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Impact of Amino Acids, Vinasse and Humic Acid as Soil Application on Fruit Quality and Quantaty of " Kalamata " Olive Trees.

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Abstract: This study was carried out during the two seasons 2013, 2014 on olive trees Kalamata 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^3) 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, "Kalamata" olive trees received humic acid soil application at 150 cm^3 per tree once at full bloom gained the highest yield (kg/tree) as while as fruit oil rather than dividing humic acid soil application dose into two or three doses. Whereas, fruit physical properties were significantly affected by the source of material (humic acid or Greenpower) as will as number of applications. Generally, humic acid applications were superior in their impact on studied fruit quality parameters than Greenpower, Moreover, humic acid or Greenpower applications at one dose resulted in higher significant values compared with two or three times of application.
Key words: olive Kalamata cv., yield, Fruit quality, fruit oil percentage, fruit acidity, organic fertilizer, Humic acid, Greenpower, Vinasse.

Introduction

Olive (*Olea europaea* L.) is one of the oldest, most widespread and important crops of the Mediterranean basin. Many different olive genotypes are cultivated and a high degree of morphological and biological variation exists[1]. The Mediterranean basin is the traditional area of olive cultivation and has 95% of the olive orchards of the world.

Egypt is the world's top producer of table olives but is limited by under developed post-harvest practices. Egypt produced an average of 413,000 tons of table olives per year from 2007 to 2011. In 2011 alone, Egypt produced more than 13 percent of the world's table olives, making Egypt the top global producer of this type of olive. From 2007 to 2011, Egypt was one of the top consumers and exporters of table olives, consuming an average of 330,000 tons and exporting an average of 89,500 tons per year. In 2011, Egypt and Argentina – Egypt's main competitor in international markets – exported 12 percent and 11 percent of the world's table olive exports, respectively. About Some 79920 feddans of Egyptian land are currently devoted to olive cultivation, 25 percent of which is located in the North Sinai governorate, according to the Central Administration for Agriculture Education. Today the tradition continues throughout six main regions of Egypt. The Alamin, Al Areesh, and Rafah regions all produce Picual, Manzanilla and Kalamata olives. Alex Desert

Road and Ismalia regions harvest Picual, Manzanilla, Kalamata, and Aggezi, while the Siwa region produces only the Hamed Siwi variety.

The olive tree productivity is generally low due to the poor soil fertility and low water holding capacity. Accordingly, it seems that trees need to organic fertilizers avoided pollution and reduced the costs of fertilization. Also, it has drowned the attention of olive growers to use the organic and bio-fertilizers that would be healthy for human and safe for environment. [2,3]Humic acid (HA) is a heterogeneous mixture of many compounds with generally similar chemical properties which performs various functions in the soil and on plant growth. One of the functions of humic acid is the positive effect on the promotion of root development. [4,5] reported that humic acid increased the root/shoot ratio as well as the production of thin lateral roots of olive plants. In addition, HA, prepared from leonardite coal, stimulated both shoot and root growth. Furthermore, humic acid is known to improve nutrient absorption and plant growth. In many soils, phosphate readily forms in almost insoluble mineral compounds, such as apatite. Experimental data conducted by [6] reported that increasing the amount of HA increased the rate of dissolution of apatite. [7]The application of humic acid has a positive influence in promoting overall tree vigor. Treated avocado trees were larger and the root system was better developed than the untreated trees.

[8]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.

Humic materials may increase root growth in a manner similar to auxins [9]. [10]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.

On other hand, the addition of organic residues from plant and/or animal origin in organic and biodynamic farming represents the basic practice to enhance overall soil fertility, thereby greatly influencing root development[11]. Humic substances such as humic acid, fulvic acid, are the major components (65-70%) of soil organic matter, increase plant growth enormously due to increasing cell membrane permeability, respiration, photosynthesis, oxygen and phosphorus uptake, and supplying root cell growth[12,13]. Humic acid is complex substances derived from organic matte decomposition. Agricultural humic acid are reputed to enhance nutrient uptake, drought tolerance, seed germination and overall plants performance[14,15]. Soil organic matter mainly consists of humic and fulvic acids which are called humin materials[16,17]. They are mainly produced from nitrogenous compounds containing decomposed amino acids and aromatic complexes[18, 19,20] reported that soil application of humus increased the N uptake of wheat, meanwhile, foliar application of humic acid increased the uptake of P, K, Mg, Na, Cu and Zn. [21]Biofertigation of microbial inoculums and humic substances could use as a complementary for mineral fertilizers to improve yield and quality of cowpea under under sandy soil conditions which protect the environment chemical pollution and its harmful effect on human and animal health. [22]Humic substances sprayed positively affect aerial part and root system of papaya seedlings and seedling quality of papaya are improved by humic acids foliar spray. [23]"Economical levels of application should be determined and should not exceed 2 g humus/kg in soil and 0.1% in foliar".

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 L of vinasse are produced by each L of alcohol obtained from cane must[24]. Vinasse has high levels of potassium, calcium and organic matter in its chemical composition as well as moderate amounts of nitrogen and phosphorus[25] and could represent an alternative to supply such nutrients in crop production [26]. Various research works carried out in other countries, particularly in Brazil,

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[27,28] 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 phytoalexincamalexin, phenyl propanoids and other related natural products in plants was recently reported[29]. Studies have proved that amino acids can directly or indirectly influences the physiological activities of plant growth and development.

The aim of this study effect of organic substances; Actosol (humic acid) and Greenpower (Vinasse + Soyabean Amino acids) as soil application on yield, fruit physical and chemical properties of "Kalamata" olive trees.

Material and Methods

The present study was conducted during two successive seasons, 2013 and 2014, on 10 years old olive trees Kalamata 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 according to the recommendations of the Ministry of Agriculture. The trees irrigated by drip irrigation system depending on wells in irrigation (Table 2). The trees were almost similar in vigor, free from any visible pathogenic symptoms and at the same bearing phase. Greenpower (Vinasse 80% + Soyabean amino acid 20%) and Actosol® (20 % humic acid + NPK 1:5:6) was added in this study to the trees as soil applications under the drippers of each tree. 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 (humic acid) and Greenpower (Vinasse + Soyabean Amino acids) as the following:

1. Without Actosol® or Greenpower application (control)
2. 150 cm^3Actosol® add to the soil once time at the first week of April (during full bloom).
3. 75 cm^3Actosol® 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^3 at full bloom and 75 cm^3 after one month from full bloom).
4. 50 cm^3 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^3 at full bloom, 50 cm^3 after one month from full bloom and 50 cm^3 after two month from full bloom).
5. 150 cm^3 Greenpower add to the soil once time at the first week of April (during full bloom).
6. 75 cm^3 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^3 at full bloom and 75 cm^3 after one month from full bloom).
7. 50 cm^3 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^3 at full bloom, 50 cm^3 after one month from full bloom and 50 cm^3 after two month from full bloom).

Fruit physical characteristics:

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

1- 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.

2- **Fruit volume:**

It was measured by water displacement method.

3- **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).

4- **Shape index**: length\diameter.

5- **Yield:**

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

Chemical Characteristics:

Moisture content

It was determined by drying the flesh in an oven at 60-80°C until a constant weight [30].

Oil percentage:

Fruit oil content was determined by means of the Soxhlett fat extraction apparatus using Hexan of 60 80°C boiling point as described [30].

Fruit acidity percentage:

Fruit juice total acidity % as Malic acid (mgs/100 gm fruit juice)[30].

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[31].

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

60cm depth	30cm depth	Surface sample	Parameters
8.11	8.70	8.02	pH
1.70	0.80	3.80	EC(dSm^{-1})
Soluble cations (meq\l)			
3.00	2.50	6.00	Ca^{++}
1.50	1.50	4.00	Mg^{++}
12.90	4.40	28.60	Na^{+}
0.78	0.14	0.12	K^{+}
Soluble anions (meq\l)			
-	-	-	$CO3^{=}$
2.00	2.40	4.40	$HCO3^{-}$
13.00	5.00	27.20	Cl^{-}
3.18	1.14	7.12	$SO4^{=}$

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

Values	parameters
7.49	pH
4.40	EC(dSm^{-1})
Soluble cations (meq\l)	
7.50	Ca++
5.00	Mg^{++}
33.10	Na^{+}
0.16	K^{+}
Soluble anions (meq\l)	
-	$CO3^{=}$
1.60	$HCO3^{-}$
40.00	Cl^{-}
4.16	$SO4^{=}$

Results and Discussion

Fruit Properties:

Concerning the effect of number of applications of humic acid and Greenpower on some physical and chemical properties of Kalamata olive fruits during first season (2013) as shown in Tables (3,5). It was clear that Actosol (humic acid) or Greenpower (Vinasse + Soyabean Amino acids) application doses treatments increased fruit physical and chemical parameters compared with the control in both seasons. It is found that, fruit physical parameters, moisture percentage and acidity percentage were affected Positively by soil application of Actosol or Greenpower in one doses during full bloom; while as, the highest values of fruit oil percentage where obtained from fruits harvested of Kalamata trees which received Actosol or Greenpower at three dose during, followed, in a descending order, by application at two and one doses respectively.

Whereas, physical and chemical properties of Kalamata olive fruits during second season (2014) as shown in Tables (4,6) it is clear that fruit physical properties i.e. fruit weight, size, pulp/pit ratio, shape index, oil % and acidity % as a chemical properties were significantly affected by the source of material (humic acid or Greenpower) as will as number of applications. Generally, humic acid applications were superior in their impact on studied fruit quality parameters than Greenpower, Moreover, humic acid or Greenpower applications at one dose resulted in higher significant values compared with two or three times of application, while pulp/pit ratio, oil% and fruit acidity % did not follow the trend obtained for fruit weight, size and shape index as affected by humic acid or Greenpower number of application treatments, noticeable that not affecting significantly fruit oil percentage and fruit acidity %. Adding soil application of Actosol or Greenpower to olive picual trees affecting significantly fruit oil percentage. So soil application of humic acid once during full bloom gave the highest oil percentage of Kalamata olives grown in Ismailia in the second season.

Also noticed that, fruit oil percentage was increased in the second season than the first one, which might be due to accumulation effects of organic treatments. However, the control treatments recorded the lowest fruit oil and acidity %. These observations are in accordance with those obtained by[32] who stated that, physical and chemical properties of 'Canino' apricot fruits were progressively increased as foliar and soil doses of humic acid increased. Also, [33]observed that, poultry manure source in inhancing fruit physical properties of olive trees. However, soil application of compost tea on Le cont pear trees with humic acid and or bio-fertilizers gave the highest level of improving all fruit physical properties[34]. Also, [2]observed that highest values of fruit physical properties of Roghiani olive trees were obtained by spraying yeast + humic acid. Where, [35]reported that, compost tea gave high significant values of Washington navel orange fruit quality

Table 3: Effect of number of application of humic acid and green power on some physical fruit properties of Kalamata olive during first season (2013).

Treatment		weight	Mean	volume	Mean	Pulp/Pit	Mean	L/D	Mean
Humic acid	Once	5.65 a	**5.28 A**	5.48 a	**5.01 A**	5.49 b	**5.49 A**	1.59 ab	**1.51 A**
	Twice	5.06 c		4.75 c		5.84 a		1.61 a	
	Three times	5.12 c		4.81 c		5.53 ab		1.54 cd	
Green power	Once	5.38 b	**5.08 B**	5.09 b	**4.86 B**	4.95 c	**5.27 A**	1.50d	**1.55 A**
	Twice	5.02 c		4.86 c		5.39 b		1.56 bc	
	Three times	4.85 d		4.62 c		5.47 b		1.58 abc	
Control		4.49 e	**4.49 C**	4.38 d	**4.38 C**	5.37 bc	**5.37 A**	1.45 e	**1.45 B**
Mean	Once	**5.17 A"**		**4.98 A"**		**5.14 A"**		**1.51 A"**	
	Twice	**4.86 B"**		**4.66 B"**		**5.53 A"**		**1.54 A"**	
	Three times	**4.82 B"**		**4.60 B"**		**5.46 A"**		**1.52 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 green power on some physical fruit properties Kalamata olive during second season (2014).

Treatment		weight	Mean	volume	Mean	Pulp/Pit	Mean	L/D	Mean
Humic acid	Once	7.78 a	**6.78 A**	7.34 a	**6.59 A**	5.41 ab	**5.30 B**	1.58 ab	**1.56 A**
	Twice	6.37 d		6.24 c		5.00 b		1.59 ab	
	Three times	6.18 d		6.18 c		5.49 ab		1.52 c	
Green bower	Once	6.95 b	**6.64 B**	6.73 b	**6.56 A**	5.13 b	**5.65 A**	1.51 cd	**1.56 A**
	Twice	6.67 c		6.53 bc		5.88 a		1.61 a	
	Three times	6.31 d		6.43 bc		5.95 a		1.55 bc	
Control		5.59 e	**5.59 C**	5.67 d	**5.67 B**	5.45 ab	**5.45 AB**	1.46 d	**1.46 B**
Mean	Once	**7.36 A"**		**7.04 A"**		**5.27 A"**		**1.55 B"**	
	Twice	**6.52 B"**		**6.39 B"**		**5.44 A"**		**1.60 A"**	
	Three times	**6.24 C"**		**6.3 B"**		**5.72 A"**		**1.54 B"**	

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

Table 5: Effect of number of application of humic acid and green power on some chemical fruit properties Kalamata olive during first season (2013).

Treatment		Moisture %	Mean	Fruit Oil %	Mean	Fruit acidity %	Mean
Humic acid	Once	61.13 a	**59.92 A**	44.95 b	**45.40 A**	1.50 a	**1.28 A**
	Twice	59.81 a		45.08 b		1.31 b	
	Three times	58.83 ab		46.17 ab		1.04 c	
Green bower	Once	59.32 ab	**58.48 A**	44.69 b	**45.66 A**	0.98 c	**1.22 A**
	Twice	58.65 ab		44.93 b		1.29 b	
	Three times	57.48 b		47.35 a		1.40 ab	
Control		54.55 c	**54.55 B**	43.06 c	**43.06 B**	0.94 c	**0.94 B**
Mean	Once	**58.33 A"**		**44.23 B"**		**1.14 A"**	
	Twice	**57.67 AB"**		**44.36 B"**		**1.18 A"**	
	Three times	**56.95 B"**		**45.53 A"**		**1.13 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 green power on some chemical fruit properties Kalamata olive during second season (2014).

Treatment		Moisture %	Mean	Fruit Oil %	Mean	Fruit acidity %	Mean
Humic acid	Once	63.74 a	**58.56 A**	40.50 cd	**42.38 A**	1.23 a	**1.05 A**
	Twice	57.37 b		41.47 c		1.07 a	
	Three times	54.56 c		45.17 a		0.85 b	
Green bower	Once	55.96 bc	**55.72 B**	36.37 f	**39.35 B**	0.80 b	**1.00 A**
	Twice	56.98 b		38.35 e		1.06 a	
	Three times	54.23 c		43.33 b		1.15 a	
Control		51.46 d	**51.46 C**	39.33 de	**39.33 B**	0.80 b	**0.80 B**
Mean	Once	**59.85 A"**		**38.43 C"**		**1.02 A"**	
	Twice	**57.17 B"**		**39.91 B"**		**1.06 A"**	
	Three times	**54.40 C"**		**44.25 A"**		**1.00 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 "Kalamata" 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 humic acid application treatments resulted in a significant increase in "Kalamata" olive yield than those received Greenpower, while both of humic acid and Greenpower treatments significantly increased yield than the control in both seasons. The highest yield was obtained from adding humic acid at a rate of 150 cm^3 at full bloom as one dose. However, increasing application dose number to two or three doses tended to significantly reduce the obtained increment in yield as compared with one dose application.

These observations are in accordance with those obtained[2] indicated that, soil application of compost tea gave the highest set and yield (Kg\ tree) of Roghiani olives grown in Libya in two seasons, followed by manure tea comparing to control trees. Also noticed that, fruit set percentage and yield were increased in the second season than the first one, which might be due to accumulation effects of organic treatments. These observations are in accordance with those obtained[36] that, the application of humic acid caused a significant increase in berry size. In particular, humic acid applied at full bloom significantly increased width and weight of berries collected at harvest with respect to the control treatment. The increase in berry size as a consequence of humic acid application at full bloom is probably ascribed to the uptake of mineral nutrients by the grapevines, but the possible hormone- like activity of the humic acid (i.e.,auxin-, gibberellin- and cytokinin-like activity) should also be taken into consideration. Also, the results are in line with [33]on olive trees, [34]on pear trees, who found that using compost tea with spraing humic acid recorded the highest values of fruit set and yield. [35] on orange reported that the combination between compost tea and chicken manure extracts at concentration (1:10 x 1:10 w\v) gave significant increase in yield per tree.

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.

Amino acids as organic nitrogenous compounds are the building blocks in the synthesis of proteins[37]. Amino acids are particularly important for stimulation cell growth, they act as buffers which help tomaintain 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. [38] 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. [39]Alter native 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 decaboxylation of a-keto acids, such as Pyruvic acid and keto-glutamic acid which has its importance in the metabolism of carbohydrates and fats [40]. 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 [41].

Table 7: Effect of number of application of humic acid and green power on yield Kalamata olive during (2013 – 2014).

Treatment		Yield 2013	Mean	Yield 2014	Mean	Average yield	Mean
Humic acid	Once	47.52 a	**42.94 A**	15.33 a	**13.15 A**	31.43 a	**28.04 A**
	Twice	42.27 c		13.50 b		27.85 c	
	Three times	39.04 e		10.63 d		24.84 e	
Green bower	Once	45.32 b	**41.32 B**	13.91 b	**12.18 B**	29.62 b	**26.75 B**
	Twice	40.65 d		12.36 c		26.51 d	
	Three times	37.98 e		10.27 d		24.13 e	
Control		29.78 f	**29.78 C**	8.39 e	**8.39 C**	19.09 f	**19.09 C**
Mean	Once	**40.87 A"**		**14.62 A"**		**26.71 A"**	
	Twice	**37.56 B"**		**12.93 B"**		**24.48 B"**	
	Three times	**35.60 C"**		**10.45 C"**		**22.69 C"**	

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

Conclusion

Soil application of humic acid once at full bloom stage at 150 cm^3\tree to "Kalamata" olive trees is recommended for high yield and oil% under Sinai condition. The increase in yield and oil% as a consequence of humic acid application at full bloom is probably ascribed to the uptake of mineral nutrients by the olive trees, but the possible hormone- like activity of the humic acid (i.e.,auxin-, gibberellin- and cytokinin-like activity) should also be taken into consideration. These results were in the same trend with those reported that[42,43, 44] 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[45,46,48]. 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.

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