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RESEARCH ARTICLE

Effect on plant growth parameters and secondary metabolite content of lettuce (*Lactuca sativa* L.), coriander (*Coriandrum sativum*), and chili pepper (*Capsicum annuum* L.) watered with disinfected water by Ag-TiO_2 nanoparticles

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Abstract

Nowadays, the use of different nanoscale structures has been introduced to a large number of research areas. One of these is the treatment and remediation of water through photocatalytic processes, seeking to reuse wastewater for agriculture. In this paper, *Lactuca sativa*, *Coriandrum sativum*, and *Capsicum annuum* were used as crop models to observe the effects in plant growth and the secondary metabolism of different water qualities and types used in the watering process. Initial results show that the photocatalytic process's water maintains a pH and ion concentration within the allowed limits, significantly reducing the number of bacteria. Along the growth process, an influence on germination times, appearance of true leaves, maturation, and fruit production depending on the type of water used is observed, obtaining the best results in both growth times and quantity of fruits, for the 50% and 70% disinfected water/tap water (DW/TAW) study groups. Secondary metabolites, such as phenols, flavonoids, and antioxidant activity, were studied to evaluate changes in the vegetables' composition, showing increased concentration for the disinfected water groups in most specimens. Additionally, no traces of metals and microorganisms were detected, concluding that the crops are viable to be consumed by human beings.

Keywords Solar photocatalysis · Nanoparticles · Vegetal stress · Wastewater disinfection · Secondary metabolites · Advanced oxidation processes

Highlights
- Solar photocatalysis by Ag@TiO_2 reduces the pathogen presence in wastewater.
- Crops irrigated with disinfected/tap water ratios shows higher growth rates.
- Secondary metabolism in lettuces and coriander shows stress.
- In lettuces and coriander, the phenol content and antioxidant activity increases.
- Chili crops produces more fruits when are watered with disinfected water ratios.

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Introduction

Among the past 10 years, several patents and products have been produced to incorporate nanoparticles (NPs) and other nanomaterials (NMs) designed for agricultural practices such as nanopesticides, nanofertilizers, and nanosensors with the objective to do the agricultural practice a sustainable one (Iavicoli et al. 2017; Liu and Lal 2015; Servin et al. 2015).

The NPs are manufactured and developed from a wide range of inorganic, organic, and combined material, generally grouped into four types, carbon-based (single-walled and multi-walled carbon nanotubes (SWCNT/MWCNT)), metal-based (Al, Cu, Au, Ag, Zn), metal oxides (Fe_3O_4, Ce_2O_3, SiO_2, ZnO, TiO_2), and dendrimers and composites (Begum and Fugetsu 2012; Boykov et al. 2018; Cai et al. 2017; Chutipaijit and Sutjaritvorakul 2017; Chutipaijit et al. 2018). Specifically, the titanium dioxide (TiO_2) nanoparticles can be synthesized by diverse types of techniques (Cortéz-Lorenzo et al. 2017; de Santiago Colín et al. 2018; Esquivel et al. 2013b) and are used in sensors, dye-sensitized solar cells, and photocatalytic processes in wastewater treatment, among others (Cortéz-Lorenzo et al. 2017; de Santiago Colín et al. 2018).

The TiO_2 to be used for wastewater treatment needs to be generated under lab conditions to modify some of its electronic, morphological, structural, and textural properties. Our research group has been dealing with these needs by several synthesis methods (Esquivel et al. 2011; Esquivel et al. 2013a; Hernández et al. 2017) for TiO_2 and doped-TiO_2 to remove dyes and microorganisms on synthetic and real wastewater effluents (Esquivel-Escalante et al. 2016; Rodríguez-Méndez et al. 2017). In the case of real or synthetic wastewater effluents treated by solar/visible/UV TiO_2, photocatalysis is possible to achieve the removal of almost 90% of the pathogen microorganisms and specifically 100% of *Escherichia coli* (Afsharnia et al. 2018; Liu et al. 2019; Mesones et al. 2020; Montenegro-Ayo et al. 2020), but no further real-life application has been described in our best of knowledge. The treated wastewater in these cases is lab-scale, and only a few in pilot-scale tests deal with the effects of microorganism's inactivation, its mechanisms, and how other emergent pollutants affect those removal mechanisms (Barakat et al. 2020; Biancullo et al. 2019; He et al. 2019; Karaolia et al. 2018; Moreira et al. 2018; Rodríguez-González et al. 2020; Uyguner Demirel et al. 2018; Wahyuni et al. 2019). The treated water obtained by photocatalysis and solar-photocatalysis is starting to raise some interest for an agricultural purpose. It shows several effects of the NMs over the various matrix such as soil, water, plant growth-promoting bacteria, nitrogen fixers, phosphate solubilizers, and biofilm formers (Chavan et al. 2020). Recent studies show that the response (beneficial or toxically) of plants towards NM application depend on the specificity of the plant and soil, as well as concentration, nature, morphology, and crystallinity of the NMs (Kibbey and Strevett 2019; Rajput et al. 2020; Peng et al. 2020; Paramo et al. 2020).

In this study, the response of three crop families (*Lactuca sativa* L., *Coriandrum sativum*, and *Capsicum annuum* L.) treated with disinfected water (DW) by Ag-TiO_2 NPs was determined. Biologically treated water (TWT), tap water (TAW), DW, and several disinfected/tap water (DW/TAW) ratios with a specific composition of the soil, humidity, and temperature were used to determine the effect on plant growth. It is known that secondary metabolite content, with beneficial effects on human health (Zeb and Muhammad 2019; Soleymani et al. 2020), increases as a response to abiotic stress (Madanayake et al. 2021; Landi et al. 2020); then, total content of flavonoids, and phenols, as well as antioxidant activity, was evaluated (Bhattacharjee and Saha 2014).

Materials and methods

Ag-TiO_2 synthesis

Synthesis and characterization of the Ag-TiO_2 material were previously reported by Rodríguez-Méndez et al. (2017). Titanium isopropoxide (97% Aldrich, TTIP) and isopropanol (99.9%, JT Baker) were magnetically stirred under a nitrogen atmosphere for 20 min. The mixture of water and 0.1%w $AgNO_3$ (99.99% Sigma-Aldrich) was added after that time. The complete solution was kept stirred, and the molar ratio of the precursors and solvents was 0.03:4.2:1 for TTIP to H_2O to isopropanol, respectively. After, the solution was transferred into Teflon-lined vessels and placed on a turntable for uniform heating using a microwave reaction system for 60 min at 215 °C (Multiwave 5000, Anton Paar). The resulting powder was filtered, washed, and dried at room temperature for 18 h to later dried at 80 °C for 12 h. A calcination process was carried out at 450 °C for 3 h to improve the material crystallinity (Pérez-Sánchez et al. 2014).

Treated wastewater, disinfected, and tap water evaluation

Treated wastewater (TWT) was obtained from an activated sludge biologic treatment at pH 7 and room temperature from the Campus Aeropuerto wastewater treatment plant installation located at 20° 35′ 28″ N, 100° 23′ 28″ O (Querétaro, México). Once the treated wastewater was collected, one part was disinfected by the solar-photocatalysis process, using 0.5 gL^{-1} of the photocatalyst bubbled with air and magnetically stirred for 30 min in the darkness before irradiation (Pérez-Sánchez et al. 2014; Rodríguez-Méndez et al. 2017). The system was illuminated using the solar radiation of a spring day at Querétaro located at 20° 35′ 28″ N, 100° 23′ 28″ O, México, in lapses from 11:00 am to 03:00 pm. Representing values between 1000 and 1200 Wm^{-2}. At the same location, the tap water (TAW) was collected. According to the Official Mexican Norm (Regulation M 1997), the water samples were evaluated in the content of fecal coliforms by quantifying the more likely number of pathogen agents to form colonies and by differentiation of those microorganisms by biochemical reactions in diverse mediums (regulation 1997; Rodríguez-Méndez et al. 2017). Using a bacteriological water test based on the microorganism growth in two presumptive mediums (Lactose and Sodium Lauryl Sulfate, BD Bioxon) at concentrations of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} mL L^{-1}, the water samples were characterized. In the presumptive test, the mediums once seeded were incubated at 36 °C for 24 h and 37 °C for another 24 h. If the samples show the presence of gas, it can be noticed as a positive sample. After the presumptive test, the positive samples were seeded inoculant 1 mL in the confirmative mediums, EC, MIO, and Green Bright Billis (BD Bioxon). The tubes were incubated for 24 h at 36 °C, and the presence of gas and a color change means a positive sample. Finally, for the differential tests, the positive samples from the confirmative tests were seeded in a petri dish containing Shigella and Salmonella medium (BD Bioxon) and Mc Conkey agar (BD Bioxon) for 24 h at 36 °C (Rodríguez-Méndez et al. 2017). The types of water used in this work were tap water as reference (TAW), treated water directly from the biologic process (TWT), disinfected water from the solar photocatalysis treatment (DW), and several ratios of disinfected and tap water (90, 70 and 50%v DW/TAW).

Also, chemical parameters in all samples such as pH (HANNA Instruments HI 98107), dissolved anions by an Ion-Exchange Chromatography System (Dionex ARSR 300), and total carbon content by total organic carbon analysis (TOC, Teledyne Tekmar TOC Fusion) were measured.

Crop seeding and growth plant

The crop seeding was carried out in a plasticized greenhouse (68 × 49 × 156 cm in length, width, and height, respectively) located at 20° 35′ 28″ N, 100° 23′ 28″ O, Querétaro, México) with an exposure to the sunlight from 7:00 to 17:00 h. The irrigation water was used as a variable parameter, having five different levels: 50%, 70%, 90% DW/TAW, DW, TAW, and TWT between the experimental groups.

Lettuce (*Lactuca sativa* L. var. *Longifolia*, *Lactuca sativa* var. *Defender*), coriander (*Coriandrum sativum*), jalapeno pepper (*Capsicum annuum* ‘Jalapeño’), and green hot pepper (*Capsicum annuum* ‘Serrano’) (Hortaflor) seeds were placed in plastic seeders with humus soil (SUNSHINE special mix #3, Canadian Plug-Grade, Sphagnum Moss Peat, Vermiculite Plug-Grade, Dolomitic Limestone) inside of the plasticized greenhouse of dimensions previously presented. Before the seeding process, the seeds were previously washed with tap water (TAW) and were kept in water for 12 h at room temperature. Then, the seeds that remain in the bottom of the vessel were selected to initiate the germination process. The sowing of the different species was carried out during March and April, initiating 15 samples from each group. Every third day the temperature and humidity inside the greenhouse were recorded with the help of a hygrometer (Uplaytek), having an average of 37 °C and 60% of temperature and humidity, respectively. Once the plant reaches 5 cm of height, it was replaced in bigger containers with the same type of fertile soil in plastic vessels of 11 × 20 cm of diameter and height. The irrigation process was sub-superficial and directly intercalated between weeks by 3 months along with the plant growth. During the growth of species, the time of appearance of the first sprouts, true leaf, height of the plant, size of leaves, death of vegetables, and harvest time was recorded among the different groups. Different harvest times were presented, with ranges for coriander from 17 to 22 days, for lettuces from 30 to 40 days, and for chilies from 100 to 130 days.

Plant characterization and statistical analysis

After the harvest process, 10 g of vegetal material for the leaf crops and 50 g of vegetal material from the fruit for the pepper’s plants were collected and kept in aluminum foil to be immersed in liquid nitrogen to prevent any chemical structural change of the samples. Then, the samples were milled and kept under refrigeration at 4 °C. The extracts were obtained by mixing for 24 h, 1 g of freeze sample, and 2 mL of ethanol. After that, the samples were centrifuged at 5000 rpm in 10 min (Thermo Scientific Biofuge Primo R). The liquid phase was kept under dark and refrigerated.

The following secondary metabolites were determined as a general basis for understanding the Ag-TiO_2-disinfected water effect over the crop’s growth (Bakshi et al. 2019). The total phenolic and flavonoid contents of the extracts were determined according to the Folin-Ciocalteu spectrophotometric method modified for use in 96-well microplate (Chen et al. 2015) results were expressed as milligrams equivalent of gallic acid per gram of sample and Oomah et al. method (Oomah et al. 2005), and the results were expressed in rutin hydrate milligrams equivalent per gram of the sample, respectively. The antioxidant activity of the extracts was evaluated by the DPPH, and the results were expressed as micrograms equivalent of Trolox per milligram of sample and as the percentage of DPPH (1:10 μL extract/μL of DPPH) discoloration (% radical inhibition) (Zenil et al. 2014). The inhibition percentage was calculated following the equation: % inhibition = $[(A_{\mathrm{DPPH}} - A_{\mathrm{S}})/A_{\mathrm{DPPH}}] \times 100$, where A_{S} is the absorbance of the solution containing the simple, and A_{DPPH} is the absorbance of the DPPH solution. All the spectrophotometric measurements were obtained in a Thermo Scientific Multiskan Go spectrophotometer (Marincaş et al. 2018).

An acidic microwave (MW flexiwave MILESTONE) digestion with an HNO_3/H_2O_2 ratio of 3 by 15 min at 180 °C was made to obtain an extract that was analyzed by X-ray fluorescence (Bruker S2 PicoFox) to observe the presence of Ti, Ag, and Fe along with the vegetable tissue.

The experiment was set up in a randomized complete block design with three replicates composed of at least three plants. The data were tested using one-way ANOVA and Tukey test for the multiple comparisons. Correlation analysis was used to establish relationships between the variables. All statistical analyses were done using JMP Trial 15.pkg software (https://www.jmp.com/).

Results and discussion

Effluent characterization

The water characterization was carried out to prove the effluents' physical, chemical, and biological quality used to irrigate the diverse crops because no nutritional solution was used. The nutrients were collected by the plants from the organic matter remained and the mineral components of the different types of waters (TAW, TWT, and DW). The pH values of the different samples are shown in Table 1. According to the Mexican regulations (Legislation 1994), water with a pH value between 6.0 and 9.0 can be used for crop irrigation. All the samples used in this research are around a pH value of 7, which leads us to use them freely in this aspect. Ionic exchange chromatography analysis was performed to obtain information about the samples' anionic content, and the anions found in the water were Cl^-, NO_2^-, NO_3^-, F^-, and SO_4^{2-} (Table 1). In this matter, Mexican regulations establish a maximum permissible content of these ions for human use or consumption of 250.0, 0.05, 10.0, 1.50, and 400.0 mg L^{-1}, respectively. As we can observe in Table 1, the TAW analysis shows that all parameters are below the permissible limits; then, it is safe for human use; however, in the case of TWT, NO_2^- concentration was 1.35 mg L^{-1}, which is above limits. In comparison, when TWT was disinfected by photocatalysis, we can observe an increment in ionic species concentration, specifically with NO_2^- and NO_3^- species, which increase from 1.35 and < detection limit (DL) to 91.33 and 136.20 mg L^{-1}, respectively. This difference is due to the oxidation of organic matter in TWT, as observed by the total organic carbon (TOC) analysis (Table 1). TOC content decreased from 122.26 mg L^{-1} in TWT to 87.21 mg L^{-1} in DW after the photocatalysis process. This decay in TOC content indicates that organic matter was being oxidized and mineralized in the presence of Ag-TiO_2 photocatalyst. Also, partial oxidation of organic matter was assessed by increasing ionic content of the identified anions in water such as Cl^- which increased from 181.96 to 196.30 mg L^{-1}.

As can be seen in Table 1, TAW has a value of < 3 bacteria/100 mL in both lactose and lauryl broth, which agrees to the Mexican regulations for the permissible limits of pathogen presence in water (NOM-003-SEMARNAT-1997) (Regulation 1997). In comparison, TWT has a value of > 1100 bacteria/100 mL for lactose and lauryl broth. According to this analysis, the photocatalysis disinfection process removes nearly half of the total bacteria found in the water, decreasing to 460 and 150 bacteria/100 mL for lactose and lauryl broth, respectively. The water can be used in public services with occasional and indirect public contact, as mentioned in the Mexican regulation (regulation 1997). On the specific reactions to identify the detected microorganisms, eight different microorganisms were found in the TWT (Table 1); however, after the photocatalysis process, only four microorganisms were able to persist, namely, *Shigella flexneri*, *Escherichia coli* (25922), *Proteus mirabilis*, and *Enterobacter cloacae*. They indicate that the increase of ion species after the disinfection process could be due to the water's microorganisms' oxidation products.

Table 1 Physical, chemical, and biologic characterization of the different water samples and disinfected water ratios

Water sample	pH value	Anion content (mgL^{-1})					Total carbon (mg L^{-1})	Most likely number (bacteria/100 mL)		Detected microorganisms
		Cl^-	NO_2^-	NO_3^-	F^-	SO_4^{2-}		Lactose	Lauryl	
Tap (TAW)	7.5	56.58	< DL	4.01	< DL	299.94	79.96	< 3	< 3	None
Treated (TWT)	7.1	181.96	1.35	< DL	< DL	333.10	122.26	> 1100	> 1100	*Salmonella typhimurium* *Shigella flexneri* *Escherichia coli (25922)* *Escherichia coli (8739)* *Klebsiella pneumoniae* *Proteus mirabilis* *Eterobacter cloacae* *Pseudomonas aeruginosa*
Disinfected (DW)	7.3	196.30	91.33	136.20	< DL	310.22	87.21	460	150	*Shigella flexneri* *Escherichia coli (25922)* *Proteus mirabilis* *Eterobacter cloacae*
90% (DW/TAW)	7.3	---	---	---	---	---	86.52	460	93	
70% (DW/TAW)	7.3	---	---	---	---	---	85.51	460	93	
50% (DW/TAW)	7.4	---	---	---	---	---	84.81	460	39	

DL, detection limit; *TAW*, tap water; *DW*, disinfected water; *TWT*, treated wastewater

90% DW/TAW = 90% disinfected water/10% tap water, 70% DW/TAW = 70% disinfected water/30% tap water, 50% DW/TAW = 50% disinfected water/50% tap water

The water samples' physical aspect was only inspected in a visual way to avoid any floating matter and color. Previously reported works show that all the waters used in this research were according to the Mexican legislation to be used as irrigation waters (Pérez-Sánchez et al. 2014; Rodríguez-Méndez et al. 2017). The TAW is entirely transparent; no floating matter and color were detected. No color and floating matter were also found for the DW, but in the TWT samples obtained after the biologic wastewater process, a light yellow color was observed, and no presence of floating matter was noticed.

Crop growth evaluation

The crop growth was evaluated in terms of time and size of the fully grown plants; results are shown in Table 2. Firstly, for coriander (*Coriandrum sativum*), when irrigation was made with TAW water, a fully grown plant was obtained after 17 days, while, for plants irrigated with TWT, the time of growth was 21 days and 22 days for DW-watered plants. There was a clear decreasing tendency in growing time when irrigating with the 90%, 70%, and 50% ratio DW/TAW, obtaining 22, 20, and 17 days. In size terms, the larger plant was 9.5 cm irrigated with 70% DW/TAW, followed by 50% DW/TAW, DW, TWT, 90% DW/TAW, and TAW water, with sizes of 9.3, 9.2, 8.9, 8.6, and 8.6 cm, respectively. It is essential to note that the plant's size was improved by irrigating with a mixture of DW and TAW.

For lettuce crops (*Lactuca sativa* L. var. *Longifolia*, and *Lactuca sativa* L. var. *Defender*), irrigation with different water types modifies growing time. The mature plants of both lettuce varieties were obtained in the following order: 50% DW/TAW: 70% DW/TAW; and 90% DW/TAW, DW, TAW, and TWT. For the *Longifolia* variety, the maturing time was 30, 30, 32, 32, 38, and 42 days, respectively. The size was also water-dependent, obtaining 14.2, 16.1, 17.4, 18.7, 17.7, and 15.3 cm, respectively, for each irrigation water. For *Defender* variety, the plants' maturing time and size were 34, 34, 38, 42, 38, and 42 days and 12.7, 14, 16.7, 17.3, 12.4, and 13.2 cm in size. It is crucial to notice that bigger plants were obtained with DW water while TWT water increases maturing time and decreases its size.

For the jalapeno pepper (*Capsicum annuum* 'Jalapeño'), the plant's maturity was measured by how many fruits it produced. In this case, only plants irrigated with TAW and 50% DW/TAW showed 1 and 4 fruits, with maturing times of 109 and 73 days, respectively. It is essential to notice that the maturing time was reduced when a 50% ratio of DW to TAW water was used. For *Capsicum annuum* 'Serrano' (green hot pepper) when irrigating plants with TAW, 70% DW/TAW, and 90% DW/TAW, 2, 3, and 7 fruits were produced with a maturing time of 102, 132, and 102 days, respectively. By changing the DW/TAW ratio, obtaining more fruits in the same maturing time was possible.

Secondary metabolite content and evaluation of the antioxidant activity

Figure 1a shows the coriander (*Coriandrum sativum*) results in a radar graph with an independent scale on each axis to appreciate its composition better. It can be observed that when it was irrigated with DW water, a significant content of phenols and antioxidant activity was reached, also displaying a lower content of flavonoids with this type of water (DW) of 0.020-mg rutin/g sample. In comparison with the rest of the

Table 2 Crop growth by the different water samples and disinfected water ratios

Studied plants	Plant growth											
	TAW		TWT		DW		90% (DW/TAW)		70% (DW/TAW)		50% (DW/TAW)	
	Days	Size (cm)	Days	Size (cm)	Days	Size (cm)	Days	Size (cm)	Days	Size (cm)	Days	Size (cm)
Coriander (*Coriandrum sativum*)	17	8.6	21	8.9	22	9.2	22	8.6	20	9.5	17	9.3
Lettuce (*Lactuca sativa* L. var. *Longifolia*)	38	17.7	42	15.3	32	18.7	32	17.4	30	16.1	30	14.2
Lettuce (*Lactuca sativa* var. *Defender*)	38	12.4	42	13.2	42	17.3	38	16.7	34	14	34	12.7
Jalapeno pepper (*Capsicum annuum* 'Jalapeño')	109	# F 1	132	# F 0	109	# F 0	102	# F 0	102	# F 0	73	# F 4
Green hot pepper (*Capsicum annuum* 'Serrano')	132	2	109	0	132	0	132	7	102	3	102	0

*Tap water (TAW), treated wastewater (TWT), disinfected water (DW). 90% DW/TAW = 90% disinfected water/10% tap water, 70% DW/TAW = 70% disinfected water/30% tap water, 50% DW/TAW = 50% disinfected water/50% tap water. # F = number of fruits generated by the plant samples, two of three parameters are improved with the DW water but not the flavonoid content, which is higher with the TAW water (Fig. 1a), showing that the metabolic stress affects the time development of the crop (Table 2) having the lowest values of phenol content but the fastest growth times. The plant stress may be attributed to the dissolved ions presented in the DW water, which helps to the nutrient absorption and keeps the crop in a continuous state of secondary metabolite production to minimize the effect of the dissolved ions (Fukumoto and Mazza 2000). In Table 3, the values of the secondary metabolite content and antioxidant activity are presented for the coriander crop. As can be noticed, the coriander

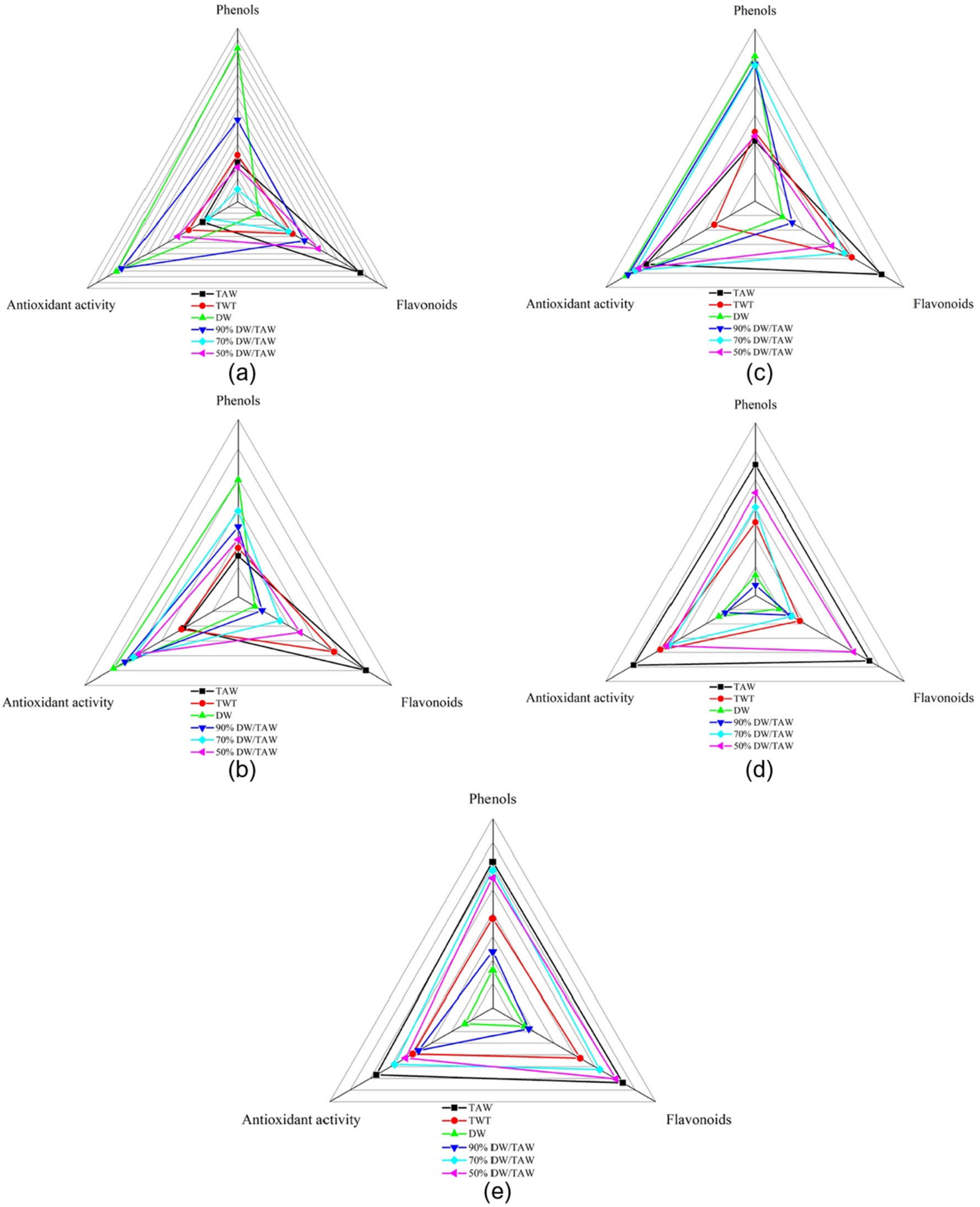

Fig. 1 Secondary metabolites evaluation for coriander **a** *Coriandrum sativum*; **b** *Lactuca sativa* L. var, *Longifolia*; **c** *Lactuca sativa* L. var. *Defender*; **d** *Capsicum annuum* 'Serrano'; and **e** *Capsicum annuum* 'Jalapeño' watered with the diverse types of waters and ratios. Tap water (TAW), treated wastewater (TWT), disinfected water (DW). 90% DW/TAW = 90% disinfected water/10% tap water, 70% DW/TAW = 70% disinfected water/30% tap water, 50% DW/TAW = 50% disinfected water/50% tap water

samples weathered with the DW, achieving the highest values in the content of phenols (27.657-mg gallic acid/g sample), antioxidant activity (2.965-μM Trolox/mg sample), and inhibition percentage (35.62%). These results are three times higher than those obtained in the same crop weathered with the TAW and TWT, showing that the coriander crops are under stress, directly affecting the secondary metabolites' presence in the plant (Fukumoto and Mazza 2000). Additionally, the statistical analysis indicates that the coriander samples with the different treatments present significant differences for the content of phenols and in the antioxidant activity, but not for the concentrations of flavonoids where a difference is observed only between the TAW treatments (0.123), DW (0.20), and 50% (DW/TAW (0.0123)) mg rutin/g sample. The latter treatment (50% (DW/TAW)), being ten times lower than the highest value 0.123-mg rutin/g sample, presented by water TAW (Table 3).

For lettuce (*Lactuca sativa* L. var. *Longifolia*), as in the coriander crop, the best results in two of the three evaluated parameters (Fig. 1b) were achieved with the DW, and once again, the highest flavonoid content was with the TAW water (0.207-mg rutin/g sample). The difference between the lettuce var. *Longifolia* and the coriander crops is that the presence of the dissolved ions in the DW induces an effect over the growth time (Table 2), and in the secondary metabolite production in both parameters, the DW generates more nutritional species in lower harvest times in comparison with the TAW and TWT. The analysis results are shown in Fig. 1b, each axis graphed independently for a better appreciation. The highest content of flavonoid was obtained when the crop was irrigated with TAW water (0.208-mg rutin/g sample), while the highest content of phenols and antioxidant activity was obtained when the plants were irrigated with DW water with values of 5.089-mg gallic acid/g sample and 2.771-μM Trolox/mg sample, respectively. These observations are supported by statistical analysis that shows a significant difference between the DW water treatment and the rest of the treatments for phenols and the antioxidant activity. While the TAW and TWT treatments do not show a significant difference for the flavonoid content, a higher value for the TAW treatment is reported compared to the rest of the treatments (Table 4).

The analysis made to lettuce crops (*Lactuca sativa* L. var. *Defender*) is shown in Fig. 1c and Table 5. It is possible to observe that the most flavonoid content was obtained with TAW irrigation with 0.051-mg rutin/g sample. Phenol content increases with the TAW water to 1.02-mg gallic acid/g sample. Also, antioxidant activity increases to 1.733-μM Trolox/mg sample for the DW water. In this crop, the statistical analysis indicates the same trend as in the case of var. *Longifolia*, observing that there is not a significant difference between TAW and TWT for the concentration of flavonoids. The same trend is observed in the antioxidant activity with DW, where this treatment presents the highest value and a statistical difference to the rest of the treatments. However, the highest concentration of phenols for treatment with TAW presents a statistical difference among the rest of the treatments (Table 5). This behavior was not observed in the two last types of crops (coriander and lettuce var. *Longifolia*). The flavonoid content is higher with the TAW water, which can be attributed to the presence of the nitrogen ion species in the DW (Table 1), which may affect the air-nitrogen fixation process by the symbiotic bacteria (Ramakrishna et al. 2020). Because in the case of the DW, the nitrogen which the plants may require is present by the dissolved ion species (NO_2^- and NO_3^-), and no air-nitrogen fixation process is needed, showing an effect over the secondary metabolisms specifically in the flavonoid production (Liu et al. 2020). This can be compared with the crops watered with the TAW, in which the flavonoid content by the air-nitrogen fixation process by the symbiotic bacteria is improved (Wu et al. 2019).

Table 3 Secondary metabolite values for the coriander crop according to the different types of waters

Coriander (*Coriandrum sativum*)

		TAW	TWT	DW	90%鳥(DW/TAW)	70%鳥(DW/TAW)	50%鳥(DW/TAW)
Phenols	mg gallic acid/g sample	8.039^{D}	9.2445^{C}	27.657^{A}	15.2324^{B}	3.2768^{F}	7.1275^{E}
Flavonoids	mg rutin/g sample	0.123^{A}	0.055^{AB}	0.020^{B}	0.055^{AB}	0.061^{AB}	0.0123^{A}
Antioxidant activity	μM Trolox/mg sample	2.277^{C}	2.390^{BC}	2.965^{A}	2.924^{A}	2.230^{C}	2.475^{B}
Inhibition percentage	% radical inhibition (1:10 mL extract/mL of DPPH)	26.98	28.38	35.62	35.11	26.39	29.51

Alpha 0.05; q* 3.3588

Different letters per row indicate statistical difference in comparison of means (Tukey)

TAW, tap water; *TWT*, treated wastewater; *DW*, disinfected water. 90% DW/TAW = 90% disinfected water/10% tap water, 70% DW/TAW = 70% disinfected water/30% tap water, 50% DW/TAW = 50% disinfected water/50% tap water

Table 4 Secondary metabolite values for the lettuce var. *Longifolia* crop according to the different types of waters

Lettuce (*Lactuca sativa* L. var. *Longifolia*)

		TAW	TWT	DW	90%鳥(DW/TAW)	70%鳥(DW/TAW)	50%鳥(DW/TAW)
Phenols	mg gallic acid/g sample	4.509^{BC}	4.736^{B}	5.089^{A}	3.897^{D}	4.297^{C}	3.577^{E}
Flavonoids	mg rutin/g sample	0.207^{A}	0.205^{A}	0.027^{D}	0.039^{D}	0.068^{C}	0.100^{B}
Antioxidant activity	μM Trolox/mg sample	0.125^{D}	0.217^{D}	2.771^{A}	2.444^{B}	2.098^{C}	1.877^{C}
Inhibition percentage	% radical inhibition (1:10 mL extract/mL of DPPH)	4.29	7.29	34.392	28.81	24.69	21.96

Alpha 0.05; q* 3.3588

Different letters per row indicate statistical difference in comparison of means (Tukey)

TAW, tap water; *TWT*, treated wastewater; *DW*, disinfected water. 90% DW/TAW = 90% disinfected water/10% tap water, 70% DW/TAW = 70% disinfected water/30% tap water, 50% DW/TAW = 50% disinfected water/50% tap water

Analysis of green hot pepper (*Capsicum annuum* 'Serrano') is shown in Fig. 1d and Table 6. As can be seen, irrigation with the TAW yields the highest values of secondary metabolites analyzed, with values of 125.433-mg gallic acid/g sample; 0.965-mg rutin/g sample; and 8.444-μM Trolox/mg sample of phenols, flavonoids, and antioxidant activity, respectively. TAW presents a statistical difference to the rest of the treatments for phenols, flavonoids, and antioxidant activity, as can be seen in Table 6. In this case, the TAW produces the stress over the plant to induce an effect over the secondary metabolism, but the highest fruit production was achieved with the 90% (DW/TAW) water; more experiments must be carried out with this crop to understand this result in the fruit production.

For the jalapeno pepper (*Capsicum annuum* 'Jalapeño'), as before, the major content of secondary metabolites was found with samples irrigated with the TAW with values of 102.025-mg gallic acid/-g sample; 0.500-mg rutin/g sample; and 8.850-μM Trolox/mg sample of phenols, flavonoids, and antioxidant activity, respectively (Fig. 1e and Table 7). However, unlike the green hot pepper, a trend that is corroborated with the statistical analysis does not present a statistical difference among all the treatments in each of the secondary metabolites analyzed. The most notable statistical difference is between TAW and the DW, and 90% (DW/TAW), where a constant statistical difference is observed in each of the values of secondary metabolites analyzed. Also, the same effect can be noticed that the green hot pepper presented; the highest stress in the secondary metabolism is with the TAW, but the highest fruit production was with the 50% (DW/TAW) water (Table 2).

According to the obtained results, a high concentration of phenolic compounds can be attributed to increased oxidative stress in the plant, as shown in the first three crops (coriander and lettuces (var. *Longifolia* and var. *Defender*). Mengel and Kirkby (2000) indicate that oxidative stress induced by high concentrations of ions keeps the plant in continuous stress by retarding the growth and development of the plant and

Table 5 Secondary metabolite values for the lettuce var. *Defender* crop according to the different types of waters

Lettuce (*Lactuca sativa* L. var. *Defender*)

		TAW	TWT	DW	90%鳥(DW/TAW)	70%鳥(DW/TAW)	50%鳥(DW/TAW)
Phenols	mg gallic acid/g sample	1.02^{A}	0.666^{C}	0.629^{C}	0.854^{B}	0.831^{B}	0.521^{D}
Flavonoids	mg rutin/g sample	0.053^{A}	0.039^{AB}	0.011^{D}	0.015^{CD}	0.036^{B}	0.031^{BC}
Antioxidant activity	μM Trolox/mg sample	1.436^{C}	0.535^{D}	1.733^{A}	1.697^{A}	1.602^{B}	1.555^{B}
Inhibition percentage	% radical inhibition (1:10 mL extract/mL of DPPH)	16.63	6.45	20.10	19.72	18.52	18.42

Alpha 0.05; q* 3.3588

Different letters per row indicate statistical difference in comparison of means (Tukey)

TAW, tap water; *TWT*, treated wastewater; *DW*, disinfected water. 90% DW/TAW = 90% disinfected water/10% tap water, 70% DW/TAW = 70% disinfected water/30% tap water, 50% DW/TAW = 50% disinfected water/50% tap water

Table 6 Secondary metabolite values for the green hot pepper crop according to the different types of waters

Green hot pepper (*Capsicum annuum* 'Serrano')

		TAW	TWT	DW	90%鳥(DW/TAW)	70%鳥(DW/TAW)	50%鳥(DW/TAW)
Phenols	mg gallic acid/g sample	125.433^{A}	105.53^{C}	87.084^{D}	83.582^{D}	110.558^{BC}	115.589^{B}
Flavonoids	mg rutin/g sample	0.965^{A}	0.498^{B}	0.349^{B}	0.431^{B}	0.443^{B}	0.859^{A}
Antioxidant activity	µM Trolox/mg sample	8.444^{A}	7.904^{B}	6.736^{E}	6.605^{F}	7.715^{D}	7.775^{C}
Inhibition percentage	% radical inhibition (1:10 mL extract/mL of DPPH)	88.47	83.48	72.73	71.54	81.77	82.29

Alpha 0.05; q* 3.3588

Different letters per row indicate statistical difference in comparison of means (Tukey)

TAW, tap water; *TWT*, treated wastewater; *DW*, disinfected water. 90% DW/TAW = 90% disinfected water/10% tap water, 70% DW/TAW = 70% disinfected water/30% tap water, 50% DW/TAW = 50% disinfected water/50% tap water

increasing the concentration of secondary metabolites. This is consistent with the characteristics presented in each of the types of water used in this work. The DW has a high concentration of anions such as nitrates and phosphates and a low density of still persistent microorganisms, and the TWT has not any of the characteristics of the other groups.

On the other hand, flavonoid type compounds' content increases in all the crops when treated with the TWT. Calvo-García (2011) indicates that a state of symbiosis between plants and nitrifying bacteria allows the fixation of nitrogen through the plant's roots, which the plant uses to meet the demand for nitrogen and, therefore, metabolic needs influencing the flavonoid synthesis route. Flavonoids are the plant's signal to interact with nitrogen-fixing bacteria, giving rise to a later symbiosis (Azcón-Bieto and Talón 2013). When the plant is found in a poorly nourished environment and has only the substrate contributing to its demand for nitrogen and nutrients, it will increase the concentration of flavonoids as chemoattractant signals and thereby try to meet its requirements.

According to García-Herrera et al. (2014), the stages where there is a greater demand for nutrients, such as nitrites and phosphates, are the stage of flowering and fruiting, which is why the crops must be nourished with nitrates and phosphates to optimize development. The results indicate that the crops of green hot pepper and jalapeno pepper irrigated with the TWT were in a period of stress because they did not have the necessary nutrients for their forced production, which was corroded by the yellowing of the leaves, which is indicative of the lack of nitrogen in the plant organism and low fruit production (Shedley et al. 1995).

The increase in secondary metabolites for lettuce and coriander species would represent an improvement in the nutritional value of these foods for the diet of the human being by increasing the concentration of polyphenols and the antioxidant activity thereof, allowing vegetables to be developed in shorter production times that contain a higher nutritional value.

Finally, the remaining trace metal analysis was made to determine if the plants retained some metals from the water

Table 7 Secondary metabolite values for the jalapeno pepper crop according to the different types of waters

Jalapeno pepper (*Capsicum annuum* 'Jalapeño')

		TAW	TWT	DW	90%鳥(DW/TAW)	70%鳥(DW/TAW)	50%鳥(DW/TAW)
Phenols	mg gallic acid/g sample	102.025^{A}	77.777^{C}	55.719^{E}	63.689^{E}	98.47^{AB}	95.119^{B}
Flavonoids	mg rutin/g sample	0.500^{A}	0.368^{AB}	0.197^{B}	0.219^{B}	0.428^{A}	0.478^{A}
Antioxidant activity	µM Trolox/mg sample	8.850^{A}	7.952^{D}	6.675^{F}	7.814^{E}	8.406^{B}	8.137^{C}
Inhibition percentage	% radical inhibition (1:10 mL extract/mL of DPPH)	92.26	84.0	72.13	82.7	88.13	85.62

Alpha 0.05; q* 3.3588

Different letters per row indicate statistical difference in comparison of means (Tukey)

TAW, tap water; *TWT*, treated wastewater; *DW*, disinfected water. 90% DW/TAW = 90% disinfected water/10% tap water, 70% DW/TAW = 70% disinfected water/30% tap water, 50% DW/TAW = 50% disinfected water/50% tap water

treated with the photocatalyst disinfection process. The analysis was made to coriander and lettuce crops irrigated with pure DW. Determination of titanium, iron, and silver content was carried out. Titanium content was below the detection limit for all samples analyzed. Iron content was 0.013, 0.13, and 0.005 mg L^{-1} for coriander, *Longifolia* lettuce, and *Defender* lettuce, respectively. Lastly, the silver content was 1.617, 0.999, and 1.523 mg L^{-1} for coriander, *Longifolia* lettuce, and *Defender* lettuce, respectively. As observed, no titanium was detected, which indicates that the crops do not absorb titanium from the photocatalyst process. Although iron and silver were detected, we can observe that they are below Mexican regulations' limits. Also, it is possible to expand the studies to cases where the NPs are not removed from the effluent and in future works to generate nanotoxicology protocols to update the Mexican regulations on the topic of water.

Conclusion

It can be evidenced that the use of disinfected water by solar photocatalysis represents a method that allows for eliminating the majority of microorganisms present in the treated wastewater. It also allows the introduction of ions such as nitrates and phosphates as a source of nutrients, opening the possibility to reduce the use of substrates and fertilizers, and also, to have a higher plant growth rate, as was possible to see in the groups of 70% and 50% disinfected water for the different species and higher production efficiency in the case of pepper fruits. The results suggest that (i) the composition of the type of water used in the treatments influences the synthesis of secondary metabolites, and (ii) the type of culture used is a variable to consider for the use of any of these treatments. It was proved that the use of different types of water gives different results in growth rate, plant size, fruit quantity, and nutritional value, and it also depends on the type of crop, allowing for starting new studies with different morphologies, crystallinity, and superficial area of nanoparticles used for the photocatalysis disinfection water.

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Declarations

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