Short Research Article

Phytotoxic Effect of Treated Wastewater on Seed Germination and Percent Root Elongation Inhibition in Some Vegetable Crops

Abstract

Aims: Summer irrigations for citrus fruits and fodder are considered as pre-irrigations for growing annual crops in Tunisia. However, given the quality of this treated wastewater, pre-irrigation effect on the seed germination capacity remains unclear. We propose to study the effect of different concentrations of treated wastewater on the germination of some vegetables.

Methodology: This laboratory experiment investigates the effect of different concentration from 0 to 100% (0%, 20%,40%, 60%, 80% and 100%) of treated wastewater on seed germination (%), germination index (Ig), root elongation and root elongation inhibition index (REI%) for some vegetable crops, the most commonly used by farmers: Radish, Okra and Pea.

Results: Pea germination rate (%G) dropped significantly from 60% to 30% with WW concentration. The germination index (Ig) decreased for Radish and Pea seeds with increasing WW concentration; it decreases from 1.03 to 0.72 and from 0.8 to 0.33 for radish and pea, respectively. While, the sprouting index for okra increased from 1.24 to 2.2 with increasing WW concentration. According to the REI% values, there is no effect of WW concentration on the root elongation of okra. On the other hand, the most pronounced REI% was noticed for Pea which has shown positive REI% values for all the concentrations with an inhibition effect of about 67% for the 100% WW treatment. For the radish, the root elongation inhibition started from the 40% WW concentration.

Conclusion: The crops tested have been arranged in the following order: Okra>Radish>Pea depending on the tolerance to treated wastewater. We conclude that the effect of treated wastewater on seed germination is depending on crops species and we should take care before using the treated wastewater for pre-sowing irrigation purposes

Keywords— Treated wastewater, germination percentage (%G), germination index (Ig), Root elongation inhibition (REI%).

1. INTRODUCTION

Water is the essential natural resource. Despite its presence in large quantities on land in the form of salt water, fresh water accessible and usable by humans is much rarer. Indeed, it represents only 0.001% of the hydrosphere (Water Information Center, 2013). In countries where water stress is a permanent problem, the question of the reuse of treated wastewater was quickly addressed. Therefore, use of wastewater is one of the many options being considered as a source of water in arid and semi-arid regions. The growing demand of water for irrigation has resulted in a marked increase in the use of treated or untreated wastewater worldwide (Ruma and Sheih, 2010). The pioneering nations in terms of reuse are for many of them on the Mediterranean rim like Tunisia, Israel and Spain. The production of reusable water today reaches about 20 million m3 / day. However, countries are not on an equal footing. In fact, in 2006, while Spain reused 347 million m3 / year of treated wastewater and Italy 233 million m3 / year (EUWI Med, 2007), only 62 million m3 / year was reused in Tunisia in 2017 (ONAS 2017 report). This low rate of reuse registered in Tunisia, is the consequence of several factors. The most important one is the poor quality of treated waste water which according to the ONAS report (2016), the rate of treated wastewater complies with the standards is only 60% and also, the prohibition by law irrigation of market gardening with high added value for the farmer. In this context, Tunisia is the first state that benefited in 2009 from an effective financing of the European Commission's Investment for Neighborhood (NIF), which aims to promote European co-financing, in this case between the German KfW and AFD. The program (2009-2015) is also part of the "Horizon 2020" initiative, which aims to clean up the Mediterranean. The program aims to renovate and expand the purification capacity of about ten wastewater treatment plants and pumping stations in 11 of Tunisia's 24 governorates. After treatment in wastewater treatment plants, wastewater can be further treated with more specific treatments, known as tertiary treatment, which will improve the quality of treated wastewater, in line with Tunisian rejection standards, similar to the main ones international standards in this area. Treated water from some treatment plants may be reused in the future for non-restrictive use. Thus, the volume of water that has benefited from tertiary treatment has increased from 17.8Mm3 in 2016 to 26Mm3 in 2017. Volume is expected to increase with the entry into operation of the ten scheduled stations. The use of such treated waste water in irrigation system provide some nutriments to enhance the fertility of soil but also deposit toxicants that change soil properties in the long run. This necessitates a detailed scientific study before any specific waste can be used for irrigation for a particular crop and environmental conditions.

Since different crop species may have different tolerance to various pollutants. Seed germination and plant growth bioassays are the most common techniques used to evaluate phytotoxicity (Kapanen and Itavara, 2001). Present study was designed for screening the suitability of different concentration of treated wastewater on seed germination and seedling growth in some vegetable crops; okra (*Abelmoscus Esculentus L*.), radish (*Raphanus sativus*) and pea (*Pisum sativum L*.).

2. MATERIALS AND METHODS

2.1.1 Experimental Design

The experiment was conducted in the laboratory of the Agricultural Experimentation Unit (Nabeul) belonging to the National Research Institute in Rural, Water and Forest Engineering. The effect of different concentration of treated wastewater (WW) on the initial growth parameters such as germination percentage, germination index, root length and root length inhibition index were studied using sterilized glass petri dishes (10 cm diameter), lined at the bottom with two layers of sterilized cotton. The cotton beds were then moistened with 10ml of distilled water for control and with the same quantity of various concentrations of WW (20% WW, 40% WW, 60% WW, 80% WW and 100% WW). The different concentrations of WW were prepared by adding a well-defined volume of distilled water to a known volume of WW (for example: for the 20% WW treatment, 20ml of WW is mixed with 80ml of distilled water to obtain a final volume of 100ml). There were three replications for each treatment and for each variety of seed (Okra, Radish and Pea); in all 54 glass Petri dishes were arranged. Then, petri dishes were placed in growth chamber at 25°C for 5 days in the dark.

2.1.2 Effluent Collection and Analysis

Treated wastewater (WW) used in the present study was collected from outlet in pre-cleaned, sterilized plastic containers directly at a wastewater terminal situated in the Agricultural

Experiment Unit of Oued Souhil – Nabeul located in the North-Est of Tunisia at 36°27'627'' North and 10°42'400'' Est. In the laboratory, water samples were filtered through Whatman paper No.1 and kept at 25°C. The various physico-Chemical properties were analyzed using standard methods. The treated Wastewater (WW) has a pH of 7.6 and a salinity of 3.1 ms / cm and contained appreciable amount of plant nutrients (Table 1).

Table 1: physico-Chemical quality of treated wastewater

Parameters	Treated wastewater	‡NT 106.03 Tunisian standard
pH	7.6	6.5-8.5
Ce (ms /cm)	3.1	7
	mg/l	
Sodium	807	-
Chloride	756	2000
Potassium	59	-
Calcium	122	
Magnesium	76	
Bicarbonate	68.34	-
Ammonia nitrogen	41.4	-
Phosphorus	3.5	-
sulfates	379	-
BOD5	34	30
COD	98	90
SM	17	30
	ppm	
Cadmium	0.009	0.01
Cobalt	0.029	0.1
Chromium	0.027	0.1
Copper	0.006	0.5
Manganese	0.04	0.5
Iron	0.217	5
Nikel	0.028	0.2
Zinc	0.03	5
Lead	0.043	1

2.1.3 Collection of Seeds

Commercially available seeds of okra (*Abelmoscus Esculentus L.*), radish (*Raphanus sativus*) and pea (*Pisum sativum L.*) were procured from the certified local seed supplier (Regional Agricultural Development Center-Nabeul), used in the study. Seeds with uniform size, color and weight were chosen for the experimental purpose (Fig 1).

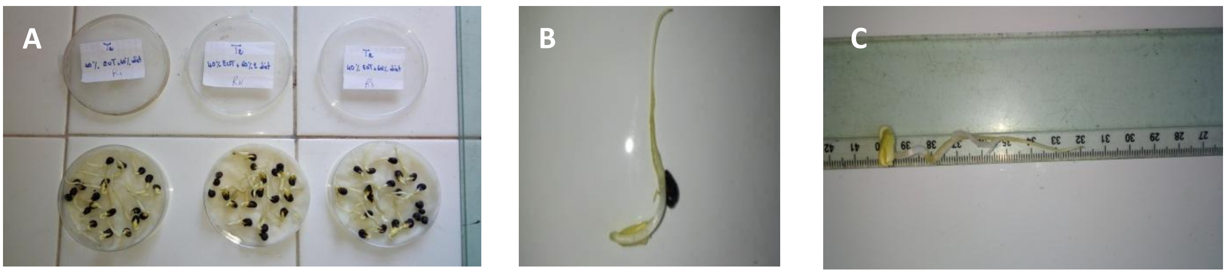


Figure 1: Germination photo for okra: A (the three repetitions at the beginning of germination), B (germinated okra seed with radicle and coleoptile), C (measurement of root length with a flat ruler)

2.1.4 Course of the Test

For the germination test, twenty healthy and equally sized seeds from each of the three varieties (Okra, Radish and Pea) were surface sterilized by dipping in ethyl alcohol for 1min and then washed with distilled water for many times. Then the seeds were incubated for two hours with distilled water for the control treatment and with the different concentrations of WW for each respective treatment. After that, they were dried on sterile filter paper. Seeds of each variety were placed in sterilized glass Petri dishes on a sterilized cotton bed, giving proper spacing

2.1.5 Measurements Collected

To assess the effect of the different concentrations of WW on the germination power we looked the visible protrusion of radical from the seed as criterion of germination as adopted by Ramana and *al.* (2002). Germinated seeds were counted each day for 5 days at the same time and percent germination was calculated as proposed by Kamlesh (2016) and expressed in percentage according to the formula:

$$\%G = \frac{n \ x \ 100}{N}$$

Where n = number of seedlings emerging during the experiment
N = total number of seed tested.

For each treatment and for each variety of seed, the cotton of each Petri dish was impregnated with the respective solution to facilitate the removal of the roots without the damaged ones. After determining the number of seeds that have sprouted for each variety, the length of the roots is measured using a rule to the nearest millimeter. In order to provide an integrative interpretation of the measurements, seed germination and root elongation were combined into an index of germination (Ig) as described by Ezenwa and *al.* (2017) according to the following equation

$$\text{Ig} = \frac{Gt \ x \ Lt}{Gc \ x \ Lc}$$

Where Gt is the number of germinated seeds
Lt is the corresponding root length mean in the WW treatments
Gc and Lc are the corresponding values for the distilled water.

Consequently, percent root elongation inhibition could be calculated as described by Zoghlemi and *al.* (2016) as follows: $\text{REI} = (1 - Ig)x100$. This index allows us to have an integrated approach to the phytotoxicity of the different concentration of WW compared to a distilled water sample taken as a control.

3. RESULTS AND DISCUSSION

3.1 Effect of WW Concentration on Early Germination

The early germination, which is the germination rate measured after 24 hours, shows a significant variation in early germination depending on the species. The lowest early germination is recorded for the pea with no germination during the first 24 hours (Fig 2).

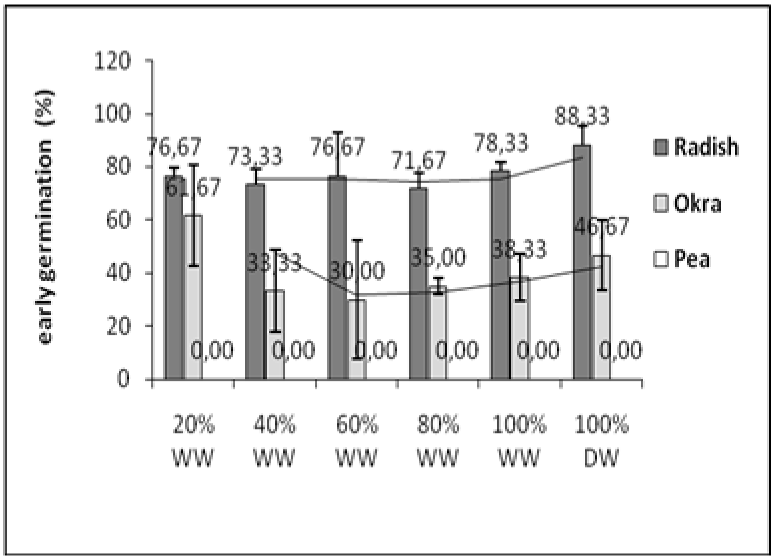


Figure 2: Early germination of okra, radish and pea seeds according to the concentration of WW.

This delay of germination is either explained by a longer imbibition's time for these seeds, especially since they are good dried, resulting in a slow germination or by a depressive effect of the inorganic salts contained in the WW which would have led to a rise in the osmotic pressure which according to Chopra et *al.* (2013) leads to the inhibition of germination. Accordingly, Benidire et *al.* (2015) showed that salinity has a negative impact on the germination and early seedling growth of faba bean (*Vicia faba L.*). Otherwise, some researcher related this delayed germination directly with the presence of higher salt/metal concentrations (Baruah et Das, 1997). Whereas, according to Leila Radhouene (2008), salt has little effect on germination rate and coleoptile emergence. However, this effect is more significant for radicular growth and between pear millet ecotypes (*Pennisetum glaucum* (L.) *R. Br*.). Similar results on the precocity of okra germination carried out in the study setting by Menouar Mohammed (2015) showed a depressive effect of the increase of salinity on sprouted seeds. Regarding the other seeds, it is noted that the radish has a better early germination than okra irrespective of the concentration of WW and with a higher germination in distilled water (88.3%) than in all WW treatments. However, for okra, it appears that treatment with a low concentration of WW (20% WW) allows for the highest early germination 61.7% against only 46.67% in distilled water. Apart from this treatment (20% WW) although the increase in the concentration of treated wastewater negatively affects the germination of okra seeds it has no effect on radish. These results are consistent with the conclusions of Calisir et *al.* (2005), who report a decrease in okra germination with increasing the concentration of treated wastewater from 0 to 100%.

3.2 Effect of WW Concentration on Germination Rate

The germination rate of the different seeds was monitored for five days and presented in Table 2. The results show that the germination rate is different from one species to another. In a similar study on germination, Ezenwa Ngozi et *al.* (2017) also noted that the percentage of germinated seeds varies for different seeds tested with better germination of sorghum seeds (*S. saccarhatum*) compared to corn, tomato and apricot. The lowest germination rate was noticed for peas that did not reach 100% regardless of the concentration of WW.

Table 2: Variation of the germination rate (%G) as a function of treated wastewater concentration

Crops	Treatment	% G At different time				
		24h	48h	72h	96h	120h
Okra	20% WW	61.7	98.3	100	100	100
	40% WW	33.3	98.3	100	100	100
	60% WW	30.0	98.3	100	100	100
	80% WW	35.0	100	100	100	100
	100% WW	38.3	100	100	100	100
	100% DW	46.7	80	86.7	91.7	91.7
Radish	20% WW	76.7	100	100	100	100
	40% WW	73.3	100	100	100	100
	60% WW	76.7	100	100	100	100
	80% WW	71.7	98.3	100	100	100
	100% WW	78.3	98.3	100	100	100
	100% DW	88.3	98.3	98.3	100	100
Pea	20% WW	0	25.0	46.6	56.6	60.0
	40% WW	0	35.0	51.7	55.0	56.7
	60% WW	0	28.3	46.7	50.0	51.7
	80% WW	0	35.0	55.0	60.0	61.7
	100% WW	0	20.0	28.3	30.0	30.0
	100% DW	0	81.7	81.7	83.3	83.3

A depressive effect of the WW was clearly noted on pea germination with treatment at high concentration of treated wastewater (100%) which yielded only 30% seed germinated after five days compared to distilled water which allowed a rate of 83.3%. This reduction in germination with high concentration of WW was attributed by Chopra et al. (2013), to the high concentration of inorganic salt and therefore the high electrical conductivity of water that would have generated a high osmotic pressure, and therefore an inhibition of germination. Our results are consistent with previous studies (Calisir et al. 2005; Chopra et al. 2013) who also observed a decrease in seed germination with increase in the concentration of WW. Further, the osmotic pressures of the wastewater also increased at higher concentrations. Rodger at al. (1957) cited by Ramana et al. (2002) reported that high osmotic pressures of the germination solution make imbibition more difficult and retard germination, while the ability of seeds to germinate under high osmotic pressure differs with variety as well as species.

However, it appears that the WW concentration did not affect the germination of okra even for the 100% WW treatment that reached 100% seed germinated after 48 hours. Paradoxically we can even assume a positive effect of the WW concentration on the germination of okra seeds which reached 100% germination with 80% EUT treatment after 48h with a maximum of germination in distilled water of 91.7% recorded after 96h. This is contrary to the results put forward by Kumar et *al.* (2017), who found a negative correlation between the germination rate of okra seeds and the different concentrations of treated wastewater, certainly in relation to the high electrical conductivity (12.2 ds/m) of the treated wastewater used in their work. Furthermore, it appears from Table 2, that WW concentration has a negative impact on the germination of radish seeds, especially for 80 and 100% WW treatments after 48 hours, unlike okra seeds which have reached 100% germination with 80% and 100% WW treatments after 48h.

3.3 Germination Index

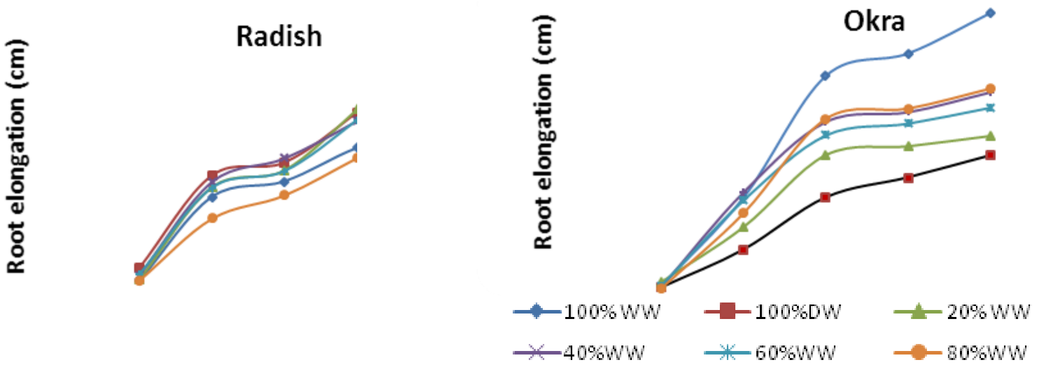
The germination index allows us to have an integrated approach to the phytotoxicity of treated wastewater compared to distilled water (Table 3). The germination index decreases with increasing the concentration of WW, this index decreases from 1.03 to 0.72 and from 0.8 to 0.33 for radish and pea, respectively. In contrast to radish and peas seeds, the sprouting index for okra calculated after 120 hours of germination increases with the concentration of treated wastewater from 1.24 to 2.2 from the low concentration to 100% EUT. These results corroborate with the previously reported results concerning the germination rate of the different seeds.

Table 3: Germination index (Ig)

% WW	**Ig=** (Gt x Lt) / (Gc x Lc)		
	Radish	**Okra**	**Pea**
20	**1.03**	1.24	0.80
40	0.89	1.59	0.93
60	0.96	1.47	0.59
80	0.74	1.61	0.78
100	0.72	**2.20**	**0.33**

3.4 Effect of WW Concentration on Root Elongation

The root elongation of the different cultures tested is shown in Fig 3.



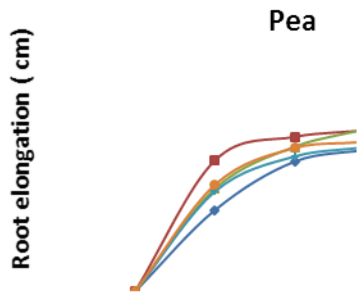


Figure 3: Effect of treated wastewater concentration on root elongation (cm) of Okra (*Abelmoscus esculentus L*.), Radish (*Raphanus Sativus*) and Pea (*Pisum sativum L.*) seeds

We note that the best root elongation was obtained with the treatment 100% DW for the pea and radish and then decreased in WW treatment with increasing WW concentration. Regarding okra, the opposite has been noticed. The root elongation of okra has been better with treatment with high WW concentration and then decreases with decreasing WW concentration. The lowest root elongation was noted for 100% DW treatment. It is likely that the fertilizer elements brought back by the treated wastewater contributed to the improvement of the root elongation observed in treated wastewater treatment. The determination of the root elongation inhibition index (%REI) for the different crops calculated after five days is shown in Table 4.

Table 4: Root elongation inhibition Index for different seeds as a function of the concentration of treated wastewater

% WW	% REI		
	Radish	Okra	Pea
20	-2.644	-24.22	20.397
40	10.658	-58.85	7.080
60	3.578	-46.51	41.273
80	25.844	-61.24	22.322
100	28.230	-120.17	66.795

According to the REI% values, there is no effect of treated wastewater concentration on the root elongation of Okra. On the other hand, the inhibition effect of the WW concentration is more pronounced in the pea which has shown positive REI% values for all the concentrations with an inhibition effect of the order of 67% for treatment at high concentration (100%).

Concerning the radish, an inhibition effect of the root elongation was noted from the WW concentration of 40%. These results are online with the germination index values which indicate a more marked effect of WW concentration on sprout inhibition in pea.

4. CONCLUSION

From the present study, it can be concluded that this work must be extended to other species to draw up a list of crops ranging from the most sensitive to the most tolerant to treated wastewater pre-irrigation. Further study could identify and monitor the impact of the elements brought by these waters on the crop germination such as the impact of trace metals or the effect of microbial and fungal load on germination. This work has shown that the response to the different concentration of treated wastewater is different from one species to another and that the bibliography is sometimes contradictory for the same species, all depends on the nature and composition of these waters whether they are urban or industrial. The use of the germination test is essential, especially in irrigation with unconventional waters loaded with solutes. It enables farmers to optimize their yield by guaranteeing maximum germination. Therefore, it is essential to take precaution as to the suitability of the species to be cultivated before pre-sowing irrigation with treated wastewater. As a result, secondary treated wastewater can be used as pre-irrigation but after appropriate dilution up to 60% and 20% for Radish and Pea, respectively.

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