

## Studies on the Effect of Vinasse, Amino acids and Humic Acid Substances as Soil Applications on Fruit Quality and Quantity of Manzanillo Olive Trees

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### ABSTRACT

This study was carried out during the two seasons 2013, 2014 on olive trees Manzanillo cv. the trees were 10 years old growing in sandy soil at a private orchard in Ismailia governorate, Egypt. This investigation was performed to study the effect of humic acid as Actosol® (contains 20 % humic acid + NPK 1:5:6) and Greenpower (Vinasse 80%+Soybean amino acid 20%) as soil application under the drippers of each tree at (150, 75 and 50 cm<sup>3</sup>) from April till June. At the end of the season, yield (kg/tree) and Fruit quality: average fruit size, weight, shape index (length/diameter) and pulp/pit ratio also fruit chemical characterizes: fruit oil and acidity percentage were recorded. The obtained results showed that, "Manzanillo" olive trees received soil application of humic acid twice ( first application 75 cm<sup>3</sup>/tree at full bloom and the second application 75 cm<sup>3</sup>/tree after one month from full bloom) or soil application of Greenpower three times (the first application 50 cm<sup>3</sup>/tree at full bloom, the second application 50 cm<sup>3</sup>/tree after one month from full bloom, while the third application 50 cm<sup>3</sup>/tree after two month from full bloom), are recommended for high yield and fruit oil% of "Manzanillo" olive trees grown under Ismailia condition.

**Key words:** Olive Manzanillo cv, yield, Fruit quality, fruit oil percentage, fruit acidity, organic fertilizer, Humic acid, Actosol®, Greenpower, Vinasse, Soybean amino acid

### Introduction

Olive (*Olea europaea* L.) is an evergreen tree grown primarily between 30 and 45° latitude in both hemispheres. Current olive groves are estimated at approximately 960 million olive trees, of which some 945 million (98% of the total), are found in the Mediterranean Basin countries where they cover approximately 9.3 million hectares. Approximately 50 million olive trees are under irrigation, but most groves are rained. Olive plantations have shown a continuous increase since 1987 with a spectacular increase of the irrigated area in many countries. The average annual production is 14 million tons of olives, of which 90% are used for oil production and 10%, are used for the table olives. In 2008 total harvested area was over 10 500 000 ha, 95.5 percent of which was concentrated in ten countries surrounding the Mediterranean Sea (FAO, 2011). Spain, Italy and Greece are the main producers of virgin oil followed by Tunisia, Syria, Turkey and Morocco (years 2002-2008). About 90 percent of the world production of olive fruit is for oil extraction, the remaining 10 percent for table olives. The world cultivated area of olives in 2009 was over 9.2 million ha with an average yield of 2.1 tonne/ha (FAO, 2011). European Union countries produce 78 percent and consume 68 percent of the world's olive oil. Benefits described to the use of humic acid and related products to increase nutrient uptake, tolerance to drought and temperature extremes, activity of beneficial soil microorganisms, and availability of soil nutrients particularly in alkaline soils and low organic matter (Senn and Kingman, 1973 and Russo and Berlyn, 1990). Also, humic materials may increase root growth in a similar manner to auxins (Senn and Kingman, 1973; O'Donnell, 1973 and Tatini, *et al.*, 1991).

There is growing interest of the use of humic acid and K-humate as a substitute to chemical fertilizers which have potential polluting effects in the environment (Senn and Kingman, 2000). However, Chunhua *et al.* (1998) showed that it is not clear how these products induce their effect, whether it is due to their increase of cation exchange capacity which affects the retention and availability of nutrients, or due to a hormonal effect, or a combination of both. Also, humic materials significantly increased orange and grapefruit trees growth and fruit production (Alva and Obreza, 1998), enhanced apple fruit weight; yield and soluble solids content (Li, *et al.* 1999) increased yield, fruit quality and grower income of peach and apple (Fathi *et al.* 2002). Aml *et al.*, (2011) indicated that treatment Chemlali olive with (Humic acid + amino acids + macro elements + trace elements) was the most effective one compared with the other treatments since this treatments gave the best results concerning plant height, brunch numbers, leaf numbers, also it increased plant diameter and leaves area comparing with control. On the other hand, this treatment raised root length and root weight than the control plant.

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Humic materials may increase root growth in a manner similar to auxins (Donnell, 1973, Tatini *et al.*, 1991). Fernández-Escobar *et al.* (1999) who mentioned that, foliar application of leonardite extracts (humic substances extracted) to young olive plants stimulated shoot growth when they were growing without the addition of mineral elements to the irrigation water, but did not promote growth when applied to plants watered with a nutrient solution, although growth of fertilized plants was greater than that of unfertilized ones. Under field conditions, foliar application of leonardite extracts stimulated shoot growth and promoted the accumulation of K, B, Mg, Ca and Fe in leaves.

Cavalcante *et al.*, (2011) demonstrated that humic substances sprayed positively affect aerial part and root system of papaya seedlings and seedling quality of papaya were improved by humic acids foliar spray. One of the main factors affecting plant growth in sandy soils is the types and amounts of fertilizers. However, the cost of mineral fertilizers has been significantly going up. As a result, it has become necessary to seek alternatives that would supply the poor soil with more economic sources of fertilizers (Rodriguez, 2000). Organic materials have the benefit or disadvantage of being slow release fertilizers and are less likely to leach into ground or surface waters. Conventional fertilization has traditionally been used because they are cheap, less bulky and easy to apply. The line between has been becoming blurred with slow release conventional fertilizers and high analysis organic fertilizers that are easily applied and less bulky.

Vinasse is a byproduct of distilleries during alcohol production. In terms of volume, approximately 13 liter of vinasse are produced by each liter of alcohol obtained from cane must (Copersucar, 1986). Represent an alternative to supply such nutrients in crop production (García, 1994; Gloria, 1985). Various research works carried out in other countries, particularly in Brazil, report that vinasse increases sugarcane productivity (Copersucar, 1980; Copersucar, 1986; Gloria, 1985) as well as they have demonstrated that under controlled conditions, it can partially or completely replace mineral fertilization.

On the other hand, Bioregulator substances were shown to enhance the biosynthesis of certain chemical constituents in plants. In this respect the amino acids which have a high integrity with different metabolic pools in plants were used to promote plant growth (Coruzzi and Last, 2000). Maxwell and Kieber (2004) indicated the link of methionine to the biosynthesis of growth regulating substances, e.g. cytokinins, auxins and brassinosteroids in plants. Whereas the link of tryptophan to the biosynthesis of auxins, the phytoalexin camalexin, phenyl propanoids and other related natural products in plants was recently reported (Tao *et al.* 2008). Studies have proved that amino acids can directly or indirectly influences the physiological activities of plant growth and development.

The aim of this study was evaluating fruit physical and chemical properties and yield of Manzanillo trees treated with of organic matter (humic acid, amino acids and Vinasse) as soil applications in Ismailia condition.

## Material and Methods

This study was conducted during two successive seasons, 2013 and 2014, on 10 years old olive trees Manzanillo cv. Grown in a private orchard in Ismailia – Egypt. The trees spaced 5 x 5 meter apart (168 trees/acre) in a sandy soil (Table1). The trees received the same cultural practices that were recommended. The farm is depending on wells in irrigation (Table 2). Greenpower (Vinasse 80% + Soyabean amino acid 20%) and humic acid as Actosol® (contains 20 % humic acid + NPK 1:5:6) was added in this study to the trees as soil applications under the drippers of each tree.

**Table 1:** Chemical characteristics of sandy soil used for the present study.

parameters	Depth of simple (cm)		
	Surface sample	30 cm depth	60 cm depth
pH	8.02	8.70	8.11
EC(dSm <sup>-1</sup> )	3.80	0.80	1.70
	Soluble cations (meq\l)		
Ca <sup>++</sup>	6.00	2.50	3.00
Mg <sup>++</sup>	4.00	1.50	1.50
Na <sup>+</sup>	28.60	4.40	12.90
K <sup>+</sup>	0.12	0.14	0.78
	Soluble anions (meq\l)		
CO <sub>3</sub> <sup>-</sup>	-	-	-
HCO <sub>3</sub> <sup>=</sup>	4.40	2.40	2.00
Cl <sup>-</sup>	27.20	5.00	13.00
SO <sub>4</sub> <sup>=</sup>	7.12	1.14	3.18

**Table 2:** Chemical characteristics of water weal used for the present study

Parameters	Values
pH	7.49
EC(dSm <sup>-1</sup> )	4.40
Soluble cations (meq <sup>-1</sup> )	
Ca <sup>++</sup>	7.50
Mg <sup>++</sup>	5.00
Na <sup>+</sup>	33.10
K <sup>+</sup>	0.16
Soluble anions (meq <sup>-1</sup> )	
CO <sub>3</sub> <sup>=</sup>	-
HCO <sub>3</sub> <sup>-</sup>	1.60
Cl <sup>-</sup>	40.00
SO <sub>4</sub> <sup>=</sup>	4.16

Complete randomized design was applied. Seven treatments were applied in three replicates; all of the 21 trees conducted in this study were vigorous and similar in growth and canopy.

The investigation aimed at studying the effect of different doses and applications time of Actosol® and Greenpower as the following:

1. Without Actosol® or Greenpower application (control).
2. 150 cm<sup>3</sup> Actosol® add to the soil once time at the first week of April (during full bloom).
3. 75 cm<sup>3</sup> Actosol® add to the soil two times during growth season the first application at the first week of April while the second application at the first week of May (75 cm<sup>3</sup> at full bloom and 75 cm<sup>3</sup> after one month from full bloom).
4. 50 cm<sup>3</sup> Actosol® drenched to the soil three times during growth season the first application at the first week of April, the second application at the first week of May, while the third application at the first week of June (50 cm<sup>3</sup> at full bloom, 50 cm<sup>3</sup> after one month from full bloom and 50 cm<sup>3</sup> after two month from full bloom).
5. 150 cm<sup>3</sup> Greenpower add to the soil once time at the first week of April (during full bloom).
6. 75 cm<sup>3</sup> Greenpower add to the soil two times during growth season the first application at the first week of April while the second application at the first week of May (75 cm<sup>3</sup> at full bloom and 75 cm<sup>3</sup> after one month from full bloom).
7. 50 cm<sup>3</sup> Greenpower drenched to the soil three times during growth season the first application at the first week of April, the second application at the first week of May, while the third application at the first week of June (50 cm<sup>3</sup> at full bloom, 50 cm<sup>3</sup> after one month from full bloom and 50 cm<sup>3</sup> after two month from full bloom).  
Samples at harvest time of 100 random mature fruits per tree were used for the determination of fruit physical and Chemical Characteristics:

#### **Fruit physical characteristics:**

These include fruit weight, volume, pit weight and flesh/ pit ratio. Fruit samples were collected at mid Sept.

##### *Fruit weight:*

It was determined by weighing the samples (100 fruits) by ordinary balance with 0.01 gm sensitivity and average weight per fruit was calculated.

##### *Fruit volume:*

It was measured by water displacement method.

##### *Pulp / Pit ratio:*

Values were calculated by dividing the weight of the flesh over the weight of the pit (Pit weight was determined by weighing the sample (100 pits) and average weight of pit was calculated).

##### *Shape index:*

length\diameter (L/D).

##### *Yield:*

At maturity stage (mid sept.), fruits of each tree were separately harvested, then weighed and yield as Kg / tree was estimated.

#### **Chemical Characteristics:**

##### *Fruit moisture percentage*

Moisture percentage of fruit in the previous fruit samples was estimated, samples were dried at 60-80 ° C in electrical air oven until constant weight, the fruit moisture percentage was calculated according to A.O.A.C., (1975).

*Oil percentage of fruit dry weight:*

Fruit oil content was determined by means of the Soxhlett fat extraction apparatus using Hexan of 60-80°C boiling point as described by (A.O.A.C. 1975).

*Fruit acidity percentage:*

Fruit juice total acidity % as Malic acid (mgs/100 g fruit juice) according to A.O.A.C (1975).

*Data Analysis:*

The obtained data during the two seasons of the study was statistically analyzed of variance method; differences between means were compared using Duncan's multiple range tests at 0.05 level according (Duncan, 1955).

**Results and Discussion**

**Fruit Properties:**

As shown in Tables (3 and 4) It is found that, fruit physical parameters where affected by dividing humic acid or Greenpower into two or three doses. Concerning the interaction between organic extracts sources (Actosol® and Greenpower) and number of soil applications (once, twice and three times), the highest values of fruit weight, size, Pulp/Pit ratio and shape index in both seasons where obtained from fruits harvested from Manzanillo trees received humic acid twice (75 cm<sup>3</sup> humic acid add to the soil two times during growth season) or three times (50 cm<sup>3</sup> humic acid drenched to the soil three times during growth season). With respect to main number of soil applications (once, twice and three times) regardless of organic extracts sources (Actosol® and Greenpower) data cleared that, fruit physical parameters of " Manzanillo " olive trees drenched organic extracts sources three times exhibited the highest significant values during first growth season, while the highest values in the second growth season acquired by drenching organic extracts sources twice only.

**Table 3:** Effect of number of application of humic acid and Greenpower on some physical fruit properties Manzanillo olive during first season.

Treatments	weight	Mean	volume	Mean	Pulp/Pit	Mean	Shape index	Mean
Humic acid	Once	5.89 a	5.49 A	5.73 a	5.34 A	5.79 b	5.63 A	1.08 ab
	Twice	5.26 b		5.09 b		5.91 a		1.10 a
	Three times	5.33 b		5.21 b		5.18 d		1.11 a
Green bower	Once	5.67 ab	5.39 A	5.26 b	5.07 A	5.97 a	5.51 A	1.09 ab
	Twice	5.22 b		5.06 b		5.50 c		1.06 ab
	Three times	5.28 b		4.89 bc		5.06 e		1.03 ab
Control	4.68 c	4.68 B	4.46 c	4.46 B	3.91 f	3.91 B	0.99 b	0.99 A
Mean	Once	5.38 A"		5.15 A"		5.22 A"		1.05 A"
	Twice	5.05 B"		4.78 A"		5.11 AB"		1.05 A"
	Three times	5.10 C"		4.85 A"		4.72 A"		1.06 A"

Means having the same letters within a column are not significantly different at 5% level.

**Table 4:** Effect of number of application of humic acid and Greenpower on some physical fruit properties Manzanillo olive during second season.

Treatments	weight	Mean	volume	Mean	Pulp/Pit	Mean	Shape index	Mean
Humic acid	Once	6.49 a	6.00 A	5.90 ab	5.72 A	5.44 c	5.28 B	1.29 ab
	Twice	5.86 b		5.73 ab		5.56 c		1.30 ab
	Three times	5.64 b		5.53 b		4.83 d		1.32 a
Green bower	Once	6.17 ab	5.99 A	6.37 a	5.97 A	6.62 a	6.09 A	1.29 ab
	Twice	5.85 b		5.70 ab		6.15 b		1.26 ab
	Three times	5.93 ab		5.85 ab		5.50 c		1.24 ab
Control	5.28 c	5.28 B	5.10 c	5.10 B	4.45 e	4.45 B	1.21 b	1.21 A
Mean	Once	6.33 A"		6.13 A"		6.03 A"		1.29 A"
	Twice	5.86 B"		5.72 A"		5.86 A"		1.28 A"
	Three times	5.79 B"		5.69 A"		5.16 B"		1.28 A"

Means having the same letters within a column are not significantly different at 5% level.

Data in Tables (5 and 6) clear that fruit chemical properties were significantly affected by the source of organic extracts sources (Actosol® or Greenpower ) as will as number of applications. It was clear that humic

acid or Greenpower application doses treatments increased fruit oil % and decreasing fruit acidity % significantly compared with the control in both seasons. Whereas, fruit Moisture % was significantly decreased in the first season and increased in the second season compared with the control.

Regarding, the effect of organic extracts sources in fruit chemical parameters (Moisture % and Oil % ) of Manzanillo cultivar, no significant different between (Actosol® and Greenpower ) was detected in the first growth season, whereas in the second growth season humic acid applications were superior in their impact on studied fruit chemical parameters than Greenpower.

**Table 5:** Effect of number of application of humic acid and Greenpower on some chemical fruit properties Manzanillo olive during first season.

Treatments		Moisture %	Mean	Oil % of fruit dry weight	Mean	Fruit acidity%	Mean
A	B						
Humic acid	Once	59.99 a	59.29 A	36.78 b	37.65 A	0.98 b	0.93 A
	Twice	59.55 ab		37.17 b		1.01 a	
	Three times	58.33 b		39.02 a		0.97 bc	
Green bower	Once	61.30 a	59.38 A	36.64 c	37.29 A	0.93 d	0.95 A
	Twice	58.40 b		37.01 b		0.95 cd	
	Three times	58.43 b		38.21 a		0.98 b	
Control		56.26 c	56.26 B	36.54 c	36.54 A	0.89 e	0.89 B
Mean	Once	59.18 A"		36.65 B"		0.93 A"	
	Twice	58.07 A"		36.90 B"		0.95 A"	
	Three times	57.67 A"		37.92 A"		0.95 A"	

Means having the same letters within a column are not significantly different at 5% level.

**Table 6:** Effect of number of application of humic acid and Greenpower on some chemical fruit properties Manzanillo olive during second season.

Treatments		Moisture %	Mean	Oil % of fruit dry weight	Mean	Fruit acidity%	Mean
A	B						
Humic acid	Once	62.31 ab	61.61 A	32.33 c	34.82 A	0.85 b	0.86 A
	Twice	61.87 ab		34.73 b		0.88 a	
	Three times	60.65 b		37.47 a		0.84 b	
Green bower	Once	63.62 a	61.84 A	31.65 cd	33.74 A	0.26 e	0.29 C
	Twice	61.16 b		32.67 c		0.30 d	
	Three times	60.75 b		36.90 a		0.31 d	
Control		58.46 c	58.46 B	30.93 d	30.93 B	0.75 c	0.75 B
Mean	Once	62.96 A"		31.64 B"		0.55 C"	
	Twice	61.51 AB"		32.78 B"		0.59 B"	
	Three times	60.70 B"		35.10 A"		0.57 A"	

Means having the same letters within a column are not significantly different at 5% level.

### Yield:

Data in Table (7) showed the effect of Actosol® or Greenpower rates (one, two and three doses) on yield of “Manzanillo” olive trees during (2013 and 2014) and average of the two seasons. Results revealed that both of Actosol® or Greenpower increased significantly yield (kg/tree) in both seasons as while as the average of the two seasons. It was clear that, both of humic acid and Greenpower treatments significantly increased “Manzanillo” olive yield than the control in both seasons, while humic acid application treatments resulted in a significant increase in “Manzanillo” olive yield than those received Greenpower. The highest yield was obtained from adding humic acid at a rate of 150 cm<sup>3</sup> at as one dose. However, increasing application dose number to two or three doses tended to no significantly increase obtained in yield as compared with one dose application.

Finally the highest yield of “Manzanillo” olive trees during (2013 and 2014) and average of the two seasons achieved by soil application of organic extracts sources (Actosol® or Greenpower ) three times, the first application at full bloom, the second application 50 cm<sup>3</sup> after one month from full bloom, while the third application after two month from full bloom.

Yield values obtained from humic acid application treatments as one, two and three doses detected (55.67, 45.67 and 40.35 kg/tree) and (55.05, 44.67 and 36.67 kg/tree) in the first and second season respectively. The corresponding values for “Manzanillo” olive yield (kg/tree) for Greenpower treatments application doses detected (47.67, 38.67 and 36.76 kg/tree) and (45.11, 36.08 and 33.94 kg/tree) for one, two and three doses in the first and second season, respectively. As for the control, the yield was 36.23 and 27.67 kg/tree in the first and second seasons, respectively.

Organic extracts Vinasse improves most factors involved in soil fertility, provides favoring conditions for nitrogen assimilation into the soil, protects nutrients against washing out in winter and maintains them as reserve

nutrients as a slow release during the vegetative period. These are the most important affect, leading to increase yield and quality of crops.

**Table 7:** Effect of number of application of humic acid and Greenpower on yield Manzanillo olive during (2013 – 2014).

Treatments		Yield 2013	Mean	Yield 2014	Mean	Average yield	Mean
Humic acid	Once	55.67 a	47.12 A	55.05 a	45.46 A	55.36 a	46.63 A
	Twice	45.34 b		44.67 b		45.01 b	
	Three times	40.35 c		36.67 c		38.51 c	
Green bower	Once	47.67 b	41.03 B	45.11 b	38.37 B	46.39 b	39.54 B
	Twice	38.67 c		36.08 c		37.38 c	
	Three times	36.76 c		33.94 c		35.35 c	
Control		36.23 c	36.23 C	27.67 d	27.67 C	31.95 d	31.95 C
Mean	Once	46.52 A"		50.01 A"		44.90 A"	
	Twice	40.41 B"		40.33 B"		38.28 B"	
	Three times	37.11 C"		34.83 C"		34.94 B"	

Means having the same letters within a column are not significantly different at 5% level.

Amino acids as organic nitrogenous compounds are the building blocks in the synthesis of proteins (Davies, 1982). Amino acids are particularly important for stimulation cell growth, they act as buffers which help to maintain favorable PH value within the plant cell, since they contain both acid and basic groups; they remove the ammonia from the cell.

This function is associated with amid formation, so they protect the plants from ammonia toxicity. They can serve as a source of carbon and energy, as well as protect the plants against pathogens. Tyrosine is hydroxyl phenyl amino acid that is used to build neurotransmitters and hormones. Hass, (1973) reported that the biosyntheses of cinamic acids (which are the starting materials for the synthesis of phenols) are derived from phenylalanine and tyrosine.

The role of Tryptophan is well known: it has an indirect role on the growth via its Influence on auxin synthesis. Phillips, (1971) reported that alternative routes of IAA synthesis exist in plants, all starting from Tryptophan. Thus, when Tryptophan was supplied to some plant tissues, IAA was formed. Thiamine (vitamin B1) could serve as coenzyme in decarboxylation of  $\alpha$ -keto acids, such as Pyruvic acid and keto-glutamic acid which has its importance in the metabolism of carbohydrates and fats (Bidwell, 1974). Thiamine is an important cofactor for the transketolation reactions of the pentose phosphate cycle, which provides pentose phosphate for nucleotide synthesis and for the reduced NADP required or various synthetic pathways (Kawasaki, 1992). They accelerate cell division, show greater root development, and decrease stress deterioration. Under the influence of humic acids, plants grow stronger and they better resist plant diseases. Humates reduce soil erosion by increasing the cohesive forces of the very fine soil particles. They improve the soil structure and improve physical properties of soil by increasing the exchange capacity and buffering qualities, promoting the chelation of many elements and making these available to plants. Humic substances can ameliorate negative soil properties; improve the plant growth and nutrients uptake. Maggioni *et al.* 1987; De Kreij & Basar 1995; Mackowiak *et al.* 2001) reported that, humic acid is especially beneficial in freeing up nutrients in the soil so that they become available to the plant as needed. In several studies, humic and folic acids preparations were reported to increase the uptake of mineral elements, and to increase the yield of crop plants (Kausar & Azam 1985; Chen *et al.* 2004 a, b). Due to the positive effect of humic substances on the visible growth of plants, these chemicals have been widely used by the growers instead of other substances such as pesticides etc. This, however, has led to growers using excessive amounts of these substances.

## Conclusion

Soil application of humic acid twice (first application 75 cm<sup>3</sup>/tree at full bloom and the second application 75 cm<sup>3</sup>/tree after one month from full bloom) or soil application of Greenpower three times (the first application 50 cm<sup>3</sup>/tree at full bloom, the second application 50 cm<sup>3</sup>/tree after one month from full bloom, while the third application 50 cm<sup>3</sup>/tree after two month from full bloom), are recommended for high yield and fruit oil% of "Manzanillo" olive trees grown under Ismailia condition. With respect to fruit physical properties i.e. fruit weight, size and pulp/pit ratio, the highest values obtained by soil application of humic acid twice, first application 75 cm<sup>3</sup>/tree at full bloom, the second application 75 cm<sup>3</sup>/tree after one month from full bloom.

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