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Production of Pepper under Different Concentrations of Treated Sewage Effluent

Henrique Fonseca E. de Oliveiraa,\*, Tiago Moreira Vidigala, Luis Sérgio R. Valea, Márcio Mesquitab, Lessandro Coll Fariac, Jairo G. Barbosa Netod

aPostgraduate Program in Irrigation in the Cerrado, Goiano Federal Institute, Ceres, Goiás, Brazil

bCollege of Agronomy, Federal University of Goiás, Goiânia, Goiás, Brazil

cCDTec/Water Engineering, University of Pelotas, Pelotas, Rio Grande do Sul, Brazil

dCollege of Agronomy, Goiano Federal Institute, Ceres, Goiás, Brazil

henrique.fonseca@ifgoiano.edu.br

The appropriate management of treated sewage effluent (TSE) represents an alternative to irrigation. When used for cultivation in a protected environment, such as a greenhouse, the stability and quality of production can be guaranteed. Thus, the present study aimed to evaluate the morphological and productive characteristics of yellow finger pepper in a protected environment, submitted to five concentrations of treated sewage effluent (T1: 100% fluvial water; T2: 75% fluvial water + 25% TSE; T3: 50% fluvial water + 50% TSE; T4: 25% fluvial water + 75% TSE and T5: 100% TSE), applied through drip irrigation. The experiment was conducted in the central region of Brazil, from June to December 2017. The experimental design was randomized completely blocks with 4 repetition. The morphological and productivity characteristics of the pepper plants were submitted to analysis of variance (F-test), at the of 5% probability level. For the variables in which there were significant treatment effects at p < 0.05, applied regression analysis. There were no statistically significant differences between treatments for the morphological characteristics: plant height, stem diameter, number of leaves, cup diameter, root fresh weight and root dry weight. Among the productive characteristics, productivity and fruit length differed statistically at p < 0.05. The microbiological analysis did not detect contamination both for fruit spares and for seeds. In the analysis of water and residual in the soil, there was a statistical difference between the concentrations of zinc. The increase in the concentration of treated domestic sewage caused a drop in the productivity.

* 1. Introduction

Water scarcity is becoming one of the largest problems worldwide. Agricultural reuse of wastewater has been considered a valuable and reliable alternative, alleviating the pressure on freshwater resources (Farhadkhani et al., 2018; Ibekwe et al., 2018; Biswas et al., 2020). According to Suarez and Gonzalez-Rubio (2017), the increasing scarcity of fresh water means we must use alternative supplies for irrigation such as treated municipal wastewaters.

Irrigation with treated wastewater has already been implemented in several countries for the cultivation of fruit, citrus, and vegetables. According to Petousi et al. (2019), the use of treated wastewater in agriculture continues because of the benefits it offers. These include: a solution to irrigation water scarcity; the availability of large amounts throughout the year; the possibility of reserving better-quality water for human consumption; the reduction of fertilizers needed due to the nutrients contained in this type of water; protection of the environment; the reduction of effluent waters in the surrounding area; avoiding marine intrusion in coastal areas and overexploitation.

Wastewater treatment and reuse are recommended in the 2030 Agenda for Sustainable Development (UN General Assembly, 2015) in target 6.3 “improve water quality by…halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally” and target 3.9 “reduce the number of deaths and illnesses from … water and soil pollution and contamination”.

In their study, Shilpi et al. (2018) demonstrated that wastewaters at appropriate dilutions could be used for irrigation in agricultural fields to enhance crop productivity. However, due to its unique characteristics, this new resource has many challenges that cannot be ignored, such as health issues, water quality, and long- and short-term effects on soils and crops (Ahmadi and Merkley, 2009).

Thus, the objective of this study was to evaluate the productive characteristics of yellow finger pepper in a protected environment, submitted to different concentrations of treated sewage effluent and investigate the effects of wastewaters on soil, plants, and fruits.

* 1. Materials and Methods

The experiment was conducted from May to December 2017, in a greenhouse located in the experimental area of the Goiano Federal Institute; Campus Ceres (UTM coordinates: zone 22, N 8302477, E 650455, an altitude of 580 m a.s.l). The climate of the region, according to the Koppen-Geiger classification, is type Aw, i.e. a tropical climate with a dry season in winter.

The greenhouse used has a low air density, transparent polyethylene cover, and a structure in an arch form. One seed per cell was planted in an expanded polystyrene tray with 200 cells containing commercial Plantmax® substrate. At 30 days after emergence (DAE), when the seedling had around four developed leaves, they were transplanted to 12 L flexible vessels. The substrate used was composed by ravine soil, sand, and bovine manure, at a ratio of 2:1:1, respectively. The soil fraction used was classified as red latosol (35.2 % sand, 6.8 % silt and 58.0 % clay) with 9.1 g/dm3 organic matter and a pH (in H2O) of 6.72. The chemical composition of the soil was: Ca = 2.8 cmolc/dm3; Mg = 1.8 cmolc/dm3; Al = 0.0 cmolc/dm3; H + Al = 1.1 cmolc/dm3; K = 0.21 cmolc/dm3; T = 5.8 cmolc/dm3; K = 81.8 mg/dm3; P = 2.0 mg/dm3 and V = 79.1%.

The effluent used for irrigation was removed from the transit box, located next to the maturation pond of the sewage treatment plant (STP) in the municipality of Ceres-GO. The primary treatment of the STP is through a sandbox, which is used to retain the solid part of the sewage. When leaving the sandbox, the effluent passes through a “biodigester”, whose function is to eliminate the organic matter present in the sewage, thereby generating a by-product termed a sludge additive. Afterward, the effluent is discharged into another pond, and finally into the maturing pond, where it is exposed to solar radiation, which concludes the process. The effluent was collected biweekly and stored in a 500-L polyethylene box, where it was then diluted in water for other treatments.

A randomized complete block design was used, with four replications. The plots were composed of five treated sewage effluent (TSE) concentrations (T1: 100 % fluvial water / no TSE; T2: 75 % fluvial water + 25 % TSE; T3: 50 % fluvial water + 50 % TSE; T4: 25 % fluvial water + 75 % TSE and T5: 100 % TSE). The levels of irrigation replenishment estimated through the daily reading of an evaporimetric pan with dimensions of 60 cm x 28 cm, installed inside the greenhouse. The blocks were composed of rows of flexible vessels of 12 L each, spaced at 0.80 m between rows, and 0.45 m between plants.

The experiment was conducted by using a drip irrigation system with a flow rate of 8 L/h, push-button drippers, and a self-compensating flow. The temperature and air humidity data were obtained by using a thermo-hygrometer, which was installed in a meteorological shelter inside the greenhouse.

During the transplanting, the moisture of the substrates contained in the vessels was elevated to vessel capacity moisture (VCM), based on the methodology used by Gamareldawla et al. (2017). The volumetric water content in the vessels was maintained during the acclimatization of the seedlings, which occurred before the differentiation of the treatments and was performed at 30 days after transplanting (DAT).

The evaluations were performed fortnightly from 100 DAT to 235 DAT. The morphological characteristics of plant height (PH, cm), stem diameter (SD, mm), number of leaves (NL), and cup diameter (cm) were evaluated. The yellow finger pepper fruits were counted and weighed, and then evaluated average productivity (PROD, Mg/ha), considering a population of 27,778 plants/ha; fruit length (FL, mm); fruit diameter (FD, mm) and pericarp thickness (PT, mm). Root fresh weight (RFW, g/seedling) and root dry weight (RDW, g/seedling) were also evaluated. The analysed variables were submitted to variance analysis (Fisher’s F test) at the 5% probability level, using the software SISVAR (Statistical Analysis System) (Ferreira, 2011). For the variables in which there were significant treatment effects at p < 0.05, a regression analysis was performed.

The multi-tube method was used to verify the number of thermotolerant coliforms, and to quantify and identify the coliforms present in the fruit and seed samples. The fruits and seed samples were submitted to the confirmatory test for the colostrum group Bright Green Lactose Broth Bile (B. G. L. B. B.) and for the differentiation of thermotolerant coliforms in the medium (T.C).

* 1. Results and Discussion
		1. Climatic data

The temperature and humidity inside the greenhouse vary a lot throughout the experimental period and ranged from a minimum of 12 °C to a maximum of 55 °C of temperature and from 10 % to 77 % of humidity.

* + 1. Morphological characteristics of the plant

Table 1 presents the Fisher's F test at the 5 % level of significance for the morphological characteristics of the pepper plants: plant height (PH, cm), stem diameter (SD, mm), number of leaves (NL), cup diameter (CD, cm), root fresh weight (RFW, g/seedling) and root dry weight (RDW, g/seedling) as a function of treated sewage effluent (TSE) concentration (0, 25, 50, 75, 100 % of TSE) at 235 days after sowing (DAS).

*Table 1. Variance analysis for morphological characteristics as a function of TSE concentration.*

|  |  |  |
| --- | --- | --- |
|  |  | MS |
|  | DF | PH (cm) | SD (mm) | NL (un) | CD (cm) | RFW (g) | RDW (g) |
| Treatment 4 | 6.15ns | 0.63ns | 0.24ns | 0.24ns | 66.24ns | 52.33ns |
| Residue | 12 | 12.02 | 1.19 | 269.86 | 0.12 | 31.2 | 24.8 |
| CV (%) |   | 5.27 | 9.90 | 8.80 | 0.46 | 21.74 | 22.32 |

\*Significant at the 5% level of significance (p<0.05); nsNo significant (p>0.05); MS - Middle Square; DF – Degrees of Freedom; CV - Coefficient of Variation; PH - Plant Height; SD - Stem Diameter; NL - Number of Leaves; CD - Cup Diameter; RFW - Root Fresh Weight; RDW - Root Dry Weight

The morphological characteristics were not significantly affected (p < 0.05) by the TSE concentration. The results obtained by Petousi et al. (2019) showed that tertiary treated wastewater had a positive impact on young grapevine growth and yield in comparison with tap water.

**3.3 Fruit analysis**

Table 2 presents Fisher's F test at the 5 % level of significance for the productive characteristics of average productivity (PROD, Mg/ha); fruit length (FL, mm); fruit diameter (FD, mm) and pericarp thickness (PT, mm) as a function of treated sewage effluent (TSE) concentration (0, 25, 50, 75, 100 % of TSE) at 235 days after sowing (DAS).

*Table 2. Variance analysis for productive characteristics as a function of TSE concentration.*

|  |  |  |
| --- | --- | --- |
|  |  | MS |
|  | DF | PROD (g/plant) | FL (mm) | FD (mm) | PT (mm) |
| Treatment 4 | 24.870\* | 27.911\* | 0.435ns | 0.003ns |
| Residue | 12 | 3.90 | 2.58 | 0,141 | 0.009 |
| CV (%) |  | 3.80 | 2.91 | 2.59 | 5.49 |

 \*Significant at the 5% level of significance (p<0.05); nsNo significant (p>0.05); MS - Middle Square; DF – Degrees of Freedom; CV - Coefficient of Variation; PROD - Average productivity; FL - Fruit Length; FD - Fruit Diameter; PT - Pericarp Thickness

Regarding the fruits, the statistical analysis indicated that there were significant differences (p < 0.05) for PROD and FL, as presented in Table 2. The PROD decreased linearly with an increase in the TSE concentration (Figure 1a). Figure 1b shows the quadratic adjustment obtained for the characteristic FL. For this characteristic, the estimated optimum effluent concentration was 41.62 % of treated sewage effluent.

  

a

b

*Figure 1. Productivity (a) and length fruit (b) of yellow finger pepper as a function of treated sewage effluent (TSE) concentration.*

According to the equation shown in Figure 1a, each 1 % increase in the TSE concentration, corresponding to a reduction of 0.36 % in PROD, under the conditions of the study. This relationship shows a 36 % reduction in productivity when using 100 % effluent in relation to the treatment without its addition.

The estimated maximum and minimum productivity (PROD) were 16.02 and 10.23 Mg/ha, respectively, which corresponded to T1 treatment (no treated sewage effluent - TSE) and T5 treatment (100 % of TSE). All the treatments adopted in this experiment presented higher productivity than the average values found by Oliveira et al. (2014) in a study using young peppercorn fruits in the northeast region of Brazil and obtained an average yield of 8.9 Mg/ha.

**3.4 Microbiological analysis of the fruits and seeds**

Table 3 presents the results of the microbiological analysis of fruits and seeds of yellow finger pepper obtained 235 days after sowing.

*Table 3. Microbiological analysis of fruits and seeds of yellow finger pepper at 235 DAS*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|   | Fruit | Seed | Fruit | Seed |
|  | (B. G. L. B. B.)1 | (B. G. L. B. B.) | T.C2 | T.C |
|   | 10-1 | 10-2 | 10-3 | 10-1 | 10-2 | 10-3 | 10-1 | 10-2 | 10-3 | 10-1 | 10-2 | 10-3 |
| T1 (0%) | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| T2 (25%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| T3 (50%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| T4 (75%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| T5 (100%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

1Bright Green Lactose Broth Bile (B. G. L. B. B.); 2Thermotolerant coliforms (T. C)

In the analysis, there was no confirmation of thermotolerant coliforms in the pulp and seed of the fruits of yellow finger pepper. Souza et al. (2013), cultivating sweet peppers under different concentrations of swine wastewater, applying drip irrigation, did not show signs of contamination by thermotolerant coliforms and *Salmonella* spp., remaining within the sanitary microbiological standards required by Brazilian standard. Feitosa et al. (2009) when analyzing the quality of the watermelon fruit produced with treated domestic sewage water, obtained negative results in the microbiological analyzes, not showing a presence of bacteria of the coliform group, suggesting that the hygienic-sanitary conditions of the product were satisfactory.

According with Farhadkhani et al. (2018) to the analyzed parameters, including total and fecal coliforms, *E. coli*, as indicator microorganisms, and E. Coli O157, *Salmonella* and *Shigella* as pathogenic bacteria, secondary treated wastewater (STWW) could be safely used as an alternative source for irrigation of root and leafy crops.

**3.4 Chemical analysis of treated sewage effluent**

Table 4 presents the chemical characteristics of the water and t of the treated sewage effluent (TSE) at different concentrations (0, 25, 50, 75 and 100 % of TSE) used in the experiment.

*Table 4. Chemical analysis of water and TSE concentrations*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   | Magnesium | Zinc | Cadmium | Lead | Copper |
| TSE  | (Cmol/dm³)  | (mg/dm³)  |  (mg/dm³)  | (mg/dm³)   | (mg/dm³)   |
|  T1 (0%) | 0 | 1.174 | 0 | 0.036 | 0 |
| T2 (25%) | 0 | 0.430 | 0 | 0.035 | 0 |
| T3 (50%) | 0 | 0.102 | 0 | 0.031 | 0 |
| T4 (75%) | 0 | 0.168 | 0 | 0.035 | 0 |
|  T5 (100%) | 0 | 0.636 | 0 | 0.031 | 0 |
| F Test |   |   |   |   |   |
| Treatment | - | 0.503\* | - | 0.036NS | - |
|  CV% | - | 6.34 | - | 9.39 | - |

\*Significant at the 5% level of significance (p<0.05); nsNo significant (p>0.05); CV - Coefficient of Variation

Among the elements analysed (Table 4), it was observed that the zinc concentration presented a significant difference at 5% probability among the evaluated treatments. However, there was not a tendency of increase or decrease with higher/lower TSE, probably due to the variability in organic matter composition in the TSE.

Figure 2 shows the quadratic trend in the zinc concentration and TSE concentration, with the lowest concentration estimated at the 57.8% TSE concentration.



*Figure 2. Zinc concentration of the water and the concentrations of treated sewage effluent*

Concentrations of magnesium, cadmium, and copper were not detected in any of the evaluated treatments, nor were statistical differences (p>0.05) observed in the concentrations of lead. The highest average value of zinc obtained in the treatment T1 (0 % TSE) with 1.17 mg/dm³ is within the acceptable range, according to guidelines for class 2 waters, whose maximum limit is 5 mg/dm³ (BRASIL, 2013).

According to Souza et al. (2011), plants grown in environments with excess lead have growth and development affected.

**3.5 Chemical composition of substrates**

Table 5 shows the residual analysis of the substrates for the different concentrations of treated sewage effluent (0, 25, 50, 75 and 100 % of TSE) performed after the last fruit harvest at 235 days after sowing (DAS). The zinc (Zn) concentration presented a significant difference (p<0.05) among the evaluated treatments. Magnesium (Mg), calcium (Ca), lead (Pb), aluminum (Al), phosphorus (P) and potassium (K) in soil did not present statistical differences (p>0.05) between treatments.

*Table 5. Residual analysis of the substrates for the different concentrations of treated sewage effluent at 235 days after sowing (DAS)*

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|   | pH | MO | Mg+ | Ca+ | Al+ | Al +H | P | K+ | Zn | Cd | Pb |
|   | H2O | g/dm3 | Cmol/dm³ | Mg/dm³ | Mg/dm³ | Mg/dm³ | Mg/dm³ | Mg/dm³ |
| T0 | 7.6 | 30.4 | 2.0 | 2.6 | 0 | 0 | 283.00 | 1714.00 | 1.86 | 0.015 | 0 |
| T1 | 7.7 | 34.8 | 2.7 | 4.3 | 0 | 0 | 305.00 | 1294.75 | 2.04 | 0.048 | 0 |
| T2 | 7.6 | 22.3 | 1.9 | 4.4 | 0 | 0 | 231.25 | 849.00 | 1.22 | 0.024 | 0 |
| T3 | 7.8 | 29.5 | 1.8 | 3.5 | 0 | 0 | 310.00 | 1194.75 | 1.71 | 0.028 | 0 |
| T4 | 7.6 | 23.8 | 1.9 | 4.3 | 0 | 0 | 266.75 | 1123.75 | 1.49 | 0.058 | 0 |
| T5 | 7.5 | 33.5 | 2.6 | 5.7 | 0 | 0 | 335.50 | 1283.00 | 1.88 | 0.043 | 0 |
| F Test |   |   |   |   |   |   |   |   |   |   |   |
| Treatment | 7.6ns | 28.5ns | 2.4ns | 4.2ns | - | - | 256ns | 1149.05ns | 1.66\* | 0.036ns | - |
| CV (%) | 5.82 | 3.11 | 4.66 | 2.33 | - | - | 16.76 | 26.08 | 21.86 | 3.83 | - |

\*Significant at the 5% level of significance (p<0.05); nsNo significant (p>0.05); T0: Substrate before differentiation of treatments

Liu et al. (2018) studying plants irrigated with wastewater effluent (WE) or deionized water (DIW) observed after 95 days, significantly higher concentrations of extractable Ti and Zn (439.2 ± 24.4 and 9.0 ± 0.5 mg/dm3, respectively) found in WE-irrigated soil than those in DIW-irrigated soil (161.2 ± 2.1 and 4.0 ± 0.1 mg/dm3).

Souza et al. (2003), studying the use of domestic effluent from an anaerobic lagoon, observed, after treatment using a furrow irrigation system, that the irrigation with effluent did not alter soil fertility. There was a small increment of P, Ca, S, Cu and Zn with the application of effluents. The organic matter content remained constant because its nature provides rapid degradation in soils under tropical conditions.

For Petousi et al. (2019), it is important to study the effect of irrigation of wastewater on the soil-plant system, crop yield, fruit quality and presence of inorganic chemical contamination (salts, elements and heavy metals), organic chemical contamination (polycyclic aromatic hydrocarbons) and contamination (E. coli, total coliforms).

* 1. Conclusions

1. The increase in the concentration of treated domestic effluent (TSE) caused a reduction in the productivity of yellow finger pepper.

2. There was no contamination on the yellow finger pepper fruits.

3. Water and residual substrate of the experiment showed significant differences in the zinc contents, the same did not occur for the other elements analysed.

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