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Agroecological Screening of Copper Alternatives for the Conservation of Soil Health in Organic Olive Production

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Abstract: The efficacy of soil conditioner (vermicompost tea), fertiliser (potassium silicate), and biological control agents (BCAs) as practical agroecological copper alternatives against olive leaf spot (*Spilocaea oleaginea (Cast.) Hughe.*) disease was investigated between 2018 and 2021 under organic management in a Mediterranean climate. In total, 9 agroecological alternatives to copper oxychloride (vermicompost tea, potassium silicate, *Bacillus subtilis* EU 007 WP, *Platanus orientalis* leaf extract, *Mycorrhiza* mix, seaweed commercial product, *Trichoderma citrinoviride* TR1, vermicompost tea+*Platanus orientalis* mix, *Penicillium* (Mouldy bread pieces)) were applied to olive trees in a randomised block design with 4 replicationsTotal water soluble phenol compounds (TWSP) were found to be the main bioindicator to assess the alternatives and their potential to phase-out copper application. Results related to TWSP indicated that copper oxychloride (control), potassium silicate and vermicompost tea showed significantly higher content of TWSP as we compared zero application of copper and other treatments. These stimulate the antioxidant capacity in olive fruits and reduce the olive leaf spot disease incidence. The pollution effect of copper was monitored during the trial to identify soil pollution in the organic in-conversion experimental land. The total annual 'active copper' application was 4.7 kg.ha^{-1}.year^{-1} and this is in accordance with the legal organic legislation of Turkey. During the conversion period from conventional to organic management, we determined approximately 50% reduced copper content in the soil 0–30 cm depth samples in 2020 (3.70 mg.kg^{-1}) as it is compared to those initial samples (6.43 mg.kg^{-1}) in 2018. We conclude that alternatives to copper that are easily accessible, e.g., vermicompost tea, have a potential for use in organic olive production to replace copper in mitigating olive leaf spots. Furthermore, we find that reduced copper application in organic management with the aim to decrease copper accumulation in soil, fruits and leaves was not yet enough to reduce copper to satisfactory levels. We conclude that further research with the aim of a total replacement of copper fungicide treatments in organic and non-organic systems is needed.

Keywords: soil pollution; copper phase-out; alternative input; total water-soluble phenol compounds; organic horticulture

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Citation: Kir, A.; Cetinel, B.; Sevim, D.; Gungor, F.O.; Rayns, F.; Touliatos, D.; Schmutz, U. Agroecological Screening of Copper Alternatives for the Conservation of Soil Health in Organic Olive Production. *Agronomy* **2022**, *12*, 1712. https://doi.org/10.3390/agronomy12071712

Academic Editor: Eli Zaady

Received: 14 June 2022
Accepted: 14 July 2022
Published: 20 July 2022

1. Introduction

In recent years there has been a great concern related to the conservation of agricultural lands [1] Sustainable farming systems increasingly question health this concept, among others, the phasing out peat, plastic and copper from existing certified organic agricultural production has been examined in the Organic-PLUS project (EU funded GA 774340) [2].

Ongoing and planned activities by the Ministry of Agriculture and Forestry of Turkey within Organic-PLUS included trials to investigate the best possible alternatives to replace copper under certified organic (=ecological; =biological) agriculture management.

The United States Environmental Protection Agency (EPA, 2021) [1] and Oorts (2012) [3] reported that copper is a metal and vital micronutrient for all living organisms that occurs naturally in rocks, soil, water and sediments, and even in very small amounts in the air [1,3]. While copper's main function is on enzymes that catalyse oxidative reactions in plant metabolism [4], agricultural management that includes copper can lead to environmental contamination with effects on plant and soil properties [1,3,5,6]. In the prevention of fungal diseases, copper preparations are widely used. Copper binds strongly to organic matter and other components in the soil, such as clay and silt. Compost also has a possible risk of toxicity as a source of this element (and other heavy metals) if the source materials are contaminated. Copper and copper compounds can lixiviate with the action of water and can be leached into groundwater [1]. As a result, copper, mixed with water sources, accumulates in sediments in rivers, lakes and other sources. Copper binds to proteins and disrupts the structure of proteins and cell membranes [1]. Thus, it causes the death of not only fungi but also algae in aquatic ecosystems. Copper is a health concern for humans, even in a small quantity [1].

In Turkey, operative "Control of Soil Pollution" legislation (Official Gazette, 31 May 2005, 25831) addressed Cu limitations in the soil (50 mg.kg^{-1} (pH 5–6) and 140 mg.kg^{-1} (pH > 6)) and groundwater (51 mg.kg^{-1} DM) [7]. However, "Organic Agriculture Law" and current related legislation (Official Gazette, 10 January 2018, 30297) limited Cu application to 6 kg.ha^{-1}.year^{-1} over a 5-year production period [8]. The case farm (İzmir-Turkey) is in a lowland suburban area where the production of olives is dominant. Although copper application at the case farm is lower than that reported in other countries [9] new EU regulation limits Cu to 4 kg.ha^{-1}.year^{-1} and 28 kg.ha^{-1}.year^{-1} over 7 years; compared to this the application is just within this legislation of Turkey, and applicable if olive products are exported to EU countries, as shown by Katsoulas et al., 2020 [9].

In Turkey, the production of olives for table consumption accounted for 513,000 tonnes and 803,000 tonnes for oil production in 2020 [10]. In Turkey table olive is about 25% of total olive production (1.5 million tonnes in 2019–2020) [11]. Table olive is one of the main components of the Mediterranean diet and consumer interest has been increasing due to its valuable nutritional content [12]. Olive holds great prominence in organic production in Turkey, with shares reaching 10.2% of the total organic crop production and 86,049 ha of the total organic area [13]. During the Horizon 2020 project Organic-PLUS, Ministry of Agriculture and Forestry of Turkey conducted an olive trial to identify alternatives to copper in olive leaf spot (*Venturia oleaginea* = *Spilocaea oleaginea* (Cast.) Hughes = *Cycloconium oleaginum* Cast.) disease which not only affects the yield and the quality of olive trees in Turkey, but is also one of the major problems of olive cultivation worldwide. This fungal plant pathogen has been reported in Australia, Mediterranean countries (France, Spain, Portugal, Italy, Cyprus, Greece, Tunisia, Turkey, Morocco, Israel), Russia's eastern Black Sea coast, South Africa, USA (California), and some of the South American countries (Chile, Peru, and Argentina). Prevalence of the olive leaf spot disease in olive cultivation lands and its economic importance in terms of yield in the world was reported in several publications ranging between 0% and 70% and have a strong relationship with climatic conditions. Integrated pest management and early warning system applications have shown promising results [14–23].

In Turkey, certified organic agricultural land is 646,247 ha which accounts for 1.7% of the total agricultural land of the country. The increased rate of converted lands from conventional to organic agriculture in the country is reported to be in the top ten countries of the world as it is in 2019 125,361 ha when compared to 2017 (520,886 ha). The total certified organic agricultural land and wild collection of the country was determined to be 809,471 ha. Additionally, Turkey is in the top ten in the World in terms of the number of organic farmers (79,563) and most of them are producing olive trees [13]. Recent changes in land use have attempted to address new problems related to previous Cu accumulations in water, compost and soil. A new generation of farmers demands different production techniques to enrich agrobiodiversity [24–31] This is a reliable ally in these farmers' quest to mitigate climate change [32,33], the intention to protect food sovereignty [34] and the aim of increasing profitability of the farmers [35]. However, biodiversity loss is a phenomenon in all over the Earth as well as in Turkey. Moreover, many olive groves were established using olive genetic resources under agroecological management.

Many parts of olive trees, including fruit flesh and fruit stone, leaves, stems, branches, bark, wood, and roots contain phenols, natural antioxidants, and other valuable compounds in different concentrations that depend on olive variety and environmental conditions of the orchard [36,37]. Phenolic compounds, which are found in almost all parts of plants and are defined as secondary metabolites, stand out in terms of their antioxidant properties. They have an aromatic ring bearing one or more hydroxyl groups and can range from a simple compound to a high molecular polymer structure. Flavonoids carrying the C6–C3–C6 structure form approximately four thousand different phenolic compounds. The antioxidant activity of phenolic compounds depends on the number and positions of the hydroxyl groups and the nature of the substitutions in the aromatic rings. The processing of agricultural by-products produces significant amounts of phenolic-rich by-products that can be valuable sources of natural antioxidants. Some of these by-products have been the subject of research and have proven to be effective sources of phenolic antioxidants. The extraction and production of abundant natural antioxidants from these agroindustrial wastes has great potential [38].

The objectives of this paper are to evaluate the effects of

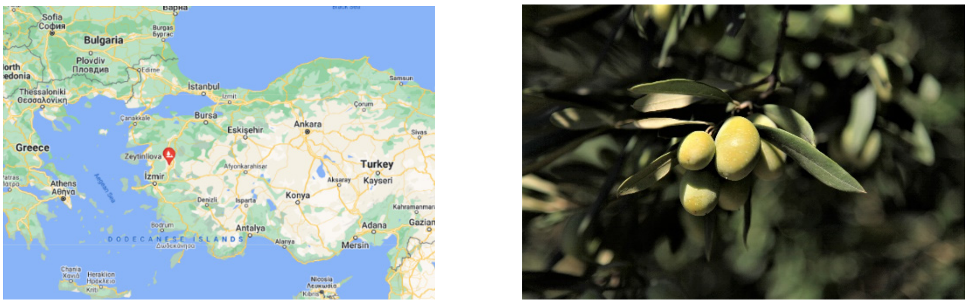
(a) Fertilisers and BCAs on copper pollution in soil;
(b) Fertilisers and BCAs on olive leaf spot disease;
(c) TWSP content in olive fruit as a preventing factor of the olive leaf spot disease, and;
(d) Copper leaf-spray application on olive leaf and fruit copper content.

We also aimed to observe copper in olive tree production during the conversion period from conventional to organic management. The target was to increase the utilisation of promising alternatives to copper in commercial organic and non-organic olive orchards but also in a suburban site.

2. Materials and Methods

2.1. Study Site

Biological control agents (BCAs) were included as possible alternatives to copper against "olive leaf spot disease". The experimental site was established in an organic olive orchard which is 28 years old planted with "Domat" table olive variety (distance between trees 5 m × 7.5 m), located in Zeytinliova, Akhisar, Manisa Province (Turkey) (38°96′3.7″ N 27°69.855′ E). The trial lasted three years from September 2018 to September 2021 (Figure 1).



(a) Trial site (b) Domat variety

Figure 1. Geographic location of the experimental site (**a**) and fruits of "Domat" variety (**b**).

2.2. Experimental Design and Field Management

In the study, N was applied annually at 300 g per tree. The fertilisation programme was based upon nitrogen dose which should be not exceeded 170 kg.ha^{-1}.year^{-1} according to legislations of organic agriculture (Table 1).

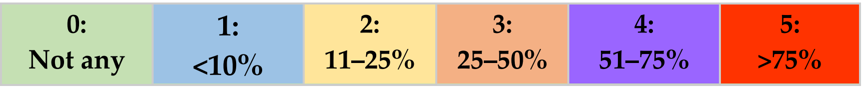
Table 1. Fertilisation programme of the copper trial.

Application of Fertiliser	**Application Dates of Fertilisers (Year/Season)**	**Contents of Applied Certified Organic Commercial Fertilisers * (A, B, and C) (*w*/*w*) in Each Year ****	**Applied Cu *** Doses to Leaves of Olive Tree for the Disease (kg.ha^{-1}.year^{-1})**
Soil	2018/Early-April 2019/Late-March 2020/Late-April	A—An annual dose of 0.8 Liters per hectare of N and P, applied 4 times. The fertiliser contain Bacilullus subtilis 1 × 10^9 KOB.mL^{-1} and Baciullus megaterium 1 × 10^8 KOB.mL^{-1}	4.7
	2018/Early-April 2019/Late-March 2020/Late-April	B—An annual dose of 1.2 Liters per hectare of K, applied in 4 times. The fertiliser contains 15% Organic matter, 6%, Organic Carbon, and 1% water soluble K_2O	
	2018/Early-April 2019/Late-March 2020/Late-April	C—An annual dose of 0.9 Liters per hectare of Compost Tea (Derived from plants), applied in 4 times. The fertiliser contains, 35% Organic matter, 24% Organic Carbon, and 2.5% Total Nitrogen	

* All fertilisers (A, B, and C) were applied each year. ** Organically Certified, liquid, in the IFOAM and OMRI list and local production. *** Applied only to copper oxychloride treatment plots.

The 44 plot trial was conducted using a randomised block design with 4 replications and 11 treatments including "copper" and "no application" control. There were two trees per plot. In this trial, several organic fertilisers were tested by MAF-ORI (Ministry of Agriculture and Forestry, Olive Research Institute (Izmir, TR). Details of the different applications in this study are shown in Table 2.

A scale from 0 to 5 was used to score the disease (Figure 2). Disease severity was calculated according to the Townsend–Heuberger Formula = [∑ (no. of plant in category × category value)] × 100/Total no. of plants × max. category value) [39]. In addition, fallen leaves were collected to quantify the fungal attack.



0: Not any	**1: <10%**	**2: 11–25%**	**3: 25–50%**	**4: 51–75%**	**5: >75%**

Figure 2. 0–5 Scale: Appearance of symptoms on the leaf area (%).

Available copper (Cu) in soil (in total 132 samples) was determined by ICP-OES (Varian-Vista) after extraction with 0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA (titratable acidity) (pH 7.3) according to Lindsay and Norvell (1978) [40].

The samples of olive fruits (in total 88 samples) and leaves (in total 88 samples) were washed with 0.1 N HCl and 2 times with deionised water, then dried in an air circulating cabinet at 65 °C for 48 h (until constant weight) and ground in a tungsten coated mill. A total of 0.3 g of the milled plant parts were taken and dissolved in 5 mL 65% HNO_3 and 3 mL distilled H_2O in a microwave oven (180 °C) (Anton Paar Multiwave Pro 5000), the final volumes were diluted to 25 mL with ultra-deionised water and filtered through blue band filter paper. The concentration of Cu in the filtrate was determined by ICP-OES.

Table 2. Periods and doses of eleven treatments in the copper field trial.

	Treatment	Details of Application
1	Copper oxychloride * (Control 1)	Total applied copper 6 kg/ha/year, WG, 4 g. 20 L^{-1} water, [700 g/L metallic copper equivalent commercial product, appl. dosage is 150 cc/100 L water], applied after early warning system alarms with "leaf spray" application system (within 48 h after Decision support system-DSS (early warning system) signal occurred)
2	*Bacillus subtilis* EU 007 WP	$1 \times 10^{4-6}$ CFU, applied after early warning system alarms with "leaf spray" application system (within 48 h after early warning signal occurred).
3	*Platanus orientalis* (extract of leaves)	Extracted in boiling water, 25 leaves. 20 L^{-1} water, home-made, applied after early warning system alarms with "leaf spray" application system (within 48 h after early warning signal occurred)
4	Vermicompost tea *	Liquid, 30 cc. 20 L^{-1} water, commercial product, applied after early warning system alarms with "leaf spray" application system (within 48 h after early warning signal occurred).
5	*Mycorrhiza* mix *	WP, 3.2 g. 20 L^{-1} water, commercial product, applied "drench from soil" application system. Application made according to the phenologic stage of the tree. *(a—1 month before the autumn leaves are seen, b—1 month before the spring leaves are seen, c—before the flower buds are seen)*
6	Seaweed® *	WP, 14 g. 20 L^{-1} water, commercial product, applied after early warning system alarms with "leaf spray "application system (within 48 h after signal early warning occurred)
7	*Trichoderma citrinoviride* TR1	1×10^6 CFU, applied "drench from soil" application system. Application made according to the phenologic sage of tree. *(a—1 month before the autumn leaves are seen, b—1 month before the spring leaves are seen, c—before the flower buds are seen)*
8	Zero application (Control 2)	Untreated control (only irrigation)
9	Potassium silicate ($KSiO_3$)	Liquid, 250 cc. 20 L^{-1} water, commercial product, "drench from soil" application system approx. 80–100 cc $KSiO_3$ per tree. Application made according to the phenologic sage of tree. *(a—1 month before the autumn leaves are seen, b—1 month before the spring leaves are seen, c—before the flower buds are seen)*
10	Vermicompost + *Platanus orientalis* (extract of leaves)	Liquid, 50% of each, applied after early warning system alarms with "leaf spray" application system
11	*Penicillium*	Mouldy bread pieces, 4 kg per tree, "mix into soil 0–20 cm depth" application system. Application made according to the phenologic sage of tree. *(a—1 month before the autumn leaves are seen, b—1 month before the spring leaves are seen, c—before the flower buds are seen)*.

* All commercial products have organic certificates according to Turkish Law and Legislations.

Analysis of the TWSP (total water-soluble phenol) content at each sapling setting was carried out. For the extraction of the samples, the methods of Bouaziz et al. (2008) [41] and Ranalli et al. (2006) [42] were modified and used. 1 g of sample was weighed, mixed with 50 mL of methanol/water 60:40 (*v/v*) and extracted by shaking for 1 h, then the mixture was filtered through blue band filter paper (S&S Filter Paper Circles 5893 blue ribbon, 125 mm, Germany). The total phenol contents (water soluble) of the extracts were determined according to Hrncirik and Fritsche (2004) [43]. According to this protocol, 0.1 mL of sample extracts was taken and placed in a 50 mL volumetric flask, then 5 mL of distilled water, 0.5 mL of Folin-Ciocalteu (Merck KGaA, Germany) solution was added to them and, after 3 min, 1 mL of sodium carbonate solution (35%, *w/v*) was added and the mixture was mixed with distilled water to 50 mL. After the solutions were kept in the dark for 2 h, the absorbance was measured against a blank solution with a spectrophotometer at 725 nm wavelength.

2.3. Sampling and Measurement

Olive soil (0–30 cm and 30–60 cm), and olive tree fruit and leaf samples were collected from each tree for preparation for laboratory analysis. Samples were collected in autumn for the three seasons (2018–2019, 2019–2020, and 2020–2021). Soil samples were collected according to Kacar (1977) [44] and leaves and fruits according to Kacar & Inal (2008) [45] and Reuter & Robinson (1986) [46].

2.4. Statistical Analysis

The statistical analysis was carried out with the software SAS (2007) JMP©, Version 7 (SAS Institute, Cary, NC, USA) [47]. A two-factor hierarchical analysis of variance with inequality of variance for copper and TWSP between the samples of the trial were carried out. Statistical differences between mean values were determined using T-Students and Tukey′s significant difference test at $p < 0.05$. A correlation matrix correlation coefficient was used to understand the relationships among the investigated parameters. Also, multiple pairwise comparisons of copper and TWSP for all materials were conducted as well as multiple pairwise comparisons within one sample.

3. Results

3.1. Copper in Soil, Leaf, and Fruit Samples in the Olive Trial

The initial soil analysis of samples (0–30 cm and 30–60 cm) showed that the trial site soil was loamy; low salinity; slightly alkaline; calcareous; very low in total organic matter; low in total nitrogen, available phosphorus, zinc and iron; high in available potassium, calcium; very high in magnesium; and sufficient in available manganese, copper and boron according to Kellogg (1952); Evliya (1964); Akalan (1965); Loue (1968); Olsen and Dean (1965); Pizer (1967); Follet and Lindsay (1970); Keren and Bingham (1985) [48–55]. N, P, K, Ca, Mg, Fe, Zn, Mn except Cu in olive leaves of the plots were determined as sufficient as classified by Anonim (1993) [56]. "Initial" (conventional) and "consecutive 2 years (2020 and 2021) (organic transition period)" results of copper content of soil, leaf and fruit samples collected in this study are presented in Tables 3 and 4.

The initially available copper contents of the "Domat" olive cultivar, determined from the soil depth of 0–30 cm, ranged between 3.50–6.46 mg.kg^{-1}, and in the soil samples taken from the soil depth of 30–60 cm, it ranged between 3.30–6.40 mg.kg^{-1}. Those initial leaf and fruit copper contents were determined between 70.51–78.96 mg.kg^{-1} and 4.40–4.79 mg.kg^{-1}, respectively.

The results of 2 consecutive years (organic conversion period 2020 and 2021) of the soil copper content at 0–30 cm depth ranged from 3.67–3.81 mg.kg^{-1} (2020), 1.12–4.23 mg.kg^{-1} (2021) and in samples taken at 30–60 cm depth, the results ranged from 1.81–3.40 mg.kg^{-1} (2020), 0.80–3.43 mg.kg^{-1} (2021). The minimum and maximum copper contents in leaves and fruits ranged between 7.80–78.43 mg.kg^{-1} (2020), 6.13–6.70 mg.kg^{-1} (2021) and 2.51–2.98 mg.kg^{-1} (2020), 2.16–2.45 mg.kg^{-1} (2021), respectively. We found very high leaf values in Cu applications as 69 mg.kg^{-1} in 2020 and 81 mg.kg^{-1} in 2021.

Table 3. Initial copper content [a] of soil, leaf and fruit samples (sampled in 2018).

No	Treatment	Soil Available Cu		Leaf Cu	Fruit Cu
		0–30 cm	30–60 cm	Initial ** (mg.kg^{-1})	
1	Copper oxychloride (Control 1) [b]	6.43	6.20	71.42	4.59
2	*Bacillus subtilis* EU 007 WP	3.50	3.45	77.45	4.40
3	*Platanus orientalis*	5.53	4.55	76.45	4.45
4	Vermicompost	4.43	3.80	74.48	4.40
5	*Mycorrhiza*	3.75	3.30	70.76	4.67
6	Seaweed	6.00	5.56	74.84	4.48
7	*Trichoderma citrinoviride* TR1	5.30	5.10	71.30	4.43
8	Zero application (Control 2)	4.30	4.23	74.86	4.76
9	Potassium silicate ($KSiO_3$)	6.46	6.40	78.96	4.79
10	Vermicompost + *P. orientalis*	4.00	3.90	70.51	4.60
11	*Penicillium*	3.50	3.00	76.20	4.41
	CV * (%)	14.2	14.5	9.4	8.1
	p-value $\leq$ 0.05: *	ns	ns	ns	ns

[a] Average of replications; [b] "Copper sulphate" was applied for many years before trials in conventional olive production. * coefficient of variation. ns = not significant. ** Logarithmic transformation is applied.

Table 4. Copper [a] content of soil, leaf and fruit sample results [b] in 2020 and 2021. Letters indicate significant differences.

No	Treatments	Soil Available Cu (mg.kg^{-1})		2020 Leaf Cu (mg.kg^{-1})	Fruit Cu (mg.kg^{-1})	Soil Available Cu (mg.kg^{-1})		2021 Leaf Cu (mg.kg^{-1})	Fruit Cu (mg.kg^{-1})
		0–30 cm	30–60 cm			0–30 cm		30–60 cm	
1	Copper oxychloride (Control 1)	3.81	3.21 A	68.63 A	2.48	0.92 A	3.24 A	80.75 A	2.17
2	*Bacillus subtilis* EU 007 WP	3.70	2.42 B	28.80 B	2.40	0.84 B	1.07 B	7.97 C–E	2.13
3	*Platanus orientalis*	3.71	2.40 B	15.94 B–D	2.21	0.84 AB	1.17 B	7.12 DE	2.05
4	Vermicompost	3.70	2.21 B	23.48 BC	2.36	0.84 AB	1.50 B	6.67 E	2.14
5	*Mycorrhiza*	3.72	2.36 B	21.92 BC	2.44	0.90 AB	1.40 B	6.70 E	2.21
6	Seaweed	3.70	2.32 B	11.61 D	2.36	0.86 AB	1.09 B	8.57 CD	2.11
7	*Trichoderma citrinoviride* TR1	3.71	2.18 B	23.61 BC	2.41	0.89 AB	1.51 B	7.12 C–E	2.27
8	Zero application (Control 2)	3.71	2.14 B	15.48 B–D	2.36	0.85 AB	1.42 B	9.02 C	2.16
9	Potassium silicate ($KSiO_3$)	3.67	2.14 B	16.34 B–D	2.24	0.92 A	1.38 B	13.19 B	2.13
10	Vermicompost + *P. orientalis*	3.71	2.66 AB	23.74 BC	2.36	0.85 AB	1.09 B	7.53 C–E	2.06
11	*Penicillium*	3.74	2.21 B	13.28 CD	2.29	0.87 AB	1.36 B	6.80 E	2.13
	CV *** (%)	0.04	0.02	0.12	0.06	0.004	0.04	0,12	0.02
	p-value $\leq$ 0.05: *	*	*	*	ns	*	*	*	ns

[a] During the trial (2019–2021) copper oxychloride was applied. [b] Logarithmic transformation is applied. *** coefficient of variation. * significant. ns = not significant.

3.2. Disease Severity and Efficiency Levels of the Treatments and Total Water Soluble Phenol Results

According to field trials, the severity of the disease was found lowest in copper oxychloride (control 1) (2.7%); potassium silicate (7.9%) and vermicompost tea (9.8%) followed the copper application, while the non-treated control (control 2) was found the highest at 23% according to visual observation in Figure 2. The *Mycorrhiza* mix *and Trichoderma citrinoviride* TR1 had 21.2% and 15.8%, respectively. The visual assessments in the lab of the treatment effects showed compatible results with the efficiency levels and TWSP.

In open field conditions, copper oxychloride (control 1) showed 88.5% efficiency and potassium silicate (66.4%) and vermicompost tea (58.4%) followed the copper control. The lowest efficiency resulted from the seaweed (7.7%). *Mycorrhiza* mix *and Trichoderma citrinoviride* TR1 had 10.5% and 33.5%, respectively. Among the 11 treatments, 4 alternative inputs to copper were shown to reduce olive leaf spot incidence in the organically managed olive growth. Those correlated with TWSP contents (Table 5). This means potassium silicate

($KSiO_3$), vermicompost tea, Mycorrhiza mix and *Trichoderma citrinoviride* TR1 applications elevated TWSP capacity and then the disease attack in those treatments decreased according to the 0–5 scale of visual observations recorded in May–June 2021. All other treatment performances were found to be worse than potassium silicate ($KSiO_3$), vermicompost tea, Mycorrhiza mix and *Trichoderma citrinoviride* TR1 plots. Results were in accordance with TWSP contents. TWSP (mg CAE. $100g^{-1}$ olive fruit) content of treatments used in this study and controls are shown in Table 5 and Figure 2.

Table 5. TWSP (mgCAE.$100g^{-1}$ olive fruit) content in the trial. Letters indicate significant differences.

No	Treatment	Average of Replications	Standard Deviation
1	Copper oxychloride (Control 1)	202.78 A	14.33
9	Potassium silicate ($KSiO_3$)	195.31 AB	29.13
4	Vermicompost tea	180.95 B	11.32
5	*Mycorrhiza mix*	178.77 B	9.94
7	*Trichoderma citrinoviride* TR1	178.31 B	13.16
3	*Platanus orientalis* (extract of leaves)	164.68 C	7.39
8	No application (Control 2)	162.86 C	10.23
10	Vermicompost + *Platanus orientalis* (extract of leaves)	160.85 C	6.50
11	*Penicillium* (Mouldy bread pieces)	156.82 C	7.11
6	Seaweed	139.59 C	11.16
2	*Bacillus subtilis* EU 007 WP	137.87 C	9.75
	CV (coefficient of variation) (%)	2.08	
	p-value ≤ 0.05: *	*	

* significant.

Lower olive leaf spot disease incidence was observed when potassium silicate and vermicompost tea was applied. In this trial, higher content of TWSP resulted from potassium silicate, vermicompost tea, Mycorrhiza mix, and *Trichoderma citrinoviride* TR1. This stimulates antioxidant capacity in olive fruit and addressed the olive leaf spot so disease incidence was decreased as compared to other treatments.

4. Discussion

4.1. Copper Content of Soil, Leaf, and Fruit

It was clearly determined that the copper content of initial (conventional) and copper oxychloride plots in 0–30 cm and 30–60 cm soil samples were higher than in the nine agroecological treatments. During the conversion period from conventional to organic management, we determined approximately 50% reduced copper content in the soil 0–30 cm depth samples in 2020 (3.70 $mg.kg^{-1}$) as it is compared to those initial samples (6.43 $mg.kg^{-1}$) in 2018. However, there is a requirement to check for contamination in deeper parts of the soil.

According to Follet and Lindsay (1970) [54] and Anonim (1993) [56] references, soil and leaf results showed that samples contain sufficient copper. However, it is reported that in the areas where pollution risk occurred in suburban olive groves the 0.2 $mg.kg^{-1}$ threshold value was greatly exceeded. When we compare the copper content in the soil, leaf, and fruit samples between the conventional and end of the organic transition period (Table 6), an inference can be made that a reduction in copper occurred even when copper continued to be applied in the form of copper oxychloride after 3 years of organic management in the trial site. Ozturk et al. (2021) [57] reported related to copper content results of pollution risk in Turkey. Likewise, our results are compatible with those reports, especially for initial samples of soil, leaf and fruit. Reports indicated that Akhisar-Manisa Province, where our trial site was located, is a copper accumulation risk area [57]. Aydogdu (2011) found average copper contents of the 'Domat' variety to be 8.13 $mg.kg^{-1}$ in leaf samples in September and 5.12 $mg.kg^{-1}$ in fruit samples; from the soil depth of 0–30 cm, it ranged between 1.2–3.3 mg.kg^{-1}, and in the soil samples taken from the soil depth of 30–60 cm, it ranged between 1.1–2.6 mg.kg^{-1} in a conventional olive growth in Akhisar-Manisa Province [58]. We obtained lower average fruit copper in initial samples than Aydogdu (2011). In contrast, in soil samples, our results were higher than the results Aydogdu reported in 2011. This suggests that the accumulation of soil copper rose over several years in the trial site, Akhisar-Manisa. However, we determined very high copper in soil samples of control 1-copper oxychloride plots. This result is one more justification for zero copper application requirement in sustainable farming systems. On the other hand, in an organic olive grove, the copper content of the garden soil taken before the experiment was determined as 0.29 mg/kg at 0–30 cm and 0.25 mg/kg at 30–60 cm in Aydın Province (Turkey) [59]. In Ayvacık-Canakkale Province, in a conventional olive grove, soil varied between 0.16–1.72 mg.kg^{-1}, while the lowest and highest values of organic conversion cultivated olive soils were found to be between 0.46–1.47 in different soil depths of the trial site [60]. We found higher reported soil copper contents than Sahin (2013) [59] in Aydın and Eryüce (2010) [60] in Çanakkale Province of Turkey. This result was probably obtained from their sloping olive orchard trial sites where the input applications are very low, as it is reported in Katsoulas et al. (2020) [9] for copper.

Table 6. Recently published results of phenol contents in raw olive fruits (expressed on a fresh weight basis).

No	Phenol Content	Publication
1	"Domat" variety: 245 mg CAE */100 g (in 2014) and 303.6 mg CAE/100 g (in 2015)	[61] Ozturk Gungor, 2020
2	144.0–674.0 mg GAE **/100g	[62] Ben Othman et al. (2008)
3	306–550.3 mg GAE/100g	[63] Piga et al. (2005)
4	206.5 mg CAE/100g	[64] Pirgün (2007)
5	110–239 mg CAE/100 g	[65] Lanza et al. (2013)
6	274.9 mg CAE/100 g	[66] Irmak (2010)
7	124–1688 mg CAE/100 g.	[67] Mettouchi et al. (2016)
8	"Domat" variety: 189.8 mg CAE/100 g	[66] Irmak et al. (2010)

* CAE: caffeic acid equivalent; ** GAE: gallic acid equivalent; Note: Both acids can be used for the phenol analysis.

4.2. Total Water Soluble Phenol

Recent reports about phenolic compound contents of "*Domat*" table olive variety under conventional farming experimental practices are listed in Table 6. Represented results reported in the literature (Table 6) are all compatible with our TWSP results and within the limits of those research findings. Ozturk et. al. (2021) [57] reported that olive growth is highly widespread in Turkey and "oleuropein" is one of the phenolic compounds of the olive tree has a protection effect against diseases [57].

In our trial, the amount of water-soluble phenols in the group treated with copper oxychloride is significantly higher than in the other groups. This group is followed by potassium silicate and vermicompost tea treated groups, respectively. In addition, although *p. orientalis* and vermicompost tea cause an increase in the amount of phenol when applied separately, they have an antagonistic effect and cause a decrease in the amount of phenol when applied together as a treatment (*p. orientalis* + Vermicompost tea). When we compare the groups with increased phenol content (copper oxychloride, potassium silicate, vermicompost tea, *Mycorrhiza mix, and Trichoderma citrinoviride* TR1) with other treatments, it can be said that these groups will be more resistant to olive leaf spot (*S. oleginea*) disease attack. The results showed that the tolerance capacity of *S. oleginea* might be related to the content of phenolic compounds in these treatments. Higher content of total phenolic reflected lower disease incidence. This might be due to the high antioxidant activity of polyphenols which is mainly due to their redox properties and neutralising reactive oxygen species (ROS) induced by *S. oleginea* allowing the plant to tolerate biotic stress It is possible to propose that the relationship between phenol capacity and plant tolerance to *S. oleginea* in fertiliser and BCA-treated olive plants supports the idea of some protection by radical scavenging. In other words, reactive oxygen species formed in *Spiloceae*-induced cells may be swept by polyphenols, allowing the plant to tolerate this stress.

5. Conclusions

The investigation has shown that copper oxychloride application against olive leaf spot disease under organic management in olive grows of the experimental site results in the risk of accumulation of copper in the soil. Furthermore, it is concluded that besides the soil accumulation risks, there is a risk of plant bioaccumulation. Olive branches with leaves can have a higher copper load. Therefore, composting olive pruning branches which, include higher copper contents in these plant parts need special attention. This is to avoid adding extra copper to the soil where it might accumulate e.g., in raised beds built with a lot of composted material. The other possible risk of losing available copper in the soil was due to additional copper washing off the leaves during the rainy season. Thus, we also conclude that alternatives to copper could be applied periodically, especially during periods of higher rainfall. This should be taken into consideration in methods and directives of organic good agricultural practices, and equally integrated management in order to make the best use of the increasingly limited amount of copper being allowed.

Some copper alternative treatments (potassium silicate, vermicompost tea, *Mycorrhiza mix*, *and Trichoderma citrinoviride* TR1) resulted in a higher content of TWSP. This stimulates antioxidant capacity in olive fruits and therefore the olive leaf spot disease incidence was decreased as compared to other BCA treatments. We conclude that alternative inputs like vermicompost tea have the potential to be advised for organic urban olive production to replace copper sulphate or copper oxychloride against olive leaf spot disease in the near future. Vermicompost tea can be made in small batches suitable for urban production, however, with larger equipment, this can also be an option for commercial scale organic orchards. Furthermore, we argue that even if the schedule of copper application is compatible with the DSS-Decision Support System (early warning system) to decrease copper application doses in organic management, even reduced copper amounts have the potential to pollute the soil, as well as to be accumulated in fruits and leaves of olive trees. Based on our practical in-field research, we conclude that the alternative inputs tested can be advised for both organic urban crop production, and commercial organic and also non-organic (integrated) production systems.

Author Contributions: Conceptualisation, A.K.; methodology, investigation and validation, A.K., B.C.; TWSP method and analysis, D.S., F.O.G.; software A.K.; writing—original draft preparation, A.K., U.S., F.R., B.C.; writing—review and editing, A.K., D.T., U.S., F.R.; research conceptualisation, budgeting and funding acquisition, A.K. and U.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research has been financially supported by the Horizon 2020 EU Funded project "Pathways to phase-out contentious inputs from organic agriculture in Europe"-Organic-PLUS (No: 774340).

Data Availability Statement: Data supporting the reported results can be found in deliverable reports of the Organic-PLUS project, grant number 774340 presented to EU Commission.

Acknowledgments: The authors are grateful to Cetinel., H.A. (at Ege University, Izmir, TR) and Aras, V. (at MAF, TR) for their contributions in software and editing the text and for the kind assistance of the organic farmer at the case farm (olive orchard). We also thank Turan, H.S. (at MAF, TR) and Aydogdu, E. (at MAF, TR) for their valuable contributions in the collection of soil, leaf and fruit samples from trial site and copper analysis of the samples of this research.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. EPA. Copper, Public Health Statement. 2021. Available online: https://www.epa.gov/ (accessed on 18 July 2021).
2. Schmutz, U.; Conroy, J. Pathways to Phase-Out Contentious Inputs from Organic Agriculture in Europe. Available online: https://organic-plus.net/ (accessed on 2 June 2021).
3. Oorts, K. Copper. In *Heavy Metals Soils*; Springer: Berlin/Heidelberg, Germany, 2012; pp. 367–394. [CrossRef]
4. Marschner, H. *Mineral Nutrition of Higher Plants*, 2nd ed.; Academic Press: London, UK, 1995; pp. 33–347.
5. Yang, X.-E.; Long, X.-X.; Ni, W.-Z.; Ye, Z.-Q.; He, Z.-L.; Stoffella, P.J.; Calvert, D.V. Assessing Copper Thresholds for Phytotoxicity and Potential Dietary Toxicity in Selected Vegetable Crops. *J. Environ. Sci. Health Part B* **2002**, *37*, 625–635. [CrossRef] [PubMed]
6. Morgan, R.; Johnston, H. The accumulation of copper in a New Zealand orchard soil. *J. R. Soc. N. Z.* **1991**, *21*, 323–327. [CrossRef]
7. Official Gazette of Republic of Turkey. 2005. 31.5.2005, No: 25831. Regulation Soil contamination. Available online: https://www.resmigazete.gov.tr/eskiler/2005/05/20050531-6.htm (accessed on 15 January 2022).
8. Official Gazette of Republic of Turkey. 2018. Organic Farming, 10.1.2018, No: 30297. Available online: https://www.mevzuat.gov.tr/mevzuat?MevzuatNo=14217&MevzuatTur=7&MevzuatTertip=5 (accessed on 15 January 2022).
9. Katsoulas, N.; Løes, A.-K.; Andrivon, D.; Cirvilleri, G.; de Cara, M.; Kir, A.; Knebl, L.; Malińska, K.; Oudshoorn, F.W.; Willer, H.; et al. Current use of copper, mineral oils and sulphur for plant protection in organic horticultural crops across 10 European countries. *Org. Agric.* **2020**, *10*, 159–171. [CrossRef]
10. Eurostat. Olive Production Statistics. 2021. Available online: https://ec.europa.eu/eurostat/web/main/data/database (accessed on 16 July 2021).
11. Conte, P.; Fadda, C.; Del Caro, A.; Urgeghe, P.P.; Piga, A. Table Olives: An Overview on Effects of Processing on Nutritional and Sensory Quality. *Foods* **2020**, *9*, 514. [CrossRef] [PubMed]
12. Faostat. Olive Production Statistics. 2021. Available online: https://www.fao.org/faostat/en/#data/QV (accessed on 16 July 2021).
13. Willer, H.; Schlatter, B.; Trávníček, J.; Kemper, L.; Lernoud, J. (Eds.) The World of Organic Agriculture. Statistics and Emerging Trends 2020. In *Research Institute of Organic Agriculture (FiBL), Frick, and IFOAM—Organics International, Bonn*; 2020. Available online: www.organic-world.net/yearbook/yearbook-2020.html (accessed on 16 January 2022).
14. Laborda, E.; Antón, F.A. Estudio de la susceptibilidad/resistencia de variedades del olivo (*Olea europaea* L.) al patógeno *Cycloconium oleaginum* (Cast.) (*Spilocaea oleaginae* Hugh. *Boletín Sanid. Vegetal. Plagas* **1989**, *15*, 385–403.
15. Azeri, T. Research on olive leaf spot, olive knot and verticillium wilt of olive in Turkey. *EPPO Bull.* **1993**, *23*, 437–440. [CrossRef]
16. Berger, S.; Sinha, A.K.; Roitsch, T. Plant physiology meets phytopathology: Plant primary metabolism and plant pathogen interactions. *J. Exp. Bot.* **2007**, *58*, 4019–4026. [CrossRef]
17. Bremer, H. *Türkiye Fitopatolojisi III*; Güney Matbaası: Ankara, Turkey, 1948.
18. Chen, S.; Zhang, J. Studies on olive peacock's eye disease, infection cycle and epidemiology. *Acta Phytopathol.-Ca Sin.* **1983**, *13*, 31–40.
19. Graniti, A. Olive scab: A review. *EPPO Bull.* **1993**, *23*, 377–384. [CrossRef]
20. Macdonald, A.; Walter, M.; Trought, M.; Frampton, C.; Burnip, G. Survey of olive leaf spot in New Zealand. *N. Z. Plant Prot.* **2000**, *53*, 126–132. [CrossRef]
21. Obanor, F.O.; Walter, M.; Jones, E.E.; Jaspers, M.V. Effect of temperature, relative humidity, leaf wetness and leaf age on *Spilocaea oleagina* conidium germination on olive leaves. *Eur. J. Plant Pathol.* **2007**, *120*, 211–222. [CrossRef]
22. Roubal, C.; Regis, S.; Philippe, C.; Nicot, P.C. OPTIPAON, a decision support system to predict the risk of peacock eye of olive in southern France. In Proceedings of the 7th Congress on Plant Protection "Integrated Plant Protection—A Knowledge-Based Step towards Sustainable Agriculture, Forestry and Landscape Architecture", Zlatibor, Serbia, 24–28 November 2014.
23. El Aabidine, A.Z.; Baissac, Y.; Moukhli, A.; Jay Allemand, C.; Khadari, B.; El Modafar, V.C. Resistance of olive-tree to Spilocaea oleagina is mediated by the synthesis of phenolic compounds. *Int. J. Agric. Biol.* **2010**, *12*, 61–67.
24. EşbahTunçay, H.; Akyol, M.; Steindl, M. Urban agriculture: Implications on Istanbul cultural Theritage. *J. Environ. Prot. Ecol.* **2014**, *15*, 1793–1800.
25. Kaldjian, P.J. Istanbul's Bostans: A Millennium of Market Gardens. *Geogr. Rev.* **2004**, *94*, 284–304. [CrossRef]
26. Official Gazette of Republic of Turkey, 2012. 6.12.2012, No: 28489. Büyükşehir Belediyesi kurulması ve sınırlarının belirlenmesi (6360, 12.11.20129). Available online: https://www.resmigazete.gov.tr/eskiler/2012/12/20121206-1.htm (accessed on 16 February 2022).
27. Official Gazette of Republic of Turkey, 2020. 16.10.2020, No: 31276. Kamu Mali Yönetimi ve Kontrol Kanunu ile Bazı Kanunlarda Değişiklik Yapılması Hakkında Kanun. Available online: https://www.resmigazete.gov.tr/eskiler/2020/10/20201016M1-1.htm (accessed on 16 February 2022).
28. Official Gazette of Republic of Turkey, 2018b. 23.2.2018, No: 30341. Tarımda Kullanılan Gübrelerin Piyasa Gözetimi ve Denetimi. Available online: https://www.mevzuat.gov.tr/anasayfa/MevzuatFihristDetayIframe?MevzuatTur=7&MevzuatNo=38682&MevzuatTertip=5 (accessed on 16 February 2022).
29. Özden, F. İzmir'de Gıda Grupları ve Topluluk Destekli Tarım. *Meltem İzmir Akdeniz Akad. Derg.* **2019**, 93–97. [CrossRef]
30. Gida Toplulukları. 2021. Available online: http://gidatopluluklari.org/ (accessed on 18 July 2021).
31. Van Der Ploeg, J.D. *The New Peasantries: Struggles for Autonomy and Sustainability in an Era of Empire and Globalization*; Routledge: Oxfordshire, UK, 2012.

32. Kazemi, H.; Klug, H.; Kamkar, B. New services and roles of biodiversity in modern agroecosystems: A review. *Ecol. Indic.* **2018**, *93*, 1126–1135. [CrossRef]
33. Kopytko, N. Supporting Sustainable Innovations: An Examination of India Farmer Agrobiodiversity Conservation. *J. Environ. Dev.* **2019**, *28*, 386–411. [CrossRef]
34. Kloppenburg, J. Impeding Dispossession, Enabling Repossession: Biological Open Source and the Recovery of Seed Sovereignty. *J. Agrar. Chang.* **2010**, *10*, 367–388. [CrossRef]
35. Di Falco, S.; Penov, I.; Aleksiev, A.; van Rensburg, T.M. Agrobiodiversity, Farm Profits and Land Fragmentation: Evidence from Bulgaria. *Land Use Policy* **2010**, *27*, 763–771. [CrossRef]
36. Japón-Luján, R.; de Castro, M.D.L. Small Branches of Olive Tree: A Source of Biophenols Complementary to Olive Leaves. *J. Agric. Food Chem.* **2007**, *55*, 4584–4588. [CrossRef]
37. Talhaoui, N.; Gómez-Caravaca, A.M.; Roldán, C.; León, L.; De La Rosa, R.; Fernández-Gutiérrez, A.; Segura-Carretero, A. Chemometric Analysis for the Evaluation of Phenolic Patterns in Olive Leaves from Six Cultivars at Different Growth Stages. *J. Agric. Food Chem.* **2015**, *63*, 1722–1729. [CrossRef] [PubMed]
38. Balasundram, N.; Sundram, K.; Samman, S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem.* **2006**, *99*, 191–203. [CrossRef]
39. Townsend, G.K.; Heuberger, J.W. Methods for Estimating Losses Caused by Diseases in Fungicide Experiments. *Plant Dis. Rep.* **1943**, *27*, 340–343.
40. Lindsay, W.L.; Norvell, W.A. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Am. J.* **1978**, *42*, 421–428. [CrossRef]
41. Bouaziz, M.; Fki, I.; Jemai, H.; Ayadi, M.; Sayadi, S. Effect of storage on refined and husk olive oils composition: Stabilization by addition of natural antioxidants from Chemlali olive leaves. *Food Chem.* **2008**, *108*, 253–262. [CrossRef]
42. Ranalli, A.; Contento, S.; Lucera, L.; Di Febo, M.; Marchegiani, D.; Di Fonzo, V. Factors Affecting the Contents of Iridoid Oleuropein in Olive Leaves (*Olea europaea* L.). *J. Agric. Food Chem.* **2005**, *54*, 434–440. [CrossRef]
43. Hrncirik, K.; Fritsche, S. Comparability and reliability of different techniques for the determination of phenolic compounds in virgin olive oil. *Eur. J. Lipid Sci. Technol.* **2004**, *106*, 540–549. [CrossRef]
44. Kacar, B. *Bitki Besleme*; Ankara University Agriculture Faculty Publication No. 367: Ankara, Turkey, 1977.
45. Kacar, B.; ve İnal, A. *Bitki Analizleri*; Şti. Yayınları, Yayın No: 1241; Fen Bilimleri: 63, (I. Basım); Nobel Yayın Dağıtım Ltd.: Ankara, Turkey, 2008.
46. Reuter, D.J.; Robinson, J.B. *Plant Analysis, An Interpretation Manual*; Inkata Press: Collingwood, VIC, Australia, 1997; 127p.
47. *SAS STAT Software, Release 2007 JMP©, Version 7*; SAS Institute Inc.: Cary, NC, USA, 2007.
48. Kellogg, C.E. *Our Gardens Soils*; The Macmillan Company: New York, NY, USA, 1952.
49. Evliya, H. *Kültür Bitkilerinin Beslenmesi*; Ankara Üniversitesi Ziraat Fakültesi Yayınları, Sayı: Ankara, Turkey, 1964; Volume 36.
50. Akalan, İ. *Soil Formation Structure and Features*; Ankara University Faculty of Agriculture: Ankara, Turkey, 1968.
51. Loue, A. Diagnostic Pétiolairem de Prospection. In *Etudes Sur La Nutrition et La Fertilisation Potassiques de La Vigne*; Sociéte Commerciale des Potasses d' Alsace Services Agronomiques: Antananarivo, Madagacar, 1968; pp. 31–41.
52. Olsen, S.R.; Dean, L.A. Phosphorus. In *Methods of Soil Analysis. Part 2*; Black, C.A., Ed.; American Society of Agronomy, Inc.: Madison, WI, USA, 1965; pp. 1035–1049.
53. Pizer, N.H. Some Advisory Aspects. Soil Potassium and Magnesium. *Tech. Bull.* **1967**, *14*, 184–186.
54. Follett, R.H.; Lindsay, W.L. Changes in DTPA-extractable zinc, iron, manganese, and copper in soils following fertilization. *Soil Sci. Soc. Am. Proc.* **1971**, *35*, 600–602. [CrossRef]
55. Keren, R.; Bingham, F.T. Boron in Water, Soils and Plants. *Adv. Soil Sci.* **1985**, *1*, 229–276.
56. *Zeytincilik Araştırma Enstitüsü, Bölge Yaprak ve Toprak Analiz Laboratuvarı Survey Çalışmaları Kesin Sonuç Raporu*; Anonim: Bornova, İzmir, 1993.
57. Ozturk, M.; Altay, V.; Gönenç, T.; Unal, B.; Efe, R.; Akçiçek, E.; Bukhari, A. An Overview of Olive Cultivation in Turkey: Botanical Features, Eco-Physiology and Phytochemical Aspects. *Agronomy* **2021**, *11*, 295. [CrossRef]
58. Aydoğdu, E. Domat ve Uslu Zeytin Çeşitlerinde Yaprakların Besin Element İçerikleri ve Bunların Mevsimsel Değişim-lerinin İncelenmesi. Toprak Bilimi ve Bitki Besleme ABD. Master's Thesis, Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Adana, Turkey, 2011.
59. Şahin, G. Organik Zeytin Yetiştiriciliğinde Farklı Gübre Dozlarının Toprak Özellikleri, Yaprak Besin Elementi Içeriği ve yağ Kalitesi Üzerine Etkileri. Toprak Bilimi ve Bitki Besleme ABD. Master's Thesis, Adnan Menderes Üniversitesi, Fen Bilimleri Enstitüsü, Aydın, Türkiye, 2013.
60. Eryüce, N. Organik ve Geleneksel Zeytin Yetiştiriciliğinde Bitki Beslenme Durumunun Meyve, Yaprak ve Zeytinyağında Önemli Kalite Ölçütleri Üzerindeki Etkilerinin Belirlenmesi. Proje No: 108O164, TUBITAK, Ankara, 2010, 132s. Available online: http://zeytindostu.org.tr/wp-content/uploads/2019/06/TVRBeU9UWTI.pdf (accessed on 13 June 2022).
61. Oztürk Gungor, F. Effects of Different Preservation Methods on Shelf Life and Qualıty of Domat Table Olive Varıety Prepared Usıng Chloride Salts (NaCl, KCl, $CaCl_2$). Ph.D. Thesis, Ege University, Izmir, Turkey, 2020.
62. Ben Othman, N.; Roblain, D.; Thonart, P.; Hamdi, M. Tunisian Table Olive Phenolic Compounds and Their Antioxidant Capacity. *J. Food Sci.* **2008**, *73*, C235–C240. [CrossRef] [PubMed]

63. Piga, A.; Del Caro, A.; Pinna, I.; Agabbio, M. Anthocyanin and colour evolution in naturally black table olives during anaerobic processing. *LWT* **2005**, *38*, 425–429. [CrossRef]
64. Pirgün, Y. Determination of Antioxidant Effects of Gemlik and Halhalı Olives Grown in Hatay. Food Engineering. No: ZF2006YL15. Ph.D. Thesis, Çukurova University, Adana, Turkey, 2007; 56p.
65. Lanza, B.; Di Serio, M.G.; Iannucci, E. Effects of maturation and processing technologies on nutritional and sensory qualities of Itrana table olives. *Grasas Y Aceites* **2013**, *64*, 272–284. [CrossRef]
66. Irmak, Ş. *A Study on the Determination of Polyphenol Content of Some Table Olive Varieties and the Effect of Processing Techniques on Poli-Phenols*; Olive Research Institute: Izmir, Turkey, 2010.
67. Tamendjari, A.; Mettouchi, S.; Sacchi, R.; Moussa, Z.E.O.; Paduano, A.; Savarese, M. Effect of Spanish style processing on the phenolic compounds and antioxidant activity of Algerian green table olives. *Grasas Y Aceites* **2016**, *67*, e114. [CrossRef]