Improving Fruit and Oil Quality of Picual Olive through Exogenous Application of Putrescine and Stigmasterol

[1]Hasnaa, S. Ayad; [2]Aml. R. M. Yousef and [1]A. El-Moursi

[1]Botany Department, National Research Centre, Giza, Egypt
[2]Horticultural Crops Technology Department, National Research Centre, Giza, Egypt
*Ahmedhms2005@yahoo.com

Abstract: A field experiment was carried out at a private orchard located at El-Saff district, Giza governorate, Egypt, during two successive seasons (2007 and 2008) to study the effect of putrescine and stigmasterol on fruit growth, quality and some chemical constituents of picual olive oil. Picual olive trees (20 years old) were sprayed with putrescine (44, 88 and 132 ppm) and stigmasterol (50, 100 and 150 ppm) before fruit harvest (one month). Applications of putrescine and stigmasterol significantly increased fruit growth characters i.e. size, weight, length and diameter during the two successive growth stages (green and purple stages). Olive fruit quality characters i.e. pulp weight and thickness, moisture content, soluble solid content and acidity were also positively affected by putrescine and stigmasterol treatments. The most effective treatments on fruit growth and quality were those of putrescine (44 ppm) and stigmasterol (150 ppm). Such treatments had the highest pronounced effect on oil production and quality of picual olive trees

[Hasnaa, S. Ayad; Aml.R.M.Yousef and A.El-Moursi. **Improving Fruit and Oil Quality of Picual Olive through Exogenous Application of Putrescine and Stigmasterol.**] New York Science Journal 2011;4(9):40-45]. (ISSN: 1554-0200). http://www.sciencepub.net/newyork.

Key words: Olive, Putrescine, Stigmasterol, Fruit, Quality, Oil characters.

1. Introduction:

Olive (*Olea europea* L.) belongs to family Oleaceae is considered as one of the most important fruit crops in Egypt. Olive leaf extract contains natural flavonoids, phenolic monoterpene glycoside (Oleuropein) as a bitter substance with antiviral, antibacterial and antioxidant properties **(Bouaziz *et al.*, 2005).** The biological properties of olive oil are related to the presence of minor components such as squaline and phytosterols, antioxidant components such as tocopherol and particularly phenols **(Owen *et al.*, 2000).** In particular, among the natural antioxidants, phenolic compounds α-tecopherol and B-carotene are reported to play a key note in preventing oxidation.

Polyamines (Putrescine, spermidin and spermin) are organic polycations and growth regulators in all plants. Plant polyamines can function as second massengers and modulating various anatomical, biochemical and physiological processes in intracellular and extracellular areas of plants under environmental stresses **(Kuznetsov *et al.,* 2006; Cavasoglu *et al.,* 2008).** The pro-oxidative role of polyamine is considered primeriley in connection with the generation of H_2O_2 in plant apoplast. In addition, they play an important role in the antioxidative system and protect membranes from oxidative damage during response to environmental stress **(Kim *et al.,* 2002; Verma and Mishra, 2005 and Kuznetsov *et al.,* 2006)** and are essential for many processes in plant growth and development **(Rao *et al.*, 2002 and Sasse, 2003).** Brassinosteroids (BRS) are steroidal sixth group of phytohormones with significant growth promoting effects. Stigmasterol is a structural component of the lipid core of cell membranes and is considered as precursor of numerous secondary metabolites including plant steroids hormones, or as carrier in acyl, sugar and protein transport **(Noguchi *et al.,* 2000)**.

The biological function of sterols conjugates, such as fatty acids or glucosides esters and steryl acylegucosides was considered as a sterol storage forms. Sterols play an important role in plant development including cell expansion, vascular differentiation etiolation and productive development (**Clouse and Sasse, 1998 and Abdel wahed *et al.*, 2001).** Similar to the effect of brassenosteroids both sitosterol, stigmasterol and typical sterols, invalued in the regulatory function of plant development and gene expression. Sterols are essential for the normal plant growth and development like the brassinosteroids **(He *et al.*, 2003)**

The aim of this investigation was to study the influence of putrescine and stigmasterol foliar spray on fruit growth, quality parameters and some chemical constituents of Picual olive oil.

2. Material and Methods

The present study was carried out during two successive seasons (2007and 2008) on Picual olive trees at a private orchard located at El-Saff district, Giza governorate, Egypt.

The olive trees were sprayed with two compounds namely, putrescine (0.5, 1.0, 1.5 m mole) to give concentration of (44, 88 and132mg/l) and stigmasterol at (50,100 and 150 mg/l) at one month before harvest date (September) while the control trees were sprayed with water only.
The experimental olive trees were 20 year old; irrigation and fertilization were carried out as usual. Three replicate trees for each treatment were used and distributed in complete randomized design. The recorded results were means of two seasons

Physical Measurements

Physical properties of the fruits determined were; fruit weight (gm),pulp weight (g), fruit length (cm) diameter (cm),specific gravity, stone weight (g),length and diameter (cm),pulp weight (g) and thickness(cm) and moisture percentage. Fruit samples were ground in an electric blender for freshly prepared juice and soluble solid content (SSC) were measured using hand refractometer

Chemical analysis

Total acidity was determined according to **A.O.A.C. (1990)** and the oil percentage in the dried flesh samples was determined according to **A.O.A.C. (1980).**

The extracted oil was analyzed for acid value (%), peroxide value (meq/kg) and saponification value according to **A.O.A.C. (1975)**
Determination of fatty acid by GlC, Methylester of fatty acids of olive oil was carried out according to **Berglund (1994)**.

Gas chromatographic analysis: Flam Ionization Detector (FID) the following condition, Capillary column Hp-INNO wax (polyethylene glycol),the column temperature was programmed from 70-220^{0}C at the rate of 4^{0}C/min, the column detector temperature was 280^{0}C.The flow carrier gas, 2ml/minN_2, 30ml/minH_2 and air 300ml/min.

Total phenol content was determined in olive fruit flesh and oil according to **A.O.A.C. (1980).**

Polyphenoloxidase (PPO) activity was assayed by using photochemical method as described by **Kumar and Khan (1982)**.Procedure: The reaction mixture was added as the following sequences 2.7ml potassium phosphate (buffer 0.1M, pH 6.2) a) 1ml of 0.1MCatechol
b) 0.5ml of enzyme extract

The increase in absorbance at 495nm and 25^{0}C was measured.

Statistical analysis:

The design of this work was Completely Randomized Design (CRD) with there replicates. Data were analyzed according the analysis of variance (ANOVA) procedure of M-Static program by **Steel and Torrie (1980).**

3. Results and discussion

Effect of putrescine and stigmasterol on physical properties of olive fruits

Data presented in Table (1) indicated that fruit volume and weight of olive (Picual variety) were significantly increased by putrescine and stigmasterol treatments, particularly putrescine at the low concentration during the two successive growth stages. The most effective treatment was that of putrescine (44m/l) for fruit weight in both stages and fruit volume in the green stage while stigmasterol (150mg/l) gave the highest value of fruit volume in the second growth stage (purple stage).Specific gravity of the fruits was also affected according to the effect of putrescine and stigmasterol on weight and volume of fruit of the treated trees. This effect was true in green as well as purple stages of fruit growth. It could be noticed from the present results that all physical characters recorded were higher in the purple stage of olive fruit than those in the green one.

Data present in Table (1) indicated also that fruit length and diameter positively affected as a result of putrescine or stigmasterol treatment. The higher effect on fruit length was that of putrescine (44mg/l) in the green stage, while stigmasterol (150mg/l) in the purple stage. Concerning putrescine treatment at the low concentration (44mg/l) resulted in the higher records for fruit diameter in both growth stages.
Stone weight, length and diameter were also increased by putrescine or stigmasterol foliar application with the tested range of concentration. The most effective treatment in this respect was that of putrescine (44mg/l).

The effect of putrescine in enhancing fruit growth was previously reported by **Malika and Singh (2006)** who reported that foliar application of polyamines (putrescine, spermiden and spermine) markedly increased fruit retention and size of mango fruits. In support, putrescine (as one of the polyamine group) had regulatory role in promoting productivity of many plants, such as sweat pepper **(Talaat, 2003)** tomato **(Cohen *et al*., 1982)** and pea plants (**Gharib and Hanafy, 2005).** The favorable effect of stigmasterol on plant growth could be attributed to the stimulated action on the phytohormones such as auxins and cytokinine, which in turn induced cell elongation and division **(Gregory and Mandaua, 1982).** Exogenous application of polyamine has bean reported to improve fruit retention and yield of apple **(Costa *et. al*., 1986)** Olive **(Rugini and Mencuceini, 1995)** Litchi **(Stern and Gazit, 2002)** and mango **(Singh and Janes, 2000).**

Table (1): Effect of putrescine and stigmasterol on olive fruit quality of Picual olive (mean of two seasons)

Stages / Treatments	Characters		Fruit Weight (g)	Fruit Volume (cm^3)	Specific Gravity	Fruit Length (cm)	Fruit Diameter (cm)	Stone Weight (g)	Stone Length (cm)	Stone Diameter (cm)
Green Stage	Control		3.4d	3.8 c	0.87 a	1.94 b	1.72b	0.67 c	1.67 g	0.76b
	Putrescine (mg/l)	44	4.98 a	5.47a	0.91 a	2.78 a	1.91 a	0.9 a	1.81 a	0.89 a
		88	4.85 ab	5.33ab	0.9 0a	2.77 a	1.89 a	0.87 ab	1.77 a	0.89 a
		132	4.45 bc	4.93b	0.91 a	2.63 ab	1.87a	0.84 ab	1.69 f	0.87 a
	Stigmasterol (mg/l)	50	4.4 c	5.07 ab	0.86 a	2.44 ab	1.68 b	0.71 c	1.70 e	0.84 a
		100	4.65abc	5.2 0 ab	0.89 a	2.66 ab	1.85 a	0.76 bc	1.73 d	0.85 a
		150	4.72abc	5.4 ab	0.89 a	2.75 a	1.89 a	0.76 bc	1.76 c	0.87 a
Purple Stage	Control		5.09 b	5.25 a	0.96 c	2.72 b	1.95 c	0.90 c	1.59 b	0.91 a
	Putrescine (mg/l)	44	6.52 a	6.27 a	1.04 a	2.83 ab	2.11 a	1.09 a	1.85 ab	0.93 a
		88	6.11 ab	6.20 a	0.98 b	2.71 b	2.05 ab	1.05 ab	1.80ab	0.93 a
		132	6.10 ab	6.16 a	0.99 b	2.79 ab	2.03 ab	0.98 bc	1.79ab	0.9 a
	Stigmasterol (mg/l)	50	5.87 ab	5.73 a	1.02 a	2.77 ab	1.96 c	0.94 bc	1.73 ab	0.88 a
		100	6.13 ab	6.07 a	1.01 a	2.83 ab	2.08 ab	0.95 bc	1.89 a	0.89 a
		150	6.25 a	6.44 a	0.97 c	2.87 a	2.05 ab	0.97 bc	1.88 a	0.91 a

Effect of putresceine and stigmasterol on olive pulp quality.

Data presented in Table (2) mentioned that pulp weight (g) and pulp thickness (cm) of olive fruit were significantly increased as a result of putresceine or stigmasterol treatments under the used range of concentrations in green as well as purple stage of fruit development. The most effective treatment in this respect was that of putresceine (44mg/l). Moisture content (%) in olive fruits was affected by putresceine or stigmasterol foliar application in green as well as purple growth stags. As for soluble solids content (SSC) in the fruit, data presented in Table (2) indicated that it markedly increased only with stigmasterol (150mg/l) in the green and purple stages and Putresceine treatment at 44 mg/l concentration during the purple growth stage of the fruit. Acidity percentage in the fruits was markedly decreased as a result of putresceine treatments at all used concentration during green stage and stigmasterol concentrations at purple stages of fruit development.

This favorable effect of putresceine on fruit quality was previously reported by **Malika and Singh (2006)** who stated that" polyamines (Putresceine, spermine and permidine) improved mango fruit through their effect on fruit firmness, total soluble solids and sugars content and increased carotinoids and ascorbic acid content."

Effect of putresceine and stigmasterol on oil production and quality

Data represented in Table (3) revealed that oil percentage in olive fruit was significantly increased as results of putresceine at (44mg/l) treatment at green stage and stigmasterol at (150mg/l) treatments at green and purple stages of fruit development. The most effective treatments in this respect were in the purple stage, since putresceine at (44 mg/l) recorded 41.49% and stigmasterol at 150 mg/l recoded 55.61%, respectively. It could be observed from the present data that stigmasterol treatments were more effective in increasing oil percentage in fruits.

Similar results were obtained by **Abdel wahed and Gamal el-Din (2004)** on the essential oil content of chamomile plants which markedly increased by spermidine and stigmasterol foliar application. The positive effect of stigmasterol on oil percentage in the plant was reported by **El-Greedly and Mekki (2005)** on sesame plants.

The acid value (%) of olive oil was negatively affected by foliar application of putresceine and stigmasterol under the used concentration. The highest decline in acid value was obtained by the low concentration of putresceine 44mg/l which recorded 2.17, 3.8% in green and purple stages compared to 4.4 and 6.21% for untreated trees (control), respectively.

Peroxide value (meq/kg) in olive oil showed the same trend of acid value and significantly decreased with foliar application of Putresceine and stigmasterol reaching their minimum values at putresceine (44mg/l) and (88mg/l) in both green and purple stages of fruit development, respectively. On the other hand, saponification value was significantly increased as a result of putresceine or stigmasterol foliar application reaching their maximum values at putresceine (44 mg/l) and (132mg/l) at both green and purple fruit stages.

Table (2): Effect of putrescine and stigmasterol on olive pulp quality of Picual variety (mean of two seasons)

Stages / Treatments	Characters		Pulp weight (g)	Pulp thickness (cm)	Moisture content (%)	S.S.C. (%)	Acidity (%)
Green Stage	Control		2.69 d	0.84 c	74.88 bc	12.53 b	2.37 a
	Putrescine (mg/l)	44	4.08 a	1.02 a	75.7 b	13.8 ab	1.64 d
		88	3.97 ab	1.01a	77.44 a	13.33 ab	1.70 cd
		132	3.60 c	1.00 a	77.98 a	13.2 ab	1.83 bcd
	Stigmasterol (mg/l)	50	3.72 bc	0.96 ab	74.93 b	13.87 ab	2.09 ab
		100	3.89 abc	1.00a	73.25 cd	15.2 ab	1.97 bc
		150	3.96 ab	1.02 a	73.05 d	15.67 a	1.93 bcd
Purple Stage	Control		4.52 c	1.17 a	63.25 cd	8.93 c	1.73 a
	Putrescine (mg/l)	44	5.44 a	1.18 a	67.98 a	10.93 a	1.28 d
		88	5.03 abc	1.12 a	67.44 a	9.67 bc	1.65 ab
		132	5.14 abc	1.13 a	65.70 b	9.00 c	1.67 ab
	Stigmasterol (mg/l)	50	4.72 bc	1.08 a	64.88 bc	9.60 bc	1.48 bc
		100	5.07 abc	1.06 a	64.88 bc	9.80 abc	1.48 bc
		150	5.23 ab	1.14 a	63.05 d	10.33 ab	1.41 cd

Table (3): Effect of putrescine and stigmasterol on olive oil production and quality of Picual variety (mean of two seasons)

Stages	Treatments / Characters		Oil content (%)	Acid Value (%)	Peroxide Value meq/kg	Saponification value
Green Stage	Control		26.78 c	4.4 a	4.49 b	171.52 c
	Putrescine (mg/l)	44	34.79 ab	2.17 d	4.1b	198.12 a
		88	31.72 bc	3.4 c	4.33 b	187.33 abc
		132	27.33 c	3.9 abc	6.63 a	178.77 abc
	Stigmasterol (mg/l)	50	27.83 c	4.3 0 ab	5.36 ab	176.18 bc
		100	36.56 ab	3.7 bc	4.49 b	189.07 abc
		150	38.41a	3.5c	4.43 b	195.79 ab
Purple Stage	Control		36.51 b	6.21 a	14.92 a	141.8 d
	Putrescine (mg/l)	44	41.49 AB	3.80 c	11.93 b	167.1 abcd
		88	37.80 AB	3.87 c	11.90 b	172.6 abc
		132	40.42 AB	4.57 bc	12.93 ab	185.4 a
	Stigmasterol (mg/l)	50	39.36 AB	5.45 ab	14.08 ab	146.4 cd
		100	39.36 AB	4.48 bc	13.37 ab	154.4 bcd
		150	55.61 A	4.2 bc	12.99 ab	178.4 ab

Effect of Putresceine and stigmasterol on phenols and Polyphenoloxidase (PPO) activity

It could be noticed from the results presented in Table (4) that phenols content in the fruits and oil of Picual olive was significantly decreased by putresceine or stigmasterol foliar application during green as well as purple developmental stages of the fruits. The lower values for fruit oil content were recorded by putresceine (44mg/l) at green stage and stigmasterol (50mg/l), while the lower values for oil content of phenols were obtained by putresceine (44mg/l) in both green and purple stages

The decrease in lipid peroxidation might be in favour of vegetative growth and oil content. It could be attributed to the protection of polysaturated fatty acids of cell membrane from oxidative damage **(Asada, 1999)** In addition, polyamines acted as antioxidants and counteracted oxidative damage in plant, which as a consequence reduced free radicals and alleviate lipid peroxidation **(Singh *et al.*, 2002).**In support **Borrel et al (1999)** suggested that inhibition of lipid peroxidation might be one of the mechanisms responsible for the antisenescence of polyamine effect in the leaves. As far the effect of putresceine and stigmasterol on the chemical constituents of Picual olive oil, data presented in Table (5) indicated that unsaturated fatty acid (oleic, linoleic, linolenic) were markedly increased according to putresceine (44mg/l) during both green and purple stages of fruit development. These increases acquired on the expense of saturated fatty acids (Palmatic and stearic acids) which recorded low valued in the oil of treated trees.

Table (4): Effect of putrescine and stigmasterol on olive phenols and Polyphenoloxidase (PPO) activity (mg/g. FW) for olive fruits and oil. of Picual variety (Mean of two seasons)

Stages / Treatments / Characters		Green stage			Purple stage		
		Phenols content (in fruit)	phenols content (in oil)	Polyphenoloxidase (PPO) activity in oil	phenols content (in fruit)	phenols content (in oil)	Polyphenoloxidase (PPO) activity in oil
Putrescine (mg/l)	44	1.929 d	0.156 e	2.25 c	2.263 d	0.135 c	0.633 b
	88	2.958 b	0.193 d	3.6 b	2.109 d	0.156 c	0.967 a
	132	3.103 a b	0.333 b	4.1a	2.88 b	0.186 c	1.143a
♀Stigmasterol (mg/l)	50	2.377 c	0.329 b	1.6d	1.685 e	0.311 b	0.866 a
	100	2.545 c	0.256 c	1.4 d	2.198 d	0.268 b	0.567 b
	150	2.077 d	0.148 f	1.37 d	2.480 c	0.166 c	0.533 b
Control		3.312 a	0.404 a	4.5 a	3.389 a	0.546 a	1.20 a

Table (5): Effect of putrescine and stigmasterol on fatty acids content (%) in oil of Picual olive

Stages	Treatments / Characters	Palmitic	Stearic	Oleic	Linoleic	linolenic	Total unsaturated fatty acid
Green stage	Putrescine (44mg/l)	12.44	0.92	61.42	14.46	4.46	80.34
	Stigmasterol (150mg/l)	18.52	2.02	62.74	4.93	2.44	70.11
Purple stage	Putrescine (44mg/l)	20.85	1.03	64.25	13.66	3.74	81.65
	Stigmasterol (150mg/l)	18.32	1.5	56.69	6.58	4.97	68.24
	Control	20.67	2.44	55.55	9.24	5.86	70.65

References

Abd El-Wahed, M.S.; Z.A. Ali; M.S. Abdel Hady and S. M. Rashad (2001). Physiological and anatomical changes on wheat cultivars as affected by sitosterol. J. Agric. Sci. Mansoura Univ., 26(8) 4823-4839.

Abd El-Wahed, M.S.A. and Krima, M. Gamal El Din (2004) stimulation of growth, flowering, biochemical constituents and essential oil of chamomile Plant(*Chamomilla recutita* l., rausch) with spermidine and stigmasterol application. Bulg. J. Plant Physiol, 30(1-2): 89-102.

A.O.A.C. (1975). Official methods of analysis of the Association of Analytical Chemists:14th Ed. Washington, D.C., U.S.A.

A.O.A.C. (1980). Official methods of analysis (13th Ed.) Association of Official Analytical Chemists. Washington, DC. U.S.A.

A.O.A.C. (1990). Official methods of analysis of the 5thed published by Association of Official Analytical Chemists.5thed Chemists. Washington, DC. U.S.A

Asada .K. (1999). The water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. Ann. Rev. Plant Physiol. plant Mol. Biol. 50:601-639.

Berglund, T.(1994). Nicotinamide, a missing link in the early stress response in eukaryotic cells: A hypothesis with special reference to oxidative stress in plant FEBS. Lett, 315:145-149.

Borrell. a.; K .Carboneel ; R. Farras; P. paio-parellada and A. F. Tiburcio (1999). Polyamines inhibit lipid peroxidation in senescing oat leave. Physiol. Plant. 99:385- 390.

Bouaziz, M.; R .J. Grayer; M. S. J. Simmonds ;M. Damak and S. Sayadi (2005). Identification and antioxidant potential of flavonoids and low molecular weight phenols in olive cultivar Chemlali grown in Tunisia. J. Agric. Food Chem.53:236-241

Cavusoglu, K.; S. Kilic and K. Kabar (2008).Effects of some plant growth regulators on stem anatomy of radish seedlings grown under saline (NaCl) conditions. Plant, Soil and Environment, 54:428-433.

Clouse, S. D. and J. ML. Sasse (1998). Brassinosteroids: Essential Regulators of Plant Growth and Development. Annu. Rev. Plant Physiol. Plants Mol. Biol., 49:427-451

Cohen, E.; S. Arad, Y. M. Heimer and Y. Mizzrhi (1982). Participation of ornithine decarboxylase in early stages of tomato fruit development. Plant Physio., 70:540-543.

Costa, G.; R. Biasi and N. Bagni (1986). Effect of putrescine on fruiting performance of apple (cv. Hi Early), Acta Hort. 179, pp. 355–361.

El-Greedly, Nadia, H.M and B.B. Mekki (2005).Growth, Yield and Endogenous Hormones of Two Sesame *(Sesamum indicumL.)* Cultivars as Influenced by Stigmasterol. J. of Applied Sci. Res., 1(1): 63-66.

Gharib, A.A. and A. H. Hanafy Ahmed (2005). Response of pea plants (*Pisum Sativum* L.) to foliar application of Putrescine, glucose, foliafeed D and silicon. J. Agric. Sci. Mansoura Unv, 21(4):1415-1423.

Gregory, L.E. and N. B. Mandaua(1982). The activity and interaction of brassinolide and gebberelic acid in mung bean epicotyl. Physiol. Plant., 53: 239 – 243

He, J.X.; S. Fujioka and T.C. Li(2003). Sterols regulate development and gene expression in Arabidopsis. Plant Physiol., 131(3): 1258-1269.

Kim. T. M.; S. K. Kim; T. I. Han; I. S. Lee and S. C. Chang (2002). ABA and polyamines act independently in primary leaves of cold-stressed tomato (*Lycopersicon esculentun*). Physiologia Plantarum, 115:370-376.

Kumar, K. B. and P.A. Khan (1982). Peroxidase and polyphenoloxidase in excised ragi (Eleusine coracana cv.PR22) Leaves during senescence. Indian J. Exp. Bot 20:412- 416.

Kuznetsov, VI. V.; N. L. Radyukina and N.I. Shevyakova (2006) Polyamines and stress: biological role, metabolism, and regulation. Russian J. of Plant Physiol., 53:583-604.

Malika, A.U. and Z. Singh (2006).Improved fruit retention, yield and fruit quality in mango with exogenous application of polyamines. Scientia Horticulturae. 2(110): 167-174.

Noguchi, T.; S. Fujioka; S. Choe; S. Takatsute; F. E. Tax; S. Yoshida and K. A. Feldmann(2000). Biosynthetic pathways of brassinolide in Arabidopsis. Plant Physiol., 124:201-209.

Owen, R. W. ;W. Mier ;A. Giacosa; W. E. Hull; B. Spiegelhalder and H. Bartsch(2000). Phenolic compounds and squalene in olive oils: The concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignans, Food and Chemical Toxicology,38:647-659.

Rao. S. S. R.; B. V. V. Vardhini; E. Sujatha and S.Anuradha (2002). Brassinosteroids-Anew class of phytohormones. Curr. Sci., 82:1239-1245.

Rugini, E. and M. Mencuccini (1985). Increased yield in the olive with putrescine treatment, Hort. Sci., 20(1):102-103.

Sasse, J. M. (2003). Physiological actions of brassinosteroids: an update. J. Plant Growth Regul., 22:276-288.

Singh, D.B.; S. Verma and N.S. Mishra (2002). Putrescine effect on nitrate reductase activity, organic nitrogen, protein and growth in heavy metal and salinity stressed mustard seedlings. Biol. Plant, 45:605-608.

Sing, Z. and J. Janes (2000). Regulation of fruit set and retention in mango exogenous Application of polyamines and their biosynthesis inhibitors. Acta Hortic., 509:675-680.

Steel, R.G. and G.H. Torrie (1980). Principles and Procedures of Statistics: A Biometrics Approach. MC. Grow Hill book Co., New York.

Stern, R. A. and S. Gazit (2002). Application of the polyamine putrescine increased yield of "Mauritius" litchi (*Litchi chinesis* sonn.) J. Hort. Sci. Biotechnol., 75:612-614.

Talaat, N.B. (2003). Physiological studies on the effect of salinity, ascorbic acid and Putrescine on sweet pepper plant. Ph.D. Thesis, Agric, Bot. Dept. Fac. Agric., Cairo Univ., pp: 286.

Verma, S. and N.S. Mishra (2005) Putrescine alleviation of growth in salt stressed Brassica juncea by inducing antioxidative defense system. J. of plant Physiol., 162:669-677.

8/22/2011