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Antioxidant Properties, Secondary metabolites and Growth as affected by application of Putrescine and Moringa leaves extract on Jojoba plants

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ABSTRACT

This work aimed to study the effect of putrescine at (50, 100 and 200 ppm) as a chemical growth regulator , moringa leaves extract (3.5%, 7% and 10 %) as a natural extract and control (distilled water) on growth, photosynthetic pigments, phytochemicals and antioxidant capacity of jojoba plants grown in earthenware pots. Application of MLE (10 %) caused an increase in plant height by 103.24%, meanwhile (7 %) of MLE led to 4.08% increment in the branches number as compared to control plants. Chlorophyll *a*, *b* and carotenoids recorded the highest concentrations by treating with MLE (10 %) followed by putrescine (50 ppm).Total phenolic, flavonoid , tannin content and antioxidant activity of jojoba plants significantly increased with putrescine treatment at 50 ppm and these increments were 28.98%, 31.33%, 74.82% and 65.44%, respectively as compared to control plants. Also, foliar spray of MLE (7 %) led to highest increment of total phenolic, flavonoid and tannins (41.67%, 85.13% and 80.50%, respectively).Moringa leaves extract had the superiority in increasing reducing power ability it increased gradually by increasing moringa leaves extract concentration. Some changes in amino acids concentration were observed with putrescine (50and200 ppm) as well as total essential amino acids.

INTRODUCTION

Jojoba (*Simmondsia chinensis Lam*) belongs to the family *Simmondsiaceae* is a semi-arid shrub native to Arizona, California and northern Mexico. The seed contains 50–60% oil. Jojoba is an extremely drought resistant species and is gaining worldwide attention, for extraction of oil which is used in cosmetics, pharmaceuticals and lubricant industries as a replacement of sperm whale oil. The total area covered by this crop throughout the world is around 18,500 hectares. The oil demand of the world is estimated at 6400-200,000 tons per year. Jojoba grows well on marginal dry and light textured soils and can utilize brackish water of arid lands **(**Benzion, 1997; Dunstone and Ball, 1996). Polyamines (PAs) namely putrescine, spermine and spermidine in different plant developmental process (Martin-Tanguy, 2001). They modulate several growth and developmental processes viz., cell division, differentiation, flowering fruit ripening, embryogenesis, senescence and rhizogenesis (Kakkar *et al*, 2000). In all these, PAs have been ascribed various roles such as that of a new class of plant growth regulators, hormonal second messengers and as one of the reserves of carbon and nitrogen at least in cultured tissues (Slocum and Flores, 1991). Polyamines are biologically active compounds involved in various physiological processes. They are cationic molecules, positively charged under intracellular pH, which are essential for plant growth and differentiation, related to aging and senescence, and usually involved in plant responses to stress (Friedman *et al.*, 1989). They regulate growth, probably by binding to negatively charged macromolecules (Messiaen *et al.*, 1997). Plant hormones can be used to increase yield per unit area because they influence every phase of plant growth and development.

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Moringa belongs to family Moringaceae. There are about 13 species of moringa of which *M. oleifera* is most widely grown. Since leaves of moringa are rich in zeatin, it can be used as natural source of cytokinin (Fuglie, 1999). In addition, moringa leaves is also rich in ascorbates, carotenoids, phenols, potassium and calcium, which have plant growth. Promoting capabilities and often applied as exogenous plant growth enhancers (Foidl *et al.,* 2001). Antioxidants such as ascorbic acid and glutathione, which are found at high concentrations in moringa chloroplasts and other cellular compartments, are crucial for plant defense against oxidative stress (Noctor & Foyer, 1998). In view of all these reports, it is hypothesized that priming with leaf extract from moringa, having a number of plant growth promoters, mineral nutrients and vitamins in a naturally balanced composition, which may promote the plant growth. Moringa leaves extract was sprayed onto leaves of onions, bell pepper, soya beans, sorghum, coffee, tea, chili, melon and maize and was shown to increase yields of these crops (Fuglie, 2000).

Therefore, the objective of this work was to investigate the effect of putrascine and moringa leaves extract application on growth, photosynthetic pigments, total phenolic, flavonoid, tannins, DPPH scavenging activity, reducing power ability and amino acids concentration of jojopa plants.

MATERIALS AND METHODS

This work aimed to study the effect of putrascine (as a chemical growth regulator) and natural extract (moringa leaves extract) on growth, photosynthetic pigments, phenolic ,flavonoid ,tannins ,DPPH scavenging activity and reducing power ability of jojoba plants grown in earthenware pots. This experiment conducted at April, 2012 and 2013 in the greenhouse of the National Research Centre, Dokki, Egypt.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide; catechin, vanillin, quercetin and $FeCl_3$ were purchased from Sigma Chemical Co. (St.Louis, MO, USA). Folin-Ciocalteus's phenol reagent, sodium carbonate and putrescine were from Merck Chemical Supplies (Damstadt, Germany). All the other chemicals used including the solvents, were of analytical grade.

Putrescine and moringa extract

The experiment included 7 treatments 3 putrascine and 3 treatment of moringa leaves extract more than the control. Putrescine in the rate of 50, 100 and 200 ppm and moringa leaves extract was prepared by the rate of 3.5%, 7% and 10% more than distilled water as a control. The design of the experiment was complete randomized blocks in 9 replicates.

Seeds of jojoba (*Sommondsia chinensis Lam*) were sown at April, 2012 and 2013 in earthenware pots.Calcium super phosphate (15.5 %P_2O_5) and Potassium sulphate (48.5% K_2O) in the rate of /pot respectively was broadcasted before sowing. Ammonium sulphate (20.5 N%) added in two equal portions, the 1st was after one month from sowing and the 2nd portion was one month later.

Photosynthetic pigments

Chlorophyll, *a, b* and carotenoids concentrations in leaves of jojoba using the method described by Saric *et al.* (1967).

Extract preparation

Plants were air dried at room temperature for 3 weeks to get consistent weight. The dried plants were later ground to powder. Two grams of ground plant material were shaken separately in methanol for 48 hrs on a shaker at room temperature. Extracts were filtered using a Buckner funnel and Whatman No 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator. Each extract was resuspended in the respective solvent, methanol, to yield a 100 mg/ml stock solution (Tylor *et al.,* 1996).

Determination of Total Phenolic

The total phenolic content of methanolic extracts of jojoba leaves were determined according to the method described by Makkar *et al.,* (1997). Aliquots of the extracts were taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the tubes were placed in the dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The amount of total phenolic was calculated as Gallic acid equivalents from a calibration curve.

Determination of Total Flavonoid

Total flavonoids content was determined spectro-photometrically using the method of Ordonez *et al.* (2006) based on the formation of a complex flavonoid-aluminum. An aliquot (0.5 ml) of aqueous extract was mixed with AlCl3 solution (2%, 0.5 ml). Then the mixture was properly mixed and allowed to stand for 30 minutes at room temperature. The intensity of colour was measured at 420 nm after filtration if it is necessary.

Total flavonoid contents were calculated as quercetin equivalent from a calibration curve and the values are presented as means of triplets analyses.Total flavonoid contents were calculated as quercetin equivalent from a calibration curve.

Determination of Tannins

Tannins of the jojoba treatments were determined using the modified vanillin hydrochloric acid (MV-HCl) as reported by Maxson and Rooney (1972). Samples of 1 gram dried jojoba treatments leaves were extracted with 1% hydrochloric acid in methanol. The mixture then were shaken for 24 hours and let to settle. A 5 ml of vanillin-HCl reagent (50:50 mixtures of 4% vanillin / 8% HCl in methanol) was quickly added to 1 ml extract. The developed color was measured at 500 nm using spectro-

photometer. The standard curve of catechin was obtained and tannins were calculated.

Antioxidant Activity (DPPH Assay)

The free radical scavenging activity using the 1.1-diphenyl-2-picryl- hydrazil (DPPH) reagent was determined according to Brand-Williams *et al.* (1995). The extracts of jojoba treatments were soluble with 85% methanol: water. To 0.5 ml of the extracts samples 1.5 ml of freshly prepared methanolic DPPH solution (20 µg ml) was added and stirred. The decolorizing processes was recorded after 5 min of reaction at 517 nm and compared with a blank control.

Antioxidant activity = [(control absorbance - sample absorbance) / control Absorbance] × 100%

Reducing power Assay

The reducing powers of methanol extracts of jojoba plants were determined according to the method of Oyaizu, (1986). 0.5 of methanol extracts of plants were added to Phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5ml). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 ml,10%) were added to the mixture, which was then centrifuged at 1000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm. Increased absorbance of their action mixture indicated increased reducing power.

Determination of amino acids

Amino acids were determined using amino acid analyzer according to Baily (1967). Dry and defatted samples containing 25 mg protein where hydrolyzed with 6 N HCl at 105 Cº for 24 hr in a sealed tube.

After cooling and filtering, the residue left after filtration was washed with distilled water and the combined filtrates were completed to 25 ml in a volumetric flask. A portion of the filtrate (5 ml) was evaporated to dryness at room temperature in desiccators under vacuum. The residue was dissolved in 5 ml buffer (0.2 N sodium citrate, pH 2.20) and the solution was filtered through 0.22 µm membrane. Twenty microliters of the final filtrate were injected in the instrument capsule for quantitative determination of the amino acids (Spachman *et al.*, 1958). The cationic exchange resins UL-tropac sodium and special programmed buffer system were used (citrate buffers 0.2 N with three different pH, at 3.2, 4.25 and 6.45 at a rate of 35 ml/hour). The effluent was met by a stream of ninhydrin reagent at a rate of 25 ml/hour. The quantitative estimation of the amino acids depends on the colorimetric determination of the blue color (Muhler, 1964).

Statistical analysis

The data obtained were subjected to standard analysis of variance procedure where as values of LSD were obtained at 0.05% as reported by Snedecor and Cochran (1980). The results were presented as mean values ±SD (standard deviations).

RESULTS AND DISCUSSION

Vegetative growth parameters

Data showed that addition of putrescine (Put.) or moringa leaves extract (MLE) significantly affected all the studied growth characters, i.e. plant height, number of branches, length of roots, fresh and dry weights of both branches and roots comparing with control plants (Table 1).Foliar application of Put. at 50 ppm was accompanied by an increase in plant height, fresh and dry weights of branches and roots. The increments were 69.37%, 4.20%, 52.22% and 29.61%, respectively as compared with control plants in the two seasons. While, the application of MLE (10 %) caused an increase in plant height by 103.24%, meanwhile (7 %) of MLE led to 4.08% increment in the number of branches as compared to control plants. The simulative effects induced by putrescine application on vegetative growth characters could be explained by Kaur-Sawhney and Galston (1979) who stated that, the anti-senescence properties of polyamines and their correlation to cell proliferation and differentiation tended to support their action as growth factors. In several dicotyledonous plants, putrescine retarded senescence of leaves by preventing chlorophyll, protein and RNA breakdown and by increasing mitotic activity in protoplasts (Kaur-Sawhney *et al.*, 1980). In this concern, Gharib and Hanafy (2005) pointed out that spraying pea plant with putrescine at 1 or 2 ppm significantly increased plant height and should dry weight.

Concerning the effect of moringa leaves extract, it contains growth, enhancing substances and can be used as natural source of growth promoter (Fuglie, 2000) which may reduce the adverse effects on maize by delaying the leaf senescence and scavenging the reactive oxygen species. Plant treated with this spray exhibited more pest and disease resistance, heavier roots, stems and leaves (Makkar and Beckar, 1996). Priming with MLE (3.5 %) not only improved seedling emergence but also enhanced the seedling vigor as indicated by higher root and shoot lengths, and seedling fresh and dry weights (Basra *et al*., 2011). Better performance of maize plants raised from seeds primed seeds with MLE 3.5 % might be due to the maintenance of tissue water contents, increase in antioxidant activities, and carbohydrate metabolism (Farooq *et al.*, 2008).

Photosynthetic pigments

The data recorded in table (2) showed that maximum chlorophyll *a, b* and carotenoids content were observed in plants treated with highest concentration MLE (10 %) followed by treatment of putrescine at concentration 50 ppm in Jojoba plants as compared with other concentrations of putrescine and MLE or control treatments. Total chlorophyll showed the same response. These results indicated that Jojoba plant content of pigments responds to putrescine and MLE positively. This could be due to putrescine capability (as polyamine) to stabiles protoplast and prevent both loss of chlorophyll during senescence in protoplast and leaves (Kaur-Sawhney *et al.*, 1980). Ma *et al.* (1996) suggested that the effect of polyamines in inhibiting chlorophyll

degradation may be related to the inhibition of peroxidase activity. Increasing carotenoids content may due to converting these substances to pyruvic acid that led to enhance biosynthesis of leaf carotenoids **(**Martin-Tanguy, 2001). Regarding the effect of MLE, Ali *et al*. (2011) observed that moringa leaves extract (MLE3.5%) increased chlorophyll a and b contents under severe drought stress. Moreover, Basra *et al*. (2011) indicated that Maximum chlorophyll contents were recorded in maize seed primed with MLE 3.5 % followed by 50 mg/L ascorbate treatments. Lower chlorophyll content and phenolic in plants raised from non-primed seeds and seeds exposed to overnight soaking might be due to absence of important hormones/nutrients or antioxidant compounds, which are abundantly present in moringa leaves.

Total phenolic, flavonoid, and tannins content

Data tabulated in Table 3 showed that total phenolic, flavonoid and tannins content of jojoba plants significantly increased with putrescine treatment at 50 ppm and these increments were 28.98% , 31.33%, 74.82% and 65.44% respectively as compared to control plants . However, foliar spray of MLE (7 %) led to highest increment of total phenoic, flavonoid and tannins (41.67%, 85.13% and 80.50%, respectively). Also Ghareib and Hanfy (2005) stating that putrescine foliar application (1or 2 ppm) increased total soluble phenols of pea shoots. Moreover, Neveen (2003) observed that putrescine foliar application 1 or 2 ppm enhanced total soluble phenols in sweet pepper shoots. Whereas, Ali *et al*. (2011) indicated that foliar spray of MLE (3.5 %) had no significant effect on the total phenolic contents of maize plants.

DPPH Radical scavenging activity

As shown in (Fig.1) Dpph radical scavenging activity of jojoba plant sprayed with the lowest concentration of putrescine (50 ppm) recorded the highest DPPH radical scavenging activity (60.85%) ,while plants treated with the moderate concentration (7%) of moringa leaves extract exhibited the highest antioxidant activity (63.05%).

It can be noticed from presented data that there was a strong relationship among total phenolic and antioxidant activity indicate that phenolic compounds were a major contributor of antioxidant activity in jojoba plants and this in agreement with Bendini *et al*. (2006) and Wojdylo *et al*. (2007) who mentioned that the same relationship, as phenols are very important plant constituents because of their scavenging ability on radicals due to their hydroxyl groups.

Therefore, the phenolic content of plants may be contributed directly to their antioxidant action, phenylpropanoid and flavonoid biosynthesis.

Reducing Power Ability

There are numerous antioxidant methods for evaluation of antioxidant activity. For *in vitro* antioxidant screening. The total antioxidant activity of an antioxidant cannot be evaluated by using one single method, due to oxidative processes. Therefore, at least two methods should be employed in order to evaluate the total antioxidant activity (Gulcin *et al.*, 2005).

Figure (2) describes the reducing power of Jojoba plants treated with several concentrations of putrescine and moringa leaves extract. As can be seen, all jojoba plant extracts exhibited an increase in reducing power ability either by application of putrescine or moringa leaves extract comparing with control extract.

Moringa leaves extract had the superiority in increasing reducing power ability it increases by about 61.81, 134.38, and 106.6% more than control with 3.5%, 7%, and 10% moringa leaves extract respectively. This increase may be attributed to that moringa leaves extract contains several photochemicals, some of which are of special interest because of their medicinal properties it contains flavonoid pigments such as kaempferol, rhamnetin, isoquercitrin and kaempferitrin. In addition,this leaves are rich in a group of the glycoside compounds , glucosinolates and isothiocyanates (Bose,2007),which may enhance the formation of the photochemical compounds which resulted in the increase in the antioxidant activity.

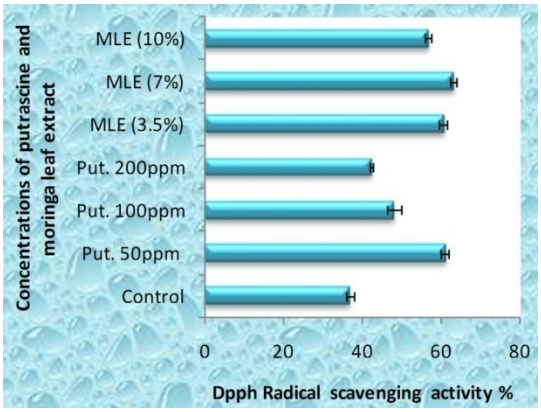

Fig. 1: Effect of putrescine and moringa leaves extract on DPPH radical scavenging activity of jojoba *(Simmondsia chinensis)* plants. (n= 3, value= mean ± SD, LSD ≤ 0.05=-1.80, Putrescine (put.), moringa leaves extract (MLE).

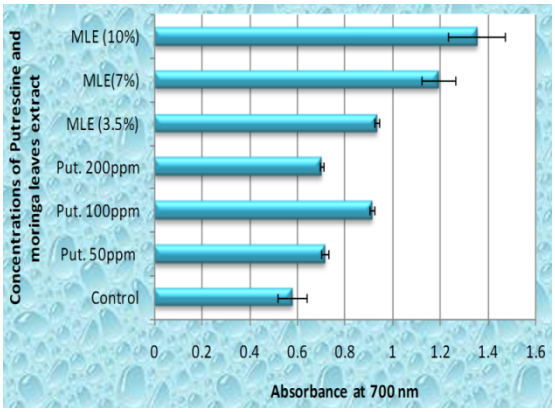

Fig. 2: Effect of putrescine and moringa leaves extract on reducing power activity of jojoba *(Simmondsia Chinensis)* plants. (n= 3, value= mean ± SD, LSD ≤ 0.05= 0.103).Putrescine (put.), moringa leaves extract (MLE).

Table 1: Effect of putrescine and moringa leaves extracts on vegetative growth of Jojoba *(Simmondria chinensis)* plants.

Treatments	Plant height	Roots D.W	Roots F.W	Branches D.W	Branches F.W	Length of roots (cm)	Number of branches
Control	41.33	2.33	3.60	5.38	11.90	58.33	8.33
Put.50ppm	70.00	3.02	5.48	7.08	12.40	31.67	3.67
Put.100ppm	47.33	2.17	3.85	4.36	8.90	38.0	3.33
Put.200ppm	49.33	2.28	3.35	4.28	10.28	60.0	4.67
MLE (3.5%)	37.33	1.70	1.83	3.43	7.12	27.67	4.67
MLE (7%)	49.33	3.00	3.80	4.03	5.82	40.0	8.67
MLE (10%)	84.00	2.65	3.79	6.32	11.65	40.33	4.67
LSD (0.05%)	6.45	0.67	0.93	1.00	2.24	4.96	1.62

(n= 3, value= mean ± SD, Putrescine (put.), moringa leaves extract (MLE).

Table 2: Effect of putrescine and moringa leaves extract on photosynthetic pigments (mg /100 g F.W) of Jojoba (*Simmondria chinensis)* plants.

Treatment	chl. *a*	chl. *b*	carotenoids	chl.*a*+chl.*b*	chl.*a*:chl.*b*	chl.*a*+chl.*b*: carotenoids
Control	153.03	65.23	69.08	218.26	2.35	3.16
Put.50ppm	160.90	94.25	102.14	255.15	1.71	2.50
Put.100ppm	111.43	73.56	78.89	184.99	1.51	2.34
Put.200ppm	56.38	24.50	23.99	80.88	2.30	3.37
MLE (3.5%)	150.77	34.15	40.72	184.92	4.41	4.54
MLE (7%)	176.09	27.79	81.62	203.88	6.34	2.50
MLE (10%)	222.16	141.32	115.24	363.48	1.57	3.15
LSD (0.05%)	17.33	17.99	5.06	23.05		

(n= 3, value= mean ± SD, Putrescine (put.), moringa leaves extract (MLE).

Table 3: Effect of putrescine and moringa leaves extract on total phenolic, falavonoid and tannins content of jojoba *(Simmondsia Chinensis)* plants (mean of the two seasons).

Treatments	Total Phenoic (mg gallic /g D.W.)	Total Flavonoids mg Querstine /gD.W.	Tannins (mg catechin /g D.W.)
Control	71.05±0.605	9.26±0.311	2.82±0.088
Put. 50ppm	91.64±0.590	12.16±0.412	4.93±0.047
Put. 100ppm	87.45±0.795	11.93±0.666	4.14±0.110
Put. 200ppm	86.36±0.688	10.38±0.321	3.41±0.250
MLE (3.5%)	95.01±1.790	12.02±0.095	5.09±0.75
MLE (7%)	100.66±2.563	14.69±0.452	5.32±0.127
MLE (10%)	91.78±1.605	10.75±0.250	4.30±0.176
LSD (0.05%)	2.492	0.691	0.250

(n= 3, value= mean ± SD, Putrescine (put.), moringa leaves extract (MLE).

Table 4: Effect of putrescine and moringa leaves extract on the proportion of amino acids in jojoba *(simmondosia chinensis)* plants.

Amino acid (%)	Control	Put.50ppm	Put.100ppm	Put.200ppm	MLE (3.5%)	MLE (7%)	MLE (10 %)
Aspartic	1.49	2.67	1.26	1.49	1.34	1.08	0.84
Threonine	0.46	1.11	0.50	0.58	0.38	0.31	0.25
Serine	0.52	1.18	0.50	0.69	0.74	0.43	0.44
Glutamic	1.79	3.38	1.61	2.27	2.04	1.32	1.58
Glycine	0.40	0.46	0.35	0.46	0.28	0.29	0.41
Alanine	1.12	1.19	1.00	1.54	0.92	0.88	1.09
Valine	0.60	0.91	0.26	0.90	0.46	0.53	0.96
Methonine	1.85	0.95	1.69	2.51	1.57	1.31	0.92
Isoleucine	0.24	0.60	0.40	0.59	0.47	0.34	0.26
Leucine	0.37	0.91	0.69	1.04	0.43	0.22	0.28
Pheylanine	0.73	0.67	0.42	1.05	0.30	0.59	0.26
Histidine	1.16	1.24	0.97	1.16	0.61	0.78	0.76
Lysine	1.01	1.12	0.72	1.06	0.74	0.63	1.10
NH4+	2.95	2.74	2.41	2.42	2.33	1.83	2.07
Arginine	2.32	1.31	1.17	1.29	1.79	0.83	0.83
Essenatial AA	6.42	7.51	5.62	8.89	4.96	4.71	4.79
Nonessential AA	7.55	10.19	8.96	7.74	4.33	4.83	5.19
Total AA	13.97	17.7	14.58	16.63	9.29	9.54	9.98

Putrescine (put.), moringa leaf extracts (MLE).

Amino acids

Data tabulated in table 4 indicated that aspartic, threonine, serin, glutamic, histidine and lysine were increased to highest ratios (2.67, 1.11, 1.18, 3.38, 1.24 and 1.12%, respectively) with putrescine treatment at 50 ppm as compared to control and other treatments. Increasing the concentration of putrescine to 200 ppm caused increasing the ratios of alanine, methionine, isoleucine, leucine and phenylalanine to highest ones (1.54, 2.51, 0.59, 1.04 and 1.05%, respectively). The highest ratio of valine (0.96%) was produced with MLE (10 %) treatment.

Data in table (4) cleared that the essential amino acids (AA) increased with the highest level with putrescine treatment (200 ppm). Meanwhile, the nonessential amino acids and total AA were increased with the lowest concentration of putrescine (50 ppm).

Concerning this, Kotzabasis *et al*. (1993) revealed that polyamines could play a role in the modulation of reduced nitrogen inside the cells and in the structure and functioning of the photosynthetic apparatus because of their richness in amine groups and their biosynthesis through amino acid decarboxylases (Walden *et al*., 1997). On the other hand, PAs have been proposed to participate in the plasticity of amino acid metabolism in both rape and tomato subjected to abiotic stresses (Aziz *et al*., 1998, 1999 and Larher *et al.,* 1998). Also pea shoots; oat leaves and soybean seedlings were shown to metabolize Putrescence and Spermidene to GABA, glutamate, aspartate, sugars, and organic acids (Rastogi and Davies, 1991) Table-4.

CONCLUSION

Addition of putrescine (Put.) or moringa leaves extract (MLE) positively affected all the studied growth charcters, i.e. plant height, number of branches, length of roots, fresh and dry weights of branches and roots as well as pigments content of jojoba plants. On the other hand secondary metbolites, DPPH radical scavenging activity and reducing power ability increased by application of both putrescine and moringa leaves extract.

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