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Response of "Le Conte" cv. Pear Trees to Some Bio-Stimulants Foliar Applications

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ABSTRACT: This study was carried out during 2021 and 2022 to investigate the role of spraying tryptophan, biozyme and fulvic acid on Le Conte pear cv. grown at a private orchard in Borg El- Arab, Alexandria, Egypt. The experiment design was a Randomized Complete Block Design (RCBD). Ten treatments with four replicates were applied three times; start of growing season, full bloom and one month after fruit set, the treatments were; control, tryptophan at 50, 100 and 150 ppm, biozyme at 2, 4 and 6 ppm and fulvic acid at 500, 1000 and 1500 ppm. The results showed that all treatments enhanced the shoot length and thickness, leaf area and total chlorophyll compared to the control, the highest value of shoot length was obtained by 1500 ppm fulvic acid in 2021 and 6 ppm biozyme in 2022, In the meantime, 1500 ppm fulvic acid caused significant increment, in shoot thickness, leaf area and total chlorophyll, fruit set (%), the number of fruits per tree, yield (kg/ tree) and the least fruit drop % in 2021 and 2022. All treatments enhanced the fruit physical and chemical properties and fulvic acid at 1500 ppm caused remarkable increment in fruit weight and length in the second season and fruit diameter in both seasons. Tryptophan at 150 ppm had the highest percentages of total soluble solids (TSS), total sugars and reducing sugars in the two seasons, while it caused the highest value of TSS/ acid in 2022 and non- reducing sugars % in the first season. In the meantime, fulvic acid at 1500 ppm significantly increased the TSS/ Acid in 2021 and 2022 seasons, in one side and reducing sugars % in the first season, on the other side. In contrast, all treatments reduced acidity (%) and ascorbic acid mg/100ml compared with control. The level of minerals improved by all treatments in comparison with the control, 6 ppm biozyme caused the highest value in phosphorus and potassium % and copper ppm in 2020 and 2021. 1500 ppm fulvic acid estimated the highest value in nitrogen (%), manganese and copper (ppm), whereas, the increase in leaf zinc ppm was obtained by 150 ppm tryptophan in the two seasons.

Keywords: pear; bio- stimulants; yield; fruit quality; mineral content

INTRODUCTION

Pear (Pyrus communis L.), belongs to family Rosaceae and is cultivated on an area of 1,379,387 hectares worldwide with the production of approximately 23,919,075 tons (FAO, 2019). In Egypt, pear cultivated area is 13439 Faddan produced 68407 Tons (Agricultural Statistics Institute, 2019). Pear is favored by consumers because of its distinctive scent, delicate aroma and sweetness, it is a common fruit found in temperate regions. Pyrus Communis and P. Serotina were crossed to create the hybrid known as Le-Conte, which is regarded as the primary pear cultivar farmed in Egypt, Le Conte pear trees, however, have difficulties in Egypt due to their poor fruit set and variable production. plant growth Environmental conditions, regulators, and nutrition are just a few of the many factors that have an impact on pear trees' fruit set and output. (Mosa et al., 2022; Jackson, 2003; Sanzol and Herrero, 2001).

Biostimulants considered to be a significant application in horticultural crops, these materials promote the plant growth under both optimal environmental conditions and when administered to species exposed to abiotic and biotic stresses (**Kisvarga** *et al.*, 2022; **Bashir** *et al.*, 2021). Recent years have seen a considerable increasing in soil fertility due to the use of biofertilization, improving the ability of plants to resist disease, as well as promoting growth, productivity, and fruit quality for a variety of fruit tree species (**Abd El Rahman and Bakr, 2022**).

Amino acids significantly influence plants' capacity to withstand abiotic stress through a variety of mechanisms, including pH regulation, maintaining cellular water by cellular osmotic change, a reservoir of nitrogen and carbon is created by increased photosynthesis and suitable giving osmolytes, plant cells mechanical toughness, and organizing stomatal opening and closing, as reported by Souri (2016) and Abd EL-Rahman (2022). Tryptophan is a fragrant amino acid produced by way of the shikimate pathway, which chorismate activates (Maeda and **Dudareva**, 2012), Having an α - amino group, an α -carboxylic acid group, side chains, Since it

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participates in the manufacture of the natural auxin, indole acetic acid, it becomes a very significant amino acid necessary for plant growth which affect plant output, growth, and chemical compound content (**Mahdi** *et al.*, **2023**).

Commercial growth stimulant Biozyme is nontoxic and environmentally safe; reported to be abundant in cytokinins and auxin precursor, enzymes, and hydrolyzed protein which influences plants physiological system at low concentrations (Sau et al., 2015). Application of biozyme, a bio-stimulant that boosts a plant's ability to significantly increase its absorption capacity, which is vital for enhancing the behaviour of trees, combining the power of natural plant extracts with naturally occurring macro and micronutrients (Mohammed and Naile, 2020). It contains minerals, that are naturally chelated, promotes cell growth and division for superior chlorophyll and increased output (Manna et al., 2012). In addition to enhancing fruit physico-chemical features, it also markedly increased plant growth, development of fruits, and yield on guava (Sau et al., 2015).

Fulvic acid is very efficient, it has a lower molecular weight than humic acid, ranging from about 1.000 to 10.000, contains important and effective molecular bonds that cause minerals and elements to disintegrate and expand into active fulvic complexes (Aiken *et al.*, 1985). It is crucial to organic and natural products in aquatic systems and very advantageous to both plants and soil because of crucial boosting microbial activity, also, it is regarded as a bio-stimulant for plant growth; as a chelating agent, it encourages nutrient uptake and improves vegetative traits, nutritional status, and leaf pigments (Mostafa *et al.*, 2017; El-Salhy*et al.*, 2023). The target in this

study to determine the impact of spraying various concentrations of tryptophan, biozyme and fulvic acid on the behavior of Le Conte cv. Pear trees.

MATERIALS AND METHODS

1. Plant Material, Treatments, Experimental Site and Design

The following study was carried out during 2021 and 2022 seasons to investigate the role of spraying tryptophan, biozyme and fulvic acid on 12- year- old Le Conte pear cultivar grown at a private orchard in Borg El- Arab at 29.55 longitude and 30.90 latitude, Alexandria, Egypt. Forty trees (5 x 5 m apart) as uniform as possible grown in calcareous soil and budded on (Pyrus communis L.) rootstock with drip irrigation, were selected for this study. The trees were uniform in their size shape and diseases free as well as received the cultural practices that are recommended by Ministry of Agriculture and were trained to the central leader system. The experiment design was a Randomized Complete Block Design (RCBD), with four replicates. The trees were sprayed three times; at the start of growing season in February, at full bloom in March and one month after fruit set. The treatments were; control (water only), tryptophan at 50, 100 and 150 ppm, biozyme at 2, 4 and 6 ppm and fulvic acid at 500, 1000 and 1500 ppm, Triton B was added as wetting agent to all spraying solutions at 0.1 %. Before the applications, soil samples were taken at a depth of 0: 30 and 30: 60 cm from different experimental sites, to determine physical and chemical properties of soil according to Piper (1950) as shown in Table (1).

 Table (1): Physical and chemical properties of experimental orchard soil

Depths	Sand	Silt	Clay	CaCo3	pН	EC	Anions (meq /L)		Cations (meq / L)						
(cm)		%		mg/L	ds	s/m	HCO ⁻³	CL-	$SO^{2}4^{-}$	NH4 ⁺	Ca ⁺²	Mg^{+2}	Na ⁺	\mathbf{K}^+		
0- 30	70.8	12	17.2	39.01	8.2	2.1	8.71	12.05	3.5	0.07	6.30	1.71	6.9	1.4		
30-60	71.5	11.9	16.6	37.65	8.2	1.9	8.13	11.88	3.4	0.08	5.70	1.67	6.2	1.2		
Average	71.15	11.95	16.9	38.33	8.2	2.0	8.42	11.96	3.45	0.07	6.0	1.69	6.5	1.3		

The molecular formula of tryptophan is $C_{11}H_{12}N_2O_2$, L-Tryptophan reagent grade, $\geq 98\%$ (HPLC) and its molecular weight is 204.23g mol-1 (Taiz and Zeiger, 2002), synonym(s): (S)-2-Amino-3-(3-indolyl) propionic acid, L-a-Amino-3-indolepropionic acid CAS Number: 73-22-3. PubChem Substance ID:24278135 -Sigma-Aldrich Company, India. Biozyme is produced by different agro-input agencies, the composition is more or less same and i.e. 78.7% biological active extracts of plant origin and growth regulators (32.2 ppm GA3, 32.2 ppm IAA and 82.2 ppm Zeatine), 1.88% plant micro-nutrients (0.49%Fe, 0.37% Zn, 0.12% Mn, 0.14% Mg, 0.30% B and 0.44% S),19.27% solvents and conditioners

(<u>https://www.agrogenial.com/</u>)(**Hassan** *et al.*, **2009**). Fulvic acid at %70 was used (100% - water soluble powder - Humate (Tianjin) International Ltd., Tianjin, China)

2. Vegetative Parameters

In the spring of each season, 20 non –fruiting shoots of spring cycle were tagged at constant height and at all direction of each tree, in the end of September, the average shoot length was measured in cm and shoot thickness (cm) was determined using a Digital Vernier Caliper. In August, leaf area (cm²) was estimated in fully mature leaves according to the formula: leaf area (cm²) = LA = 0.70 (L × W) – 1.06; LA = leaf area,

L is the length, and W is the width of the leaves (**Demirsoy, 2009**), while, total chlorophyll index was measured by chlorophyll meter (SPAD- 502, Minolta Co. Japan), an average of 4 measurements from different spots of leaf was considered as reported by **Yadava** (1986).

3. Fruit set, drop and number and the Yield

Eight branches in different sides of each replicate were tagged for determining the fruit set (%) and fruit drop (%) according to formula:

Fruit set (%) = Number of developed fruitlets/ Total number of flowers at full $bloom \times 100$.

Fruit drop (%) = Number of fallen fruits / Number of set fruitlets \times 100

Fruit drop (%) was measured by counting the number of dropped fruits after fruit set (mid of March) till the harvesting time (August).

Yield (Kg/ tree) was determined by collecting the fruits at maturity stage late of August from each tree and yield was estimated by multiplying the number of fruits with average fruit weight.

4. Fruit Physical Parameters

For each tree, 20 fruits were selected for determining fruit weight (g/ fruit); fruit samples were weighed in grams, average fruit length (cm) and diameter (cm) were measured by using Hand caliper. Length was measured in maximum vertical point, while diameter was measured from the widest point. Firmness (Ib/inch²) was estimated by the **Magness and Taylor (1925)** pressure tester of 5/16 inch plunger (mod. FT 02) (0–2 Lb., Via Reale, 63, 48011 Alfonsine, Italy).

5. Fruit Quality

The total soluble solids TSS (%) were determined in pear fruit juice using a hand refractometer (A.O.A.C. 2005). Acidity was measured colorimetrically based on estimated malic acid using five milliliters of the fruit juice of each fruit sample and titrated with sodium hydroxide solution of a known normality using phenolphthalein as an indicator (A.O.A.C. 2005), the results of these titrations were converted to percent of titratable acidity, where: 0.067= mille equivalent factor of malic acid, then TSS/ Acidity ratio was calculated. Estimated ascorbic acid in mg/100 ml juice was measured by utilizing 2,6dichlorophenol indophenols and 3% oxalic acid (Malik and Singh, 1980). Finally, total sugars (%) colorimetrically measured using phenol and sulphuric acid extracted from 5-gram pulp (Malik and Singh, 1980), reducing sugars (%) using the Nelson arsenate molybdate colorimetric method (Dubois et al., 1956). The difference between total sugars and reducing sugars used to compute non-reducing sugar (%).

6. Foliage Minerals Composition

Samples of 20 leaves sited in the middle of the shoots (Chuntanaperb and Cummings, 1981), were randomly chosen in each replicate during the last week of August, then cleaned, dried in oven at 70° C to a constant weight, grind and sample of 5 g was digested by H₂SO₄ and H₂O₂ according to Cottenie (1980). Total nitrogen and phosphorus the digested material were measured in colorimetrically according to Evenhuis and De waard (1980) and Jackson (1958), respectively. Potassium content was estimated by flame photometer (SKZ International Co., Ltd., Jinan, China) according to Estefan et al. (2013). Foliage Fe, Zn, Mn and Cu contents were identified by the atomic absorption (3300), Johnson and Ulrich (1959). Leaf nutrient elements content were expressed as a ratio of the leaf dry weight, i.e., percentage for N, P, and K and as a part per million (ppm) for Fe, Zn, Mn and Cu.

7. Statistical Analysis

The experiments designed in randomized complete block (RCBD) with four replications was used. Using the MSTAT software (Microcomputer Statistical Package) for analysis of variance (ANOVA) by **Russel**, (1986), the results of the measured parameters were subjected to computerized statistical analysis, and the means of the treatments were compared using LSD at 0.05, **Snedecor and Cochran (1990)**.

RESULTS AND DISCUSSION

1. Vegetative Growth Properties

Tryptophan, biozyme, and fulvic acid application significantly improved the vegetative traits of Le Conte pear trees; shoot length and thickness, leaf area, and total chlorophyll content in both growing seasons (**Table 2**). The highest significant value of shoot length was obtained by fulvic acid at 1500 ppm (50.05 cm) in the first season, and 6 ppm biozyme (56.41 cm) in the second season. It was clear that 1500 ppm fulvic acid caused significant increment, in shoot thickness (1.186 and 1.280 cm), leaf area (34.14 and 35.16 cm2) and total chlorophyll (49.41 and 52.09 SPAD) in 2021 and 2022, respectively.

El-Kenawy (2022), found that the increase in shoot length, leaf area, and leaf total chlorophyll of Red Roumy grapevines caused by the various amino acid treatments such as tryptophan, that may be due to the fact that as organic nitrogenous substances, amino acids, are used to create the structural building blocks for proteins, that is catalysed by ribosomes and results the polymerizing of amino acids to **Opik and Rolfe** (2005). According to **Maeda and Dudareva** (2012), It has an impact on the synthesis of auxin. Spraying tryptophan acid enhanced vegetative development (**Abd Elkader** *et al.*, 2020) The major role that amino acid is promoting cell division and elongation, glucose absorption, photosynthesis, nucleic acid synthesis, and protein synthesis, which improved vegetative growth and physiological state (El Sese *et al.*, 2020; Barghout and Mohammed, 2023).

Using biozyme in plant has a favorable effect on the development and vigor to tree leading to increased nutrient uptake and accelerated plant biozyme metabolite movement; whereas, application significantly increased vegetative traits; shoot length (cm), the number of leaves, leaf area (cm2) and leaf total chlorophyll content (SPAD) of pear trees as compared to control treatment, Sau et al. (2015) found the same results in guava, with the application of biozyme, leaf pigments (chlorophyll A, chlorophyll B, and total chlorophyll) has greatly risen. Such bio stimulants increase the photosynthesis and nutrient uptake; Iron and magnesium (Rieset al., 1978), those are necessary ingredients for the production of chlorophyll.

Fulvic acid has a positive impact on the length of shoots, the size of leaves, and the amount of chlorophyll in leaves. It's competent of quickly bind minerals to its molecular structure, which causes them to dissolve and be mobilized (Aiken et al.,1985;El-Salhyet al., 2023). Additionally, Chen et al. (2004) showed, fulvic acid is one of the primary components of premium foliar spray fertilizers, due to its ability to promote substance penetration to plant tissues and sections. Antioxidants, hormones including IAA and GA3, and cytokines are all made more readily available by fulvic acid, including a number of vitamins, such as Vit. B, which may explain why it has a positive influence on vegetative growth (Abd El-Hameed et al., 2014). Fulvic acid energizes and balances cells, fostering the best conditions for growth and replication while accelerating cell division and elongation (Mostafa et al., 2017). In addition, increasing apple shoot length and leaf area was more successful when fulvic acid was applied topically (Taha et al., 2016; Khan et al., 2019).

Table (2): Effect of tryptophan, biozyme and fulvic acid on shoot length(cm), shoot thickness, leaf area (cm²) and total chlorophyll (SPAD) of " Le Conte" pear cv. during 2021 and 2022

Treatments		Length m)		hickness m)	Leaf (cn	Area n ²)	Total chlorophyll (SPAD)		
	2021	2022	2021	2022	2021	2022	2021	2022	
Control	42.23 ^j	45.18 ^f	0.880 ^g	0.933 ^g	24.13 ^j	27.22 ⁱ	40.12 ^j	43.55 ⁱ	
Tryptophan (50 ppm)	44.49 ^g	48.68 ^{de}	0.936 ^f	0.980^{f}	27.7 ^g	29.87 ^f	42.39 ^h	45.30 ^g	
Tryptophan (100 ppm)	46.28^{f}	50.10 ^{cd}	1.013 ^d	1.04 ^e	2908 ^e	31.17 ^e	44.73 ^f	49.11°	
Tryptophan (150 ppm)	48.55°	53.91 ^b	1.083°	1.116 ^d	32.81°	34.95 ^b	46.2 ^e	49.89 ^b	
Biozyme (2 ppm)	44.4 ^h	48.24 ^e	0.94^{f}	0.983 ^f	26.97 ^h	29.15 ^b	42 ⁱ	44.93 ^h	
Biozyme (4 ppm)	47.82 ^d	51.02°	0.983 ^e	1.176°	30.15 ^d	33.13 ^d	46.62 ^c	48.39 ^e	
Biozyme (6 ppm)	49.51 ^b	56.41ª	1.166 ^a	1.250 ^b	33.65 ^b	35.15 ^a	46.53 ^d	48.65 ^d	
Fulvic acid (500 ppm)	44.21 ⁱ	47.39 ^e	1^{de}	0.976 ^f	25.16 ⁱ	29.67 ^g	42.39 ^h	46.08 ^f	
Fulvic acid (1000ppm)	46.81 ^e	50.06 ^{cd}	1.11 ^b	1.666°	28.94^{f}	33.7°	48.58 ^b	49.78 ^b	
Fulvic acid (1500ppm)	50.05 ^a	51.65°	1.186 ^a	1.280ª	34.14 ^a	35.16 ^a	49.41 ^a	52.09ª	

Same letters within a separate column indicate non-significant difference among treatments in each season at 0.05 level of probability.

2. Fruit Set and Drop (%), Number of fruits /tree and Yield(kg/ tree)

Fruit set (%), yield as Kg/ tree significantly were enhanced and the percentage of fruit drop was reduced by the spraying of, tryptophan, biozyme and fulvic acid compared to the control, **Table** (**3**). The greatest worth for fruit set (11.99 and 13.04 %), number of fruits/ tree (299.72 and 301.79) and yield (47.00 and 49.23 kg/ tree) and the least percentage of fruit drop was (88 and 86.95) for the 2021 and 2022, respectively. were obtained by the treatment of 1500 ppm fulvic acid. Productivity of pear was indeed dependent on proper conditions; especially temperatures, which negatively affect fruit set and yield, biostimulants may be crucial for increasing yield while lowering the ecological impact of fertilizer output and nutrient leaching into aquifer. Spraying tryptophan enhanced yield, according to **Abd-Elkader** *et al.* (2020). It enhances berry set by stopping the early flower and berry decline is crucial for the operation of the enzyme generation catalyses auxin synthesis response and as a result, berry set is improved (Saburi *et al.*, 2014). Concerning the action of tryptophan, endogenous IAA is thought to prevent ethylene from initiating abscission processes (Kim *etal.*, 2001).

Consistent with the results of the current study, the collaborative effects of biozyme recorded an increase in fruit yield in apple cv. Red Delicious (**Rieset al., 1978; Sharma, 1990**). In the current study in Table (3), the reduction in flower dropping and fruit sets being more plentiful may result from the influence of cytokinins and auxins, which delays abscission by preserving the loss of pectin material in the middle lamella, (**Kachave and Bhosale, 2007**) and strengthen resilience to nutritional stress and water stress (**Fujioka and Sakurai, 1997**), This increased the blooms' metabolite mobilization and photosynthesis (**Bhatia and Kaur, 1997**), also, the favorable impact of using biozyme on the yield might be due to increase in number of leave shoot and shoot length, consequently, the plants could use more sunshine to produce more. photosynthesis (**Zubair** *et al.*, **2017**)

Harhashet al. (2021) demonstrated that humic acid or fulvic acid boosted vine output. In the meantime, the application of 75% mineral-N plus humic or fulvic acids significantly improved yield/vine (El-Salhyet al., 2023). Molecules of fulvic acid (FA) can easily infiltrate plant stems, leaves, and roots. They convey traces from surfaces into tissues of the plant as they enter these plant components. It can easily bond the minerals and others in to its molecular component, which causes them to decomposed and become mobilized in fulvic complexes cells, which in turn affects plant development and yield (El-Salhyet al., 2023).

Table (3): Effect of tryptophan, biozyme and fulvic acid on fruit set (%), fruit drop (%), yield (kg/ tree) and the number of fruits/tree of " Le Conte" pear cv. during 2021 and 2022

	ci oi ii uito		pear ev. during 2021 and 2022							
						Number of Fruits/ tree				
2021	2022	2021	2022	2021	2022	2021	2022			
7.02 ^j	7.24 ^j	92.97ª	92.76 ^a	39.89 ^h	42.65^{f}	284.68^{j}	289.48^{i}			
8.14 ^h	8.94 ⁱ	91.85°	91.05 ^b	41.70 ^g	44.31 ^e	287.02 ⁱ	291.67 ^h			
8.95 ^g	10.14 ^f	91.04 ^d	89.85°	44.30 ^d	46.34 ^d	289.95^{f}	293.25 ^f			
10.51 ^d	12.05 ^c	89.49 ^g	87.94 ^h	46.36 ^c	48.50 ^b	293.58°	295.94 ^e			
8.95 ⁱ	9.28 ^h	91.94 ^b	90.72°	41.62 ^g	44.20 ^e	287.46 ^h	292.73 ^g			
9.06 ^f	10.71 ^e	90.93 ^e	89.29 ^f	43.65 ^e	46.42 ^d	291.03 ^e	297.07°			
11.15 ^b	12.91 ^b	88.84 ⁱ	87.08 ⁱ	46.68 ^b	48.65 ^b	298.85 ^b	300.06 ^b			
9.36 ^e	9.94 ^g	90.63 ^f	90.05 ^d	41.59 ^g	44.31 ^e	289.04 ^g	293.33 ^f			
10.73 ^c	11.57 ^d	89.26 ^h	88.43 ^g	43.02^{f}	46.71°	292.21 ^d	296.82 ^d			
11.99ª	13.04ª	88 ^j	86.95 ^j	47.00 ^a	49.23ª	299.72ª	301.79ª			
	(* 2021 7.02 ^j 8.14 ^h 8.95 ^g 10.51 ^d 8.95 ⁱ 9.06 ^f 11.15 ^b 9.36 ^e 10.73 ^c	$\begin{array}{c cccc} 7.02^{j} & 7.24^{j} \\ \hline 8.14^{h} & 8.94^{i} \\ \hline 8.95^{g} & 10.14^{f} \\ \hline 10.51^{d} & 12.05^{c} \\ \hline 8.95^{i} & 9.28^{h} \\ \hline 9.06^{f} & 10.71^{e} \\ \hline 11.15^{b} & 12.91^{b} \\ \hline 9.36^{e} & 9.94^{g} \\ \hline 10.73^{c} & 11.57^{d} \\ \end{array}$	$(\%) \qquad (\%) $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	(%)(%)(Kg/tree)2021202220212022202120227.02 ^j 7.24 ^j 92.97 ^a 92.76 ^a 39.89 ^h 42.65 ^f 8.14^{h} 8.94^{i} 91.85 ^c 91.05 ^b 41.70 ^g 44.31 ^e 8.95^{g} 10.14 ^f 91.04 ^d 89.85 ^e 44.30 ^d 46.34 ^d 10.51 ^d 12.05 ^c 89.49 ^g 87.94 ^h 46.36 ^c 48.50 ^b 8.95^{i} 9.28 ^h 91.94 ^b 90.72 ^c 41.62 ^g 44.20 ^e 9.06 ^f 10.71 ^e 90.93 ^e 89.29 ^f 43.65 ^e 46.42 ^d 11.15 ^b 12.91 ^b 88.84 ⁱ 87.08 ⁱ 46.68 ^b 48.65 ^b 9.36 ^e 9.94 ^g 90.63 ^f 90.05 ^d 41.59 ^g 44.31 ^e 10.73 ^c 11.57 ^d 89.26 ^h 88.43 ^g 43.02 ^f 46.71 ^c	(%)(%)(Kg/tree)Number of2021202220212022202120222021 7.02^j 7.24^j 92.97^a 92.76^a 39.89^h 42.65^f 284.68^j 8.14^h 8.94^i 91.85^c 91.05^b 41.70^g 44.31^e 287.02^i 8.95^g 10.14^f 91.04^d 89.85^e 44.30^d 46.34^d 289.95^f 10.51^d 12.05^c 89.49^g 87.94^h 46.36^c 48.50^b 293.58^c 8.95^i 9.28^h 91.94^b 90.72^c 41.62^g 44.20^e 287.46^h 9.06^f 10.71^e 90.93^e 89.29^f 43.65^e 46.42^d 291.03^e 11.15^b 12.91^b 88.84^i 87.08^i 46.68^b 48.65^b 298.85^b 9.36^e 9.94^g 90.63^f 90.05^d 41.59^g 44.31^e 289.04^g 10.73^c 11.57^d 89.26^h 88.43^g 43.02^f 46.71^c 292.21^d			

Same letters within a separate column indicate non-significant difference among treatments in each season at 0.05 level of probability.

3. Physical Characteristics of Fruits

Data in **Table 4**, showed a general improvement in fruit physical parameters; fruit weight (FW), length (FL), diameter (FD) and firmness (FF) by foliar tryptophan, biozyme and fulvic acid applications in compared to control. The application of tryptophan at 150 ppm resulted in a greatest increment of FW, FL and FD in the first season, and fruit FF in the two seasons. In the meantime, fulvic acid at 1500 ppm caused remarkable increment in both FW and FL in the second season and FD in both seasons.

L-tryptophan (TRP), an amino acid and a precursor of IAA, is used to promote citrus fruit set and size. Application of DL-TRP (10-7 M) on 'Frost' nucellar navel orange trees grown for commercial purposes at the end of cell division stage in fruit development (July), when fruit were at most peel thickness, greatly enhanced the 4-year cumulative total yield and yield of

commercially valuable large size fruit (6.9-8.8 cm), which is consistent with a positive effect of L-TRP on fruit set and size (**Pillitteri** *et al.*, **2010**). **Mosa** *et al.* (**2021**) found a high increase in fruit hardness by treatment of tryptophan and glycine on apple fruits.

Biozyme regulates the plant bio-physiological activities, which maintained higher photosynthetic activity even during the period of fruit growth and development, resulting in higher yield and yield characteristics (**Zubair** *et al.*, **2017**). Moreover, it might increase the uptake of elements along with water and thereby, raised the chlorophyll

concentration, which in turn enhanced the production; a positive linear association was discovered between the total number of fruits per tree and the weight of the fruits with the total chlorophyll content (Sau *et al.*, 2015). The improve in size of fruits by biozyme treatment could attribute to cell divide and cell elongate in growth process due to auxins, indols and cytokinins (Zubair *et al.*, 2017).

75% mineral-N plus fulvic and humic acids mostly improved berry weight (El-Salhyet al., 2023).

 Table (4): Effect of tryptophan, biozyme and fulvic acid on fruit weight (g), length (cm), diameter

 (cm) and firmness (Ib/ inch²) of " Le Conte" pear cv. during 2021 and 2022

<u>em) una minic</u>		Weight	•	Length	0	Diameter	Fruit Firmness		
Treatments	((g)	(cm)		(cm)	(Ib /	inch ²)	
	2021	2022	2021	2022	2021	2022	2021	2022	
Control	140.15 ⁱ	145.34 ⁱ	7.13 ^h	7.32 ^h	5.12 ^f	5.22 ^g	12.42 ^f	12.46 ^h	
Tryptophan (50 ppm)	145.3 ^g	151.93 ^g	7.36 ^e	7.78 ^e	5.27 ^d	5.41 ^e	12.56 ^e	12.67 ^e	
Tryptophan (100 ppm)	152.78 ^d	158.03 ^d	7.76 ^{bc}	7.93°	5.4 ^b	5.61 ^d	12.71°	12.77 ^d	
Tryptophan (150 ppm)	157.91ª	163.91 ^b	7.87ª	8.18 ^b	5.47ª	5.69 ^b	12.93ª	13.01 ^a	
Biozyme (2 ppm)	144.81 ^g	151.02 ^h	7.31 ^f	7.73 ^f	5.20 ^e	5.4 ^e	12.45 ^f	12.60 ^f	
Biozyme (4 ppm)	150 ^e	156.27^{f}	7.77 ^b	7.81 ^e	5.31°	5.59 ^d	12.58 ^e	12.84 ^c	
Biozyme (6 ppm)	156.22°	162.15 ^c	7.76 ^{bc}	8.17 ^b	5.41 ^b	5.65°	12.72 ^c	12.95 ^b	
Fulvic acid (500 ppm)	143.90 ^h	151.02 ^h	7.27 ^g	7.68 ^g	5.22 ^e	5.36 ^f	12.43 ^f	12.55 ^g	
Fulvic acid (1000ppm)	147.24^{f}	157.39 ^e	7.63 ^d	7.88 ^d	5.31°	5.59 ^d	12.63 ^d	12.79 ^d	
Fulvic acid (1500ppm)	156.84 ^b	168.13ª	7.73°	8.29ª	5.48 ^a	5.77ª	12.83 ^b	12.95 ^b	

Same letters within a separate column indicate non-significant difference among treatments in each season at 0.05 level of probability.

4. Fruit quality

The results in Table (5) showed that, the foliar spray of tryptophan, biozyme and fulvic acid had a beneficial effect on the fruit biochemical parameters in comparison to control treatment. The exogenous application of tryptophan at 15 ppm considerably increased the total soluble solids (11.63, 11.74 %), total sugars (8.06, 8.23 %), reducing sugars (5.12 and 5.57 %) in 2021 and 2022, respectively, while had the highest significant increase in TSS/ Acid (50.73) in 2022 and non- reducing sugars (2.93%) in the first season. In the meantime, fulvic acid at 1500 ppm significantly increased the TSS/ Acid (40.62 and 50.48 in 2021 and 2022 resp.), in one side and reducing sugars 5.14 % in the first season, in the other side. Acidity (%) and ascorbic acid (mg/100 ml) showed a reduction pattern as a result of application with tryptophan, biozyme and fulvic

acid compared with control. In 2021, the lowest significant value was 0.283 %, which obtained by 1500 ppm fulvic acid and 0.290 % obtained by the foliar application of 6 ppm biozyme, while the least value was 0.256 % obtained by tryptophan at 150 ppm and fulvic acid at 1500 ppm, in the second season. Whereas, in the first season, the least value in ascorbic acid was 13.18 and 13.20 mg/ 100 ml resulted from 150 ppm tryptophan and 6 ppm biozyme treatments respectively, while, the lowest value was14.07 mg/100 ml obtained by 1500 ppmfulvic acid in the second season.

Due to their vital role in producing chlorophyll molecules, which in turn had an impact on carbohydrates, amino acids had a promoting effect on the overall amount of carbohydrates (Ahmed *et al.*, 2017). Data reported by Mosa *et*

al. (2021) cleared that spraying glycine and tryptophan amino acids at 25, 50, and 100 ppm and their combinations over the course of two seasons significantly enhanced the fruit content of "Anna" apple as TSS %, total sugars, and reduced sugars, in contrast, they found a significant reduction in fruit acidity in comparison with control.

In the meantime, biozyme application stimulates the functioning of number of enzymes, higher TSS and total sugars content was the outcome of physiological mechanisms that hydrolyzed starch and assisted in the metabolic activity during the conversion of available starch into sugar, which had been cleared by **Jain** (2006). Our data showed that biozyme significantly improved the fruit quality (**Table 5**). Similarly, (**Sau** *et al.*, 2015) revealed that biozyme application increased TSS, total sugars and ascorbic acid content in guava. The improved fruit quality in higher TSS, total sugars and ascorbic acid and lower fruit acidity possibly a result of the treated plants

growing more vegetatively, which led to more photosynthates being produced (starch, carbohydrates etc.), the transfer to the fruits, raising the concentration of several fruit quality parameters (**Dutta** *et al.* **2014**).

Utilizing fulvic acid increased SSC% and SSC/acidity ratio while lowering the proportion of total acidity (Abd El-Hameed et al., 2014), it resembles the auxin hormone in plants, which is important for potassium absorption and starch In grapevines, fulvic metabolism. acid significantly enhanced the percentages of total sugars (El-Salhyet al., 2023). Like our findings, Zancaniet al. (2011) found that fulvic acids can play a beneficial role in the transport of hormones within plants and can increase intercellular ATP and glucose-6-phosphate levels, which have a positive correlation with the promotion of Greek fir cell culture growth. It promotes specific physiological processes that increase the pH of juice, and the vitamin C content of lemons (Citrus limon).

Treatments	Total soluble solids TSS (%)		Acidity (%)		TSS/Acidity		Ascorbic acid (mg/ 100 ml)		Total sugars (%)		Reducing sugar (%)		Non- reducing sugar (%)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
Control	10.64 ^h	10.73 ⁱ	0.453 ^a	0.426 ^a	23.48 ^g	29.21 ^e	13.93ª	14.89 ^a	7.14 ⁱ	7.23 ⁱ	4.80 ^g	4.90 ^g	2.34 ^e	2.33 ^f
Tryptophan (50 ppm)	10.95 ^f	11.07 ^f	0.416 ^b	0.380 ^b	26.29 ^f	33.35 ^d	13.62 ^c	14.76 ^b	7.35 ^g	7.46 ^g	4.89 ^{de}	5.13 ^e	2.45 ^d	2.32 ^f
Tryptophan (100 ppm)	11.25 ^d	11.29 ^e	0.383°	0.330°	29.36 ^e	38.72 ^c	13.37 ^d	14.52 ^d	7.75 ^d	7.83f	4.96 °	5.28 ^d	2.78 ^b	2.54 ^d
Tryptophan (150 ppm)	11.63ª	11 .74 ª	0.310 ^f	0.256 ^e	37.55 ^b	50.73 ^a	13.18 ^f	14.35°	8.06 ^a	8.23 ^a	5.12 ^a	5.57ª	2.93ª	2.66 ^b
Biozyme (2 ppm)	10.87 ^g	10.92 ^g	0.390°	0.386 ^b	27.90 ^{ef}	3261 ^d	13.62 ^c	14.64 ^c	7.29 ^h	7.39 ^h	4.85 ^{ef}	5.05 ^f	2.44 ^d	2.34 ^f
Biozyme (4 ppm)	11.14 ^e	11.35 ^d	0.356 ^d	0.323°	31.23 ^d	39.74 °	13.39 ^d	14.32 ^e	7.59 ^e	7.95 ^e	4.93 ^{cd}	5.34°	2.65 ^c	2.60 ^c
Biozyme (6 ppm)	11.43°	11.57 ^b	0.290 ^g	0.276d	39.47 ª	46.88 ^b	13.20 ^{ef}	14.24 ^f	7.95 ^b	8.07 ^c	5.01 ^b	5.51 ^b	2.94 ^a	2.56 ^d
Fulvic acid (500 ppm)	10.94 ^f	10.87^h	0.413 ^b	0.390 ^b	26.50 ^f	32.21 ^d	13.74 ^b	14.66 ^c	7.45 ^f	7.37 ^h	4.82 f ^g	4.93 ^g	2.63 ^c	2.43 ^e
Fulvic acid (1000ppm)	11.26 ^d	11.50 ^c	0.333 ^e	0.336 ^c	33.82 ^c	38.02 ^c	13.41 ^d	14.27 ^f	7.80 ^c	8.02 ^d	4.97 ^{bc}	5.16 ^e	2.83 ^b	2.86 ^a
Fulvic acid (1500ppm)	11.49 ^b	11.61 ^b	0.283 ^g	0.256 ^e	40.62 ^a	50.48 ª	13.24 ^e	14.07 ^g	7.98 ^b	8.12 ^b	5.14 ^a	5.51 ^b	2.84 ^b	2.61°

Table (5): Effect of tryptophan, biozyme and fulvic acid on fruit TSS(%), acidity (%), TSS/Acidity, ascorbic acid (mg/ 100ml) and total, reducing and non-reducing sugars of "Le Conte" pear cv. during 2021 and 2022

Same letters within a separate column indicate non-significant difference among treatments in each season at 0.05 level of probability

5. Foliage Mineral Composition

The influence of tryptophan, biozyme and fulvic acid as foliar applications on minerals in leaf of Le Conte trees were shown in Table 6. In general, all treatments improved the levels of minerals in comparison with the control, biozyme at 6 ppm treatment had the increment and significant value in P (0.710 and 0.770 %), K (1.81 and 1.91 %) and Cu (10.44 and 11.41 ppm) respectively. in 2020 and 2021. Fulvic acid at 1500 ppm estimated the highest value of N (2.33 and 2.43 %), Mn (142.89 and 149.56 ppm) and Cu (10.84 and 11.73 ppm), the increment value in leaf Zn ppm (119.03 and 123.52) was obtained by 150 ppm tryptophan in the two seasons.

Tryptophan appeared to be beneficial in increasing the N, P, K and Mg (%) in leaves (El-Kenawy, 2022). Tryptophan clearly improved the vegetative behavior and nutrient absorption, the plant uses acid ions quickly, allowing them to enter its cells where they activate the carbonmethane cycle and increase photosynthesis by allowing them to enter cells where they can install photosynthesis-specific enzymes (Villarreal et al., 2012; Khalil et al., 2023). According to Souri and Hatamian (2019), most amino acids form chelates with certain nutrients and have an affinity for these nutrients. They added that these characteristics help them to improve the uptake and delivery of micronutrients, particularly Fe, in plants (Basanth and Mahesh 2018; Souriet al. 2017). According to Radkowski et al. (2018), foliar applied with an amino acid preparation boosted the concentration of the investigated macro components. In the meantime, Mosa et al. (2021) treated Anna apple trees with 25, 50 and 100 ppm glycine or tryptophan and their combinations, and found a significant increase in N, P, K, Ca, Fe, Zn, Mn, and B.

Considering the findings of our study, the collaborative effects of biozyme as biostimulants in improving the macro and micro elements is shown in Table (5). Similar results were observed by **Sau** *et al.* (2015), they noticed a significant increase in macro and micro-nutrient at guava leaves, the enhancement in leaf nutrient content as a result larger flow of these nutrients from the source to sink (Mohammed and Naile, 2020). Foliar spray of biozyme (bio growth regulators) merges the properties of natural extract from living organisms and mineral content improves the absorption of the plant to significantly boost its uptake capacity (Kumar *et al.*, 2021).

In comparison to the control, the foliar application of FA increased the leaf mineral content of phosphorous, potassium, nitrogen, zinc. manganese, and iron in apricot (Haggag et al. 2016), it typically contains 70 or more minerals and trace elements in its molecular complexes (Aiken et al., 1985), which increases nutritional status, and leaf pigments while acting as a chelating agent for nutrients (Mostafa et al., 2017; El-Salhyet al., 2023). According to Bocanegra et al. (2006), fulvic acid has the power to chelate nutrients like Fe and transport them through cell membranes. They also noted that it is more acidic overall, contains more carboxyl groups than humic acid, has stronger adsorption and cation exchange capabilities, and may act as a natural chelator in the movement and transfer of micronutrients.

	N (%)	P(%	()	K(*	%)	Fe(p	pm)	Mn(j	opm)	Zn (j	opm)	Cu (j	ppm)
Treatments	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
Control	1.72 ^g	1.82 ^h	0.530^{f}	0.576 ^e	1.31 ^h	1.45 ^h	106.96 ^j	110.32 ^h	102.62 ^h	106.35 ⁱ	78.73 ^g	82.51 ⁱ	6.14 ^d	7.48 ^d
Tryptophan (50 ppm)	1.85 ^f	1.94 ^f	0.580 ^{def}	0.626 ^d	1.44 ^g	1.55 ^g	112.82 ⁱ	118.21 ^g	114.15 ^g	117.51 ^h	97.59°	103.83 ^e	7.48 ^c	9.31°
Tryptophan (100 ppm)	1.96 ^e	2.05 ^d	0.606 ^{bcde}	0.683°	1.68 ^d	1.71 ^d	129.69 ^f	131.22 ^e	120.73 ^e	125.08 ^g	103.85 ^b	107.27 ^d	9.14 ^b	10.51 ^b
Tryptophan (150 ppm)	2.12 ^c	2.19°	0.643 ^{bc}	0.720 ^b	1.73 ^{bc}	1.81°	160.46ª	165.1 ^b	126.17 ^d	133.8 ^d	119.03 ^a	123.52 ^a	10.50 ^a	11.25ª
Biozyme (2 ppm)	1.86 ^f	1.89 ^g	0.560 ^{ef}	0.586 ^e	1.51 ^f	1.61 ^f	117.47 ^h	124.54 ^f	117.79 ^f	126.30 ^f	85.11 ^f	88.58^{h}	7.61 ^c	8.92 ^c
Biozyme (4 ppm)	2.04 ^d	1.97 ^e	0.660 ^{ab}	0.706 ^b	1.70 ^{cd}	1.78°	144.93 ^d	157.72°	125.19 ^d	133.37 ^d	91.80 ^e	96.88^{f}	9.05 ^b	10.62 ^b
Biozyme (6 ppm)	2.19 ^b	2.35 ^b	0.710 ^a	0.770 ^a	1.81 ^a	1.91ª	125.19 ^g	131.22 ^e	133.03°	139.96°	103.07 ^b	118.19 ^b	10.44 ^a	11.41 ^a
Fulvic acid (500 ppm)	1.86 ^f	1.92 ^{fg}	0.613 ^{bcde}	0.570 ^e	1.46 ^g	1.56 ^g	137.44 ^e	145.84 ^d	120.08 ^e	128.59 ^e	85.55 ^f	93.10 ^g	7.36 ^c	9.03°
Fulvic acid (1000ppm)	2.07 ^d	2.17°	0.600 ^{cde}	0.676°	1.63 ^e	1.67 ^e	150.41°	157.68 ^c	137.67 ^b	142.96 ^b	91.82 ^e	103.64 ^e	8.79 ^b	10.28 ^b
Fulvic acid (1500ppm)	2.33ª	2.43ª	0.636 ^{bcd}	0.766ª	1.74 ^b	1.85 ^b	157.36 ^b	166.86ª	142.89ª	149.56ª	95.79 ^d	110.25°	10.84ª	11.73ª

Table (6): Effect of tryptophan, biozyme and fulvic acid on foliage macro (N, P and K %) and micro (Fe, Mn, Zn and Cu ppm) elements of "Le Conte" pear cv. tree during 2021 and 2022

Same letters within a separate column indicate non-significant difference among treatments in each season at 0.05 level of probability.

CONCLUSION

Tryptophan, biozyme and fulvic acid applications enhanced significantly the shoot length and thickness, leaf area and chlorophyll, as well as, fruit set (%), the number of fruits per tree, yield (kg/ tree) and decreased the fruit drop (%),in the meantime, they improved the fruit physical and chemical parameters, in addition to leaf mineral content compared to the control in 2021 and 2022. The most noticeable result was obtained by 1500 ppm fulvic acid, 6 ppm biozyme, and 150 ppm tryptophan comparing with other treatments and the control in the two experimental seasons.

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الملخص العربى

استجابة اشجار الكمثرى صنف ليكونت لمعاملات الرش ببعض المنشطات الحيوية ريحاب محمد عوض قسم الانتاج النباتي (البساتين- الفاكهه) كلية الزراعة - سابا باشا - جامعة الاسكندرية

تمت هذه التجربة خلال عامي 2021 و 2022 لدراسة دور الرش بالتربتوفان و البيوزيم وحامض الفولفيك على اشجار الكمثرى صنف الليكونت و المنزرعة في مزرعة خاصة – يرج العرب – الاسكندرية. تصميم التجرية قطاعات عشوائية كاملة. تم تطبيق 10 معاملات بأربع مكررات في ثلاث مواعيد: في بداية موسم النمو– أثناء الازهار الكامل وبعد عقد الثمار بشهر. وكانت المعاملات هي: الكنترول– التربتوفان بتركيز 50 و 100 و 150 جزء في المليون- البيوزيم بتركيز 2 و4 و6 جزء في المليون- وحامض الفولفيك بتركيز 500 و 1000 و 1500 جزء في المليون. وكانت النتائج كالاتي: أدت جميع المعاملات تحت التجرية الي تحسين طول وسمك الفرخ الخضرى (سم) والمساحة الورقية سم² و الكلوروفيل الكلى SPAD مقارنة بالكنترول. وكانت أعلى قيمة لطول الفرخ الخضري نتجت من المعاملة 1500جزء في المليون حمض الفولفيك في عام 2021 و المعاملة 6 جزء في المليون بيوزيم لعام 2022. كما أعطت المعاملة 1500 جزء في المليون حمض الفولفيك أعلى قيم لسمك الفرخ (سم) - المساحة الورقية (سم²) و الكلوروفيل الكلي (SPAD) -النسبة المئوية لعقد الثمارو عدد الثمار لكل شجرة و المحصول بالكجم/ شجرة بينما ادى الى اقل نسبة مئوبة لتساقط الثمار خلال موسمي الدراسة. أدت جميع المعاملات الى تحسين الصفات الفيزيائية و الكيميائية: حيث أدت المعاملة 1500 جزء في المليون حامض الفولفيك الي اعلى زيادة في وزن (جم) وطول الثمار (سم) في الموسم الثاني وقطر الثمار (سم) في الموسمين. المعاملة 150 جزء في المليون من التريتوفان ادت الي اعلى قيمة كنسبة مئوية للمواد الصلبة الذائبة الكلية و السكريات الكلية و المختزلة في الموسمين و النسبة بين المواد الصلبة الذائبة الكلية الى الحموضة في موسم 2022 و السكرات الغير مختزلة للموسم الاول. كما ادت المعاملة 1500 جزء في المليون حامض الفولفيك الى اعلى زيادة معنوية لنسبة المواد الصلبة الذائبة الكلية الى الحموضة للموسمين من جانب وايضا كاعلى نسبة مئوية للسكريات المختزلة خلال الموسم الاول من جانب اخر . وعلى العكس: ادت جميع المعاملات الى انخفاض النسبة المئوية للحموضة وحمض الاسكورييك (مجم/ 100 مل) مقارنة بالكنترول. ايضا جميع المعاملات عملت على تحسين محتوى الاوراق من العناصر مقارنة بالكنترول حيث ان اعلى محتوى من الفوسفور و البوتاسيوم و النحاس خلال الموسمين ناتجة من المعاملة 6 جزء في المليون من البيوزيم كما ان اعلى قيمة من النيتروجين و المنجنيز و النحاس ناتجة من المعاملة 1500 جزء في المليون حمض الفولفيك بينما اعلى قيمة لمحتوى الاوراق من الزنك كانت من المعاملة 150 جزء في المليون تربتوفان خلال الموسمين.

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