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Effect of Organic Fertilizers on Avocado Trees (Cvs. Fuerte, Hass, Lamb Hass) in Western Crete, a Cool Subtropical Region

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Abstract: The market for avocado is one of the fastest expanding worldwide, inclduing the Mediterranean basin. Organic farming systems cannot make use of synthetic fertilizers and therefore rely on several cultural techniques to maintain vigorous young trees and for quick shoot development, satisfactory yield, and fruit quality. We studied the effect of three different organic products (Terra Insecta® (Aeiphoria-Sustainable products of Crete, Chania, Greece), Fruit-Fix®, and AMINO-16® (both products manufactured by EVYP, Sindos, Thessaloniki, Greece) on avocado plants in the cool subtropical area of southern Greece. Three experiments were carried out, two of them on young avocado trees, and the third one on mature, fully productive trees. The establishment success (ES) (%), plants with shoot induction (SI) (%), plants with shoot growth $\geq$ 3 cm (SG) (%), number of sprouted buds $\geq$3 cm per plant (SB), mean shoot length (cm) per plant (SL), total shoot length (cm) per plant (TSL), mean leaf number per plant (LN), and total leaf number per plant (TLN) were measured in the first and second experiments, while in the third experiment, fruit growth parameters (length, width, fruit weight, fruit weight increase (FWI)) and fruit quality parameters (dry matter, oil concentration, dry matter, and oil concentration increase) were measured. The application of 0.2 K of Terra Insecta® to the planting hole did not have any statistically significant effect on plant growth, but when added around the trunk, statistically higher values were observed for SB, SL, LN, TSL, and TLN in the Terra Insecta® treatment compared to the control. Fruit-Fix® application to Lamb Hass avocado trees resulted in significant differences in ES, SI, SG, SL, TSL, LN, and TLN, and in the Hass variety, in SB. In the AMINO-16® experiment, the fruit dimensions, quality parameters, and yield of the Hass variety were not recorded as significantly different. However, in the Fuerte variety, FW increased by 119.3% in the AMINO-16® treatment. The effect of the organic fertilizers used in this research showed noticeable results requiring studies to be carried out over more seasons, different tree ages, cultivation methods, and stress conditions.

Keywords: amino-acid fertilizer; *Ascophyllum nodosum*; avocado; insect manure; Mediterranean; organic fertilizer

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1. Introduction

The market for avocado is one of the fastest expanding worldwide, and the marked increase in the consumption of this fruit worldwide has caused environmental and socio-economic issues in the producing countries, which are often far from the final commercial destination of the product [1]. In Europe alone, avocado consumption increased with yearly rates up to 179 percent in the last decade [2]. However, in the last years, due also to higher popularity and media coverage, European consumers have started showing a preference towards locally and sustainably grown avocados, rather than imported ones, and the fruit has increasingly taken part in the Mediterranean diet [3–5]. Locally grown fruits can, in general, be harvested at a ripening point closer to the optimum, which allows one to offer the consumer a higher quality, in terms of both taste and nutritional and organoleptic characteristics. Moreover, many of the most important avocado varieties worldwide can be grown with satisfying results in the hot-summer Mediterranean climate [6], which characterizes many areas of the Mediterranean basin.

Dry matter and oil concentration are significant parameters of fruit maturation of avocado trees and determine the harvesting time [7]. These parameters can be affected by fertilization protocols [8,9]. Dry matter has also been observed to be linked to the consumer's liking and intent to buy. During an experiment when one hundred and seven consumers tasted avocados with a range of dry matter levels, the higher the dry matter content, the higher the preference [10].

Organic farming systems cannot make use of synthetic fertilizers and therefore rely on several cultural techniques to maintain vigorous young trees and for quick shoot development, satisfactory yield, and fruit quality. Alternative organic fertilizers can be used and different, natural sources of nitrogen are increasingly demanded by the producers following the protocols of organic agriculture [11,12]. Among others, amino acid application, sometimes paired with other organic material such as seaweed, has been reported to affect several parameters related to fruit-set and the yield of several crops, as it represents an alternative, organic source of nitrogen [13–19]. Little is known about the possibility of using insect manure as an organic source of nitrogen fertilizer and about the effective nutritional value of this product for the plants [20]. However, some authors affirm that this byproduct of edible insects farming systems could work similarly to poultry manure, with a smaller environmental impact [21]. With this in mind, we tested the effects of the application of different organic products (Terra Insecta® (Aeiphoria-Sustainable products of Crete, Chania, Greece), Fruit-Fix®, and Amino16® (both products manufactured by EVYP, Sindos, Thessaloniki, Greece)) on young and mature avocado trees in the cool subtropical area of the island of Crete, southern Greece.

2. Materials and Methods

To study the effect of various organic products, i.e., Terra Insecta®, Fruit-Fix®, and AMINO-16® on avocado trees, three experiments were carried out, two of them on young avocado trees and one on mature trees. In all experiments, additional fertigation of 15-15-15 was applied monthly.

Soil analysis: Before the three experiments were implemented, soil samples were taken from each experimental field, from 30 cm depth, at 40 cm distance from the tree trunk. Three samples were taken from each treatment (18 trees), one from each replication with 6 trees included. Soil samples were air-dried, crushed, and then sieved through a 10 mm and a 2 mm mesh. Soil pH and electrical conductivity (EC) were determined by preparing a 1:2.5 soil/distilled water (w/v) suspension, and the respective measurements were made with a Multi multimeter (Mettler-Toledo, Greifensee, Switzerland) using the relevant electrodes. Soil particle size analysis was determined by the Bouyoucos hydrometer method (Bouyoucos, 1962) [22]. The modified Walkley–Black wet combustion method was used to determine the soil organic matter (OM) content (Nelson and Sommers, 1982) [23]. Available P was measured using the Olsen method (Olsen et al., 1954) [24], while the carbonate content ($CaCO_3$% w/w) was analyzed by the Bernard calcimeter method (Horton and Newsom, 1953) [25]. Exchangeable cations in soil (Ca, Mg and K) were extracted using 1 N ammonium acetate at 1:20 dilution, while the bioavailable fraction of micronutrients (Fe, Mn, Zn, and Cu) was determined by extraction with 0.005 M DTPA (pH 7.3) (Lindsay & Norvell, 1978) [26] and quantified by Inductively Coupled Plasma—Optical Emission Spectrometry (ICP-OES) (Optima 8300, Perkin Elmer, Waltham, MA, USA) Nitrate nitrogen in the soil was measured colorimetrically using the Cd reduction method with Nitraver reagent (Hach-Lange, Germany), after extraction with 1 M KCl for 1 h.

2.1. Experiment 1. The Effect of Terra Insecta® Organic Insect Manure on Young Avocado Trees

The fertilizer used was Terra Insecta®, a natural soil improver consisting of insect frass and exuviae derived from the mass rearing of *Tenebrio molitor* (Coleoptera: Tenebrionidae), containing 3% N, 2% P, and 2.5% K. Two treatments were applied as follows: a) Control: no application and b) Terra Insecta®: three applications, once every two months (May, July, September). A complete block design was applied per sub-experiment (1.1, 1.2) and cultivar, including 3 replications with 6 trees per replication. In total, 18 trees per treatment in each experiment and cultivar were used. Sub-experiment **1.1**: Fuerte variety, application of 200 g/tree in the planting hole, during planting. Sub-experiment **1.2**: Hass and Lamb Hass varieties, application of 500 g/tree superficially around the trunk. This was the first time that Terra Insecta® has been applied in avocado trees, so different application types were necessary to determine the most appropriate method. The G6 avocado seedling was used as rootstock for all experimental trees.

At the end of the experiments, the following parameters were measured: establishment success (ES) (%), plants with shoot induction (SI) (%), plants with shoot growth $\geq$ 3 cm (SG) (%), number of sprouted buds $\geq$3 cm per plant (SB), mean shoot length (cm) per plant (SL), total shoot length (cm) per plant (TSL), mean leaf number per plant (LN), and total leaf number per plant (TLN).

2.2. Experiment 2. The Effect of Fruit-Fix® Organic Mixture on Young Avocado Trees

Fruit-Fix® is a product obtained by the combination of an AMINO-16® hydrolyzed protein solution (see below) with *Ascophyllum nodosum* algal extract. Two treatments were applied as follows: (a) Control: No application, (b) Fruit-Fix®: Two applications of Fruit-Fix® through the fertigation system, with the first immediately after transplanting (October). The dose of Fruit-Fix® was 20 L/hectare/application. A complete block design was applied in both sub-experiments (2.1, 2.2), including 3 replications with 6 trees per replication, i.e., 18 trees per treatment in each sub-experiment. Measurements were taken at the end of spring (May). Sub-experiment **2.1**: Lamb Hass variety grafted on Duke7 clonal rootstock, 2nd application in April, in a field in Souda-Chania. Sub-experiment **2.2**: Hass variety grafted on G6 seedling rootstock, 2nd application in November, in a field in Vatolackos-Chania.

The parameters measured were the establishment success (ES) (%), plants with shoot induction (SI) (%), plants with shoot growth $\geq$ 3 cm (SG) (%), number of sprouted buds $\geq$3 cm per plant (SB), mean shoot length (cm) per plant (SL), total shoot length (cm) per plant (TSL), mean leaf number per plant (LN), and total leaf number per plant (TLN).

2.3. Experiment 3. The Effect of AMINO-16® Organic Mixture on Mature Avocado Trees

AMINO-16® is a hydrolyzed protein solution containing 11.3% L-amino acids (alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine), 3% total N, and 33% organic matter, produced of raw plant materials.

For this experiment, a field with nine-year-old Fuerte and Hass avocado trees grafted on Zutano seedling rootstock in western Crete was chosen. Two treatments were applied as follows: (a) Control: no application, (b) AMINO-16®: six applications of AMINO-16® through the fertigation system (10th, 13th, 16th, 37th, 40th, and 43rd week of the year). The dose of AMINO-16® was 30 L/hectare/application. A complete block design was applied, including 3 replications with 6 trees per replication, i.e., 18 trees for each variety (Hass, Fuerte) per treatment in total. From each treatment of 18 trees, 3 fruit samples consisting of 5 fruit each were taken (one from each replication). Twenty days before the first spring and autumn AMINO-16® application (7th week of the year for Hass and 34th week for Fuerte), fruit dimensions (length, width), fruit weight, dry matter, and oil concentration were recorded as initial measurements. At harvesting time (21st week for Hass and 49th week for Fuerte), the following parameters were recorded as final measurements: fruit growth parameters (length, width, weight, and fruit weight increase) and fruit quality parameters (dry matter, oil concentration, dry matter increase (DMI), and oil concentration increase (OCI)). The "increase" results were calculated by the equitation

$$\text{Increase} = ((\text{final measurement} - \text{initial measurement})/\text{initial measurement}) \times 100$$

During harvesting, the fruit yield was recorded. Leaf analysis was recorded in September (38th week of the year). The experiment was repeated twice (two-ear-d uration), in 2019 and 2020. All measurements presented are the mean numbers of the two-year results. Additional measurements were taken in the third year on flowering and fruiting.

2.4. Leaf Analysis

Leaf samples were collected in the 38th week of the year. A sample of 50 leaves per replication and cultivar, consisting of 5–6-month-old leaves, was collected, washed with distilled water, and dried at 65°C to a constant weight prior to fine grinding with a mill. Total N was determined colorimetrically after wet digestion (Kjeldhal method) of a subsample of 0.1 g of dry tissue (Gaines and Mitchell, 1979) [27]. Ground samples (1 g) were dry ashed (520 °C for 5 h) and dissoluted with 1 M HCl (1:4) under mild heating (50–60 °C) prior to being filtered and diluted to 25 mL. The concentrations of K, Ca, Mg, Fe, Zn, Mn, and Cu were determined by ICP-OES (Optima 8300, Perkin Elmer, Waltham, MA, USA). The total P content was determined colorimetrically in the same solution, using the ammonium vanadate method. The total B concentration was determined after the addition of an azomethin-H/EDTA reagent and colorimetric determination at 410 nm, using a visual spectrum photometer (PhtoLab 6100, WTW, Oberbayern, Germany).

2.5. Dry Matter Analysis

At the harvesting period of each cultivar (21st week for Hass and 49th week for Fuerte) the avocado fruits obtained from the farm were immediately taken to the laboratory (within an hour) for dry matter analysis. One fruit was kept as the control. The rest had the seed, seed coat, and skin removed. The flesh of the fruit was cut into smaller pieces and with a mechanical blender, turned into paste. The fresh sample-paste was put in three pre-weighed ceramic pods, and the initial weight of the fresh sample was recorded. Samples were then placed in an oven at 103.5 °C overnight, to dry. The final weight of the dried sample was measured, and the dry matter was calculated according to the following equation:

$$DM\ (\%) = (1 - ((FW - DW)/FW)) \times 100$$

where *DM*: Dry Matter, *FW*: Fresh Weight, *DW*: Dry Weight.

2.6. Oil Concentration Analysis

Extraction was performed using the Soxhlet method with n-hexane. After the extraction, the crude oil samples were placed in a rotary evaporator to remove the remaining solvent and after that in an oven at 103.5 °C overnight, for the final n-hexane evaporation. The oil yield was computed as follows:

$$OC\ (\%) = (1 - ((FW - OW)/FW)) \times 100$$

where *OC*: Oil Concentration, *FW*: Fresh Weight, *OW*: Oil Weight.

2.7. Statistical Analysis

The data of all the three experiments were statistically analyzed by the SPSS 21 statistical program (SPSS, Inc., Chicago, IL, USA. Student's *t*-test, $p \leq 0.05$, was used to compare the means since all experiments consisted of the comparison of only two groups (control and treatment).

3. Results

3.1. Soil Analysis

The detailed soil data of the three experiments are presented in Table 1. Briefly:

i. Experiment 1 took place on a sandy loam soil, with a sand content of 55.8%, clay 16.2%, silt 28.0%, pH of 7.41, total organic matter of 4.29%, high N-NO_3, and low $CaCO_3$.
ii. Sub-experiment 2.1: The soil where Lamb Hass trees were planted (Souda experiment) had a sand content of 47.3%, clay 21.1%, silt 31.6%, pH of 7.54, total organic matter of 1.88%, high N-NO_3, and low $CaCO_3$.
iii. Sub-experiment 2.2: Soil analysis of the experimental field (Vatolackos experiment) showed that it contained 59.8% sand, 10.2% clay, 30.0% silt, pH 6.27, total organic matter of 2.41%, N-NO_3 at 18.10, and low $CaCO_3$.
iv. For the third experiment, the experimental plot consisted of silty-clay soil, with a sand content of 45.5%, clay 27.7%, silt 26.7%, pH of 8.3, total organic matter of 6.42%, high N-NO_3 (30.71%), and high $CaCO_3$ (20.70%).

Table 1. Soil analysis of the three experimental fields before the application of Terra Insecta® (Experiment 1), Fruit-Fix® (Sub-experiment 2.1 & 2.2), and AMINO-16® (Experiment 3).

Soil Analysis									
	Sand	**Clay**	**Silt**	**pH**	**Conductance (mS/cm)**	**Total Organic Matter (%)**	**Total $CaCO_3$ (%)**	**mg N-NO_3 /K Soil**	**P (mg/K Soil)**
Exp. 1 Terra Insecta®	55.8	16.2	28.0	7.41	0.17	4.29	0.37	52.80	6.04
Sub-exp. 2.1 Fruit-Fix®	47.3	21.1	31.6	7.54	0.37	1.88	1.09	39.90	80.00
Sub-exp. 2.2 Fruit-Fix®	59.8	10.2	30.0	6.27	0.11	2.41	0.25	18.10	24.00
Exp. 3 AMINO-16®	45.5	27.7	26.7	8.30	0.53	6.72	20.70	30.71	31.52

3.2. Experiment 1: The Effect of TERRA INSECTA® on Young Avocado Trees

3.2.1. Sub-Experiment 1.1

Terra Insecta® insect organic manure is a new product that was first applied to avocado trees. The application of 0.2 K to the planting hole of the Fuerte variety did not have a statistically significant effect on plant growth, as shown in Table 2. The establishment success was 100%, and all plants developed shoots longer than 3 cm. In the Control treatment, the mean length of new shoots per plant was 6.5 cm, the total length of new shoots per plant was 52.9 cm, the mean number of new leaves per plant was 5.3, and the total number of new leaves per plant was 44.1. In the Terra Insecta® treatment, the results were 6.7 cm, 56.4 cm, 4.9, and 41.1, respectively.

Table 2. Sub-experiment 1.1; effect of Terra Insecta® insect organic manure on young Fuerte avocado trees, grafted on G6 seedling avocado rootstock. The insect manure was added to the planting hole during tree establishment in one dose of 0.2 K/plant. The following parameters were measured: establishment success (ES) (%), plants with shoot induction (SI) (%), plants with shoot growth ≥ 3 cm (SG) (%), number of sprouted buds ≥3 cm per plant (SB), mean shoot length (cm) per plant (SL), total shoot length (cm) per plant (TSL), mean leaf number per plant (LN), and total leaf number per plant (TLN).

The Effect of TERRA INSECTA® on Young Fuerte Avocado Trees								
	ES %	**SI (%)**	**SG (%)**	**SB**	**SL (cm)**	**TSL (cm)**	**LN**	**TLN**
Control	100.0a ± 0.0	100.0a ± 0.0	100.0a ± 0.0	8.1a ± 2.345	6.5a ± 1.758	52.9a ± 6.190	5.3a ± 1.673	44.1a ± 4.975
Terra Insecta®	100.0a ± 0.0	100.0a ± 0.0	100.0a ± 0.0	8.5a ± 2.101	6.7a ± 1.967	56.4a ± 5.036	4.9a ± 2.015	41.1a ± 5.064

Values (mean ± SD) within a column followed by the same letter are not significantly different, according to Student's *t*-test, at $p \leq 0.05$.

3.2.2. Sub-Experiment 1.2

To study the effect of Terra Insecta® further, it was applied, in addition to the planting hole, as a fertilizer to two avocado varieties (Hass, Lamb Hass). In this case, although plant growth was observed in all plants, in the Terra Insecta® treatment, statistically higher values were observed (Tables 3 and 4). Specifically, in the Hass variety (Table 3), the number of sprouted buds per plant was 11.6, compared to 8.2 in the Control treatment. The mean length of new shoots per plant and the total length of new shoots per plant were also higher in the Terra Insecta® treatment (9.2 and 107.9 cm, respectively) compared to the Control (6.6 and 55.2 cm, respectively). The mean number of new leaves per plant and the total number of new leaves per plant were also higher in the Terra Insecta® treatment than in the Control treatment (7.4 and 86.8 vs. 5.6 and 46.1) (Table 3). The results for the Lamb Hass variety were similar to those of Hass, where the number of sprouted buds, mean and total length of new shoots per plant, and mean and total number of new leaves per plant were also higher in the Terra Insecta® treatment (Table 4).

Table 3. Sub-experiment 1.2; the effect of Terra Insecta® insect organic manure on young Hass avocado trees, grafted on G6 seedling avocado rootstock. The insect manure was added round the tree trunk after tree establishment in two doses of 0.5 K/dose/plant. The following parameters were measured: establishment success (ES) (%), plants with shoot induction (SI) (%), plants with shoot growth ≥ 3 cm (SG) (%), number of sprouted buds ≥3 cm per plant (SB), mean shoot length (cm) per plant (SL), total shoot length (cm) per plant (TSL), mean leaf number per plant (LN), and total leaf number per plant (TLN).

The Effect of Terra Insecta® on Young Hass Avocado Trees								
	ES (%)	**SI (%)**	**SG (%)**	**SB**	**SL (cm)**	**TSL (cm)**	**LN**	**TLN**
Control	100.0a ± 0.0	100.0a ± 0.0	100.0a ± 0.0	8.2a ± 1.078	6.6a ± 1.450	55.2a ± 10.835	5.6a ± 0.643	46.1a ± 12.986
Terra Insecta®	100.0a ± 0.0	100.0a ± 0.0	100.0a ± 0.0	11.6b ± 1.972	9.2b ± 1.069	107.9b ± 18.740	7.4b ± 1.087	86.8b ± 18.301

Values (mean ± SD) within a column followed by the same letter are not significantly different, according to Student's *t*-test, at $p \leq 0.05$.

Table 4. Sub-experiment 1.2; the effect of Terra Insecta® insect organic manure on young Lamb Hass avocado young trees, grafted on G6 seedling avocado rootstock. The insect manure was added round the tree trunk after tree establishment in two doses of 0.5 K/dose/plant. The following parameters were measured: establishment success (ES) (%), plants with shoot induction (SI) (%), plants with shoot growth ≥ 3 cm (SG) (%), number of sprouted buds ≥3 cm per plant (SB), mean shoot length (cm) per plant (SL), total shoot length (cm) per plant (TSL), mean leaf number per plant (LN), and total leaf number per plant (TLN).

The Effect of Terra Insecta® on Young Lamb Hass Avocado Trees								
	ES (%)	**SI (%)**	**SG (%)**	**SB**	**SL (cm)**	**TSL (cm)**	**LN**	**TLN**
Control	100.0a ± 0.0	100.0a ± 0.0	100.0a ± 0.0	10.2a ± 1.703	6.3a ± 0.923	66.4a ± 19.478	4.7a ± 1.179	52.4a ± 14.296
Terra Insecta®	100.0a ± 0.0	100.0a ± 0.0	100.0a ±0.0	14.2b ± 2.108	8.7b ± 1.086	123.8b ± 34.926	7.2b ± 1.056	103.0b ± 31.914

Values (mean ± SD) within a column followed by the same letter are not significantly different, according to Student's *t*-test, at $p \leq 0.05$.

3.3. Experiment 2: The Effect of Fruit-Fix® on Young Avocado Trees

Fruit-Fix® was tested in two experimental fields where Lamp Hass and Hass avocado varieties were planted. It was applied according to the directions of the producing company, by drip irrigation at a dose of 20 L per hectare.

3.3.1. Sub-Experiment 2.1

According to the results (Table 5), in Lamb Hass avocado trees, there were statistically significant differences in all parameters measured except sprouted buds per plant. When Fruit-Fix® was applied, 100% of the installed plants survived, but only 87.5% of the Control plants. The plants that grew (SI) accounted for 87.5% in response to Fruit-Fix® compared with 50.0% in the Control treatment. In addition, plants with shoot growth of at least 3 cm long accounted for only 37.5% in the Control, compared with 87.5% in Fruit-Fix®. Moreover, the mean length of new shoots per plant (2.6 cm), the total length of new shoots per plant (3.1 cm), the mean number of new leaves per plant (2.1), and the total number of new leaves per plant (2.5) showed significantly higher values with Fruit-Fix® application in contrast to the Control (0.7 for both parameters) (Table 5).

Table 5. Sub-experiment 2.1; the effect of Fruit-Fix® organic mixture on young Lamb Hass avocado trees grafted on Duke 7 clonal avocado rootstock in the Souda region. The organic mixture was added by drip irrigation after tree establishment in two doses (autumn and spring) of 20 L/dose/hectare. The following parameters were measured: establishment success (ES) (%), plants with shoot induction (SI) (%), plants with shoot growth ≥ 3 cm (SG) (%), number of sprouted buds ≥3 cm per plant (SB), mean shoot length (cm) per plant (SL), total shoot length (cm) per plant (TSL), mean leaf number per plant (LN), and total leaf number per plant (TLN).

The Effect of Fruit-Fix® on Young Lamb Hass Avocado Trees								
	ES (%)	**SI (%)**	**SG (%)**	**SB**	**SL (cm)**	**TSL (cm)**	**LN**	**TLN**
Control	87.5a ± 0.0	50.0a ± 13.742	37.5a ± 17.812	1.0a ± 0.281	0.9a ± 0.033	0.9a ± 0.072	0.7a ± 0.056	0.7a ± 0.286
Fruit-Fix®	100.0b ± 0.0	87.5b ± 16.876	87.5b ± 18.923	1.2a ± 0.796	2.6b ± 0.819	3.1b ± 1.014	2.1b ± 0.991	2.5b ± 1.003

Values (mean ± SD) within a column followed by the same letter are not significantly different, according to Student's *t*-test, at $p \leq 0.05$.

3.3.2. Sub-Experiment 2.2

In the Hass avocado trees, the number of sprouted buds per plant (3.4) was statistically higher in the Fruit-Fix® treatment than in the Control treatment (2.4). No significant differences were recorded for the other growth parameters (Table 6).

Table 6. Sub-experiment 2.2; the effect of Fruit-Fix® organic mixture on young Hass avocado trees grafted on G6 seedling avocado rootstock in the Vatolackos region. The organic mixture was added by drip irrigation after tree establishment in two doses (autumn) of 20 L/dose/hectare. The following parameters were measured: establishment success (ES) (%), plants with shoot induction (SI) (%), plants with shoot growth $\geq$ 3 cm (SG) (%), number of sprouted buds $\geq$3 cm per plant (SB), mean shoot length (cm) per plant (SL), total shoot length (cm) per plant (TSL), mean leaf number per plant (LN), and total leaf number per plant (TLN).

The Effect of Fruit-Fix® on Young Hass Avocado Trees								
	ES %	**SI (%)**	**SG (%)**	**SB**	**SL (cm)**	**TSL (cm)**	**LN**	**TLN**
Control	95.8a ± 6.028	83.3a ± 13.765	50.0a ± 9.740	2.4a ± 0.279	4.2a ± 1.148	10.0a ± 3.902	3.9a ± 1.820	9.3a ± 3.901
Fruit-Fix®	100.0a ± 0.0	83.3a ± 16.953	45.8a ± 8.710	3.4b ± 0.586	4.1a ± 1.891	14.0a ± 3.109	3.6a ± 1.342	12.1a ± 4.867

Values (mean ± SD) within a column followed by the same letter are not significantly different, according to Student's *t*-test, at $p \leq 0.05$.

3.4. Experiment 3: The Effect of AMINO-16® on Mature Avocado Trees

Leaf analysis after AMINO-16® application showed that in the Hass variety, manganese increased significantly compared to the Control. There were no statistical differences for other leaf analyses, in the Fuerte variety or in Hass (Table 7).

Table 7. Experiment 3; Leaf analysis of Hass and Fuerte avocado trees after AMINO-16® application. The organic mixture was added by drip irrigation in six doses of 30 L/dose/hectare.

Avocado Leaf Analysis											
		N % d.m.	**P %**	**K %**	**Ca %**	**Mg %**	**Fe ppm**	**Zn ppm**	**Mn ppm**	**Cu ppm**	**B ppm**
Hass	Control	1.42a ± 0.826	0.101a ± 0.045	0.53a ± 0.274	2.28a ± 0.872	0.657a ± 0.328	86.2a ± 18.836	15.7a ± 5.924	43.6a ± 8.982	5.9a ± 3.921	10.30a ± 4.643
	AMINO-16®	1.49a ± 0.319	0.104a ± 0.031	0.51a ± 0.134	2.26a ± 1.283	0.666a ± 0.271	75.3a ± 17.286	15.4a ± 6.712	61.5b ± 7.016	5.7a ± 3.008	9.85a ± 3.864
Fuerte	Control	1.63a ± 0.582	0.122a ± 0.627	0.73a ± 0.245	2.68a ± 0.786	0.548a ± 0.301	77.8a ± 17.825	25.8a ± 6.901	55.8a ± 12.975	7.2a ± 4.862	12.2a ± 5.981
	AMINO-16®	1.73a ± 0.971	0.128a ± 0.386	0.71a ± 0.416	2.55a ± 1.834	0.561a ± 0.418	70.3a ± 18.925	24.7a ± 8.430	65.3a ± 14.234	6.9a ± 3.986	13.2a ± 5.096

Values (mean ± SD) within a column for each variety followed by the same letter are not significantly different, according to Student's *t*-test, at $p \leq 0.05$.

The measurements of the fruit growth parameters (dimension and weight) were taken at the beginning and at the end of the experiment. According to the results, AMINO-16® did not have any significant effect on the fruit dimensions in either of the two varieties studied (Figure 1A, Table 8). However, in the Fuerte variety, fruit weight increased by 54.7% (from 97.9 to 151.8 g) in the Control treatment and by 119.3% (from 86.8 to 190.5 g) in the AMINO-16® treatment (Table 8, Figures 1B and 2A).

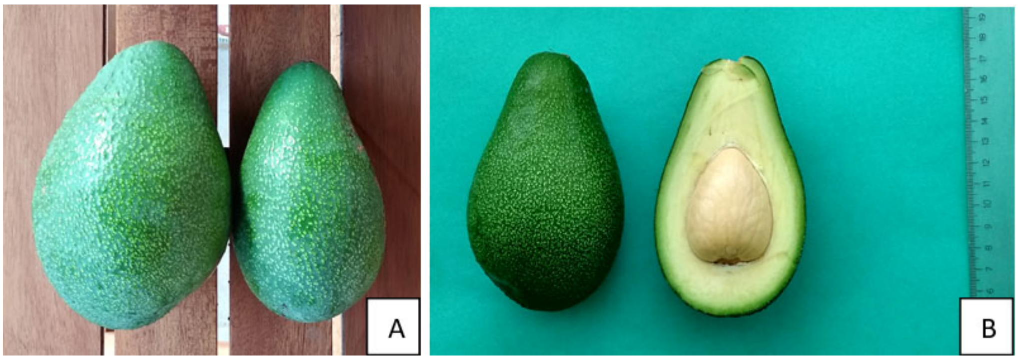

Figure 1. (**A**) Avocado Hass fruits during harvest. AMINO-16® treatment (**left**) and Control treatment (**right**), (**B**) Avocado Fuerte fruit of the AMINO-16® treatment during harvest.

Table 8. Experiment 3; Growth parameters of fresh Hass and Fuerte avocado fruit, before (Initial) and after (Final) AMINO-16® application, at harvest. The organic mixture was added by drip irrigation in three doses of 30 L/dose hectare. The length, width, and fruit weight were measured at the beginning of the experiment (Initial) and during harvest (Final), while the fruit weight increase was measured only at harvest.

Fresh Fruit Growth Parameters								
		Length (cm)		Width (cm)		Fruit Weight (cm)		Fruit Weight Increase %
		Initial	Final	Initial	Final	Initial	Final	
Hass	Control	9.8a ± 4.982	10.3a ± 4.240	6.1a ± 3.156	6.2a ± 2.897	180.0a ± 45.825	182.4a ± 54.753	1.3a ± 0.354
	AMINO-16®	9.9a ± 4.824	10.9a ± 3.752	6.1a ± 2.714	6.3a ± 3.132	176.8a ± 38.262	180.5a ± 43.875	2.1a ± 0.846
Fuerte	Control	9.0a ± 5.134	11.0a ± 5.824	4.6a ± 2.815	6.4a ± 3.712	97.9a ± 43.753	151.8a ± 19.764	54.7a ± 23.976
	AMINO-16®	8.4a ± 4.831	12.0a ± 5.217	4.5a ± 3.143	6.9a ± 3.985	86.8a ± 36.219	190.5b ± 17.363	119.3b ± 38.826

Values (mean ± SD) within a column for each variety followed by the same letter are not significantly different, according to Student's *t*-test, at $p \leq 0.05$.

The fruit quality parameters, which determine fruit ripeness (dry matter, oil concentration), were measured before AMINO-16® application and during harvest. The resulting values showed that the final percentages of the dry matter, dry matter increase, oil concentration, and oil concentration increase, although higher in the AMINO-16® treatment as compared to the Control, were not significantly different (Table 9). In particular, in the Fuerte variety, dry matter in the Control treatment increased by 22.8% (from 19.8% to 24.3%), while in the AMINO-16® treatment, by 35.1% (from 18.8% to 25.4%) (Figure 2B, Table 9); the oil concentration in the Control treatment increased by 112.6% (from 5.8% to 12.4%) and by 135.1% (from 5.0% to 13.7%) with AMINO-16® (Figure 2C, Table 9).

AMINO-16® application resulted in a 71.3 K fruit yield per tree for the Hass variety compared with 69.1 K for the Fuerte variety, which were higher than that in the Control but not statistically significant (Table 10).

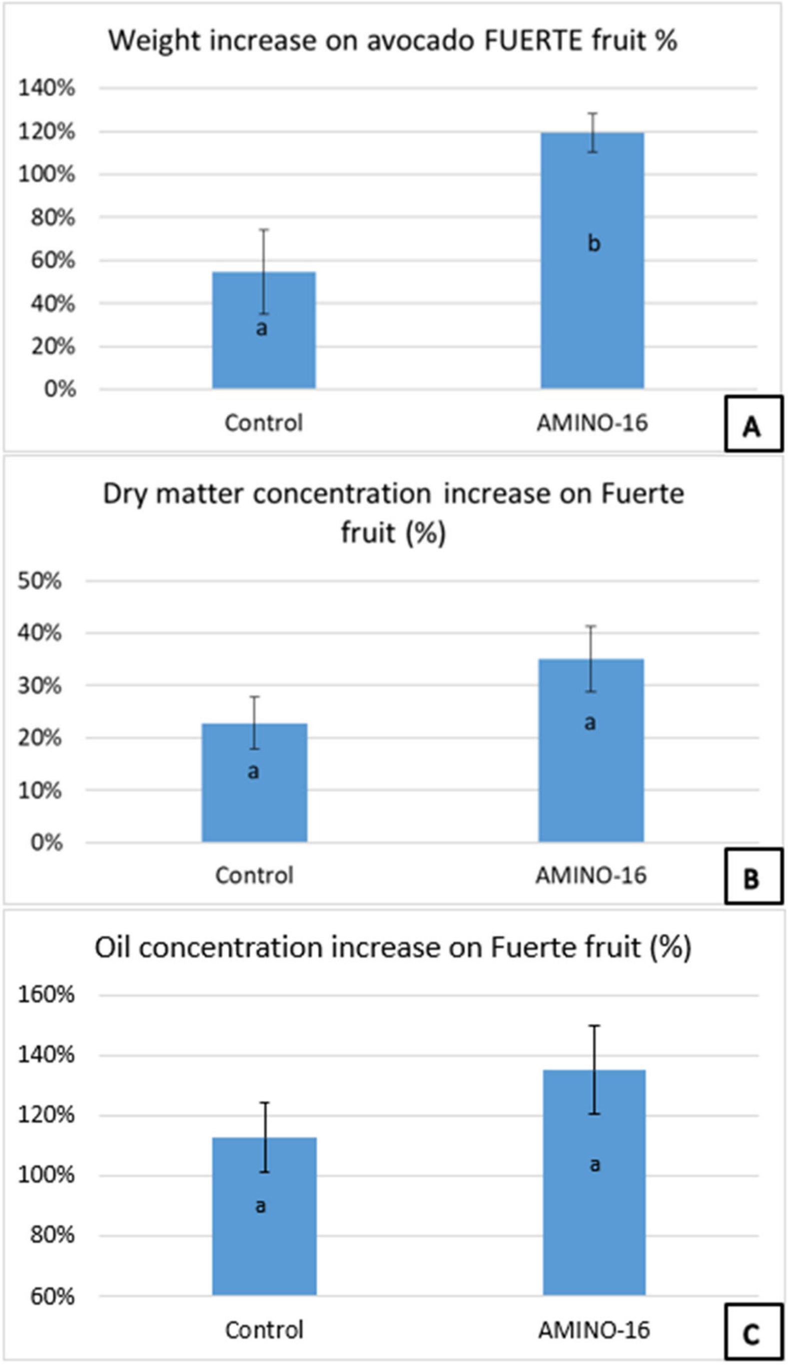

Figure 2. Experiment 3; (**A**) Weight increase in fresh Fuerte avocado fruit in the Control and AMINO-16® treatment; (**B**) Dry matter concentration increase in fresh Fuerte avocado fruit in the Control and AMINO-16® treatment; (**C**) Oil concentration increase on fresh Fuerte avocado fruit in the Control and AMINO-16® treatment. The organic mixture was added by drip irrigation, in three doses of 30 L/dose/hectare. The weight, dry matter, and oil concentration were measured at the beginning of the experiment and during harvest. For each parameter, different letters indicate differences between the values (mean ± SD) of the Control and AMINO-16® treatments, according to Student's *t*-test, at $p \leq 0.05$.

Table 9. Experiment 3; Quality parameters of fresh Hass and Fuerte avocado fruit, before (Initial) and after (Final) AMINO-16® application, at harvest. The organic mixture was added by drip irrigation in three doses of 30 L/dose/hectare. The length, width, and fruit weight were measured at the beginning of the experiment (Initial) and during harvest (Final), while the fruit weight increase was measured only at harvest.

Fresh Fruit Quality Parameters							
		DM (% f.w.)		**DMI**	**OC (% f.w.)**		**OCI %**
		Initial	**Final**		**Initial**	**Final**	
Hass	Control	21.8a ± 2.143	30.9a ± 2.016	41.4a ± 0.765	11.0a ± 0.876	17.0a ± 1.905	54.8a ± 5.125
	AMINO-16®	22.0a ± 1.987	31.2a ± 2.175	41.7a ± 0.825	11.2a ± 0.648	18.2a ± 1.574	62.4a ± 3.301
Fuerte	Control	19.8a ± 2.857	24.3a ± 2.012	22.8a ± 5.298	5.8a ± 0.968	12.4a ± 1.768	112.6a ± 11.543
	AMINO-16®	18.8a ± 2.098	25.4a ± 1.989	35.1a ± 7.286	5.0a ± 0.829	13.7a ± 2.125	135.1a ± 12.391

Values (mean ± SD) within a column for each variety followed by the same letter are not significantly different, according to Student's *t*-test, at $p \leq 0.05$.

Table 10. Experiment 3; the effect of AMINO-16® on Hass and Fuerte avocado tree yield. The organic mixture was added by drip irrigation in three doses of 30 L/dose/hectare.

Avocado Yield (K/Tree)		
Hass	Control	64.0a ± 8.125
	AMINO-16®	71.3a ± 6.016
Fuerte	Control	63.9a ± 5.097
	AMINO-16®	69.1a ± 4.134

Values (mean ± SD) within a column for each variety followed by the same letter are not significantly different, according to Student's *t*-test, at $p \leq 0.05$.

In the Hass variety, there was more flowering and fruiting the in the following spring, 3 years after the continuous use of AMINO-16®, even in trees where low production was expected.

4. Discussion

Since avocado is a highly developed crop, many companies would be interested in offering effective fertilizers to avocado producers for better quality and yield. The three organic fertilizers used in our research were tested for the first time in an avocado crop, and the results are optimistic.

4.1. Experiment 1

Since the application of Terra Insecta® insect manure in the planting hole (Sub-experiment 1.1) was not effective, we assumed that either the amount was inadequate (0.2 K/plant) or applying it before planting is not an effective technique. However, the application of Terra Insecta® round the tree trunk of young trees yielded interesting effects on plant growth, with several parameters related to the vegetative activity of the plants reaching significantly higher values than the Control. In fact, the treated plants of the Hass and Lamb Hass varieties produced more new shoots, which were also longer than those of the Control, and had a higher total number of leaves than the Control. Indeed, due to its rapid mineralization and the presence of nutrients in a readily available form, insect frass and compost obtained via insect digestion have previously been observed to be an efficient natural NPK fertilizer, especially effective in increasing the vegetative biomass of plants, also thanks to the stimulation of soil microbial activity [28–30].

4.2. Experiment 2

In the Fruit-Fix® experimental fields, the total $CaCO_3$ and pH levels were within the optimum range for avocado cultivation, although the organic matter concentration was moderate. However, the trees reacted differently in each field. In Lamb Hass (sub-experiment 2.1), the trees were severely stressed throughout the winter (long periods of flooding) and recovered by either the autumn Fruit-Fix® application or the spring application (limited growth but significant differences). Treated plants produced longer shoots and more leaves than the Control group. According to Tzatzani et al. (2020) [31], prolonged exposure of avocado plants to high soil water content conditions (waterlogging) reduced the total nutrient content (absolute quantity) of almost all of the nutrients in the scion's tissues (leaves and stems) of the Hass and Fuerte cultivars. On the other hand, in Hass (sub-experiment 2.2), no flooding problem was faced by the young avocado trees, which were not stressed. Therefore, except for the sprouted bud number, no other growth parameters were affected by the Fruit-Fix® application. Products that contain *A. nodosum*, the same seaweed present in Fruit-Fix®, improved the stress tolerance and growth of other woody plants, even though the mode of action of such compounds is still not well known [32,33]. The fundamental review by De Saeger et al. [34] concludes that *A. nodosum* extracts exert their effect by modulating hormonal homeostasis, stimulating and protecting photosynthetic machinery, and mitigating plant responses induced by stress factors. However, the molecular basis of the effect of *A. nodosum* extracts on plants is unclear due to the great complexity of the processes involved in such responses. Most of the research regarding the application of such products in agriculture has been carried out on herbaceous crops. We hope that our results can contribute to enrich the knowledge of the effect of *A. nodosum* extracts on woody perennial crops and help the diffusion of biostimolant products in all sectors of agriculture. In the Fruit-Fix® Hass experiment (sub-experiment 2.2), the plants were not stressed; they grew normally, and the organic mixture had a positive effect on the number of sprouted buds. A similar effect on flowering and the subsequent number of fruits per plant was observed in several crops treated with mixtures of *A. nodosum* extract [35,36].

4.3. Experiment 3

The biological fruiting cycle in avocados begins in spring with flowering, and immediately after successful pollination, fruiting follows. Fruit growth continues until the beginning of autumn, and then ripening takes place, which includes the final increase in fruit size, dry matter, and oil concentration, until harvest [37].

In the AMINO-16® experimental field, the total $CaCO_3$ was 20.7% and the pH was 8.3. Although these parameters are not ideal for avocado cultivation, the trees were vigorous and productive, possibly thanks to adequate fertigation and a high percentage of organic matter (6.7%). The harvest season for the Hass variety in Greece is late winter to late spring. This means that the harvest period coincides with flowering. The experiment started in March and coincided with these two periods. As the fruits of the Hass variety were in the final stage of ripening, the effect of AMINO-16® did not show significant differences in the initial application (spring–summer). The higher manganese content found in the leaves of the AMINO-16®-treated plants can be seen as a positive result, as a higher manganese content is linked to the minor incidence of the avocado disorder known as mesocarp discoloration [38].

For the Fuerte variety, it was observed that the fresh fruit weight and dry matter were not significantly higher in the AMINO-16® treatment. In fact, there are reports of an effective increase in fruit dimensions [39] and lettuce crop uniformity [40] after the application of amino acid mixtures. In conclusion: A. AMINO-16® application to the Hass and Fuerte avocado trees did not affect the nutritional status of the plants, which was overall satisfactory. This may be due to good fertigation applied to the experimental field. B. AMINO-16® did not affect fruit ripening. C. The dry matter, oil content, and yields of avocados from treated plants gave interesting results in the AMINO-16® treatments but were not always statistically significant.

The above results were the reason for the extension of the experiment, for another year. The AMINO-16® application followed a new protocol that included four applications: 1. early spring (March) during flowering, 2. late spring (April–May) during fruiting, 3. early summer (June) during fruit growth, and 4. early autumn (September) during the increase in the dry matter and oil content.

It is important to mention that in avocado trees, biennial bearing occurs quite often [41], i.e., a year with high production, followed by a year with low production, alternately. In the Hass variety, according to our observations, there was more flowering and fruiting the following spring three years after the continuous use of AMINO-16® in trees even where low production was expected. This may be an indication of a reduction in biennial bearing and would confirm for avocado what was observed for herbaceous plants such as sweet pepper treated with mixtures of amino acids [42]. The results revealed that AMINO-16® has a positive effect on fruit quality, but further experimentation is needed in order to verify if this can lead to a statistically higher yield. Other studies have shown similar effects of the application of amino acids on tropical fruits, resulting in an increase, though not always statistically significant, of vegetative and reproductive activities of the plants [43]. This happens because of the capacity of amino acids of being readily absorbed and utilized in protein synthesis, while as chelating agents, they help in the absorption and transport of micronutrients within a plant [44,45]. As suggested by the grower's observations and highlighted by other authors [46], the effect of the application of amino acids and the other organic fertilizers used in this research could bear noticeable results in the medium to long term, requiring studies to be carried out over more seasons, in the different tree ages, and under stress conditions.

5. Conclusions

- In the present study, the application of an organic mixture and insect manure to soil with satisfactory specifications led to many indices of plant growth reaching higher values than those of the control, although these results are not always statistically significant.
- Stress from environmental conditions throughout the winter (long periods of flooding) in young avocado trees can be addressed with an appropriate organic fertilizer mixture, as indicated by the Fruit-Fix® application.
- The positive results of the application of amino acids, seaweed extract, and insect frass, three sustainable sources of organic nitrogen, which were observed in other crops, were confirmed for avocado, under specific circumstances.
- The repeated application of organic nitrogen sources to young avocado trees during the stem growth period contributed to their better growth and development.

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