



## Effect of Molybdenum and *Azospirillum brasiliense* Inoculation on Growth and Yield of Pear Trees under Calcareous Soil Condition

Laila Y. Mostafa<sup>1</sup> and Eman M. EL. M. Abd EL-Mageid<sup>2</sup>

<sup>1</sup>Tropical Fruit Res. Dept., Hort. Res. Inst., Agric. Res. Center, Giza, Egypt.

<sup>2</sup>Deciduous Fruit Res. Dept., Hort. Res. Inst., Agric. Res. Center, Giza, Egypt.

Received: 03 January 2021

Accepted: 15 March 2021

Published: 20 March 2021

### ABSTRACT

This investigation was conducted during two successive seasons 2019-2020 on Le- Conte pear trees grown in calcareous soil at AL- Atebaa orchard EL-Nubaria region, EL-Beheira Governorate, Egypt. It intended to find out the possibility of enhancing Le-Conte pear trees under the newly reclaimed land .Thus , this study aimed to evaluate the performance of *Azospirillum brasiliense*, a nitrogen -fixing bacterium and efficiency of molybdenum for stimulating nitrogen fixation during fertilization .The trees received the recommended dose of mineral fertilizer only or combined with biofertilizer with or without molybdenum in order to study their influence on the yield and fruit physical and chemical quality characteristics. The results revealed that, Le-Conte pear trees treated with molybdenum (Mo) either with 500g ammonium sulphate +150g *Azospirillum* or 1000g ammonium sulphate +100g *Azospirillum* produced the highest number of fruit/tree, yield and improved leaf mineral content of N,P,K as compared with control. It was also noticed that combined treatment of 500g ammonium sulphate +150g *Azospirillum* +Mo is the superior treatment for increasing shoot length, leaf area, total chorophyll, fruit weight ,length and diameter, TSS%,TSS/acid and total sugars content and decreasing fruit acidity. All treatments with biofertilizers with or without Mo resulted in decreasing fruit lead, cadmium and nitrite content more than mineral fertilization. Hence, it could be concluded that, minimizing the use of chemical nitrogen fertilizer through biofertilizer addition and also, as promising alternative for chemical fertilizers to avoid pollution and reduce costs.

**Keywords:** mineral, molybdenum, biofertilizers, pear, yield, vegetative growth, fruit quality

### 1. Introduction

“Le-Conte” pear (*Pyrus communis*) is one of the deciduous fruits successfully grown and widely spread in newly reclaimed land in Egypt. The cultivated area with pear in Egypt has enormously increased through the last decades reaching about 12182 fed. The fruiting area reached 9515 fed. Producing about 58344 tons with average of 6.13 tons/fed. (Annual Reports of Statistics of Ministry of Agriculture, 2014). The pears investigated in the present study were grown in calcareous soil. These conditions strongly influence the amount of nutrient uptake. Therefore, the adaptation of a proper fertilization program, including adequate methods and timing are important strategies for obtaining better yield and fruit quality (Fageria and Baligar 2005). It is well known that nitrogen fertilizers are lost via nitrate reduction, denitrification and ammonia volatilization. Moreover, some nitrogenous fertilizer can be leached to the underground water causing environmental pollution. Pollutants are transferred through the plants to human and animals causing serious diseases. Thus environmental pollution as a result of exaggeration in the application of chemical fertilizers is reported by Nijjar, (1985), Casale *et al.*, (1995) and Ram Rao *et al.*, (2007). Recently, great attention has been focused on the possibility of using bio fertilizers for enhancing growth and fruit quality and depressing pollution occurring in the Egyptian environment.

Molybdenum is a trace element in the soil essential for plants. Nitrogen fixing microbes need molybdenum during fixation processes as it is a constituent of nitrogenase enzyme. Moreover,

**Corresponding Author:** Laila Y. Mostafa, Tropical Fruit Res. Dept., Hort. Res. Inst., Agric. Res. Center, Giza, Egypt. E-mail: layla\_yehya2009@yahoo.com

molybdenum is needed for nitrate reduction, nitrogen fixation and general metabolism in plants (Togay *et al.*, 2008). Previous studies emphasized the necessity of using molybdenum for stimulating nitrogen fixation during the application of mineral and bio fertilization (Yagodin, 1990 and Miller *et al.* 1990). Adjusting the uptake of N by biofertilization as well as application of molybdenum was accompanied with enhancing growth as well as yield quantitatively and qualitatively at the same time reducing nitrite pollution in our environment (Abada *et al.*, 2010 and Abd- El-Aziz, 2011).

*Azospirillum* is a rhizosphere microorganism which has potential use to stimulate growth and increase yield in apple, citrus, olive, pomegranate and apricot (Aslantas *et al.*, 2007, Hafez., 2013 and Esitken *et al.*, 2010). In this respect, Boddy *et al.*, 1991 found that about 60-80% of total nitrogen came from nitrogen fixation by *Azospirillum* on sugar cane plants. Moreover, phytohormones, like auxins, cytokinins, gibberellins and ethylene can be synthesized by beneficial microorganisms (Esitken *et al.*, 2006). These plant hormones regulate multiple physiological processes. During the last decade, bio fertilizers were used to improve soil fertility status and for the enhancement of crop production by their biological activity in rhizosphere( El-Haddad *et al.*, 1993, Casale *et al.*, 1995 and Ram Rao *et al.*, 2007). Therefore, the present investigation was planned and conducted to evaluate the effect of using some types of bio fertilizers with or without molybdenum on reducing the recommended doses of chemical fertilizers to achieve better growth, yield, fruit quality and leaf mineral content of Le-Conte pear trees grown in calcareous soil. The final target is to produce a safe and healthy food free of chemical pollutants.

## 2. Materials and Methods

The present investigation was conducted during the two growing seasons of 2019 and 2020 on 21 uniform in vigor 15- years old Le-Conte pear trees (*Pyrus communis* cultivar) budded on *Pyrus betulaefolia* rootstock and grown in a private orchard situated at Alexandria Egypt Desert Road, Nubaria region. The chosen trees were spaced at 5× 5 m apart. The texture of the soil is Loamy sand. Drip irrigation system was followed. Soil analysis was done according to the procedure of Wilde *et al.*, (1985) and the obtained data are shown in Table (1):

**Table 1:** Analysis of the tested soil.

Characters	Values
Sand %	88.0
Silt %	7.5
Clay %	4.5
Texture	Loamy Sand
O.M. %	0.11
pH	8.09
E.C ds/m	8.20
CaCO <sub>3</sub> %	12.3
Total N %	1.68
Available P	12.6
Available K	116.0

The chosen trees received all horticultural practices that were commonly applied in the orchard except those dealing with mineral, organic and bio fertilization. The present experiment included the following seven treatments:

T1- Control 2000 g ammonium sulphate / tree/ yr.

T2- 1500 g ammonium sulphate / tree/ yr + 50 g *Azospirillum brasiliense* without molybdenum (MO).

T3- 1500 g ammonium sulphate/ tree/ yr + 50g *Azospirillum brasiliense* + 5 g ammonium molybdate (MO).

T4- 1000 g ammonium sulphate/ tree/ yr + 100 g *Azospirillum brasiliense* without molybdate (MO).

T5- 1000 g ammonium sulphate/ tree/ yr + 100 g *Azospirillum brasiliense* + 5 g ammonium molybdate.

T6- 500 g ammonium sulphate/ tree/ yr + 150 g *Azospirillum brasiliense* without molybdenum (MO).

7- 500 g ammonium sulphate/ tree/ yr + 150 g *Azospirillum brasiliense* + 5 g ammonium molybdate (MO).

Each treatment was replicated three times, one tree per each. After adding the farm manure in the winter, *Azospirillum* was added (in the form of Nitrobin bio fertilizer) with ammonium molybdate in the first week of February in two seasons almost over the farm manure in large hole, covered and left for two months before applications. This mixture was added once in  $0.5 \times 0.5 \times 0.5 \text{ m}^3$  dimensions digs around each tree about 75 cm far from trunk. Ammonium sulphate (20.6 %N) as a source of mineral N was added at three equal batches on the third week of Apr., Jun and Agust far from the place of addition of *Azospirillum* and Mo.

Molybdate was added in large hole, covered and left to stand for two weeks before application. This mixture was added once at the last week of Jan. in  $0.5 \times 0.5 \times 0.5 \text{ m}^3$  dimensions digs around each tree about 75 cm far from trunk.

The following parameters were measured for both seasons:

- Leaf area ( $\text{cm}^2$ ): Samples of twenty leaves from the middle part of the shoots according to Summer (1985) were selected at randomly from each tree (1st week of June) to measure their area ( $\text{cm}^2$ ) according to Ahmed and Morsy (1999).
- The average total chlorophyll content: leaves were tested at the end of August in field using Minolta meter SPAD-502.
- Length of the new developed shoots (cm): Ten of one year old shoots were collected from around the canopy representing the four main directions were tagged for measuring new developed shoots length at the end of growing season in September.
- Macronutrients in leaves: at the end of the experimental season (at the end of September), twenty leaves/tree were collected and NPK was colorimetrically determined according to Wilde *et al.*, (1985).
- The leaf nitrate reductase activity was assayed as described by Perez and Kliewer (1978) in 300 mg finely chopped leaf material. The enzyme activity was expressed as  $\mu\text{g NO}_2$  produced per gram fresh weight per hour.
- The tree yield (Kg /tree), yield (ton/feddan) and number of fruits /tree were recorded.
- Fruit parameters: fruits sample was taken at the harvest time to be used for determining the physical properties (i.e., fruit weight (gm), fruit length (cm), fruit diameter (cm))
- Fruit quality: a sample of 10 mature fruits of each tree was taken at the harvest time to be used for determining the chemical properties i.e., the total soluble solids percentage (TSS%) was measured by using a hand refractometer, the acidity % as citric acid content using fresh juice with titration against 0.1 NaOH, and total sugars content were determined according to A.O.A.C (1995). Also, pulp content of nitrite as ppm determined according to Ridnour- Lisa *et al.*, (2000), the concentration of Cd and lead were determined by Perkin Elmer Atomic Absorption Spectrophotometer Model 305 B and expressed as part per million (on dry weight basis).

### Statistical analysis

The obtained data were subjected to analysis of variance in a randomized complete block design (RCBD), according to Snedecor and Cochran (1980). The averages were compared by using the method of new least significant differences (New L.S.D.) described by Waller and Duncan (1969).

## 3. Result and Discussion

### 3.1. Vegetative growth

Data presented in Table (2) clearly showed that the combined applications of ammonium sulphate and *Azospirillum* with or without molybdenum (Mo) induced high positive effect on all the vegetative growth parameters compared to the control treatment in both seasons. It was clear that the highest value for leaf area and shoot length were achieved by applying 500g ammonium sulphate + 150g *Azospirillum* either with Mo or without. While the lowest values for these parameters were obtained by control in both seasons. These results are in the same direction with those of Abdul Jabbar *et al.*, (2014) who found that the interaction between *Azospirillum brasiliense* and molybdenum was beneficial for plant biomass of soybean plant. The increment of biomass was related to the ability of *Azospirillum* in fixing nitrogen and producing growth regulators which promoted root and shoot development (Wani *et al.*, 1988). Increased nitrogen availability in plant rhizosphere created sponge effect for root and also synthesised indol-3-acetic acid (Tien *et al.*, 1979). As a consequence, plant biomass was increased through increasing root length surface area and the number of root hairs ( Kapulnik *et al.*, 1985).

### 3.2. Total chlorophyll

Table (2) illustrated that all tested treatments succeeded in improving total chlorophyll content in 2019 and 2020 seasons. Briefly, Mo treatments either with T7 (500 g ammonium sulphate + 150g *Azospirillum*) or with T5 (1000g ammonium sulphate + 100g *Azospirillum*) exerted similar and higher pronounced effect on total chlorophyll content and proved to be the superior treatments in this concern. While control treatment (T1) gave the lowest one. These results are in accordance with those reported by Abou- El Khashab (2002), on olive seedlings. The increase of chlorophyll content might be a result of balanced nutritional environment in the soil and thus kept iron physiologically active for chlorophyll synthesis in certain plants (El-Morshedy, 1997 and Abou – El Khashab, 2002).

**Table 2:** The effect of molybdenum along with mineral N and biofertilizers on leaf area, shoot length and total chlorophyll of Le-Conte pear in 2019 and 2020 seasons.

Treatments	Leaf area (cm <sup>2</sup> )		Shoot length (cm)		Total chlorophyll	
	2019	2020	2019	2020	2019	2020
T1 : Control	24.87	24.87	35.40	36.83	48.77	49.07
T2 : 1500g ammonium sulphate + 50g <i>Azospirillum</i>	34.39	31.93	38.63	40.23	53.90	53.63
T3 : 1500g ammonium sulphate + 50g <i>Azospirillum</i> + Mo	35.47	36.63	41.93	42.77	55.0	55.20
T4 : 1000g ammonium sulphate + 100g <i>Azospirillum</i>	36.10	36.83	40.40	40.33	54.77	54.23
T5 : 1000g ammonium sulphate + 100g <i>Azospirillum</i> + Mo	39.63	42.0	46.30	46.63	56.10	56.83
T6 : 500g ammonium sulphate +150g <i>Azospirillum</i>	40.80	41.70	49.80	48.53	55.53	55.50
T7 : 500g ammonium sulphate +150g <i>Azospirillum</i> + Mo	42.90	41.77	49.83	50.33	59.0	58.63
New LSD 0.05	0.88	2.12	1.33	1.36	0.90	0.55

### 3.3. Leaf Nitrate Reductase Activity (NR)

Regarding the effect of different levels and combinations of mineral and bio fertilizers with or without molybdenum (Mo) during 2019 and 2020 seasons Table (3), the obtained results indicated that the highest value of NR activity (4.03 and 4.07 µg NO<sub>2</sub> (g.fw.hr)<sup>-1</sup>) was noticed by applying 500g ammonium sulphate + 150 g *Azospirillum* + Mo (T7) comparing with all other treatments. In the meantime, T5 (1000g Ammonium sulphate + 100 g *Azospirillum* + Mo) and T6 (500g ammonium sulphate +150g *Azospirillum*) gave the second highest NR activity values on the other hand, treatment T2 (1500g Ammonium Sulphate + 50g *Azospirillum*) gave the lowest value (1.47 and 1.47 µg NO<sub>2</sub> (g.fw.hr)<sup>-1</sup>) among treatments except T1 (control). These results partially agreed with those reported by Khan *et al.*, (1995) who reported that inoculation of N either as NO<sub>3</sub> or NH<sub>4</sub> to the nutrient medium in substantial enhancement of NR activity of Alfalfa plants. El-Komy *et al.*, (2004) indicated that the positive role of cyanobacteria inoculated organic manure could be associated with its effect on enhancement of nitrate reductase (NR). Likewise, Anbuselvi *et al.*, (2011) revealed that, maximum amounts of NR were found to be higher in plant which inoculated with bio fertilizer. Furthermore, the enhancement of NR activity by the Mo may be attributed to that it is an element indispensable in the process of nitrogen fixation and plant metabolism in general, because of its effect on the nitrogenase and nitrate reductase activities (Sagi *et al.*, 2002, Pollock, *et al.*, 2002 and Mendel and Hansch, 2002). In addition, the stimulatory effect of Mo application was associated with increased Mg<sup>++</sup> content which was accompanied with significant elevation of nitrate reductase activity (Abd-El-Samad *et al.*, 2005).

### 3.4. Leaf mineral content

Concerning the results in Table (3), nitrogen, phosphorus and potassium contents in the leaves were significantly affected by all tested treatments in both seasons. However, applying trees with T7 (500g ammonium sulphate +150g *Azospirillum* + Mo) gave the best leaf nitrogen content (1.81% and 1.81%), phosphorus (0.194 and 0.192%) and potassium (1.83 and 1.82%) in 2019 and 2020 seasons, respectively followed by applying trees with T5 (1000g ammonium sulphate + 100g *Azospirillum* + Mo) and T6 (500g ammonium sulphate + 150g *Azospirillum*). On the other hand, control was the lowest in leaf nitrogen content (1.37 and 1.31%), phosphorus (0.112 and 0.118%) and potassium (1.29 and 1.23%, respectively). These results could be due to the important role of biofertilizers in fixing bacteria to supplying the soil and plants by available nitrogen to be absorbed and metabolized by plant. (Gogoi *et al.*, 2004 and Zeid *et al.*, 2009). Also, releasing organic acids and enzymes, decreased soil pH and enhanced the availability of nutrients. (Abd-El-Matty 2010 and Ahmed *et al.*, 2011). The great benefits of molybdenum on enhancing the activity of dehydrogenase as well as growth of bacteria were obtained by Kannaiyan, (2002), Hammam *et al.*, (2003) and Morsi (2009).

**Table 3:** The effect of molybdenum along with mineral N and biofertilizers on leaf Nitrate Reductase (NR) activity and leaf content of N, P, K of Le-Conte Pear in 2019-2020.

Treatments	Leaf NO <sub>2</sub> µg (g.fw.hr) <sup>-1</sup>	Leaves							
		N%		P%		K%			
		2019	2020	2019	2020	2019	2020	2019	2020
T1 : control	0.85	0.94	1.37	1.31	0.112	0.118	1.29	1.23	
T2 : 1500g ammonium sulphate + 50g <i>Azospirillum</i>	1.47	1.47	1.41	1.42	0.121	0.128	1.42	1.41	
T3 : 1500g ammonium sulphate+ 50g <i>Azospirillum</i> + Mo	1.77	1.77	1.57	1.53	0.143	0.146	1.50	1.52	
T4 : 1000g ammonium sulphate + 100g <i>Azospirillum</i>	2.80	2.73	1.43	1.48	0.148	0.146	1.60	1.58	
T5: 1000g ammonium sulphate+ 100g <i>Azospirillum</i> + Mo	3.19	3.18	1.62	1.66	0.168	0.170	1.78	1.75	
T6 : 500g ammonium sulphate + 150g <i>Azospirillum</i>	3.55	3.17	1.74	1.65	0.165	0.168	1.74	1.71	
T7 : 500g ammonium sulphate + 150g <i>Azospirillum</i> + Mo	4.03	4.07	1.81	1.81	0.194	0.192	1.83	1.82	
New LSD 0.05	0.32	0.35	0.07	0.07	0.012	0.006	0.04	0.03	

### 3.5. Fruit physical characteristics

The data in Table (4) showed the fruit weight of Le-Conte pears as affected by different levels and combinations of mineral and bio N fertilizers with or without molybdenum (Mo) during 2019 and 2020 seasons. The obtained results indicated that the fruit weight markedly increased with treatment (1000g ammonium sulphate + 100g *Azospirillum* + Mo) and reaching their maximum value (207.17 and 220.23 g) as compared with control in both seasons. Le Conte pear trees with T5 (1000 g ammonium sulphate + 100g *Azospirillum* + Mo) produced the highest fruit diameter (6.53 and 6.70 cm) as compared with control which recorded the lowest fruit diameter (5.43 and 5.60) in 2019 and 2020 seasons, respectively. Data in Table (4) indicated that fruit firmness was significantly increased by all treatments compared with control treatment in both seasons. The highest fruit firmness (16.4 and 16.6 lb/in<sup>-2</sup>) was recorded by applying T7 (500g ammonium sulphate + 150g *Azospirillum* + Mo) in 2019 and 2020 seasons, respectively and T5 (1000g ammonium sulphate + 100g *Azospirillum* + Mo) in the both seasons. On the other hand, trees sprayed with T1 control (2000g ammonium sulphate) had the lowest fruit firmness.

**Table 4:** The effect of molybdenum along with mineral N and biofertilizers on fruit weight, fruit length, fruit diameter and fruit firmness of Le-Conte pear in 2019 and 2020 seasons.

Treatments	Fruit weight (gm)		Fruit length(cm)		Fruit Diameter (cm)		Fruit firmness (lb.in <sup>-2</sup> )	
	2019	2020	2019	2020	2019	2020	2019	2020
T1 : Control	148.27	151.0	7.20	7.40	5.43	5.60	13.3	13.6
T2 : 1500g ammonium sulphate + 50g <i>Azospirillum</i>	155.8	158.6	7.9	7.93	6.0	5.83	15.07	15.0
T3 : 1500g ammonium sulphate + 50g <i>Azospirillum</i> + Mo	157.6	163.8	8.07	8.20	5.9	6.03	15.43	15.6
T4 : 1000g ammonium sulphate + 100g <i>Azospirillum</i>	162.4	173.5	8.87	8.83	6.1	6.23	15.93	16.03
T5 : 1000g ammonium sulphate + 100g <i>Azospirillum</i> + Mo	207.17	220.23	9.03	9.20	6.53	6.70	16.33	16.17
T6 : 500g ammonium sulphate + 150g <i>Azospirillum</i>	183.8	189.27	8.73	8.60	6.1	6.17	15.7	16.0
T7 : 500g ammonium sulphate + 150g <i>Azospirillum</i> + Mo	196.5	203.17	8.70	8.57	6.40	6.50	16.4	16.6
New LSD 0.05	5.4	3.8	0.41	0.39	0.31	0.39	0.34	0.39

### 3.6. Fruit chemical properties

#### 3.6.1. Total soluble solid content TSS, acidity, TSS/acid ratio

Data in Table (5) showed the effect of mineral and biofertilizers with or without molybdenum on total soluble solids (TSS), acidity % and T.S.S/acid ratio of Le-Conte pear fruits in 2019 and 2020 seasons. The value of TSS markedly increased with treatment T5 (1000g ammonium sulphate + 100g *Azospirillum* + Mo) and reaching their maximum value (14.03 and 14.0%). Furthermore, treatment T4 (1000g ammonium sulphate + 100g *Azospirillum*) came in second rank (13.73 and 13.53) and the control gave the lowest values (10.23 and 9.93%) in 2019 and 2020, respectively. Regarding the relation between the investigated treatments and total acidity percentage, data presented in Table (5) cleared that the treatments with molybdenum either with T4 (1000g ammonium sulphate + 100g *Azospirillum* (T5) or ammonium sulphate + 150g *Azospirillum* (T7) decreased the fruit total acidity and reached the lowest values (0.20, 0.21, 0.22 and 0.22 %, respectively). On the other hand, the highest value of fruit acidity % was recorded with untreated trees (control, 0.29 and 0.28, respectively). Data presented in Table (5) illustrated that all tested treatments induced high positive effect on T.S.S/acidity ratio as compared with control in both seasons. Generally, 1000g ammonium sulphate + 100g *Azospirillum* + Mo exerted the highest ratio (70.15 and 66.7) against the 35.28 and 35.46 for the control treatment in 2019 and 2020 seasons, respectively.

### 3.6.2. Total sugars content

Concerning the impact of different levels and combinations of mineral, bio N fertilizers with or without molybdenum in 2019 and 2020 seasons, the data presented in Table (5) showed that all treatments succeeded in improving fruit total sugars content of Le-Conte pear as compared with control in 2019 and 2020 seasons. Briefly, 1000g ammonium sulphate + 100g *Azospirillum* + Mo caused the highest values of total sugars (8.8 and 8.63 %) as compared with control, which gave the lowest one (6.07 and 6.03), respectively. The other remaining treatments gave intermediate values with significant differences among them in some cases. The effect of biofertilizers on increasing the T.S.S/acidity ratio and total sugars and decreasing acidity could be due to their beneficial effect on increasing leaf area, which reflected in more carbohydrates production through photosynthesis process that improved fruit chemical properties (Hegab *et al.*, 1997 and Abd-El-Moniem *et al.*, 2003). Moreover, the improvement occurred in the fruit quality due to supplying trees with Mo could be attributed to their effect on enhancing uptake of mineral nutrients like potassium which is necessary for basic physiological function such as formation of sugars and starch, synthesis of proteins, flavor and color of fruit (Obreza, 2003 and Abbas and Fares, 2008).

**Table 5:** The effect of molybdenum along with mineral N and biofertilizers on TSS, acidity TSS/acid, and total sugars of Le-Conte pear in 2019 and 2020 seasons .

Treatments	TSS%		Acidity%		TSS/acid		Total sugars %	
	2019	2020	2019	2020	2019	2020	2019	2020
T1 : control	10.23	9.93	0.29	0.28	35.28	35.46	6.07	6.03
T2 : 1500g ammonium sulphate + 50g <i>Azospirillum</i>	11.17	10.90	0.26	0.26	43.0	41.9	6.50	6.7
T3 : 1500g ammonium sulphate + 50g <i>Azospirillum</i> + Mo	11.53	11.43	0.25	0.25	46.12	45.72	6.53	6.8
T4 : 1000g ammonium sulphate + 100g <i>Azospirillum</i>	13.73	13.53	0.23	0.24	60.0	56.38	8.17	8.1
T5: 1000g ammonium sulphate + 100g <i>Azospirillum</i> + Mo	14.03	14.0	0.20	0.21	70.15	66.7	8.8	8.63
T6 : 500g ammonium sulphate + 150g <i>Azospirillum</i>	12.47	12.60	0.26	0.27	46.12	45.72	7.17	7.37
T7 : 500g ammonium sulphate + 150g <i>Azospirillum</i> + Mo	12.9	12.8	0.22	0.22	48.0	46.7	7.90	8.2
New LSD 0.05	0.57	0.54	0.015	0.017	2.9	2.60	0.37	0.30

### 3.7. Yield measurements

It is clear from data in Table (6) that all studied treatments significantly enhanced the number of fruit/tree and yield as Kg/tree or ton/feddan than the control in both seasons. The obtained results indicated the highest significant number of fruits/tree (16.67 and 159.67) and yield as Kg/tree (31.77 and 32.42 Kg) and (5.34 and 5.45 ton /feddan) were noticed with treatment of 500g ammonium sulphate + 150g *Azospirillum* + Mo (T7) comparing with other treatments. In the meantime, T5 (1000g ammonium sulphate + 100g *Azospirillum* + Mo) and T6 (1000g ammonium sulphate + 100g *Azospirillum*) were the second highest values in this respect. On the other hand, control treatment gave the lowest values (106.67 and 115.33) and 15.80 and 17.41 Kg/ tree or (2.66 and 2.92 ton/ feddan in 2019 and 2020 seasons, respectively. The pronounced effect of bio fertilizers and molybdenum on yield may be attributed to increasing nutrient uptake which plays an important role in producing favorable balance between growth and fruit production and increasing cell division and enlargement and consequently increasing vegetative growth which reflected on increasing the yield as finally result of the physiological processes (Geetha and Nair 2000 and Shaheen *et al.*, 2009). In addition, bio fertilizer increased the soil content of growth regulator such as IAA and cytokinins (Li *et al.*, 1998).

**Table 6:** The effect of molybdenum along with mineral N and biofertilizers on No. of fruits /tree, yield /tree (kg) and yield/ feddan (ton) of Le-Conte pear in 2019 and 2020 seasons.

Treatment	No. of fruits/tree		Yield/tree (kg)		Yield/feddan(ton)	
	2019	2020	2019	2020	2019	2020
T1 : Control	106.67	115.33	15.80	17.41	2.66	2.92
T2 : 1500g ammonium sulphate + 50g <i>Azospirillum</i>	122.0	123.0	18.99	19.45	3.19	3.27
T3 : 1500g ammonium sulphate + 50g <i>Azospirillum</i> + Mo	130.67	135.67	20.57	22.22	3.45	3.73
T4 : 1000g ammonium sulphate + 100g <i>Azospirillum</i>	131.0	136.0	21.27	23.60	3.57	3.97
T5 : 1000g ammonium sulphate + 100g <i>Azospirillum</i> + Mo	142.67	146.0	29.56	32.17	4.97	5.41
T6 : 500g ammonium sulphate +150g <i>Azospirillum</i>	151.67	149.0	27.88	28.20	4.96	4.74
T7 : 500g ammonium sulphate +150g <i>Azospirillum</i> + Mo	161.67	159.67	31.77	32.42	5.34	5.45
New LSD 0.05	5.18	3.58	1.14	0.79	0.19	0.13

### 3.8. Fruit nitrite (ppm)

The data in Table (7) indicated that mineral nitrogen fertilizer alone resulted in higher average value (3.95 and 3.94 ppm) of fruit nitrite of Le-Conte pear compared with other treatments. On the other hand, all treatments with bio fertilizers or molybdenum significantly reduced fruit nitrite residues of Le-Conte pear comparing with analogous treatment without them. Treatment No.7 (500g ammonium sulphate + 150g *Azospirillum* + Mo) caused the lowest value of fruit nitrite content (0.64 and 0.63 ppm) in both seasons followed by No. 6 (500g ammonium sulphate + 150g *Azospirillum*). While the remaining treatments gave intermediate values. It is well known that adding ammonium to the media increased the induction of nitrate reductase enzyme and this reflects on producing nitrite reductase in the plant, (Schader, 1978). Consequently, increasing ammonium cations as a result of fertilizing with ammonium sulphate in the present study raised the nitrate synthesis and nitrite content of the fruit. These results were supported by the results of Shahein *et al.*, (2003), Hossam El-Dein and Boshra (2008) and Ahmed *et al.*, (2011).

### 3.9. Fruit content of heavy metals

#### 3.9.1 Cadmium and Lead (ppm)

The data recorded in Table (7) exhibited the influence of mineral, bio fertilizers and molybdenum on the concentrations of cadmium and lead in the Le-Conte pear fruits in 2019 and 2020 seasons. Regarding the value of fruit cadmium and lead under control was significantly higher (0.011 and 0.011 ppm) and (1.14 and 1.11 ppm) in 2019 and 2020, respectively as compared with those of the remaining treatments. These results agreed with those reported by Marzouk and Kassem (2011), on date palm and Lukeguo (2003) on Fuji apple, the latter reported that, bio fertilizer contained plentiful of organic matter, which reduce effective content of heavy metal ions through adsorption and chelation, through strengthening stage, the adsorption to heavy metal ion of soil, the dissociation of cadmium and lead of soil reduced and then the adsorption of apple to ion of heavy metals (HMS) dropped. Likewise, heavy metals, like Pb and Cd in a salt form, are much more available for plant uptake and potentially toxic than those held inorganic matrix and bio ( Kabata-Pendias and Adriano 1995) and Bhattacharyya *et al.*, (2005). The phenolic and carboxyl functional groups on organic matter form stable complexes with metals through the cation exchange property or chelating the metals Kabata-Pendias (2001). Therefore, applying organic and biofertilizers could result in the fixation of these elements and lowering their available amount for plant uptake (Bell *et al.*, 1991). Furthermore, the formation of soluble organic forms of heavy metals and their mass transport through channelization may result in their movement from the surface layers (Berti and Jacobs 1998), where most of the absorbing roots of date palm are present Oihabi (1991). The previous discussion might explain the lower values of Pb and Cd content in the fruit by application of organic and bio fertilizers than mineral fertilization only.

**Table 7:** The effect of molybdenum along with mineral N and biofertilizers on fruit NO<sub>2</sub>, Cd, Pb content of Le-Conte pear in 2019 and 2020 seasons .

Treatments	Fruit dry weight (ppm)					
	NO <sub>2</sub>		Cd		Pb	
	2019	2020	2019	2020	2019	2020
T1 : Control	3.95	3.94	0.011	0.011	1.14	1.11
T2 : 1500g ammonium sulphate + 50g <i>Azospirillum</i>	2.98	2.64	0.006	0.005	1.05	1.05
T3 : 1500g ammonium sulphate + 50g <i>Azospirillum</i> + Mo	2.66	2.56	0.008	0.007	0.95	0.88
T4 : 1000g ammonium sulphate + 100g <i>Azospirillum</i>	2.43	2.71	0.006	0.007	0.86	0.89
T5 : 1000g ammonium sulphate + 100g <i>Azospirillum</i> + Mo	1.75	1.76	0.008	0.008	0.73	0.75
T6 : 500g ammonium sulphate +150g <i>Azospirillum</i>	0.97	0.97	0.005	0.007	0.69	0.70
T7 : 500g ammonium sulphate +150g <i>Azospirillum</i> + Mo	0.64	0.63	0.007	0.007	0.67	0.67
New LSD <sub>0.05</sub>	0.30	0.28	0.002	0.002	0.03	0.05

### Conclusion

According to the overall results ,it could be concluded that using 500g ammonium sulphate +150g *Azospirillum* + Mo) is the most superior treatment for increasing vegetative growth, yield as well as fruit quality and reducing fruit pollutants (nitrite, lead and cadmium) in order to produce safe and healthy fruits that satisfy the human requirements.

## References

- Abada, M.A.M., A. Ibrahim-Asmaa and A. Bondok-Sawsan, 2010. How to reduce problems of soil and irrigation water salinity in Superior vineyards. Minufiya. Agric. Res., 35(4)2: 1977-1997.
- Abbas, F. and A. Fares, 2008. Best management practices in citrus production. Tree For. Sci. Biotech., 3: 1-11.
- Abd El-Aziz, Y.Z., 2011. Response of Thompson seedless grapevines to application of organic fertilizer humic acid and some bio fertilizers. Ph. D. Thesis, Fac. of Agric., Minia Univ., Egypt.
- Abd EL-Moniem, E.A.A. and S.M.A. Radwan 2003. Response of Williams banana plants to bio fertilization in relation to growth, productivity and fruit quality. Arab. Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 11(1): 751-763.
- Abd EL-Matty, E.Z., M.F.M. Shahin, M.H. EL-Shiehk and M.M. Abd EL-Migeed, 2010. Effect of algae extract and yeast application on growth, nutritional status, yield and fruit quality of Keit mango trees. Agric. Biol. J. Am., 1(3): 421- 429.
- Abd EL-Samad, H.M., H.M.EL-Komy, M.A.K. Shaddad and A.M. Hetta, 2005. Effect of molybdenum on nitrogenase and nitrate reductase activities of wheat inoculated with *Azospirillum brasiliense* grown under drought stress. Gen. Appl. Plant Physiology, 31(1-2),43-54.
- Abdul Jabbar, B.K., H.M. Saud, R. Othman, S.H. Habib, H. Kausar and S.H. Bhuiyan, 2014. Effect of *Azospirillum* in association with molybdenum on enhanced biological nitrogen fixation, growth, yield and yield contributing characters of soybean .Journal of Food, Agriculture and Environment, 12 (2): 302-306.
- Abou El-khashab, A.M., 2002. Growth and chemical constituents of some olive cultivars as affected by biofertilizers and different water regime. Egypt. J. Agric., NRC.1 (2): 243-265.
- Ahmed, F.F. and M.H. Morsy, 1999. A new method for measuring leaf area in different fruit species. Minia. J. of Agric .Rec. and Dev., 19: 97-105.
- Ahmed, F.F., A.M. Akl, E.A.H. EL-Mamlouk and H.H. Mohamed, 2011. Reducing inorganic N fertilizers partially in Sakkoty date palm orchards by application of organic and biofertilization. Minia of Agric. Res. and Develop., (31):189- 203.
- Anbuselvi, S., M.S. Kumar, M. Vikram and D.P. Pattanaik, 2011. Effect of molybdenum on nitrogen fixing enzymes of black gram using *Anabaena Azollae* sp. treated coir waste manure under drought stress. Aust. J. Basic and Appl. Sci., 5(4): 252- 256.
- Annual Reports of Statistical Institute and Agricultural Economic Research in Egypt, 2014.
- AOAC, 1995. Benjamin Franklin Station Washington, D.C, U.S.A., 490-550.
- Aslantas, R., R. Cakmakci and F. Sahin, 2007. Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. Scientia Horticulturae 111(4):371 – 377. Association of official Agriculture Chemists (1990).
- Bell, P., B.R. James and R.L. Chaney, 1991. Heavy metal extractability in long-term sewage sludge and metal salt amended soils. J. Environ. Qual., 20:481- 486.
- Berti, W.R. and D. Jacobs, 1998. Distribution of trace elements in soil from repeated sewage applications. J. Environ. Qual., 27:1280-1286.
- Bhattacharyya, P., A. Chakraborty, K. Chakrabarti, S. Tripathy and M.A. Powell, 2005. Chromium uptake by rice and accumulation in soil amended with municipal solid waste compost. Chemosphere, 60: 14810-1486.
- Boddey, R.M., S. Urquiaga, V. Reis and J. Döbereiner, 1991. Biological nitrogen fixation associated with sugar cane. Plant Soil 137:111–117.
- Casale, W.L., V. Minassian, J.A. Menge, C.J. Lovatt, E. Johnason and F. Guillement 1995. Urban agricultural wastes for use as delivery of microbial biocontrol agents. J. Hort. Sci., 70(2): 315- 332.
- El- Morshedy, F.A., 1997. Organic manure and sulphur interaction influence vegetative growth and element concentration of sour orange seedlings. J. Agric. Sci. Mansoura Univ. Egypt 22(12): 4599- 4616.
- EL-Haddad, M.E., Y.Z. Ishac and Mostafa 1993. The role of biofertilizers in reducing agricultural costs, decreasing environment pollution and raising crop yield, Arab Univ. Agric. Sci. Ain Shams Univ., Cairo, 1(1): 147- 195.

- EL-Komy, M.H., M.A. Hamdia, M.A. Hetta and A.B. Barakat, 2004. Possible roles of nitrogen fixation and mineral uptake induced by Rhizobacterial inoculation on salt tolerance of maize. Polish J. Microbiology, 53: 53-60.
- Esitken, A., H.E. Yildiz, S. Ercisli, M.F. Donmez, M. Turan and A. Gunes, 2010. Effects of plant growth promoting bacteria (PGPR) on yield, growth and nutrient contents in organically grown strawberry. Scientia Horticulturae, 124:62 – 66
- Esitken, A., L. Pirlak, M. Turan, F. Sahin, 2006. Effect of floral and foliar application of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrition of sweet cherry. Scientia Horticulturae, 110: 324– 327. 1873
- Fageria, N.K. and V.C. Baligar, 2005. Enhancing nitrogen use efficiency in crop plants. Adv. Agron., 88: 97-185.
- Geetha, K. and R.R. Nair, 2000. Integrated plant nutrition system (IPNS) for banana. Ann. of Agric. Res., (India) 21 (4): 499- 503.
- Gogoi, D., U. Kotoky and S. Hazarika, 2004. Effect of biofertilizers on productivity and soil characteristics in banana. Indian J. Hort., 61(4):354- 356.
- Hafez, O.M., M.A. Saleh and S.R. El-Lethy, 2013. Response of some seedlings olive cultivars to foliar spray of yeast and garlic extracts with or without vascular arbuscular mycorrhizal fungi. World Applied Sciences Journal 24(9):1119 – 1129. <http://astonjournals.com/lsmr>
- Hammam, M.S., E.G. Ibrahim and A.E.M. Mansour, 2003. Response of Williams banana to some organic nitrogen fertilizers. Egypt. J. Hort., 30 (1-2):51- 65.
- Hegab, M.Y., F.F. Ahmed and A.H. Ali, 1997. Influence of spraying active dry yeast on growth and productivity of Valencia orange trees (*Citrus sinensis*). Proc. First. Sci. Conf. Agric., Fac. Agric., Assiut. 1: 73-84.
- Hosam EL-Dein, A.S. and E.S. Boshra 2008. Effect of different sources of organic fertilizers as a partial substitute for mineral nitrogen fertilizer of Williams banana. J. Agric. Sci., Mansoura Univ., 33(6): 4369-4381.
- Kabata-Pendias, A., 2001. Trace Elements in Soils and Plants. 3<sup>rd</sup> ed., Press, INC. Boca Raton, FL-p 4103.
- Kabata-Pendias, A. and D.C. Adriano, 1995. Trace metals. In: Rechcigl, J.E.E. (ed.). Soil Amendments and Environmental Quality. Lewis Publishers, Boca Raton, FL, pp. 139- 168.
- Kannaiyan, S., 2002. Biotechnology of Biofertilizers. Alpha Sci. Inter. Ltd., P.O. Box 4067 Pangbourne R. 68 U.K., 1-275.
- Kapulnik, Y., R. Gafni, and Y. Okon, 1985. Effect of *Azospirillum* spp. inoculation on root development and NO<sub>3</sub> uptake in wheat (*Triticum asativum* cv. *Miriam*) in hydroponic systems. Can. J. Bot., 63:627-631.
- Khan, M.G.; M. Silberbush and S.H. Lips, 1995. Physiological studies on salinity and nitrogen interaction in alfalfa plants. III- Nitrate reductase activity. J. Plant Nutr., 18(11): 2495- 2500.
- Li, X.J., S.F. Dong and Y.S. Liu, 1998. Determination of IAA and cytokinins in the soil with different organic manure for pot cultured apple. Plant Physiology Communications, 34 (3): 183- 185.
- Lukeguo, Z.Z., 2003. The effect of bio-fertilizer on soil and absorption to cadmium and copper of fuji apple root. M.Sc. Thesis, Fac. of Agric., Univ. of Shandong.
- Marzouk, H.A. and H.A. Kassem, 2011. Improving fruit quality, nutritional value and yield of Zaghloul dates by the application of organic and/ or mineral fertilizers. Scientia Horticulturae, 127: 249- 254.
- Mendel, R. R. and R. Hansch, 2002. Molybdoenzymes and molybdenum cofactor in plants. J. Exp. Bot., 53, 1689-1698.
- Miller, R.W., R.L. Donahve and J.U. Miller, 1990. Soils. An Introduction to Soil and Plant Growth" Prentice Hall Inter Increase. Englewood Cliffs, New Jersy, 380-339.
- Morsi, M.E., 2009. Response of date palm "Sewy cv." grown in new reclaimed land to organic and inorganic nitrogen sources. Fayoum J. Agric. Res. and Dev., 33(1): 160- 172.
- Nijjar, G.S., 1985. Nutrition of Fruit Trees. MrsUsha Raj Kumar, Kalyani, Publishers, New Delhi. India, pp. 10-52.
- Obreza, T.A., 2003. Importance of potassium in a Florida citrus nutrition program. Better Crops, 87: 19-22

- Oihabi, A., 1991. Effect of vesicular arbuscular mycorrhizae on Bayoud disease and date palm nutrition. Ph.D. Thesis University of Marrakech, 199.
- Perez, J.R. and W.M. Kliewer, 1978. Nitrate reduction in leaves of grapevine and other fruit trees. *J. Amer. Soc. Hort. Sci.*, 103 (2): 246- 250.
- Pollock, V. V., R.C. Conover, M.K. Johnson and M.J. Barber, 2002. Bacterial expression of the molybdenum domain of assimilate nitrate reductase: Production of both the functional molybdenum-containing domain and the nonfunctional tungsten analog. *Arch. Biochem. Biophys.*, 2: 237-248.
- Ram Rao, D.M., J. Kodandaramaiah, M.P. Reddy, R.S. Katiyar and V.K. Rahmathulla, 2007. Effect of AM fungi and bacterial biofertilizers on mulberry leaf quality and silkworm cocoon characters under semi arid conditions. *Caspian J. Env. Sci.*, 5(2): 111- 117.
- Ridnour, Lisa, A., E. Sim-Julia, A.H. Michael, A.W. David, M.M. Scan, R.P. Garry, and R.S. Douglas, 2000. A spectrophotometric method for the direct and quantitation of Nitric oxide, nitrite and nitrate in cell culture Media. *Analytical Biochemistry*, 281: 273-229.
- Sagi, M., C. Scazzocchio, and R. Fluhr, 2002. The absence of molybdenum cofactor sulfuration is the principle cause of the flacca phenotype in tomato plants, *Plant J.*, 31: 301-317.
- Schrader, L.E., 1978. Critique-of " Factors influencing nitrate acquisition by plants: Assimilation and fate of reduced nitrogen". Nitrogen in the environment. Academic Press Rapid Manuscript Reproduction, 101: 137.
- Sendecor, G.W. and W.G. Cochran, 1980. Statistical Methods. Oxford and J. B. H. publishing. Com. 6 edition
- Shaheen, M.A., M.M. Saad and S.M. Mohamoud, 2009. Influence of organic and biofertilization on growth, yield and fruit quality of Williams Banana. *J. Agric. Sci. Mansoura Univ.*, 34(7): 8013-8025.
- Shahein, A.H., A.M. Attalla, H.A. Kassem and Hoda S.H. Aly 2003. Effect of applying different organic and inorganic nitrogen sources to Zaghloul and Samany date palm cultivars on: II- Yield, fruit quality and fruit content of some pollutants. Proceedings of the International Conference on Date Palm, 16-19 Sept. 2003, College of Agric. and Vet. Med., King Saud Univ., Qassem Branch, Kingdom of Saudi Arabia, 195-207.
- Summer, M.E., 1985. Diagnosis and Recommendation Integrated System (DRIS) as a Guide to Orchard Fertilization. *Hort. Abst.*, 55(8): 7502.
- Tien, T.M., M.H. Gaskin and D.M. Hubbell, 1979. Plant growth substances produced by *Azospirillum brasiliense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). *Appl. Environ. Microbiol.*, 37:1016-1024.
- Togay, Y., N. Togay and Y. Dogan 2008. Research on the effect of phosphorus and molybdenum applications on the yield and yield parameters in lentil (*Lens culinaris* Medic.). *African Journal of Biotechnology*, 7(9):1256-1260.
- Waller, R.A. and D.B. Duncan, 1969. A buyes rule for the symmetric multiple comparison problems. *Amer. state. Assoc. J.*, 64: 1484-1503
- Wani, S.P., S. Chandrapalaih , M.A. Zamber, and K.K. Lee, 1988. Association between N2-fixing bacteria and pearl millet plants: Response, mechanisms and persistence. *Plant and Soil*, 110:289-302.
- Wilde, S.A., R.B. Corey, J.G. Layer and G.K. Voigt, 1985. Soils and Plant Analysis for Tree Culture. 3rd Ed. Oxford and IBH publishing Co., New Delhi, pp: 9-100. Yagodin, B.A., 1990. Agricultural Chemistry. Mir Publishers Moscow, 278-281.
- Yagodin, B.A., 1990. Agricultural Chemistry. Mir Publishers Moscow, 278-281.
- Zeid, F.A., M.R.A. Nesiem, A.M.M. Abd EL-Kader and S.S.A. Hosny, 2009. Effect of mineral and bio-organic fertilization on growth, yield and chemical composition of banana plants. *J. Agric. Sci. Mansoura Univ.*, 34(7):7953-7966.