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Effect of Different Substrates, and Irrigation with Water with Different Saline Concentrations, on the Development of Tomato Fungal Diseases in an Almería-Type Greenhouse

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Abstract: The aim of this research was to assess the effect of both the salinity level and the type of growing system on the vegetative health of a tomato crop (*Solanum lycopersicum*). The study was carried out in Almería (Spain) in a local typical greenhouse. Two different growing media were tested: (i) the artificial soil "*enarenado*" and (ii) a coconut fiber substrate. Each of these growing media was irrigated with water with three different saline concentrations: (i) T1 with an electrical conductivity of 0.6 dS/m, (ii) T2 with 1.5 dS/m, and (iii) T3 with 3.0 dS/m. Using the European and Mediterranean Plant Protection Organization (EPPO) regulations, two diseases were identified: (i) powdery mildew (*Leveillula taurica*) with a lower disease incidence in tomato plants grown in soil and in plants irrigated with decreasing salinity treatments and (ii) crown and root rot in tomato (*Fusarium* f. sp. *radicis-lycopersici*) with a lower incidence in tomato plants grown in soil and a higher incidence in tomato plants grown in coconut substrate. A higher yield was observed in tomato plants transplanted in *enarenado* than in coconut substrate, although a higher level of Brix degrees was observed in the crops with higher disease severity and salinity stress.

Keywords: protected crop; EC electric conductivity; nutrient solution; crown and root rot; powdery mildew

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1. Introduction

The province of Almería is the Mediterranean region with the highest concentration of greenhouses in Europe. This type of agriculture is developed using low-cost solar greenhouses, with passive climate control systems [1], based on natural ventilation and bleaching of the plastic covers to control the amount of radiation in the interior of the greenhouses. Sophisticated systems such as the use of heating, forced ventilation, humidification and other active systems are difficult to amortize in low-cost solar greenhouses [2].

One of the largest greenhouse horticultural production areas in the world can be found on the coast of Almería, and the water supply varies significantly depending on the type of crop that is produced, as crops are watered by drip irrigation systems [3]. The water resources mostly come from underground catchments through wells or boreholes, in which some contamination of the underlying aquifers has been detected [4].

Irrigation with good-quality water increases plant production [5]. The use of localized irrigation is widely extended to greenhouses in Almería, representing 99.9% of the surface of greenhouse crops [2,6]. Localized irrigation systems are usually associated with fertirrigation, which is the application of fertilizer through irrigation water [7].

Fertirrigation regularly maintains a low nutrient input to the soil as an irrigation solution and allows for automation in fertilizer application, with a high initial investment cost [8].

It is known that the irrigation of crops with highly saline water produces an imbalance in the nutrients taken up by the plant, causing low concentrations of macronutrients in its tissues, which negatively affects growth [9], as well as fruit size and yield [10,11].

Crops irrigated with highly saline water are, for the most part, sensitive to this parameter (Na^+ contents), leading to expected reductions in the production and yields of crops with moderate levels of salinity in the root solution [12]. In the case of tomato crops, in Southeastern Spain in particular, it is necessary to know the response to salinity of these crops in soilless areas in order to optimize yield and production quality [13].

Sodium ions (Na^+) that accumulate in excess in plant tissues can cause a decrease in fruit production in various crops, and a smaller fruit size has also been observed in crops with higher Na^+ content in their tissues [14].

In tomato cultivation, improvements in fruit quality have been documented in cultivars irrigated with water with a higher salt concentration [15,16]. Water with a higher salt concentration improves the quality of tomato fruit due to the increase in sugars and soluble solids in the fruit. Some authors found that a higher solid concentration could be the result of a decrease in water transferred to the fruit, which did not affect dry matter transport, leading to higher sugar concentrations in the fruit [15,17], although other studies showed an increase in dry matter and glucose in fruits irrigated with more highly saline water [9]. A greater transport of assimilates from tomato leaves to fruit and an increase in their transformation to starch was also found [18]. Tomatoes irrigated with saline water exhibit an increased rate of dry matter transport, thereby decreasing the water transport rate [19]. On the other hand, excess fertilization in the irrigation nutrient solution usually makes plants more susceptible to disease attacks [20].

About 500 species of downy mildew pathogens have been found to be pathogenic to crop plants and can affect more than 1500 plant species [21]. Powdery mildews are one of the most important diseases affecting agricultural production [22].

Leveillula taurica is a hemiendophytic pathogen that causes clear visual symptoms on crop leaves. In severe attacks, a large number of leaf lesions cause a weakening of the plants. It is a common disease of greenhouse tomato crops worldwide [23]. The disease causes severe yield losses in both outdoor and greenhouse crops. Symptoms of *L. taurica* include the growth of white mycelium on leaves, leading to the development of chlorotic lesions that eventually lead to necrosis in those areas [24].

Fusarium oxysporum f. sp. *radicis-lycopersici* (FORL) is a saprophytic fungus that develops in the rhizosphere of various plant species. It is a pathogen with a large number and diversity of hosts, although it displays a preference for specialization on specific hosts. Isolates of the fungus that are considered pathogens of the same species are assigned the form "specialist" [25].

This saprophyte can reach a growing area or soil via contaminated seeds, infected soil or compost containing the fungus [26]. The effects of *F. oxysporum* f. sp. *radicis-lycopersici* (FORL) on the plants it infects include the wilting of the plant, which may result in plant death or the weakening of the crop, leading to a loss in yield and fruit quality [27,28]. Visual symptoms are observed at the stem level above ground, with necrotic lesions appearing on the root collar and stem base.

The objective of this study is to highlight the effects of different crop systems irrigated using water with different saline concentrations and different nutrient solutions, applied through fertigation, on the development of fungal diseases in a tomato crop. In addition, we investigate the consequences of the different stresses generated by the salt concentration of irrigation water and fungal diseases, which are derived from this use of irrigation, on a tomato crop under Mediterranean greenhouse conditions.

2. Materials and Methods

2.1. Experimental Greenhouses and Cropping System

The trials were carried out in an Almería-type greenhouse "*Raspa y Amagado*" (Figure 1) located in the UAL-ANECOOP Experimental Station "*Catedrático Eduardo Fernandez*" of the University of Almería (36°51′ N, 2°17′ W and 87 m.a.s.l.).

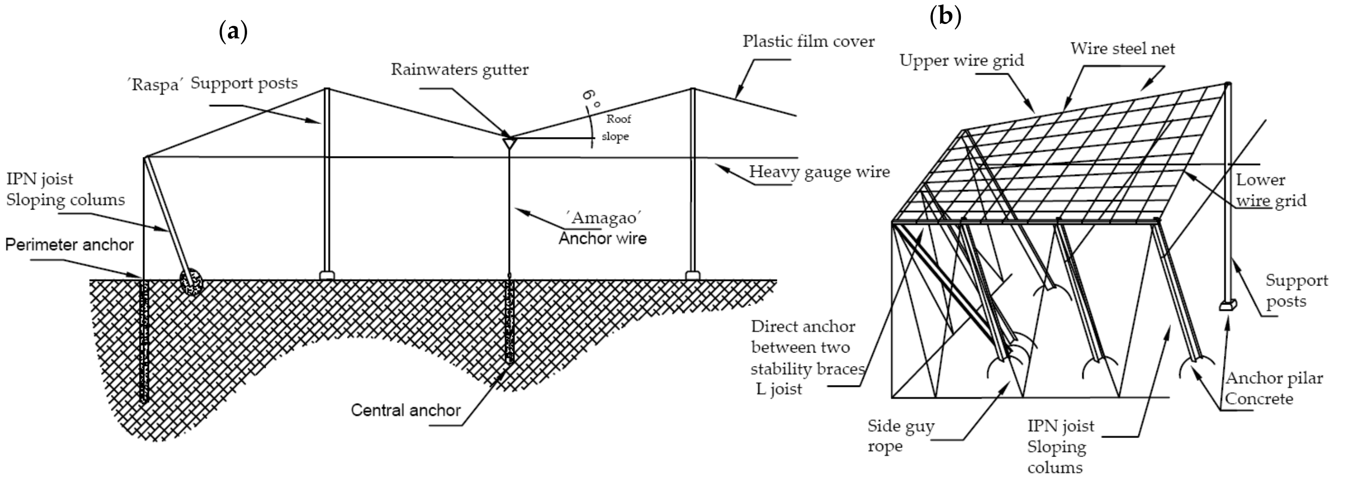

Figure 1. "*Raspa y Amagado*" greenhouse: (**a**) section; (**b**) corner greenhouse details.

The "*Raspa y amagado*"-type greenhouse is a typical Almería greenhouse consisting of gabled modules with interior modules that have symmetry with respect to the ridge [2]. This structure is composed of two basic elements (Figure 1): (i) The vertical structure consists of rigid supports that can be differentiated according to their location along the perimeter or indoors. (ii) The flexible cover structure is composed of two overlapping galvanized grids (upper and lower) that support and hold the sheet of plastic between them [2].

In the trial, we applied three different treatments with different electric conductivity levels to the nutrient solution. Treatment T1 was a low-salinity treatment consisting of irrigation water with an electrical conductivity (EC) of 0.6 dS m^{-1} (desalinated seawater). The final EC of the nutrient solution was 2.2 dS m^{-1} after the addition of the fertilizers. The irrigation water of treatment T2 had an initial EC of 1.5 dS m^{-1}, which increased to 2.5 dS m^{-1} after the fertigation. The electrical conductivity of treatment T3 was 3.0 and 3.5 dS m^{-1} for the raw water and the nutrient solution, respectively, as shown in Table 1. Each of these three treatments was applied by fertirrigation, both in the "*enarenado*" system and in the coconut fiber system.

Table 1. Electric conductivity in different irrigation areas in the experiment. T, treatment; S, typical soil substrate "enarenado"; H, coconut fiber substrate.

Greenhouse	Soil	EC dS/m		
Irrigation		**EC Water**	**ΔEC Fertilization**	**EC in Nutrient Solution**
T1-S	"*Enarenado*"	0.6	1.6	2.2
T2-S		1.5	1	2.5
T3-S		3	0.5	3.5
T1-H	coconut fiber substrate	0.6	1.6	2.2
T2-H		1.5	1	2.5
T3-H		3	0.5	3.5

Table 2 shows the ion composition of the different types of irrigation water.

Table 2. Water ion composition of the three types of irrigation water. EC, electric conductivity for treatment (dS m^{-1}); ions and anions of interest for irrigation (mmol l^{-1}).

Salinity Levels	EC	Ions and Anions									
		NO_3^-	SO_4^{2-}	$H_2PO_4^-$	HCO_3^-	Cl^-	K^+	Ca^{2+}	Mg^{2+}	Na^+	NH_4^+
1	0.6	0.00	0.06	0.01	0.75	4.48	0.12	0.37	0.13	4.39	0.00
2	1.5	0.00	1.47	0.01	3.55	7.28	0.12	3.17	1.54	5.91	0.00
3	3.0	0.00	3.58	0.01	7.75	11.48	0.12	7.37	3.65	8.18	0.00

The fertilizer supply method was adapted from Sonneveld and Straver's ideal solution [29]. Fertilizer was supplied by simulating the situation in the field so that, in the case of desalinated water, the final EC with fertilizers would be 2.2 dS·m^{-1}. In the case of water with intermediate salinity, this increased to 2.5 dS·m^{-1}, and in the case of water with higher salinity, it increased to 3.5 dS·m^{-1}. Table 3 shows the ion characteristics of the fertilization irrigation solution.

Table 3. Theoretical final irrigation nutrient solution composition for each treatment. EC, electric conductivity for treatment (dS m^{-1}); ions and anions of interest for irrigation (mmol l^{-1}).

Salinity Levels	EC	Ions and Anions									
		NO_3^-	$H_2PO_4^-$	SO_4^{2-}	HCO_3^-	Cl^-	NH_4^+	K^+	Ca^{2+}	Mg^{2+}	Na^+
1	2.2	12.00	1.51	1.31	0.75	4.48	0.50	7.12	3.92	1.08	4.39
2	2.5	9.50	1.51	1.47	3.55	7.28	0.00	7.62	4.17	1.54	5.91
3	3.5	4.50	2.01	3.58	7.75	11.48	0.00	4.62	7.37	3.65	8.18

The leaching fraction of the coconut fiber treatments was 20% since this is a common management practice in the area; the leaching fraction was not recirculated.

These EC treatments were applied to both growing media: the "*enarenado*" sandy soil (S) (Figure 2) and the coconut fiber substrate (H) (Figure 3c). The "*enarenado*" is a common layered artificial soil typically used in greenhouses in Almería [2].

First, the original soil was covered with an initial half meter layer of soil with a high clay content. This layer prevents major losses of irrigation water caused by deep percolation; this is the main area where the roots develop. The second layer of manure or organic matter was placed over the clayey soil. Finally, the plot was covered with a fine layer of sand (average particle diameter < 3 mm) [2].

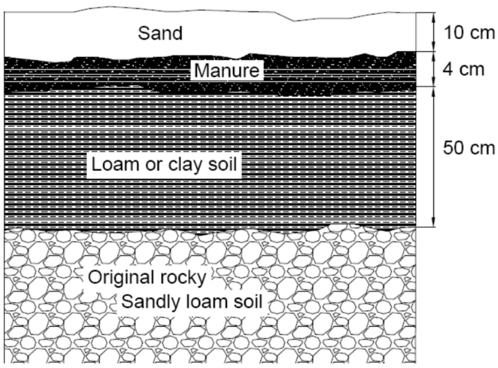

Figure 2. "*Enarenado*" experiment details.

The different treatments were fertirrigated independently. Fertirrigation was applied to each treatment depending on the nutrient concentration of the irrigation water, so the same final ideal solution, adapted from Sonneveld and Straver [29], was applied to each treatment. To accomplish this goal, the irrigation system was composed of an automated fertirrigation head and one independent irrigation network for each treatment.

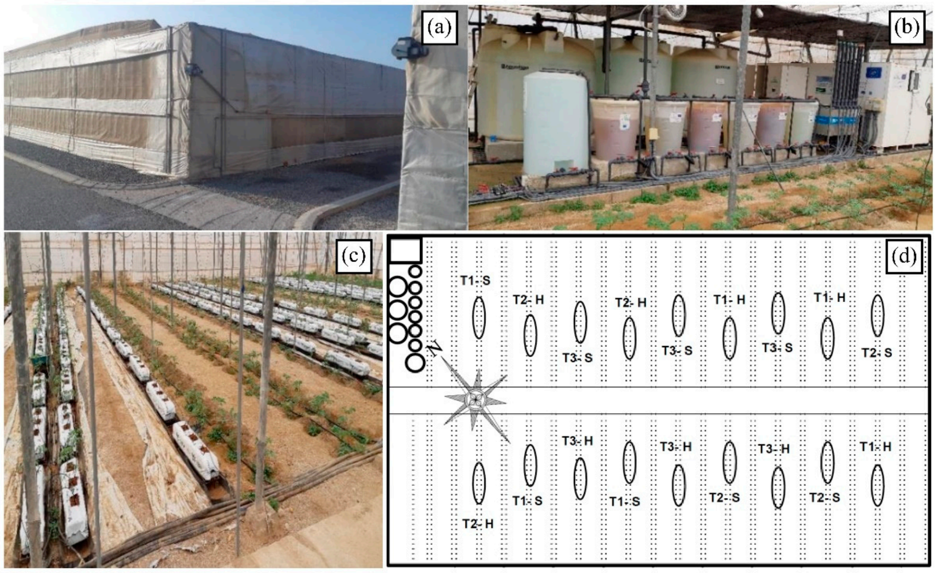

Figure 3. Experimental "*Raspa y Amagado*" greenhouse: (**a**) irrigation installation; (**b**) plants in typical soil substrate and in coconut fiber substratep (**c**) orrigation treatments and powdery mildew plot assessment distribution; (**d**) treatments T1, T2 and T3; S, typical soil substrate "*enarenado*"; H, coconut fiber substrate.

2.2. Plant Material

The study was carried out in an autumn–winter tomato (*Solanum lycopersicum*) cropping cycle, with the commercial variety "Ramyle RZ F1" (Rijk Zwaan Ibérica, S.A., Almería, Spain), a long-life tomato of indeterminate size, which is harvested as a bouquet. Seeding was carried out in coconut fiber substrate and a typical soil substrate ("*enarenado*") from the area with localized drip irrigation at a density of 2 plant/m^2. The planting design was a double row of plants seeded 0.5 m apart, with a distance between rows of 2 m.

The seeds were germinated in a nursery in optimal conditions for their development. All the seeds used in the trial were germinated under the same conditions and irrigated with the same nutritive solution. The crop was transplanted on 7 September 2020 to the experimental greenhouse (until 14 March 2021) and after this date was irrigated with the different waters and the nutrient solutions shown in Table 1. The plants were transplanted with the third true leaf on the main stem, unfolded (BBCH 13).

2.3. Plant Disease Development Quantification

During the development of the experiment, the diseases identified in this study appeared naturally; in this trial, we did not inoculate the pathogen. From the beginning of the appearance of both diseases, differences were observed in their development on the plants. These differences in disease development speed varied depending on the substrate used, and differences were even observed in the development of both diseases between the plants irrigated with the different types of nutritional solutions studied, so a disease assessment was performed. The first symptom of the disease was noticed in December 2020. The identification of the fungi, which cause the disease, was carried out through the direct observation and observation of mycelia, spores and conidia under a microscope [30].

For the design of the trial and the evaluation of disease incidence, EPPO standards were applied. For powdery mildew and Fusarium crown and root rot diseases, EPPO Standard PP 1/181 (Conduct and reporting of Efficacy Evaluation Trials), EPPO Standard PP 1/152 (Design and Analysis of Efficacy Evaluation Trials) and EPPO PP 1/57 (Powdery Mildews in Cucurbits and Other Vegetables) are applicable, along with EPPO PP 2/29 (Solanaceae Crops under Protected Cultivation), EPPO 1/163 and EPPO 1/121.

The percentage of affected leaf area on the upper and lower surfaces of at least four leaves on each plant was evaluated. For each experimental sector of the greenhouse, assessments plots were established in the different irrigation zones (Table 1). In each assessment plot, 12 plants were evaluated. For each plant analyzed, a minimum of 4 uniformly distributed leaves were evaluated (a total of 50 leaves were evaluated by each assessment plot). In total, for each treatment (T1-H, T1-S, T2-H, T2-S, T3-S and T3-H), 150 leaves were analyzed for every different irrigation zone.

Figure 4 shows an example of the quantification of the infection percentage index on a cucurbit leaf according to the EPPO regulations. The evaluation of the level of disease (powdery mildew) was carried out every 7 days from the beginning of the disease until the end of the crop cycle. A final evaluation was performed 7 days before the end of the crop cycle.

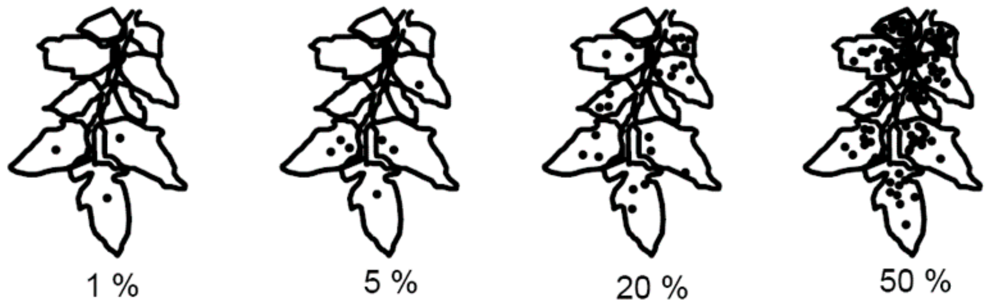

Figure 4. Example assessment of % leaf area infected.

For the Fusarium analysis, a count was conducted of plants affected by the disease that showed symptoms in the vascular bundles, which, over time, wilted the tomato plants (Figure 5). In each irrigation zone and different substrate, 50 plants (total of 150 plants for every combination of substrate and irrigation) were analyzed. An evaluation of the level of disease (Fusarium crown and root rot) was carried out every 7 days from the beginning of the disease until the end of the crop cycle. A final evaluation was performed 7 days before the end of the crop cycle.

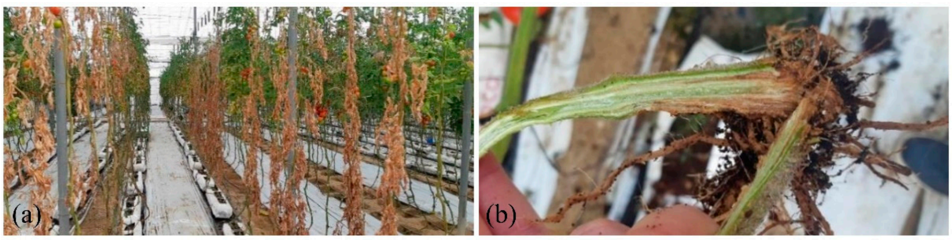

Figure 5. *Fusarium oxysporum* f. sp. *radicis-lycopersici* damage (**a**) and root detail (**b**).

2.4. Fruit Production and Quality Measurements

Production data (marketable and non-marketable yield) were registered separately for each experimental unit. An EKS Premium electronic scale (EKS Spain, S.A., Barcelona, Spain), with a sensitivity of 10 g and a maximum capacity of 40 kg, was used for this purpose.

Fruit weight was measured using a PB3002-L DeltaRange scale, with a resolution of 0.1 g and a span of 0.5 to 3100 g. A digital caliper was used to monitor the fruit diameter. A refractometer (PAL-1, Atago Co., LTD., Saitama, Japan), with a range of 0.0 to 53.0% Brix°, a resolution of 0.1% Brix° and accuracy of ±0.2% Brix°, and a digital penetrometer (PCE-FM 200, PCE-Ibérica S.L., Albacete, Spain), with a range of 0 to 200 N, a resolution of 0.1 N and an accuracy of ±0.5%, were used to measure the Brix degrees and firmness of the fruit, respectively. The tomato was harvested on the branch, and the harvest was carried out when the tomato branch was fully ripe, on the following dates: 13 January 2021, 8 February 2021, 18 February 2021 and 9 March 2021.

2.5. Statistical Analysis

The experimental greenhouse was divided into 18 experimental units. For the trial design, we used a randomized block design, with three replications for every irrigation type and every crop system (two crop system and three salinity levels). The different treatments were distributed randomly, resulting in the layout depicted in Figure 3d. The plots of six square meters (3 m plot length and 2 m plot width) of every single replication were formed for 12 tomato plants. The assessment of the disease was carried out over 50 subsamples for each plot. These subsamples consisted of 50 leaves distributed randomly in the 12 plants that formed the plots. We, therefore, analyzed 150 subsamples for every crop system and irrigation solution for powdery mildew assessment. For Fusarium crown and root rot, the design had three replications, and the assessment was performed using 50 random plants in each replication, with 150 plants evaluated for each treatment.

The statistical analysis of the data was performed with the software Statgraphics Centurion v.19 (Manugistics Inc., Rockville, MD, USA) using an analysis of variance (considered significant if the p-value is ≤ 0.05), comparing the mean values with the procedure of minimum significant difference of Fisher (LSD). Bartlett, Cochran and Hartley tests were used to determine whether a sector had a similar variation. For parameters with different variance, we performed a nonparametric analysis with the Friedman test, with each row representing a block (the evaluation date), using averages graphs.

Multiple sample comparison was used to compare the data. The F-test in the ANOVA table tests whether there are any significant differences amongst the means. The method used to discriminate among the means was Fisher's least significant difference (LSD) procedure.

3. Results

3.1. Fusarium Crown and Root Rot

Fusarium crown and root rot symptoms were first observed on 24 December before the first harvest. The disease quantification was monitored every 7 days, until 14 March 2021. At this kinetic point, some plots of the trial reached infection rates higher than 60%.

As shown in Figure 6 and Table S1, the areas irrigated in coconut substrate showed a high prevalence of crown and root rot compared to the areas irrigated in soil, and these differences were statistically significant.

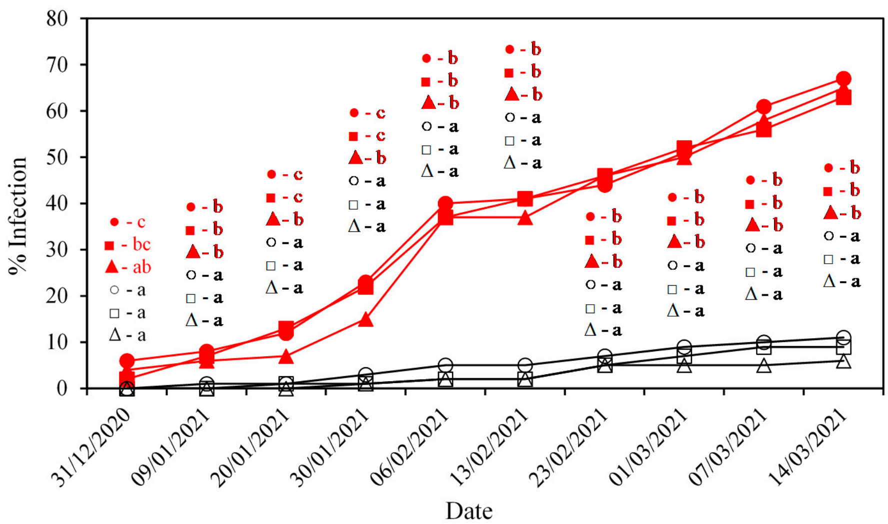

Figure 6. Development of Fusarium crown and root rot percentage of infection over time in the sectors with different irrigation and substrates. Treatments: T1-H (■), T1-S (□), T2-H (▲), T2-S (Δ), T3-H (●) and T3-S (○). S, typical soil substrate "*enarenado*"; H, coconut fiber substrate. Different letters in the same column indicate statistically significant differences with a confidence level of 95.0% (p-value ≤ 0.05).

The infection and development of the Fusarium crown and root rot were faster in the areas irrigated in coconut substrate than in "*enarenado*" soil areas, as shown in Figure 6. In our case, the coconut substrate had a lower volume than the "*enarenado*" soil, so the roots of the plants were nearer than in the "*enarenado*" soil, which could increase the infection by root-to-root contact in coconut substrate.

Figure 6 shows the mean values of the percentage of infection in the plots affected by Fusarium crown and root rot, as well as the statistically differentiated groups obtained in this trial.

Figure 6 shows no clear differences from the severity of Fusarium crown and root rot correlated with the salt concentrations in the different irrigation applications. Statistically, no difference was observed for the *enarenado* system, possibly due to the very low level of development of the disease. In the case of the coconut fiber system, from the beginning of the evaluations, statistical differences were observed depending on the salinity with which the crop was irrigated, but upon reaching a certain level of disease, when the percentage of infection was over 30%, no statistically significant differences were observed. We could observe in some evaluation periods that the plots irrigated with treatment 2 (T2-H) presented statistically lower rates of Fusarium crown and root rot infection; this may be due to the fact that this treatment did not present extreme values of salinity concentrations, due to both the addition of fertilizers and the excess of salt, mainly Na^+, in the irrigation water.

Figure 7 shows the difference in vigor between plants in coconut fiber substrate and those in "*enarenado*" soil, mainly due to the attack of *F. oxysporum* f. sp. *radices-lycopersici*, which was more pronounced in coconut fiber, while that of plants sown in "*enarenado*" soil was almost inappreciable.

Figure 7. Difference in vigor between plants in coconut fiber substrate (**left**) and those in "*enarenado*" soil (**right**) for treatment T2 at 23 February 2021.

3.2. Powdery Mildew

As for the Fusarium analysis, the areas with coconut substrate showed a high level of powdery mildew (Table S2). For powdery mildew, we observed statistical differences between different substrates and different irrigation treatments. A decrease in the percentage of infection was observed on 6 February 2021; this is due to a leaf pruning carried out on the lower leaves of the tomato plant. The leaf pruning took place between 11 February and 15 February. This is a normal procedure in this type of crop. Plants with hangers always grow vertically once they reach the height limit of the structure.

By removing the leaves at the bottom of the plant, the oldest and most affected by the disease, the level of powdery mildew that we visually appreciated decreased, as can be seen in Figure 8.

With the continuous development of the plant, the disease continued to develop after pruning, reaching levels of development similar to those that existed before pruning. In Figure 8, we observed how the trend of disease before pruning was maintained after this in a similar way.

We can see in Figure 8 how treatment T3-S in "*enarenado*" soil always had a lower percentage of infection than other treatments and for many periods of the statistically differentiated trial of treatment T1-S in "*enarenado*" soil. These two treatments were extreme both in the use of irrigation water and salinity and in the use of nutritional solution. At this time, the expression of the disease in the leaves (powdery mildew) was the highest in the experiment.

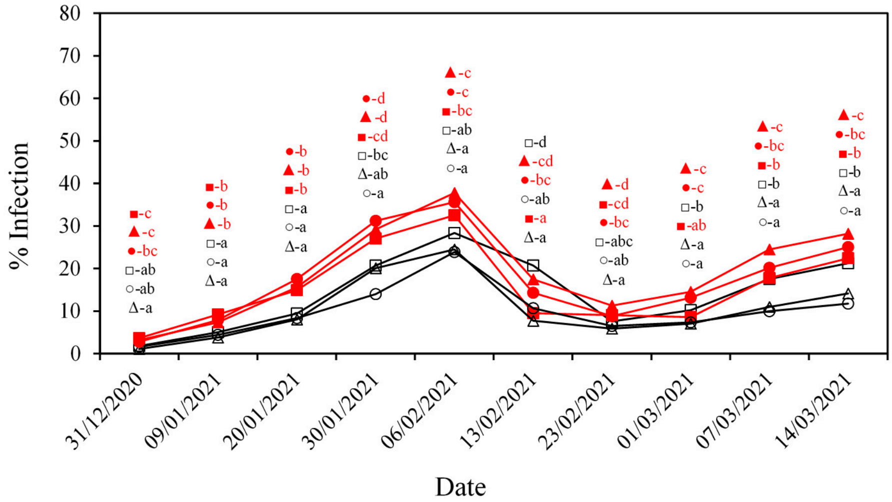

Figure 8. Development of powdery mildew percentage of infection over time in the sectors with different irrigation and crop systems. Treatments: T1-H (■), T1-S (□), T2-H (▲), T2-S (Δ), T3-H (●) and T3-S (○). S, typical soil substrate "*enarenado*"; H, coconut fiber substrate. Different letters in the same column show statistically significant differences with a confidence level of 95.0% (p-value ≤ 0.05).

We can observe that during the evolution of this trial, in "*enarenado*" soil, treatment T3-S, which presented a lower amount of nutrient solution and, therefore, fewer nitrates, presented in turn a lower percentage of powdery mildew infection. It was also observed that as the nutritional concentration increased, the expression of the disease in the leaves also increased.

Similarly, it was observed that the higher the concentrations of salts in irrigation water (due to NaCl and Ca^{+} and Mg^{++} ions), the lower the percentage of powdery mildew infection observed, in direct contrast to the results observed for the nitrate concentration.

3.3. Effect of Salinity and Diseases on Production and Quality of Tomato Harvest

Table 4 shows the production obtained in this trial. It can be seen that in the first harvest, which coincides with the onset of the diseases (powdery mildew and Fusarium crown and root rot), there were neither statistical nor numerical differences in the yields obtained, with results being very similar for all irrigation systems and substrates used.

The largest harvest took place on 8 February 2021, just before the second occurrence of the Fusarium infection, coinciding with the day of the largest losses due to plant deaths in the crop affected by Fusarium.

However, from the harvest of 18 February 2021 to 9 March 2021, production decreased. These findings coincide with the second increase in disease severity for powdery mildew and Fusarium crown and root rot. The lower production levels were observed in plants grown in coconut fiber (Figure S1). This is due to the losses caused by both diseases in the areas in which the coconut substrate was used, particularly from Fusarium crown and root rot, which killed many plants.

At the first harvest (13 January 2021), the disease should not have had an influence because the disease levels on that date were very low. There were no clear differences between treatments, although a higher numerical incidence of non-marketable fruits was observed in the plots planted in coconut fiber (Table S3). At the second harvest, in which the disease levels were also low, no differences were observed between the treatments; only treatment 1, sown in "*enarenado*", was statistically different from the rest, obtaining better results (lower percentage of non-marketable fruits). At the third harvest, where the disease began to be noticed, it appeared as though, numerically, the plots sown in "*enarenado*" presented better values and fewer non-marketable fruits, although the lowest value of this crop was found in treatment 3 of those sown in coconut fiber due to the very low yield in these plots.

Table 4. Production and fruit at different harvest times.

Greenhouse	Date				
Irrigation	13/01/2021	08/02/2021	18/02/2021	09/03/2021	Total
			Production (kg/m^2)		
T1-S	1.01 ± 0.12 [a]	2.04 ± 0.18 [a,b]	1.55 ± 0.17 [c]	0.94 ± 0.10 [c]	5.53 ± 0.31 [c]
T2-S	1.03 ± 0.12 [a]	1.70 ± 0.18 [a]	1.42 ± 0.17 [b,c]	0.77 ± 0.10 [b,c]	4.93 ± 0.31 [a,b,c]
T3-S	1.15 ± 0.12 [a]	2.47 ± 0.18 [b,c]	1.00 ± 0.17 [a,b]	0.50 ± 0.10 [a,b]	5.13 ± 0.31 [b,c]
T1-H	1.06 ± 0.12 [a]	1.84 ± 0.18 [a]	0.74 ± 0.17 [a]	0.37 ± 0.10 [a]	4.02 ± 0.31 [a]
T2-H	1.14 ± 0.12 [a]	2.73 ± 0.18 [c]	1.02 ± 0.17 [a,b,c]	0.20 ± 0.10 [a]	5.10 ± 0.31 [b,c]
T3-H	1.17 ± 0.12 [a]	1.76 ± 0.18 [a]	1.09 ± 0.17 [a,b,c]	0.24 ± 0.10 [a]	5.26 ± 0.31 [a,b]
			Number of fruits harvested		
T1-S	309 ± 45 [a]	771 ± 92 [a,b]	648 ± 86 [b]	501 ± 50 [c]	2176 ± 191 [b]
T2-S	373 ± 45 [a]	618 ± 92 [a]	684 ± 86 [b]	356 ± 50 [b,c]	2002 ± 191 [a,b]
T3-S	379 ± 45 [a]	972 ± 92 [b,c]	466 ± 86 [a,b]	204 ± 50 [a,b]	2014 ± 191 [ab]
T1-H	317 ± 45 [a]	726 ± 92 [a,b]	328 ± 86 [a]	159 ± 50 [a]	2175 ± 191 [b]
T2-H	369 ± 45 [a]	1191 ± 92 [c]	527 ± 86 [a,b]	109 ± 50 [a]	2223 ± 191 [b]
T3-H	364 ± 45 [a]	867 ± 92 [a,b]	650 ± 86 [b]	157 ± 50 [a]	2189 ± 191 [b]

T1-S, 0.6 dS m^{-1} EC irrigation water + 1.6 dS m^{-1} EC fertilization in typical soil substrate "*enarenado*"; T2-S, 1.5 dS m^{-1} EC irrigation water + 1 dS m^{-1} EC fertilization in typical soil substrate "*enarenado*"; T3-S, 3 dS m^{-1} EC irrigation water + 0.5 dS m^{-1} EC fertilization in typical soil substrate "*enarenado*"; T1-H, 0.6 dS m^{-1} EC irrigation water + 1.6 dS m^{-1} EC fertilization in coconut fiber substrate; T2-H, 1.5 dS m^{-1} EC irrigation water + 1 dS m^{-1} EC fertilization in coconut fiber substrate; T3-H, 3 dS m^{-1} EC irrigation water + 0.5 dS m^{-1} EC fertilization in coconut fiber substrate. Values with different letters in the same column show statistically significant differences with a confidence level of 95.0% (p-value ≤ 0.05).

The last two harvests showed the effects of the saline water on the yield due to the salt accumulation in the soil. Soil showed a trend towards obtaining a higher tomato yield in the areas irrigated with less saline water (salinity mainly due to NaCl). This was observed in soil mainly because in soil salts accumulate in superficial areas and are more difficult to leach, thereby increasing the concentration of salts above those applied in the irrigation solution. In the coconut substrate areas, this trend was not particularly observed, with the exception of a slight presence in the last harvest, probably due to the fact that the washing fraction in the coconut fiber was more efficient than in the soil, thereby not producing the same accumulation of salt as in the soil.

Figure 9a also shows the number of fruits harvested. As for production, the effect of salinity and disease stress continued the same trend as in the total production table. In Figure 9a, a high loss in terms of tomato yield is observed in the last harvest in the coconut substrate, mainly due to disease stress.

On the last day of harvest, there was a great loss in the number of fruits collected in the plots in the coconut fiber substrate due to the influence of Fusarium, which, at this time of the experiment, showed very high infection percentage values in coconut fiber systems. In addition, the effect of the accumulation of salts on the soil was observed in the "*enarenado*" soil plots, leading to a lower number of fruits in the plots irrigated with more saline water and less fertilizer because, in these plots, the influence of Fusarium was low and there was no difference between irrigation areas.

In Figure 9a, we can see how the production obtained on 9 March 2021 varied depending on the type of treatment and the cultivation system. It was observed that in the case of tomatoes planted in "*enarenado*", the production decreased, while the conductivity of the irrigation water of the treatments increased. The production of tomatoes planted in coconut fiber seemed to follow the same trend, but not as clearly as in the case of "*enarenado*". In this graph, a lower level of production is observed in the tomatoes planted in coconut fiber, due largely to the greater presence of plants affected by *F. oxysporum* f. sp. *radicis-lycopersici* in the coconut fiber plots.

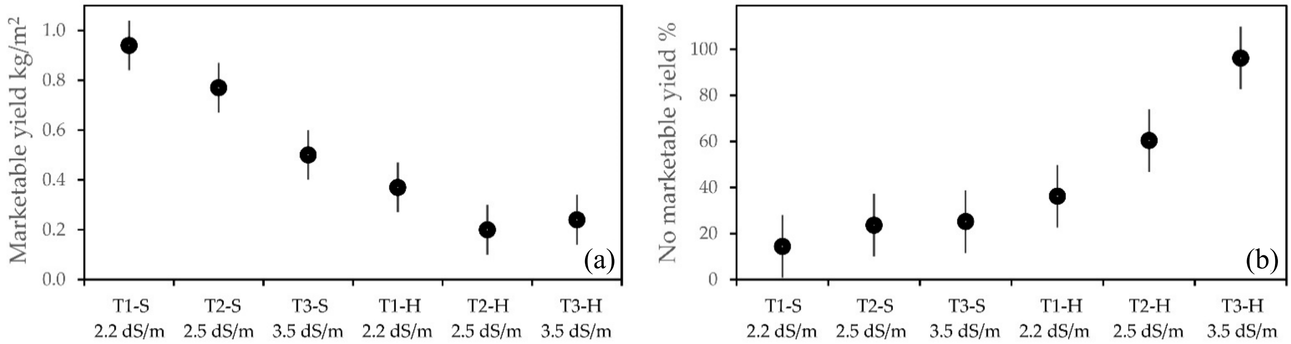

Figure 9. Comparison of the marketable (**a**) and non-marketable (**b**) yield in different treatments and crop systems in harvest at 9 March 2021. T1-S, 0.6 dS m^{-1} EC irrigation water + 1.6 dS m^{-1} EC fertilization in typical soil substrate "*enarenado*"; T2-S, 1.5 dS m^{-1} EC irrigation water + 1 dS m^{-1} EC fertilization in typical soil substrate "*enarenado*"; T3-S, 3 dS m^{-1} EC irrigation water + 0.5 dS m^{-1} EC fertilization in typical soil substrate "*enarenado*"; T1-H, 0.6 dS m^{-1} EC irrigation water + 1.6 dS m^{-1} EC fertilization in coconut fiber substrate; T2-H, 1.5 dS m^{-1} EC irrigation water + 1 dS m^{-1} EC fertilization in coconut fiber substrate; T3-H, 3 dS m^{-1} EC irrigation water + 0.5 dS m^{-1} EC fertilization in coconut fiber substrate.

When comparing the two culture systems ("*enarenado*" and coconut fiber) that we used in this trial, without differentiating irrigation treatments, it is worth discussing whether the higher incidence of both diseases affected the yield of the crop. When comparing the cropping system, both total and marketable yields were lower for the coconut substrate treatment than for the "*enarenado*" soil treatment. The difference was significant at the 95% confidence level, however. The method used to discriminate between the means was the Fisher's least significant difference (LSD) procedure. The marketable yield for the substrate culture was 4.46 kg/m^2, whereas for "*enarenado*" soil culture, it was 5.20 kg/m^2 (Figure S1). One of the most likely contributing factors to this lower yield in coconut fiber system treatments was the considerably higher incidence of both diseases, especially in the case of Fusarium crown and root rot in this type of cropping system.

The development of fungal diseases also influences the quality of the tomatoes obtained. The increased influence of these diseases over time causes production losses due to the appearance of damaged fruits that cannot be sold. In Figure 9b, we can see how the percentage of non-commercial fruits increased over time, while the incidence of both diseases (powdery mildew and Fusarium crown and root rot) also increased their development. More non-marketable fruits were observed in the plots sown in coconut fiber than in "*enarenado*" soil, being statistically differentiated in some final periods of the harvest (for example, see the three treatments in "*enarenado*" soil and coconut fiber on 9 March 2021).

On the last day of harvest analyzed, the harvest most affected by the disease, we could also observe an increase in non-marketable production with the increase in the concentration of salts that were introduced to the different substrates by irrigation. Higher percentages of non-commercial fruits were observed in treatment T3 than in treatment T2 and, in turn, in treatment T1. This may be related to the increased salinity, as described earlier in this study.

In the case of non-marketable tomatoes, again, Figure 9b shows the highest values for plots grown in coconut substrate compared to "*enarenado*" soil, which seems to be clearly related to the severity of the percentage of infection of the analyzed diseases in these areas.

Regarding the quality of the production, the variables related to the size of the fruit (weight and diameter of the fruit) were significantly lower in the coconut fiber culture treatment than in "*enarenado*" soil culture (Table 5), which seems to indicate that the higher incidence of both diseases affected the development of the fruit.

Table 5. Fruit weight, diameter and Brix degrees in different harvest times and average value for the crop cycle.

Greenhouse	Date				
Irrigation	**13/01/2021**	**08/02/2021**	**18/02/2021**	**09/03/2021**	**Average**
			Fruit weight (g)		
T1-S	126.69 ± 3.83 ^{c}	102.32 ± 4.36 ^{c}	92.23 ± 4.04 ^{c}	72.15± 3.07 ^{a}	98.35 ± 2.65 ^{c}
T2-S	106.19 ± 3.83 ^{a}	108.04 ± 4.36 ^{c}	79.71 ± 4.04 ^{a,b}	82.46± 3.07 ^{b}	94.10 ± 2.65 ^{c}
T3-S	118.65 ± 3.83 ^{b,c}	99.17 ± 4.36 ^{b,c}	84.10 ± 4.04 ^{b,c}	95.52± 3.07 ^{c}	99.36 ± 2.65 ^{c}
T1-H	114.63 ± 3.83 ^{a,b}	89.37 ± 4.36 ^{a,b}	81.31 ± 4.04 ^{a,b,c}	84.47± 3.07 ^{b}	92.45 ± 2.65 ^{b,c}
T2-H	113.52 ± 3.83 ^{a,b}	84.49 ± 4.36 ^{a}	71.82 ± 4.04 ^{a}	68.33± 3.07 ^{a}	84.54 ± 2.65 ^{a}
T3-H	112.02 ± 3.83 ^{a,b}	88.51 ± 4.36 ^{a,b}	72.69 ± 4.04 ^{a}	68.99± 3.07 ^{a}	85.55 ± 2.65 ^{a,b}
			Fruit diameter (mm)		
T1-S	66.67 ± 0.79 ^{c}	59.96 ± 0.91 ^{b}	57.44 ± 0.99 ^{b}	51.88± 0.82 ^{a}	58.99 ± 0.68 ^{b,c}
T2-S	62.71 ± 0.79 ^{a}	61.39 ± 0.91 ^{b}	55.15 ± 0.99 ^{a,b}	54.48± 0.82 ^{b}	58.43 ± 0.68 ^{b,c}
T3-S	65.36 ± 0.79 ^{b,c}	60.26 ± 0.91 ^{b}	55.81 ± 0.99 ^{b}	57.68± 0.82 ^{c}	59.78 ± 0.68 ^{c}
T1-H	63.77 ± 0.79 ^{a,b}	56.81 ± 0.91 ^{b}	54.99 ± 0.99 ^{a,b}	55.51± 0.82 ^{b,c}	57.67 ± 0.68 ^{a,b}
T2-H	64.85 ± 0.79 ^{a,b,c}	55.71 ± 0.91 ^{b}	52.61 ± 0.99 ^{a}	50.99± 0.82 ^{a}	56.04 ± 0.68 ^{a}
T3-H	64.49 ± 0.79 ^{a,b,c}	56.82 ± 0.91 ^{b}	52.71 ± 0.99 ^{a}	51.51± 0.82 ^{a}	56.38 ± 0.68 ^{a}
			Fruit Brix°		
T1-S	4.94 ± 0.12 ^{a}	6.23 ± 0.19 ^{a}	6.40 ± 0.17 ^{a}	6.51± 0.12 ^{a}	6.01 ± 0.11 ^{a}
T2-S	5.30 ± 0.12 ^{b,c}	5.93 ± 0.19 ^{a,b}	6.26 ± 0.17 ^{a}	6.89± 0.12 ^{b}	6.09 ± 0.11 ^{a,b}
T3-S	5.58 ± 0.12 ^{c}	6.78 ± 0.19 ^{c}	6.57 ± 0.17 ^{a}	6.49± 0.12 ^{a}	6.35 ± 0.11 ^{b,c}
T1-H	5.45 ± 0.12 ^{c}	7.08 ± 0.19 ^{c}	6.48 ± 0.17 ^{a}	7.04± 0.12 ^{b,c}	6.51 ± 0.11 ^{c,d}
T2-H	5.02 ± 0.12 ^{a,b}	6.78 ± 0.19 ^{c}	7.73 ± 0.17 ^{c}	7.33± 0.12 ^{c,d}	6.71 ± 0.11 ^{d}
T3-H	5.29 ± 0.12 ^{b,c}	6.73 ± 0.19 ^{b,c}	7.22 ± 0.17 ^{b}	7.59± 0.12 ^{d}	6.72 ± 0.11 ^{d}

T1-S, 0.6 dS m^{-1} EC irrigation water + 1.6 dS m^{-1} EC fertilization in typical soil substrate "*enarenado*"; T2-S, 1.5 dS m^{-1} EC irrigation water + 1 dS m^{-1} EC fertilization in typical soil substrate "*enarenado*"; T3-S, 3 dS m^{-1} EC irrigation water + 0.5 dS m^{-1} EC fertilization in typical soil substrate "*enarenado*"; T1-H, 0.6 dS m^{-1} EC irrigation water + 1.6 dS m^{-1} EC fertilization in coconut fiber substrate; T2-H, 1.5 dS m^{-1} EC irrigation water + 1 dS m^{-1} EC fertilization in coconut fiber substrate; T3-H, 3 dS m^{-1} EC irrigation water + 0.5 dS m^{-1} EC fertilization in coconut fiber substrate. Values with different letters in the same column show statistically significant differences with a confidence level of 95.0% (p-value $\leq$ 0.05).

In Table 5, we can observe the evolution over time of the average weights obtained per fruit in each harvest. We can see how the weight of the fruits decreased over time, which coincided with the increase in the level of fungal diseases in the crop during the development of the cultivation cycle. A lower fruit weight was observed in plants grown in coconut fiber substrate (Figure S2a), which was also the cultivation system with the highest level of fungal diseases described in this study.

Table 5 shows the size of the fruit diameter after harvest, an important parameter in fruit quality, as it influences the commercial classification of these fruits.

The fruit obtained in this crop decreased in size over time. One of the possible causes of this could be the increase in the development of the fungal diseases described in this trial. As in the case of weight, in the plots with the "*enarenado*" cultivation system, a larger diameter of harvested fruit was generally observed (Figure S2b).

On the contrary, the Brix° of the fruit was significantly higher in the coconut substrate treatment. The trend in Brix degrees was contrary to what was observed in for diameter and fruit weight, as shown in Table 5; in this case, the Brix° of the harvested tomato increased over time and increased with greater plant stress. Table 5 shows how the levels of Brix° were higher in yields obtained in coconut fiber substrates, which, as previously described, are those that present higher stress in the crop due to the increased development of fungal diseases.

4. Discussion

Tomatoes and most greenhouse vegetables are sensitive to salinity. In many areas in the Almería province, the irrigation water has poor quality, and its electrical conductivity is much higher than 3 dS m^{-1} [2]. However, the aim of this experiment was to accurately analyze the optimal EC when using a blend of desalinated and groundwater.

The farmers of the Almería area usually use their own well water. The water obtained in this manner has high electrical conductivity, between 2.8 and 6.5 dS m^{-1} [2]. The farmers who use the well water mix this resource with water from irrigation communities, with a lower conductivity of 1 dS m^{-1}, when they use seawater in the desalination plant of 1.6 or 2 dS m^{-1}, the normal electrical conductivity supplied by the area's irrigation communities [2].

The fresh fruit yield of tomato grown in simple plastic greenhouses on the Mediterranean coast was reduced by increasing salinity, in accordance with the Maas and Hoffman model [12]. The average EC value for marketable yield was 3.3 dS m^{-1}. Maas and Hoffman [12] reported, for open-field tomatoes grown in soil, an EC of 2.5 dS m^{-1} in saturated soil extract, which is equivalent to 3.8 dS m^{-1} in the soil solution.

Statistically significant differences among treatments were observed for both diseases, powdery mildew and Fusarium crown and root rot. In both cases, higher levels of disease were observed in plants grown in coconut fiber than in "*enarenado*" soil. This may be due to the fact that there is a greater amount of watering in coconut fiber substrata than in "*enarenado*" soil areas due to more frequent irrigation in hydroponic, soil-free systems than in irrigation with "*enarenado*" soil. This can cause constant humidity in areas with coconut fiber substrates, which favors the development of fungal diseases.

The development of *F. oxysporum* f. sp. is favored when soil and air temperatures are high, as well as excess irrigation or rainwater, which negatively affect a host's ability to cope with the disease [31]. The pathogen grows rapidly in arid soils, whereas in soils habited by different saprophytic organisms, *F. oxysporum* f. sp. does not develop in a way that risks the crop [32]. In coconut fiber substrates, we find fewer saprophytic organisms with which to compete for space than in the soil, which would favor a faster development of the disease. The damaged roots can also be colonized by secondary pathogens [30,33], which could be the reason for the higher percentage of powdery mildew infection in areas irrigated with coconut fiber substrate, with more Fusarium problems than in areas irrigated in "*enarenado*" soil.

In the case of soilless growing, the sources of primary infection are microconidia transferred from the air [34]. Plants that are aerially infected may still be colonized by *F. oxysporum* f. sp. *radicis-lycopersici* (FORL) and may infect tomatoes by root-to-root contact and increased inoculum in the substrate for the next season [20]. In our case, the coconut substrate had a lower volume than the "*enarenado*" soil, so the roots of the plants were nearer than in the "*enarenado*" soil, which could increase the infection by root-to-root contact in coconut substrate.

It is known that the disease develops rapidly in cool soil [25]. In our trial, the coconut substrate was cooler than soil because the former loses the heat collected during the day by the greenhouse more quickly than the latter, which can encourage the development of the disease.

The excessive use of fertilizers usually causes plants to become more susceptible to disease outbreaks [34]. Treatment T2 is the most balanced in terms of the use of fertilizer because treatment T1 provides more fertilizer than the others, and treatment T3 provides a higher electrical conductivity in the water (possibly due to an excess of NaCl), which weakens the plant's defense against disease. High concentrations of Na^+ or high Na^+/Ca^{2+} ratios in the root media imply a low K^+ content in the plant, which has a negative effect on growth and yield in several crops, including tomatoes [35].

The highest level of powdery mildew percentage of infection in the coconut substrate irrigation areas could be due to the fact that the damage that *F. oxysporum* f. sp. *radicis-lycopersici* causes in the tomato plants helps secondary pathogens colonization [33].

In addition, according to Koch's postulates, for the correct development of a disease, a healthy host is needed. The plants in coconut substrate in this trial were weaker than those planted in "*enarenado*" soil. Therefore, the analysis of powdery mildew was better suited to the irrigation "*enarenado*" soil areas.

For powdery mildew, in general, higher soil nitrogen availability leads to an increase in disease infection [36]. The nitrogen source also affects plant metabolism, changing susceptibility to a pathogen [20,37]. In this trial, we can affirm that a higher concentration of salts (probably NaCl) in the irrigation water and lower nitrogen fertilization decrease the development of *L. taurica* in tomato. Salinity can also directly affect nutrient uptake, such as by Na^+ reducing K^+ uptake or by Cl^- reducing NO^{-3} uptake [33].

The tomato variety planted in this trial is a long-life variety, which is harvested as a bouquet. The tomatoes were harvested in winter, so they took a longer time to ripen. Harvesting was performed once the bouquet was fully ripe, and it was considered that there was a sufficiently large harvest from all replications to evaluate; for this reason, the harvest dates were irregular.

Compared with soil-based cultivation, hydroponics culture can be more cost-effective [38], producing higher yields and prompter harvests from smaller areas of land. Hydroponics culture also has higher water and nutrient use efficiencies in general [39]. In this trial, however, Figure 9 and Table 5 show that the "*enarenado*" soil irrigation areas obtained higher production that the coconut fiber substrate; this is due to the losses caused for the different diseases, which showed more severity in the areas of the coconut substrate, especially Fusarium crown and root.

Soil showed a trend towards obtaining a higher tomato yield in the areas irrigated with less saline water (salinity mainly due to NaCl), as described by other authors [9–11]. This was observed in soil mainly because in soil salts accumulate in the superficial part and it is more difficult to leach, increasing the concentration of salts above those applied in the irrigation solution. In the coconut area, this trend was hardly observed, with the exception of a slight presence in the last harvest, probably due to the fact that the washing fraction in the coconut fiber was more efficient than in the soil, not producing the same accumulation of salt as in the soil. We can also assume that diseases did not influence this trend within each substrate, as explained above, if there were clear differences when comparing soil production with that of coconut fiber. In an analysis separating the substrates, soil and coconut fiber, in the last harvest, the influence of the diseases did not affect this previously exposed trend, since for Fusarium there were no statistical differences between the same substrates. In the case of powdery mildew, the infection percentages were very close, and the production values were higher in the areas that presented higher values of powdery mildew. The incidence of both diseases influenced a reduction in the harvest in both cultivation systems, but the trend described above can be attributed to the concentration of salinity in the irrigation water.

Mounet et al. [40] explored transcriptional and metabolic changes in expanding fruit tissues. This tissue expansion process lasts for up to 35 days, so in the last harvest carried out, we could observe the effect of the diseases on the total development of the fruits. From 35 days before the last harvest, the disease infection percentages of the disease increased greatly. This is why the effects of the diseases were especially reflected in the last days of the harvest.

Table 5 shows a reduction in fruit quality parameters that affect market value, since they negatively influence the size classification of marketable fruits [32]. It was clearly observed how the size of the fruits decreased, while the incidence of both fungal diseases studied in this trial increased.

On the contrary, the Brix° of the fruit was significantly higher in the coconut substrate treatment. The stress for the high level of salts in irrigation water increased fruit sugar [32], which could be due to a decrease in water transported to fruits, with no change in dry matter transport, resulting in higher concentrations of sugar [15,17]. *F. oxysporum* f. sp. *radicis-lycopersici* is a pathogen that mainly attacks the root and vascular plant system [28], which could affect the transport of water to the fruits; this could explain the higher level of Brix degrees observed in coconut fiber plots.

In some local Almería areas, to obtain high-quality tomato fruits from local cultivars, high values of electrical conductivity (EC) in the nutrient solution and hydric stress should be maintained throughout the crop cycle [2]. Coconut system plots showed a very high level of Fusarium crown and root rot symptoms, higher than the plots in "*enarenado*", in which the effect of Fusarium was very minor. In "*enarenado*" plots, there was also an increase in Brix degrees over time, which could be due to the accumulation of salts that occur in soils, where the washing of salts is more difficult than in substrates such as coconut fiber.

Brassinosteroids are essential hormones for plants and have multiple roles to induce tolerance to both biotic and abiotic stresses in plants, such as tolerance at high or low temperatures, excess humidity, damages from pesticides, excess salinity and drought [41,42]. The damage caused by these stresses in plants is similar to that caused by fungal diseases, so these hormones also act on the damage caused by the diseases studied in this trial. Brassinosteroids increase biomass, chlorophyll contents, photosynthesis and antioxidants to improve the resistance of plants to stress [43,44]. An excess of Cd ions, increasing brassinosteroid activity, has been documented in tomatoes [45]. The effect of these hormones could be the cause of the increase in Brix° in the tomato crops studied, as the stress produced by fungal diseases that developed on the crop increased. Although in this work no data were recorded that can validate this hypothesis, it is an aspect that should be studied in the future.

5. Conclusions

In this trial, the areas irrigated with coconut fiber favored the development of *F. oxysporum* f. sp. *radicis-lycopersici* and *L. taurica*.

The use of a higher fertilization concentration in the nutrient solution in irrigated areas favored the development of *L. taurica*.

The higher concentration of salts in irrigation water (due to NaCl, and Ca^{2+} and Mg^{2+} ions) caused a diminution in the severity of powdery mildew.

F. oxysporum f. sp. *radicis-lycopersici* caused a loss in the production of the areas irrigated in coconut fiber as these were the most affected by the disease.

F. oxysporum f. sp. *radicis-lycopersici* and *L. taurica* caused a reduction in fruit quality parameters that affect market value.

F. oxysporum f. sp. *radicis-lycopersici* and *L. taurica* caused a higher percentage of non-marketable tomato in the areas with major disease severity.

The salinity stress and the stress caused by *F. oxysporum* f. sp. *radicis-lycopersici* and *L. taurica* increased the Brix° in tomato harvest.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12051050/s1. Table S1: Percentage of infection of Fusarium crown and root rot in different moment of the trial. Table S2: Percentage of infection of Powdery mildew in different moment of the trial. Table S3: Percentage of no marketable yield in harvest moments. Figure S1: Comparison of the total marketable yield means and 95 percent LSD differences for Coconut Substrate treatment (TH) grouping all the treatments and Soil treatment (TS) grouping all the treatments. Figure S2: Comparison of the Fruit weight (a) and diameter (b) means and 95 percent LSD differences for Coconut Substrate treatment (TH) and Soil treatment (TS).

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