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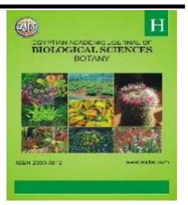

Vegetative Growth and Nutritional Status of Pear as Influenced by Putrescine, Glycine Betaine and Some Micronutrients

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ABSTRACT

The current study was conducted on twelve-year-old 'Le-Conte' pear trees plants budded on 'Butialefola' pear rootstock, planted in sandy soil under drip irrigation in a private Farm located at El-Nubaria, EL-Beheira Governorate, Egypt, throughout 2021 and 2022 season, to study the effect of foliar application of putrescine at 20, 40 and 60 ppm, and glycine betaine at 30, 60 and 90 compared to water only as a control. The trees were also sprayed by 250 ppm Fe + 250 ppm Zn + 250 ppm Mn + 100 ppm H_3BO_3, 500 ppm Fe + 500 ppm Zn + 500 ppm Mn + 200 ppm H_3BO_3 and 750 ppm Fe + 750 ppm Zn + 750 ppm Mn + 300 ppm H_3BO_3 on vegetative growth, and leaf mineral content from macro and micronutrients of 'Le-Conte' pear trees. Fifty uniform trees were chosen for this investigation, and they were subjected to identical cultural practices throughout seasonal times. Five replicates for each treatment were organized in a randomized complete block design (RCBD). The treatments were sprayed three times at; swollen buds, 15 days later and the third spray was 30 days after the second one. The results showed that foliar application of putrescine increased shoot length, thickness, leaf area, and leaf total chlorophyll content, followed by spraying of glycine betaine and certain micronutrient treatments. Sprayed treatments considerably also enhanced the leaf mineral contents of nitrogen, phosphorus, and potassium, iron, zinc, manganese, and boron.

INTRODUCTION

Pear (*Pyrus communis* L.) is a member of the family of Rosaceae, and its genus is Pyrus, which contains twenty-two species that exist in Asia, and Europe, as well as northern Africa. Le-Conte is the essential pear cultivar grown in Egypt and it resulted from the hybridization between *Pyrus communis* X *Pyrus serotina*. Besides, it is one of the greatest important pear trees in whole the world, where it is cultivated in whole temperate-zone countries. The cultivated area was 5455.38 hectares (12989 feddans), which produced 82746 tonnes (FAO, 2021).

Putrescine sprayed on mango trees' leaves improved vegetative development indices as well as the nutritional status of the trees (Malik and Singh, 2003; Malik *et al.*, 2006). Spraying Karna khatta (*Citrus karna* Raf.) with putrescine at a concentration of 50 mg/L resulted in a notable rise in the leaf levels of nitrogen, phosphorus, and potassium compared

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to untreated plants (Sharma *et al*., 2011). Spraying of putrescine at 1, 2, and 3 mM on peach v. Florida King led to enhanced vegetative growth, including increased leaf area, shoot length, and shoot diameter (Ali et al., 2014). Spraying of putrescine on 'Zebda' mango trees during the 2013 and 2014 seasons was examined by Ali *et al*. (2017) increased the levels of leaf total chlorophyll, and the leaf mineral composition in terms of phosphorus and potassium. The foliar application of putrescine on sour orange seedlings at 50, 100, and 150 ppm considerably raised the shoot length, shoot thickness, and leaf area, as well as elevated levels of leaf total chlorophyll, leaf N, P, K, Fe, Zn, and Mn on opposed to not treated trees (Mohamed *et al*., 2018).

Glycine betaine can quickly penetrate the leaves and be transported to the different plant organs where it can lead to enhancing environmental stress tolerance (Yang *et al*., 2005). Spraying of glycine betaine at 10 and 20 mM on Fortuna and Albion strawberry cultivars had a positive effect on plant growth, as evidenced by raised crown diameter and leaf area increased levels of leaf chlorophyll and enhanced content of nutrients such as nitrogen, phosphorus, potassium, zinc, boron, manganese, and iron (Adak, 2019). The foliar spray of glycine betaine at 2 and 4g/L on cherries cvs. Lapins and Regina increased the leaf mineral content, including nitrogen, phosphorus, potassium, iron, zinc, and manganese ppm (Li *et al*., 2019). Spraying of glycine betaine grape cv. King Ruby at 10, 20, and 30 mM led to increased vegetative growth such as the leaf chlorophyll, leaf number per plant, leaf area, the number of newly emerged branches per plant, and leaf mineral content, including iron, zinc, and manganese ppm (Hasnain *et al*., 2021).

Micronutrients are important for a variety of biological processes that may be linked to the development, productivity, and fruit quality attributes of fruit trees like pears in calcareous or alkaline soils. Although most micronutrients are inserted in the operation of many enzyme systems, there is wide diversity in the particular roles of the micronutrients such as Fe, Zn, Mn and B (Asaad, 2014; Atallah *et al*., 2010). Spraying of 2 kg/ha boron on pears at various growth stages, including before full bloom at green and white bud stage, full bloom, after flowering, and postharvest in the fall. The findings indicated that the application of boron at a rate of in spring or 0.8 kg/ha, resulted in increased tree vigor, and leaf content from K, and B (Wojcik and Wojcik, 2003). Spraying of boric acid at 200 and 400 ppm on 'Canino' apricot trees at full bloom stage raised the leaf content from N, P and K as compared with the control (Hassan *et al*., 2005). Using zinc at 0.25% or 0.5% zinc sulfate on papaya, two months after transplanting, resulted in a significant enhancement in plant growth, the leaf number per plant and the length of petioles (Singh *et al*., 2005).

Therefore, this study aims to study and compare the effect of foliar application of putrescine, glycine betaine and some micronutrients on improving vegetative growth and nutritional status of 'Le-Conte' pear tree cultivar.

MATERIALS AND METHODS

This experiment was carried out during the two successive seasons 2021 and 2022 on twelve years old 'Le-Conte' pear trees (*Pyrus communis L.*) budded on 'Butialefola' pear rootstock, planted at 4 x 6 meters apart (175 tree/feddan) grown in sandy soil under drip irrigation system in a private orchard located at El-Nubaria, EL-Beheira governorate, Egypt.

Fifty uniform trees were selected for this study and all of them were subjected to the same cultural practices during both successive seasons. The treatments were as follows: Control (distilled water), Putrescine at 20, 40 and 60 ppm, Glycine betaine at 30, 60 and 90 ppm, 250 ppm Fe + 250 ppm Zn + 250 ppm Mn +100 ppm H_3BO_3 and 500 ppm Fe + 500 ppm Zn + 500 ppm Mn + 200 ppm H_3BO_3 and 750 ppm Fe + 750 ppm Zn + 750 ppm Mn + 300 ppm H_3BO_3. The treatments were sprayed at three application times; at swollen buds, 15 days after the first and the third application 30 days after the second.

The above-mentioned treatments were applied and arranged in a Randomized Complete Block design (RCBD). Each treatment included five replicates with one tree for each replicate, and their effect was investigated by evaluating their influence on the following parameters:

Vegetative Growth:

At the end of growing seasons, the ten selected shoots were measured: The average of shoot length (cm), and shoot diameter (cm) using hand caliber. Leaf area (cm^2). Leaf chlorophyll indication (SPAD units): by chlorophyll meter apparatus in ten leaves from each plot according to the method described by Moran (1982).

Leaf Mineral Content from Macro and Micronutrients:

As for the influence of different used treatments on the leaf elements percent, samples consisting of twenty mature leaves were collected at random on August 20th, 2021 and August 23^{rd,} 2022 years. The leaves were washed several times with tap water, rinsed three times in distilled water, and then dried at 70-80°C in an electric air-drying oven. The dried leaves of each sample were ground in a porcelain mortar to avoid contamination with any minerals; 0.5 g from the ground dried material of each sample was digested with H_2SO_4 and H_2O_2 according to Evenhuis and Dewaard (1980). The digested solution was used for the determination of each nitrogen using the micro Kjeldhal method (Wang *et al.*, 2016), phosphorus by the vanadomolybdo method (Weiwei *et al.*, 2017) and potassium was determined by flame photometer according to the method described by Mutalik *et al.* (2011). The concentration of nitrogen, phosphorus and potassium were expressed as a percent, on a dry weight basis. Iron, manganese and zinc by a perkin-Elmer atomic absorption spectrophotometer model 305-B. Boron was determined colorimetrically by the carmine method (Hatcher and Wilcox, 1950). Iron, manganese, zinc and Boron were expressed as parts per million (ppm) on a dry weight basis.

Statistical Analysis:

The obtained data were subjected to one-way ANOVA according to (Ott and Longnecker, 2015) and the least significant difference (LSD) at 0.05% was used to compare the means of the treatments using CoHort Software (Pacific Grove, CA, USA).

RESULTS AND DISCUSSION

Vegetative Growth Parameters:

Shoot Length, Shoot Thickness and Leaf Area:

Contrasting to untreated trees, the data in Table 1 indicates that foliar applications of putrescine at 20, 40, and 60 ppm, glycine betaine at 30, 60, and 90 ppm, and micronutrients 1, 2, and 3 extremely enhanced shoot length, shoot thickness, and leaf area contrasted to the untreated trees in both testing times. The most favorable results were observed with the foliar application of putrescine at 60, 40, and 20 ppm, followed by glycine betaine at 90, 60, and 30 ppm, outperforming the other treatments in both experimental periods. Specifically, spraying putrescine at 60 ppm yielded superior results as to 40 or 20 ppm of putrescine and also surpassed the effects of spraying glycine betaine at 30, 60, and 90 ppm in both seasonals times.

These results were previously confirmed by the findings of Liu *et al.* (2006), who noticed that the enforcement of putrescine plays a pronounced role in alleviating the stress tolerance effect, promoting cell division and morphogenesis. Besides, putrescine can enhance the vegetative growth attributes in pears (Franco Mora *et al.*, 2005), apples (Malik *et al.*, 2006), pistachio trees (Talaie *et al.*, 2010), peach (Ali *et al.*, 2014), sour orange (Mohamed *et al.*, 2018), olive (Abd-Alhamid *et al.*, 2019). Additionally, many authors proved the positive effect of glycine betaine in improving the vegetative growth of grapes (Hasnain *et al.*, 2021) and oranges (Kheder and Abo-Elmagd, 2021). Additionally, it was noticed by many authors that the exterior spraying of Zn greatly improved the shoot length, shoot thickness and leaf area in papaya (Singh *et al.*, 2005), and sweet orange (Yadav *et al.*, 2007). Khafagy (2007) documented that spraying apple trees cv. Anna, with zinc, manganese, and $FeSO_4$ at 1.0%, along with boric acid at 400 ppm in March, April, and May included increased shoot length, diameter, and leaf area. Besides, the foliar application of 1.00 g of micronutrients per litre at complete flowering and fruit set resulted in a remarkable increment in shoot length, shoot diameter, leaf area, and chlorophyll content of leaves, in 'Le-Conte' pear trees (Asad *et al.,* 2013). Moreover, the application of boric acid considerably raised the shoot length, diameter and leaf area in apples (Sharma, 2016). Moreover, the application of the B + Zn + Mn + Fe combination greatly raised the apple growth parameters (Kuresova *et al.*, 2019).

Table 1: Spraying effect of putrescine, glycine betaine and some micronutrients on the shoot length, shoot thickness and leaf area of pear trees cv. Le-Conte during 2021 and 2022.

Treatments	Shoot length (cm)		Shoot thickness (cm)		Leaf area (cm^2)		Leaf chlorophyll (SPAD)	
	2021	2022	2021	2022	2021	2022	2021	2022
Control	60.4e	62.64e	0.62e	0.64d	36.56d	34.86d	43.72d	40.16d
20 ppm Putrescine	90.4ab	90.72ab	0.98abc	1.00ab	45.52ab	44.34ab	52.34a	51.1ab
40 ppm Putrescine	92.46ab	93.16a	1.04ab	1.10 a	46.18a	44.63ab	52.82a	53.16a
60 ppm Putrescine	96.02a	94.14a	1.08a	1.14a	46.27a	45.35a	53.34a	54a
30 ppm Glycine betaine	83.2bc	82.76bc	0.88bcd	0.88bc	42abc	41.30abc	50.16ab	47.56bc
60 ppm glycine betaine	84.02bc	83.9bc	0.90 abcd	0.90bc	42.21abc	41.33abc	50.36ab	47.74bc
90 ppm Glycine betaine	85.4bc	84.35bc	0.92abcd	0.98abc	44.48ab	44.25ab	50.52ab	48bc
Micronutrients 1	72.1d	70.2d	0.76d	0.78c	39.92c	38.63c	46.72c	44.2c
Micronutrients 2	74.14d	73.2d	0.80cd	0.84bc	41.28bc	39.48bc	46.76c	44.7c
Micronutrients 3	77.12cd	75.88cd	0.84cd	0.88bc	41.32bc	40.73abc	48.82bc	45c
$LSD_{0.05}$	6.55	6.67	0.13	0.14	2.91	3.46	2.199	3.40

The differences between the treatments that share the same letters in the same column are not statistically significant at a 0.05 level of probability.

Leaf Mineral Content from Macronutrients; N, P, and K Percentages:

Table 2 shows the increase in leaf macronutrients such as N, P, and K when putrescine, glycine betaine, and other micronutrients were sprayed with foliar as the control throughout the two seasonal periods. Putrescine therapies applied topically resulted in the highest increase in leaf nitrogen and phosphorus, followed by glycine betaine treatments.

Putrescine at 20, 40, and 60 ppm and glycine betaine at 30, 60, and 90 ppm were sprayed foliar to provide the best results as compared to the other required treatments in both seasons. Moreover, the application of specific micronutrients substantially increased the potassium content of the leaves compared with untreated trees in both seasons.

The best results were generally obtained from foliar spraying putrescine at 20, 40, and 60 ppm and glycine betaine at 30, 60, and 90 ppm, in comparison to the other treatments during both testing seasons. Furthermore, in comparison to trees that were not sprayed, the administration of certain micronutrients during both experimental seasons greatly increased the potassium content of the leaves.

These findings show a similar pattern to that observed by Abd-Alhamid *et al.* (2019) on 'Picual' olives and Naser *et al.* (2016) on date palms. They found that the putrescine spray enhanced the amounts of phosphate, potassium, and nitrogen in the leaves.

Spraying glycine betaine on 'Lapins' and 'Regina's cherries increased the leaf mineral content from N, P, and K (Li *et al.*, 2019). Furthermore, 'Deveci' pear plants' leaf content from nitrogen, phosphorus, and potassium rose with foliar application of glycine betaine (Kucukyumuk, 2021).

The application of some micronutrients raised the leaf nutritional content from N, P and K in 'Canino' apricot trees (Hassan *et al.*, 2005; El-Badawy, 2013), apple trees (Khafagy, 2007; Kuresova *et al.*, 2019), pomegranate (Hasani *et al.*, 2012). Furthermore, spraying of apple cv. Rubinola trees with B, Zn, Mn, and Fe significantly enhanced growth parameters such as shoot length, shoot thickness, and leaf area, and the levels of nitrogen, phosphorus, potassium, zinc, boron, manganese, and iron in both leaves and fruit compared to the control treatment (Kurešová et al., 2019). Spraying of boron, zinc, and iron at 0.5 and 1 g/L on 'Deveci' pear trees increased the the leaf mineral content from nitrogen, phosphorus, and potassium, when as to the control treatment (Serhat and Haluk, 2016).

Table 2: Spraying effect of putrescine, glycine betaine and some micronutrients on the leaf mineral content from nitrogen, phosphorous and potassium of pear trees cv. Le-Conte during 2021 and 2022.

Treatments	Nitrogen (N %)		Phosphorus (P %)		Potassium (K %)	
	2021	2022	2021	2022	2021	2022
Control	2.00e	1.95e	0.25d	0.26e	1.24e	1.25d
20 ppm Putrescine	2.27ab	2.30b	0.35ab	0.34b	1.37d	1.41c
40 ppm Putrescine	2.33a	2.33b	0.36a	0.35b	1.40cd	1.43c
60 ppm Putrescine	2.37a	2.39a	0.36a	0.37a	1.46bcd	1.47bc
30 ppm Glycine betaine	2.18b-d	2.20cd	0.29c	0.30d	1.43cd	1.44c
60 ppm Glycine betaine	2.20b-d	2.21c	0.30c	0.31d	1.42cd	1.47bc
90 ppm Glycine betaine	2.22bc	2.23c	0.30c	0.32cd	1.45b-d	1.52bc
Micronutrients 1	2.00d	2.14d	0.31bc	0.32cd	1.50bc	1.56b
Micronutrients 2	2.11cd	2.15d	0.31bc	0.33c	1.55b	1.65a
Micronutrients 3	2.17b-d	2.19cd	0.33a-c	0.34b	1.66a	1.72a
$LSD_{0.05}$	0.087	0.05	0.028	0.009	0.084	0.082

The differences between the treatments that share the same letters in any column are not statistically significant.

Leaf Mineral Content From Micronutrients; Iron, Zinc, Manganese and Boron:

Table 3 illustrates the impact of putrescine, glycine betaine, and some micronutrient applications on the leaf mineral content derived from micronutrients of 'Le-Conte' pear trees in the 2021 and 2022 growing seasons. When compared to the control, the iron, zinc, manganese, and boron mineral contents in the leaves were much higher after all the applied treatments. Throughout both experimental seasons, the administration of micronutrients resulted in the largest increase in the content of leaf micronutrients, followed by putrescine treatments and glycine betaine treatments.

These findings concur with those of Abd-Alhamid *et al.* (2019), who discovered that putrescine treatment raised the levels of Fe, Zn, Mn, and B in the leaves and flowers of 'Picual' olive trees.

Hasnain *et al.* (2021) discovered that glycine betaine markedly raised the Fe, Zn, and Mn content of the leaves of the grape cultivar King Ruby, following a similar pattern. Furthermore, according to Kucukyumuk (2021), pear trees cv. Deveci had higher leaf content of Fe, Zn, Mn, and B after applying glycine betaine.

The application of a mixture of B, Zn, Mn, and Fe enhanced the leaf content of Zn, B, Mn, and Fe in olive (Sourour *et al.*, 2011), 'Deveci' pear (Serhat and Haluk, 2016), and apple cv. Rubinola (Kuresova *et al.*, 2019). The application of micronutrients: 5% Fe, 2.48% Zn, and 3.5 % Mn on eleven-year-old 'Le Conte' pear trees in March, June, and August improved the leaf micronutrients like Fe, Mn, Zn, B ppm (Zaen El-Deen *et al.*, 2018).

Table 3: Spraying effect of putrescine, glycine betaine and some micronutrients on the leaf mineral content from iron (Fe), zinc (Zn), manganese (Mn) and boron (B) of pear trees cv. Le-Conte during 2021 and 2022.

Treatments	Fe ppm		Zn ppm		Mn ppm		B ppm	
	Seasons							
	2021	2022	2021	2022	2021	2022	2021	2022
Control	136.2e	130d	24e	24.4d	45.6f	46.4f	40.4e	41.2e
20 ppm Putrescine	153.4cd	160.2bc	30.8d	33bc	55.2cd	57.4cd	51c	51cd
40 ppm Putrescine	154.6cd	163bc	33.2c	34.4bc	56.4cd	58.2cd	51c	52.4c
60 ppm Putrescine	158.8c	166.6b	34c	36.4b	57.6c	59.2c	52c	54c
30 ppm Glycine betaine	144.4d	144.8c	29.4d	29.6c	50e	51.2e	44.6d	46d
60 ppm Glycine betaine	146.4d	147.8bc	30d	30c	52.6de	52.4e	46.2d	46.4d
90 ppm Glycine betaine	150cd	148bc	30.4d	30.4c	53.6cde	53.6de	47d	47.2d
Micronutrients 1	177.6b	183.6a	40.2b	42.8a	70b	71b	63.2b	65.4b
Micronutrients 2	187.2a	191a	43a	45.4a	75a	73.2b	65.8b	69.4ab
Micronutrients 3	192.4a	197a	45a	46.2a	77.2a	79a	69.4a	73a
$LSD_{0.05}$	7.36	14.20	2.02	4.51	3.52	3.99	3.28	4.30

The differences between the treatments that share the same letters in any column are not statistically significant**.**

Conclusion

Based on the results, it was concluded that spraying putrescene at 60 ppm and then at 40 ppm was the optimum application for producing the best results in terms of shoot length and thickness, leaf area, and leaf total chlorophyll. Furthermore, they significantly reduced fruit drop percentages during the test study seasons while improving fruit set percentages, fruit number, yield in kg per tree or tonnes per hectare, and leaf mineral content from nitrogen and phosphorus. Moreover, in both seasons, the surface application of putrescine at concentrations of 60 and 40 ppm demonstrated superior results in fruit physical characteristics, including size, length, diameter, and firmness when compared to other applications. Furthermore, the application of micronutrients 3, in comparison to other treatments during the two seasons, markedly improved various aspects, including fruit content of soluble solids, TSS-acidity ratio, as well as total, reduced, and non-reduced sugars, vitamin C, and leaf content of K, Fe, Zn, manganese, and boron.

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