Colegio de Postgraduados

Effect of pig farming wastewater on the physiological quality of habanero pepper (*Capsicum chinense* Jaq.) seeds

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ABSTRACT

Objective: to evaluate the effect of pig farming wastewater on the viability and vigor of habanero pepper seeds.

Design/Methodology/Approach: treatments (T) applied were T1: 20% wastewater+80% tap water; T2: 40% wastewater+60% tap water; T3: 60% wastewater+40% tap water; T4: 80% wastewater+20% tap water; and T5: 100% wastewater; T6: was the control (100% tap water). The experiment was established in a completely randomized design with five replicates. Physiological variables and vigor in the seeds were evaluated through the calculation of seed germination rate and seedling emergence rate, as these express the germination capacity and vigor of seeds. Also, morphological variables in the seedlings were measured.

Results: in germination and emergence, seeds in T1 treatment and the control T6 were superior to T5. A similar trend was also observed in the emergence rate, T1 treatment and the control T6 reached the highest values. T1 and control T6 recorded the greatest plant height compared to the other treatments. Similar results were obtained for root length among all treatments, except T5, which showed seedlings with the lowest growth and vigor.

Limitations/Implications of the study: only five fixed percentages of pig farm wastewater were used in this study; So, it remains unknown about any effect that other concentrations of wastewater may have on the evaluated attributes of seed and plants of *C. chinense*.

Findings/Conclusions: pig farming wastewater at 100% decreased seed physiological attributes. In contrast, treatments with wastewater dilutions increased seed viability, vigor, and seedling growth.

Keywords: germination, emergence, growth, wastewater.

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INTRODUCTION

In recent years, population growth has led to a greater demand for food. Under this scenario, agricultural and livestock production systems have increased their productivity based on increasingly larger planting areas, a greater number of reproductive females, and larger numbers of animals in production, among other strategies (Rodrigues de Souza *et al.*, 2023). On the other hand, the increase in animal load in confinements increased the amount of waste generated in production (Rodrigues de Souza *et al.*, 2023). So, these

strategies had a negative impact on the environment due to the amount of manure and effluents generated by animals, which generally end deposited in agricultural soils and underground water bodies (Shashvatt *et al.*, 2017).

Waste from pig farming typically contains excrement, urine, water, food scraps, dust, among others. These generally contain nutrients and chemical elements such as nitrogen, phosphorus, potassium, calcium, magnesium, and some others, such as iron, copper, and zinc, which can be used in crop production and to improve the physical composition of soils (Guardini *et al.*, 2012; Silva *et al.*, 2015; Loss *et al.*, 2017; Mergen Junior *et al.*, 2019). Considering the above, some alternatives have been searched to try mitigating and reducing the impact of waste on the environment and on human health. One among those alternatives is to reuse wastewater for agriculture irrigation, which can benefit both the environment and the producer (Matos & Matos, 2017).

Due to the mineral content present in wastewater, it can be used as a fertilizer for soils and crops (Bastos, 2016; Debortolli *et al.*, 2018). Among the benefits of reusing wastewater in the soil is that it can reduce production costs by decreasing the use of commercial fertilizers for crops. Crops, in turn, can function as a natural bio-filter, which would improve the treatment of waste. Likewise, by using wastewater as a fertilization method in crop production, results similar to conventional management, or even an increase in production can be obtained. As, in contrast to the conventional form of production, wastewater use would represent a reduction in environmental pollution and an improvement of those agronomic characteristics that are required in a crop (Barbosa *et al.*, 2014).

On the other hand, inappropriate management of pig farm wastewater can have the opposite of expected effects, since it is usually used without considering the crop to be produced and its requirements. In many cases, an excess of use can originate poisoning and a reduction in crop yield, also, it can cause greater environmental pollution (Matos, 2014; Rodrigues de Souza *et al.*, 2023). In this context, it is imperative to generate information on the interaction of wastewater with plants and to evaluate possible forms of production by reusing pig production waste. Therefore, the objective of the research was to evaluate the effect of pig farming wastewater on the viability and vigor of habanero pepper seeds.

MATERIALS AND METHODS

Experimental site and plant material

The experiment was conducted in the laboratory of the Faculty of Environmental Engineering under the Autonomous University of Yucatán. Seeds of a commercial variety of habanero pepper (*Capsicum chinense*) were used.

Experimental design and treatments

This research was developed with a completely randomized experimental design. The treated pig wastewater used in the experiment was obtained from a pig farm that has a wastewater treatment plant. This facility consists of a sump, an anaerobic bio-digester, and oxidation lagoons, composed of a part with aeration and another with ozonation where the treated wastewater for agricultural use is obtained. Seeds and seedlings were produced and irrigated with treated wastewater and with wastewater dilutions based on the following treatments T1: 20% wastewater + 80% tap water; T2: 40% wastewater + 60% tap water; T3: 60% wastewater + 40% tap water; T4: 80% wastewater + 20% tap water; and T5: 100% wastewater; T6: was the control (100% tap water). Seedlings produced in the control were supplemented with 190 mg L^{-1} of NPK (Poly Feed, 19N-19P-19K, Mexico City, Mexico). Each treatment consisted of five replicates.

Variables evaluated

Physicochemical parameters (pH, electrical conductivity, chemical oxygen demand, total nitrogen and total phosphorus) of the wastewater and the dilutions corresponding to each treatment were analyzed, as well as the tap water used in the control treatment, in accordance with the Mexican Official Standard NOM-001-SEMARNAT-2021.

Physiological variables of the seeds were determined as effects in relation to seed imbibition with the dilutions of wastewater. Germination and germination rate (GR) of the seeds were evaluated according to Hernández-Pinto *et al.* (2020); the latter was counted for 14 days and a seed was considered germinated when the radicle was observed. The seeds were placed in Petri dishes of 90 mm diameter, in each dish 25 seeds were placed on paper towels, moistened with the dilutions (4 mL each) based on the established treatments. Four Petri dishes were established per treatment, each one represented a replicate; at a temperature of 22±1 °C, 60% of relative humidity, and placed in total darkness.

The seedlings emergence test was done in polystyrene trays with germinating substrate (Sunshine mix 3), placing one seed per cavity. Emergence was counted daily for seven days after sowing (das). The percentage (%E) and emergence rate (ER) were calculated from the data obtained (Hernández-Pinto *et al.*, 2020). Once the seedlings emerged (at 4 das), trays were moved to a greenhouse for growth. Irrigation with the wastewater dilutions and inorganic fertilization began at 12 das. The experiment was arranged in a completely randomized design with five replicates.

Morphological variables of the seedlings were also measured; height, which was measured with a tape measure from the base of the root ball to the apical bud; stem diameter was obtained with a digital Vernier caliper 1 cm apart from the root ball; and root length was measured using the ImageJ software. These variables were evaluated at 40 days. Fifty seedlings were evaluated as the experimental unit.

Statistical analysis

Data obtained as percentage were transformed with the arcsine square root. An analysis of variance ($p \leq 0.05$) was performed for all variables; where significant statistical difference was found among treatments, a multiple comparison of means was applied (Tukey, $p \leq 0.05$). The analyses were performed using Statistica© v.7 (Statsoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Chemical analysis of the water showed significant statistical differences among treatments. Treatments with a higher proportion of wastewater (T5 and T4) had a higher pH and a much higher electrical conductivity (EC), than the other treatments. In contrast, treatment T1 and control T6 had significantly lower EC and pH. The trend was similar for most of the parameters evaluated and for chemical oxygen demand (COD) (Table 1). Dilutions of the wastewater reduced the chemical parameters. The lower EC of the dilutions in treatment T1 and the control T6 allowed for better assimilation of the mineral content present in the wastewater, which enhanced seedling growth.

In this regard, Sambo *et al.* (2019) indicated that EC and pH affect and influence the absorption of nutrients required by plants and consequently their growth and development. Nitrogen content was significantly higher in those treatments with a higher amount of wastewater (T4, 491 mg L^{-1} and T5, 544 mg L^{-1}), this concentration decreased in relation to the percentage of wastewater present in the treatments. On the other hand, a higher concentration of phosphorus was observed in those treatments T1 (10.32 mg L^{-1}) and T2 (9.62 mg L^{-1}) with a lower percentage of wastewater, compared to the rest of the treatments. The concentration of these elements positively influenced the viability and vigor of seeds.

Treatments with higher nitrogen concentrations had lower results in the variables evaluated, which could be due to interference with the functions of phosphorus in the seeds and seedlings evaluated in those treatments. In addition to increasing the EC, which hindered the interaction and action of minerals on the physiological attributes of the seeds. In this regard, Bilal *et al.* (2020) mentioned that an EC greater than 3000 μS cm^{-1} reduces the absorption of water and nutrients necessary for plant growth and development. In contrast, treatments T1 and T2, and the control T6 with lower mineral concentrations recorded better results in the evaluated variables.

Regarding COD, treatments T1, T2 and the control T6 presented a lower degree of contamination in comparison to treatments T4 and T5, which were higher compared to the others. The dilutions of the wastewater made it possible to improve the quality of the irrigation water used for seeds and seedling production, as well as decreasing contamination. As indicated by Banach *et al.* (2009) who mentioned that COD quantifies the amount of total organic matter present in a liquid sample and it is used to determine the level of water contamination.

Table 1. Chemical parameters of wastewater and wastewater dilutions in treatments.

Treatments	pH	EC (μS cm^{-1})	COD (mg L^{-1})	TKN (mg L^{-1})	TP (mg L^{-1})
T1	7.73±0.015 NS	2041±0.041 e	152±0.01 c	100.3±0.31 d	10.32±0.30 a
T2	8.6±0.020 NS	3207±0.079 d	215±0.02 b	203±0.39 c	9.62±0.46 a
T3	8.72±0.027 NS	4488±0.105 c	233±0.05 b	294±0.51 b	8.7±0.76 b
T4	8.87±0.041 NS	5680±0.109 b	440±0.13 a	491±0.75 a	5.84±0.80 d
T5	8.96±0.052 NS	6620±0.130 a	503±0.33 a	544±1.02 a	6.48±0.91 c
T6 (Control)	6.56±0.011 NS	971±0.034 f	≤20±0.01 d	6.72±0.27 e	6.17±0.14 c

T1: 20% wastewater + 80% tap water; T2: 40% wastewater + 60% tap water; T3: 60% wastewater + 40% tap water; T4: 80% wastewater + 20% tap water; and T5: 100% wastewater; T6: was the control (100% tap water); pH: hydrogen ion concentration in water; EC: Electrical conductivity; COD: Chemical oxygen demand; TKN: Total Kjeldahl nitrogen; TP: Total phosphorus. Data are means + standard error. Different letters in the same column indicate significant statistical difference (Tukey, $p \leq 0.05$). NS: non-significant.

Seed viability and vigor

Highly significant differences in seed germination were found starting on the fourth day. Control T6 (control) and treatment T1 had the highest germination (98% and 96%, respectively), compared to the other treatments; whereas treatments T2 and T3 were statistically similar. In contrast, treatments T4 and T5 had the lowest germination, 52% and 31%, respectively; T5 was at least 69% lower than T1 and the control T6 (Figure 1 A). A similar trend was observed for seedling emergence; treatment T1, 94% and the control T6, 98% were statistically superior to the rest. Most treatments were similar in seedling emergence, except treatment T5, which had the lowest emergence rate (72%) (Figure 1 B).

It was evident that pig farming wastewater influenced the germination capacity and vigor of the seeds. The dilutions of wastewater enhanced seed germination and seedling emergence in a shorter time (4 and 1 day), statistically similar to the control T6 (100% tap water). In contrast, treatments that had a higher percentage of wastewater in their composition decreased viability and vigor in the evaluation. The high viability of the seeds and the emergence in treatments with a low percentage of wastewater is likely due to a higher concentration of phosphorus in relation to total nitrogen, compared to the other dilutions. This could enhance the greater germination and emergence by promoting the decrease of abscisic acid and the activity of metabolic enzymes that initiated the germination process in less time.

It is known that some minerals such as N, Mg, K, Ca, P, among others, are related to the viability of seeds, and participate in the synthesis of proteins and concentration of abscisic acid (Lott *et al.*, 1995). Also, the minerals K, Ca, Mg, Mn, P and Fe are initiators of enzyme activators, promoting protein synthesis during seed development and germination (Xu *et al.*, 2002; Iwai *et al.*, 2012). This allows for greater availability of reserves to the embryo during the germination and emergence process, which in turn enhanced germination capacity and vigor in less time.

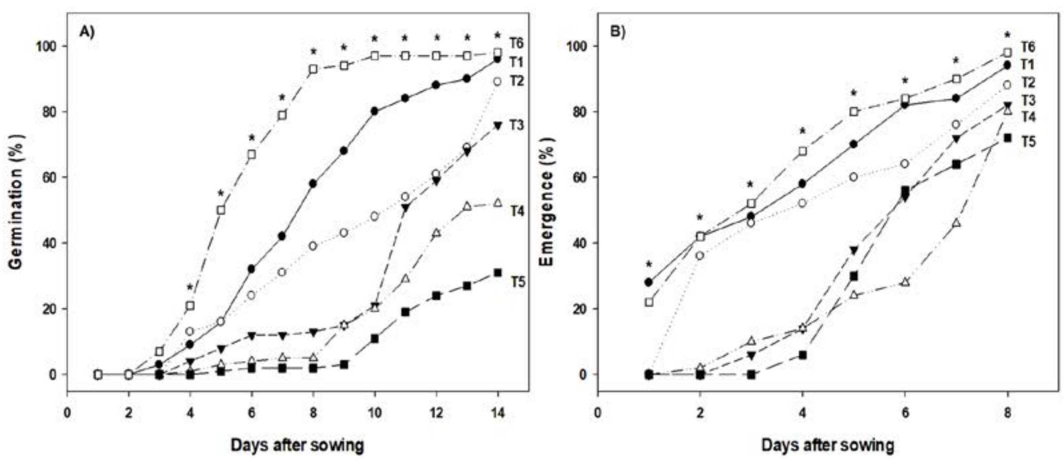

Figure 1. A: seed germination dynamics and B: seedling emergence of Habanero pepper (*Capsicum chinense*) produced with pig farm wastewater dilutions T1: 20% wastewater + 80% tap water; T2: 40% wastewater + 60% tap water; T3: 60% wastewater + 40% tap water; T4: 80% wastewater + 20% tap water; T5: 100% wastewater; T6: 100% tap water (control). Data are means. * indicates significant statistical difference (Tukey, $p \leq 0.05$); n=100.

Highly significant differences were found in seed germination and plant emergence rates of habanero pepper seeds. In the GR, treatment T1 and control T6 recorded the highest values (18.86 and 10.19 seeds germinated per day), respectively 89% and 88% higher than T5, which obtained the lowest value (2.19 seeds germinated per day) (Table 2). Similarly, in ER, T1 and the control T6 (with 13.09 and 13.24 seedlings per day) statistically surpassed the rest of the treatments; closer behind was T2, which obtained 9.08 seedlings per day; whereas T3, T4, and T5 only were statistically similar to each other (4.26, 3.98, and 3.49 seedlings per day, respectively) (Table 2).

There was evidence that the dilutions of wastewater made it possible to enhance and homogenize seed germination and seedling emergence due to the presence of minerals in the wastewater. Xu & Kafkafi (2003) and Mori *et al*. (2012) mentioned that the presence and concentration of minerals in contact with the seeds, increase the activity of enzymes and metabolic reactions. Consequently, there is a greater availability of reserves and energy during the germination process, improving the viability and vigor of the seeds.

The aforementioned is in agreement with the homogenization of germination and emergence of seedlings in less time observed in this study, contrary to what was observed in treatments with a higher concentration of wastewater, which negatively influenced the percentages obtained (Table 2).

In growth variables, diluted wastewater significantly influenced by inducing plant growth. Control T6 (8.53 cm) and treatment T1 (7.78 cm) were superior to all others, T2 with 7.13 cm was closer. However, the high concentration of wastewater was not favorable for vegetative growth, as observed in T3, T4, and T5, which had the lowest values (respectively 6.59, 6.59, and 3.57, cm) (Table 3). In stem diameter, control T6 had the greatest thickness (2.07 mm), treatment T1 with 2.02 mm was statistically similar. The low concentration of wastewater that was present in that treatment increased stem thickness in seedlings by at least 74%. In contrast, a 100% concentration of wastewater decreased this variable (T5, 0.97 mm) by up to 66% (Table 3).

Table 2. Seed germination rate and seedling emergence rate of Habanero pepper (*Capsicum chinense*) watered with pig farming wastewater dilutions.

Treatments	GR (seeds per day)	ER (seedlings per day)
T1	18.86±0.025 a	13.09±0.32 a
T2	10.19±0.27 b	9.08±0.67 b
T3	6.38±0.35 c	4.26±0.71 c
T4	4.07±0.38 d, e	3.98±0.73 c
T5	2.19±0.80 e	3.49±1.07 c
T6 (Control)	20.63±0.22 a	13.24±0.27 a

T1: 20% wastewater + 80% tap water; T2: 40% wastewater + 60% tap water; T3: 60% wastewater + 40% tap water; T4: 80% wastewater + 20% tap water; T5: 100% wastewater; T6: 100% tap water (control). Data are means ± standard error. Different literals in the same column indicate significant statistical difference (Tukey, $p \leq 0.05$); $n=100$.

Table 3. Morphological responses of Habanero pepper (*Capsicum chinense*) seedlings produced with dilutions of pig farm wastewater.

Treatments	Plant height (cm)	Stem diameter (mm)	Root length (cm)
T1	7.78±0.08 a	2.02±0.02 ab	6.04±0.27 a
T2	7.13±0.08 b	1.90±0.02 bc	5.23±0.20 b
T3	6.59±0.06 c	1.84±0.02 cd	4.19±0.24 c
T4	6.59±0.10 c	1.73±0.02 d	3.30±0.16 d
T5	3.57±0.32 d	0.97±0.09 e	1.47±0.09 e
T6 (Control)	8.53±0.06 a	2.07±0.01 a	6.33±0.20 a

T1: 20% wastewater + 80% tap water; T2: 40% wastewater + 60% tap water; T3: 60% wastewater + 40% tap water; T4: 80% wastewater + 20% tap water; T5: 100% wastewater; T6: 100% tap water (control). Data are means ± standard error. Different letters in the same column indicate significant statistical difference (Tukey, $p \leq 0.05$).

In root length, the presence of minerals in the wastewater favored greater root growth as observed in treatments T1 with 6.04 cm and the control (T6, 6.33 cm) (Table 3). The wastewater allowed greater root growth which increased the height of the seedlings and improved stem development; as a consequence, seedlings have a greater probability of survival in the field after transplantation. In this regard, Villar-Montero *et al.* (2004) indicated that the presence of minerals such as N, P and K in interaction with the seedlings influences quality and tolerance because of a greater amount of reserves and elements available to survive the transplant. Mineral composition of the wastewater with the prepared dilutions promoted vegetative growth and development of seedlings because they had mineral elements available for assimilation.

Likewise, Gorbe and Calatayud (2010) and Torres-García *et al.* (2019) mentioned that N and P are essential during plant growth due to their role in the various photosynthetic activities and energy transfer in metabolic processes. Higher available energy is expressed in plant growth, as increases in height, stem diameter, root length, number of leaves, among others. Overall, an inversely proportional relationship was observed in regard to seed viability and the concentration of pig farm wastewater. That is, a high concentration of pig farm wastewater decreased the germination capacity of the seeds, whereas a low concentration of it (pig farm wastewater in dilution) increased seed germination capacity and seedling emergence (Figure 2).

CONCLUSIONS

Pig farm wastewater contains a higher amount of chemical elements. In contrast, wastewater dilutions decreased mineral content and increased seed attributes. Treated pig farm wastewater (100%) decreased seed viability and vigor. In contrast, treatments with wastewater dilutions increased and enhanced seed germination and seedling emergence. Wastewater dilutions influenced seedling morphology by increasing height, stem diameter, and root length. Therefore, pig farm wastewater diluted is a viable alternative that modifies and enhances seed physiological quality, and improves seedling production of Habanero pepper in a shorter time.

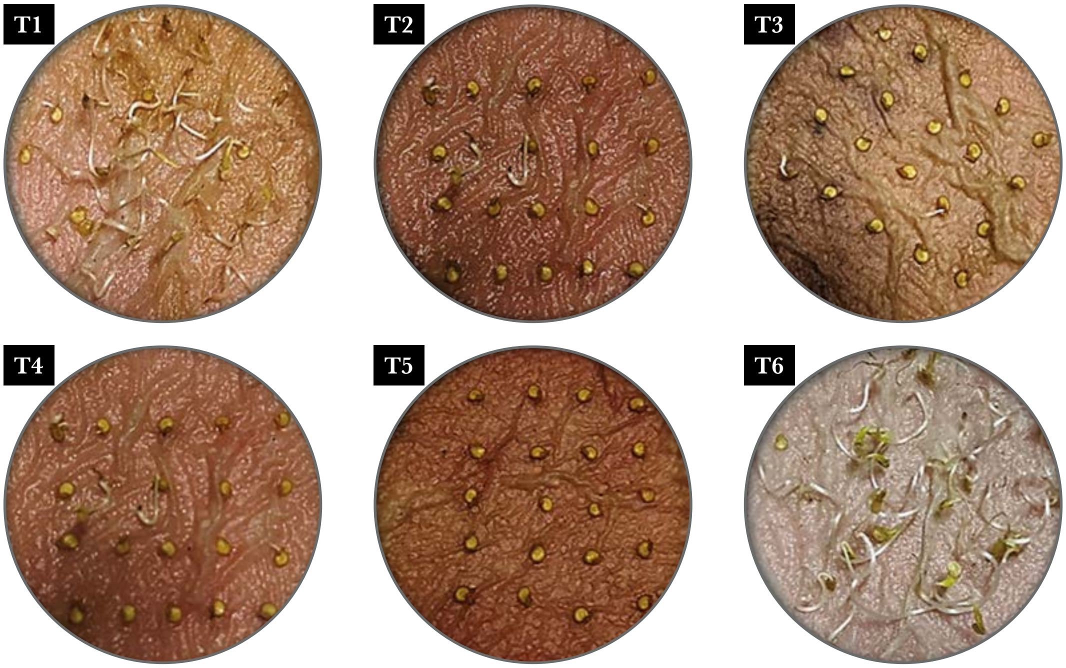

Figure 2. Impact of treated pig farm wastewater on the physiological quality of Habanero pepper (*Capsicum chinense*) seeds. T1: 20% wastewater + 80% tap water; T2: 40% wastewater + 60% tap water; T3: 60% wastewater + 40% tap water; T4: 80% wastewater + 20% tap water; T5: 100% wastewater; T6: 100% tap water (control).

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