150	37.33	40.50	9.17	1.88
control	27.25	28.75	3.75	0.93
LSD (5%)	3.07	3.18	0.94	0.32

Table 2. Physiological effect of chelated zinc and stigmasterol on growth of moringa plants at full vegetative stage.

Treatments (mg/L)	Plant height (cm)	No. of leaves	No. of branches	Fresh weight of branches (g/plant)	Dry weight of branches (g/plant)	Fresh weight of leaves (g/plant)	Dry weight of leaves (g/plant)
Zn 100	106.67	12.33	6. 33	54.35	16.04	58.08	15.74
Zn 200	117.67	14.67	7.00	60.10	19.74	65.95	16.94
Zn 300	143.33	17.00	7.00	89.61	28.41	74.94	18.11
Stigma 50	119.00	14.92	6.00	57.81	17.38	65.79	14.63
Stigma100	122.00	16.00	7.00	62.92	22.00	73. 05	16.90
Stigma150	122.33	14.00	7.33	64.70	24.26	73. 13	17.72
control	99.33	10.00	6.00	32.97	10.06	50.01	17.67
LSD (5%)	6.87	1.84	N.S.	9.48	3.03	5.61	3.97

stigmasterol led to significant increases in total sugars% in all treatments, except that treatment 50 mg/L stigmasterol was non-significant. Treatments 300 mg/L chelated zinc and 150 mg/L stigmasterol recorded the highest values of total sugars% (3.80 and 3.46%, respectively) compared with 2.68% in control plants (Table 3).

Data presented in Table 3 also indicate that foliar spraying moringa plants with chelated zinc or stigmasterol significantly increased total protein%, except treatments 50 and 100 mg/L stigmasterol which were not significant. Treatments 300 mg/L chelated zinc and 150 mg/L stigmasterol recorded the highest values of total protein% (3.95 and 3.50%, respectively), compared to 2.45% in control plants.

The positive effect of chelated zinc and stigmasterol on chemical constituents was previously reported on lemongrass plant (Gamal El-Din et al. 1997), sugar beet plants (Abdel-Wahed and Ali 2001), fenugreek plant (Gamal El-Din 2005), *Tagetes erecta* L. Plant (Balbaa et al. 2008), geranium (Ayad et al. 2009), flax plant (El-Lethy et al. 2010) and *Matthiola incana* plants (Mahgoub et al. 2011).

Protein pattern

Table 4 reveals the changes in the pattern of protein electrophoresis (SDS-PAGE) extracted from the newly formed leaves of moringa plants treated with different concentrations of chelated zinc (Zn) or stigmasterol (stigma.). The molecular weights of the proteins ranged between 9.82-108.57 kDa and exhibited a maximum number of 17 bands. The scanning profile of such detected protein bands revealed that the band number 9 having the molecular weight of 31.87 kDa produced the highest intensity of protein which recorded 19.36% in plants treated with chelated Zn at concentration equal 100 mg/L.

Treatment of moringa plants with chelated Zn at 100 mg/L led to the appearance of 6 protein bands ranging between 11.86-108.57 kDa. Comparing with the features of protein banding pattern obtained from the untreated plants, it is evident that such treatment induced the appearance of 1 newly protein band having the molecular weights 31.87 kDa.

The electrophoretic pattern of the plants treated with 200 mg/L chelated Zn showed the presence of 7 protein bands ranging between 11.86-108.57 kDa. It is evident that this treatment induced the appearance of 2 new protein bands having molecular weights of 66.03 and 31.87 kDa. These two new bands have protein intensity of 6.05 and 7.39%, respectively.

Application of chelated Zn at 300 mg/L showed the existence of 6 protein bands with the molecular weights ranging from between11.86-108.57 kDa as compared to the protein bands obtained from the control plants. The data show that this treatment induced the appearance of 1 newly protein band having the molecular weight of 31.87 kDa and protein intensity 7.84%. Meanwhile, one protein band disappeared having molecular weight of 18.40 kDa.

Table 3. Physiological effect of chelated zinc and stigmasterol on chemical constituents of moringa plants.

Treatments Mg/L	K (mg/100 g dry matter)	Ca (mg/100 g dry matter)	Fe (mg/100 g dry matter)	P (mg/100 g dry matter)	Total sugars%	Total protein%
Zn 100	43.00	105.68	69.00	98.67	3.47	3.32
Zn 200	43.67	120.66	74.33	110.00	3.70	3.48
Zn 300	45.00	133.35	83.67	113.33	3.80	3.95
Stigma 50	44.00	108.10	72.50	101.00	2. 80	2.80
Stigma100	45.33	124.11	76.00	103.00	3.35	2.95
Stigma150	51.00	130.38	82.00	111.17	3.46	3.50
Control	41.00	87.10	63.50	100.00	2. 68	2.45
LSD (5%)	4.24	11.06	5.54	5.66	0.55	0.82

Table 4. Comparative analysis of relative area (%) of each band of the Coomassie blue-stained gels of moringa plants treated with different concentrations of chelated Zn and Stigmasterol.

Band number	Mol. weight (kDa)	Control	Zn at 100 mg/L	Zn at 200 mg/L	Zn at 300 mg/L	Stigma at 50 mg/L	Stigma at 100 mg/L	Stigma at 150 mg/L
1	108.57	7.13	10.12	8.88	7.03	7.42	7.09	8.98
2	101.88							
3	87..81	3.13	4.63	5.43	3.16	4.10	3.71	4.47
4	74.23							
5	66.03	-	-	6.05*	8.36*	9.36*	7.832*	8.81*
6	49.95							
7	42.92	5.13	9.20	6.40	4.74	5.74	7.23	7.44
8	34.62							
9	31.87	-	19.36*	7.39*	7.84*	7.09*	6.31*	10.32*
10	28.51							
11	22.96					8.10*		
12	20.83							
13	18.40	10.43	11.60	11.77	-	-	-	-
14	16.61							
15	14.50							
16	11.86	6.87	12.01	3.95	10.80	11.80	9.51	11.55
17	9.82	-						7.83*
Total no. of bands	-	5	6	7	6	7	6	7
New bands	-	-	1	2	2	3	2	3
Disappear bands	-	-	-	-	1	1	-	-

Note: Zn = chelated Zn, Stigma = stigmasterol, *= new bands

The electrophoretic banding pattern of proteins resulted from the application of stigmasterol at 50 mg/L on moringa plants showed the appearance of 7 protein bands with molecular weights ranging from from11.86-108.57 kDa. Three new bands were shown to be induced as a result of this treatment having molecular weights of 66.03, 31.87 and 22.96 kDa. The three new bands have protein intensity 9.36, 7.09 and 8.10, respectively. Meanwhile, one protein band disappeared having molecular weight of 18.40 kDa.

Spraying the plants with 100 mg/L stigmasterol resulted in the induction of two new protein band having molecular weights of 66.03 and 31.87 kDa. This treatment also resulted in the disappearance of one protein bands of molecular weight of 18.40 kDa.

Application of stigmasterol at 150 mg/L on moringa plants led to the appearance of 17 protein bands ranging between 9.82-108.57 kDa and induced the appearance of 3 newly protein bands having the molecular weights of 66.03, 31.87 and 9,82 kDa, respectively. It is necessary to mention here that, this treatment led to appearance of 1 new band having molecular weight 9.82 kDa, which did not appear in the control and all other treatments with protein intensity 7.83%. Meanwhile, one protein band disappeared having molecular weights of 18.40 kDa.

The outcome of the obtained results clearly indicates that spraying moringa plants with different concentrations of chelated Zn or stigmasterol led to the appearance of new protein bands which varied according to the applied concentration. The existence of such newly formed protein bands in treated moringa plants might be explained basing on the potentiality of chelated Zn and stigmasterol to trigger the expression of specific genes along DNA molecule in the target cells, a process which appears to play a key role in regulating a cascade of biochemical reactions which might determine the ultimate appearance of growth patterns and yield of the produced plants. This might be accompanied by a persistent effect carrying over to the progeny via alteration of DNA-binding protein receptors mechanism which might amplify the signal-transduction pathway, this suggestion is reinforced by the findings of Jacobsen and Beach (1985) and Abdel-Hamid (2002).

Bekheta (2004) showed that application of paclo-putrazol accompanied with gibberellic acid (GA_3) on wheat plants changed the electrophoretic profile of protein patterns. In addition, Bekheta and Talaat (2009) showed changes in the pattern of protein electrophoresis (SDS-PAGE) extracted from the newly formed leaves of mung bean plants treated with different concentrations of salicylic acid, glutathione or paclobutrazol. The molecular weights of the proteins ranged between 8.094-2455.534 kDa and exhibited a maximum number of 21 bands. The scanning profile of such detected protein bands revealed that the band number 20 having the molecular weight of 8.291 kDa produced the highest intensity of protein which recorded 42.13% in plants treated with 100 mg/L glutathione.

In conclusion, foliar treatments of moringa plants with different concentrations of chelated Zn or stigmasterol had beneficial influences for improving plant growth, leaves quality and quantity.

CONCLUSION

From the obtained data, it could be concluded that stigmasterol or chelated zinc might play an important role in plant phytochemical mechanism through its effect on the electrophoretic pattern of protein electrophoresis and/or on the mineral ions content, but further studies are needed to learn more about these mechanisms.

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