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# WASTEWATER EFFECTS ON GERMİNATİON AND SEEDLİNG GROWTH OF TWO FORAGES, *TRİGONELLA FOENUM-GRACUM* AND *TRİTİCUM SECALE*

**N. Marzougui [1]**
[1] Researcher, Laboratory of Valorization of Unconventional Waters, Institut National de Recherches en Génie Rural eaux et Forêts, Ariana, Tunisia

**S. Rejeb [2]**
[2] Professor, Laboratory of Valorization of Unconventional waters, Institut National de Recherches en Génie Rural eaux et Forêts, Ariana, Tunisia

## ABSTRACT

*Given the water scarcity around the world many states adopted a water preservation policy through increasing efficiently the unconventional water reuse in agriculture. Here we studied the effects of Tunisian urban treated wastewater on germination and seedling growth of two forages seeds, Trigonella foenum-graecum (fenugreek) and Triticum secale (triticale). Germination was performed at 25°C for 7 days under light and darkness conditions. The seeds were irrigated with distilled water and well water (as controls) and raw and treated urban wastewaters. Seeds moisture content, shoot length, root length and lateral root number were measured. The statistical study was performed using R software. Quantitative parameters variability was assessed through a two factors ANOVA. Changes of shoot length was explained and predicted using a multiple linear regression analysis involving the other studied parameters. Results showed that irrigation water quality affected significantly fenugreek germination percentage, seeds moisture content and triticale germination index. Seeds incubation conditions affected significantly only triticale shoot length and had no effect on the other studied germination and seedling growth parameters of the two tested forages. Treated wastewater constitutes potential water for the irrigation of fenugreek and triticale.*

**KEYWORDS:** *Germination, R software, seedling growth, Trigonella foenum-greacum, Triticum secale, Tunisia, urban wastewater.*

## INTRODUCTION

Fresh water is fundamental for life, humans well being and the natural environment. This resource is becoming rarer meet the growing demands of the populations. The control of water resources in many arid and semi arid regions constituted a big challenge for long time due to water scarcity problems[1]. The United Nations estimates that 1.8 billion people will live in regions with water shortage by 2025, in addition population growth and climate change will continue to exert more stress on fresh water resources, and this occurs with great frequency and intensity, even in developed countries[2]. Faced with this growing problem, many countries such as Tunisia have introduced adaptation policies with two kinds of options, those focused on improving supply and those aimed at demand management. Improving supply passes through increased access to conventional water resources, reuse of unconventional water, transfers between basins, desalination and pollution control[3]. Recourse to reuse of treated wastewater began in Tunisia in the early sixties. This practice has become increasingly important in water resources management for both environmental and economic reasons. It was mainly applied in agriculture, but progressively, industrial, municipal and urban applications are being developed[4, 5]. Actually this country has 112 wastewater treatment plants with a water capacity of 300 million $m^3$ / year and producing, during the year 2015, 243 million $m^3$ of treated wastewater of which 60 million were reused; 33% of the reused water volume was intended for agricultural irrigation[4].

The quality of wastewater, in terms of its physico-chemical and biological characteristics, determines its environmental impacts and its effects on crops when it is reused in agriculture irrigation. Even after wastewater undergoes the purification processes, the treated effluent is not entirely free of undesirable constituents such as metallic trace elements (MTE) and pathogenic microorganisms[6]. For this some countries have developed their own standards for reuse. Tunisia is one of them; it has developed its standard NT 106.03 since 1989[7]. Reuse of wastewater for irrigation may have also many beneficial effects as it increases the organic matter content of the soil and have several advantages for crop production[8]. Taking into account the benefits and drawbacks of the irrigation with treated wastewater leads to consider that some crops are more prone to contamination by pathogens than others. For example, crops which the edible parts are exposed to the soil contaminated with irrigation water like leafy vegetables or root crops will be more prone to contamination with MTE and pathogens[9]. WHO in its guidelines for the safe use of wastewater in agriculture recommends restrictions for crops, especially those eaten raw[10]. So in several countries around the world, these waters are used mainly for irrigation of green areas, forage and industrial plants[11] and also for human food crops knowing that the degree of pre-application treatment is an important factor in the planning, design, and management of wastewater irrigation systems[12]. In Tunisia, the 1994 ordnance from the Ministry of Agriculture and Water Resources listed the crops that can be irrigated with treated effluents. These crops are industrial ones, cereals (wheat, barley and oat), fodder, fruit trees (date palms, citrus trees and vines) provided that they are not irrigated by spraying, forest trees, flowers and herbs[13]. Wastewater reuse in agricultural irrigation should be preceded by studying the environmental impact of this water. Germination is often used as a biological parameter when assessing the effects of environmental pollutants like urban effluents; especially as these waters are a potential alternative to fresh water in water shortage areas. Scientists showed that exposure to urban effluents had an inhibitory effect on the germination percentage of several plant species like *Solanum lycopersicum*, *Trifolium pratense*, *Triticum aestivum*, *Secale cereale* , *Pisum sativum*, *Trigonella foenum-graecum*, *Hordeum vulgare*, *Brassica juncea*, *Brassica napus*, *Coriandrum sativum*, *Nigella sativa*: irrigation with these waters delayed germination and led to reduce the fresh weight of seedlings[14, 15].

Here, we present the results of a study in which we evaluate the effects of irrigation with urban raw and treated wastewater on the germination and early growth of the seeds of two forages species, *Trigonella foenum-graecum* (fenugreek) and *Triticum secale* (triticale), pretreated differently and in different incubation conditions.

## MATERIALS AND METHODS

### Plant material and water samples

Fenugreek and triticale seeds used in this study belong to local varieties cultivated by farmers in Beja region in northwestern Tunisia. Irrigation water qualities used in the germination tests are distilled water (DW) used as control with pH = 7.07 and electrical conductivity = 0.38 m*S*/cm, well water (WW), raw (RUW) and secondary treated urban wastewater (TUW). Well water was taken from the region of Oued Souhil in Nabeul city. Urban wastewater was taken from the wastewater treatment plant of Hammamet city (activated sludge) with supply water of 90% domestic and 10% from tourism activity. Table 1 shows the characterization of the water qualities used for the germination tests.

**Table .1 Mean concentrations of physico-chemical parameters of the water samples used for germination tests.**

| Parameters \ Water samples | Well water | Raw urban wastewater | Secondary treated urban wastewater |
|---|---|---|---|
| pH | 8.08 | 7.01 | 7.12 |
| EC (m*S*/cm) | 4.6 | 2.83 | 2.77 |
| COD (mg/L) | NS | 425 | 45 |
| $BOD_5$ (mg/L) | NS | 139 | 7 |
| TSS (mg/L) | NS | 196 | 7 |
| $Na^+$ (mg/L) | 547.56 | 636.18 | 616.3 |
| $Mg^{2+}$ (mg/L) | 2.82 | 36.29 | 30.75 |
| $Ca^{2+}$ (mg/L) | 12.252 | 26.05 | 25.05 |
| $K^+$ (mg/L) | 25.76 | 26.1 | 24.5 |
| $NH_4^+$ (mg/L) | 3.26 | 37.03 | 21.78 |
| $Cl^-$ (mg/L) | 390 | 500 | 450 |
| $HCO_3^-$ (mg/L) | 504.9 | 1285.2 | 1101.6 |
| $Cd^{2+}$ (mg/L) | 0.005 | 0.006 | 0.006 |
| $Co^{2+}$ (mg/L) | 0.023 | 0.015 | 0.014 |
| $Cr^{2+}$ (mg/L) | 0.024 | 0.026 | 0.024 |
| $Cu^{2+}$ (mg/L) | 0.016 | 0.007 | 0.001 |
| $Fe^{3+}$ (mg/L) | 0.028 | 0.274 | 0.14 |
| $Mn^{2+}$ (mg/L) | 0.004 | 0.0084 | 0.01 |
| $Ni^{2+}$ (mg/L) | 0.032 | 0.024 | 0.022 |
| $Pb^{2+}$ (mg/L) | 0.036 | 0.038 | 0.037 |
| $Zn^{2+}$ (mg/L) | 0.042 | 3.55 | 3.35 |

EC: Electrical conductivity; COD: Chemical oxygen demand; $BOD_5$: Biochemical oxygen demand during 5 days; TSS: Total suspended solids.

## Germination and seedling growth studies

The water samples were filtered through Whatman paper No.1 (filter mesh= 11 µm) and kept at 25°C. The healthy and uniform seeds of each species (fenugreek and triticale) were selected and sterilized with a solution of $HgCl_2$ at 0.1% and thoroughly washed with sterilized distilled water to avoid surface contamination. Forty seeds were placed equidistantly on soaked filter paper in Petri dishes and were irrigated with equal volumes of the different water samples described above. The seeds irrigated with distilled water were taken as control. Six replicate were taken for each irrigation water quality. Then Petri dishes were placed in growth chamber (memmert, Germany) at 25°C for 7 days in the dark and for 7 days in the light. The seeds were considered germinated when their radicles had lengths of at least 5 mm (USEPA, 1992). Germinated seeds were counted and total germination was calculated and expressed in percentage according to the formula: G = [n x 100]\ N, where n= number of seedlings emerging in a certain time and N= total number of seedlings emerging during the experiment[16]. The mean time to germination (MTG) of the seeds was calculated as following: $MTG = \sum \frac{nd}{N}$, where n is the number of seeds that germinated between scoring intervals, d is the incubation period in days at that time point and N is the total number of seeds germinated in the treatment[17]. The root and shoot lengths, fresh weight and lateral root number were recorded after 7 days. Dry weight of seedlings was taken after keeping them in hot air oven at 80°C for 24 h. These data were used to calculate the moisture content[15] (MC) and the germination index[17] as following:

$$\text{Moisture content (MC) (mg)} = \text{fresh weight (mg)} - \text{dry weight (mg)}$$

and

$$\text{Germination index (GI)} = 100 \times \frac{Gs}{Gc} \times \frac{Ls}{Lc}$$

where Gs and Gc are the number of seeds germinated in the sample and the control, and Ls and Lc are the root lengths in the sample and the control, respectively; the germination index is measured as % of control.

## Statistical analyses

The studied statistical model included two qualitative variables (quality of irrigation water and incubation condition) considered as factors explaining the variations of the quantitative variables germination percentage, germination index, mean time to germination, root and shoot lengths, lateral root number and moisture content. The data normality of all studied variables was assessed through the Shapiro-Wilks test. A two classification factors ANOVA according to the quality of irrigation water and incubation condition was performed to compare variations of the quantitative parameters. All tests were done by version 3.3.2 of R software[18]. The strength of the relationship between quantitative variables was evaluated by Pearson correlation analysis.

## RESULTS AND DISCUSSION

### Effects of the water quality and incubation conditions on fenugreek germination and seedling growth

Results showed significant variations of fenugreek germination percentage according to irrigation water quality (ANOVA; $p < 0.05$) and no significant variations depending on incubation conditions ($p = 0.3$). *T. foenum-graecum* species presented high germinative capacity. The highest value of germination percentage (98.88%) was obtained during irrigation with TUW under light condition while the lowest value of this parameter (91.11%) was recorded when irrigating with WW under light condition (Fig.1). These high germination percentages detected for *T. foenum-graecum* species, where the seed reserves are located in the endosperm[19], were not consistent with the findings of Gardarin et al. (2016)[20] which confirmed that germination rates were faster for species with seed reserves located principally in the embryo (rather than the endosperm or perisperm). The findings of the experiment showed also that light has no effect on the fenugreek germination and this is not consistent with the subsequent work of scientists who considered light to be an agent inducing germination[21, 22]. Variations of the mean time to germination (MTG) parameter was not significant according to water quality (ANOVA, $p = 0.9$) and incubation conditions ($p = 0.5$). The highest value of MTG was 57.4 hours obtained when fenugreek seeds were irrigated with TUW after 72 hours of incubation under light condition; whereas the lowest MTG value (48.9 hours) was recorded with the seeds irrigated with DW after 48 hours of incubation under darkness condition (Fig. 1). The moisture content parameter had not significant variations according to the incubation conditions (ANOVA, $p = 0.32$) and significant variations depending on the irrigation water quality ($p < 0.05$). The highest value of moisture content (5.6 mg) was obtained during irrigation with RUW under darkness conditions and the lowest value (4.3 mg) was recorded when irrigating with TUW in light condition (Fig.1). These values are higher than the values of fenugreek seeds moisture content recorded by Huma et al. (2012)[15] which were 1.57 mg when the seeds are irrigated with distilled water, 1.22 mg for domestic wastewater and 1.04 mg with pharmaceutical industry waste water. Statistical analysis did not revealed significant variations of germination index (GI) parameter according to water quality (ANOVA, $p = 0.8$) and incubation conditions ($p = 0.1$). The highest GI value (107.8%) was observed when fenugreek seeds are irrigated with TUW under light conditions and the lowest value (67.9%) appeared with seeds irrigation by RUW in darknesst conditions. Germination index is considered as rapid tool to assess wastewater phytotoxicity[17] and showed a similar response to the plant growth experiments. This has been confirmed by our results since for the seedling growth parameters, shoot length varied from a minimum of 2.8 cm obtained by irrigation with RUW under darkness condition and a maximum of 8.09 cm with TUW under light conditions (Fig. 1). The root length varied from 2.5 cm following irrigation with WW under darkness conditions and 5.3 cm with TUW under light conditions (Fig. 1). During the germination tests the fenugreek seeds did not develop lateral roots even by varying irrigation water and incubation conditions during the experiment (Fig. 1). Shoot and root lengths varied significantly according to the incubation conditions ($p < 0.05$) and not according to the irrigation water quality (ANOVA, $p > 0.05$). While in other works, it has been shown that treated wastewater has a toxic effect inhibiting seed growth of rice[23].

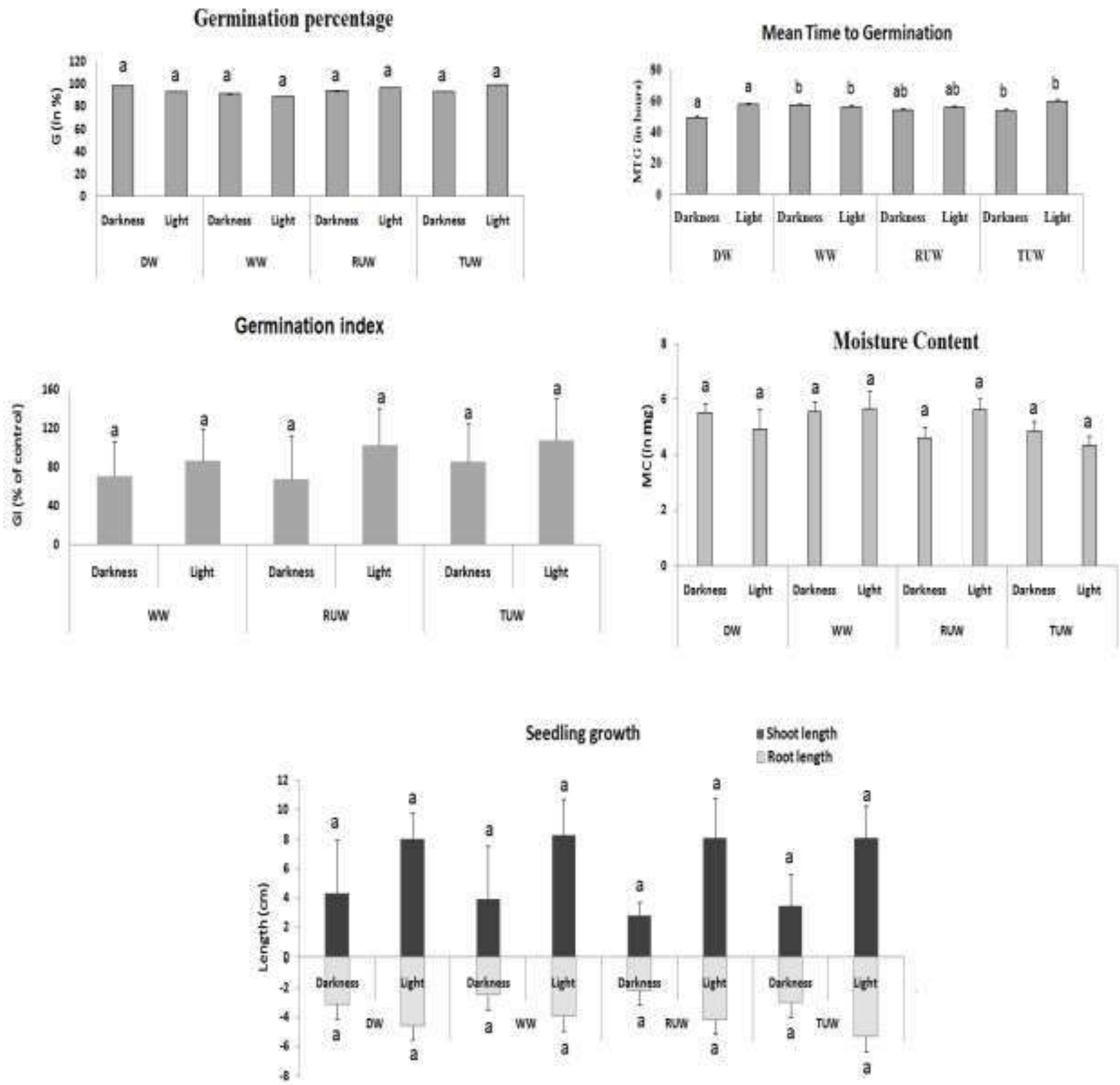


**Fig. 1 Variations of Fenugreek Germination percentage, Mean time to germination, germination index, moisture content and seedling growth parameters according to irrigation water quality and incubation conditions.**

Seedling growth includes the parameters shoot and root lengths and lateral root number. DW: distilled water, WW: well water, RUW: raw urban wastewater, TUW: treated urban wastewater. Means (±S.E.)followed by the same letter are not significantly different at the $P < 0.05$ level, as determined by Tukey's HSD test.

In an attempt to develop a rapid assessment tool, correlations test has been made between seed germination and seedling growth parameters[17]. While germination and vegetative growth involve different physiological processes[24], the seedling growth experiment incorporated the germination stage, and therefore may be considered to be linked to the germination experiments. There was positive significant correlation between fenugreek seeds germination and shoot length and between shoot length and root length.

### Effects of the water quality and incubation conditions on triticale germination and seedling growth

Results showed that the highest value of germination percentage (97.77%) was obtained with TUW irrigation under light condition and the lowest value (93.88%) was showed with WW irrigation under light condition (Fig.2). Germination percentage of triticale seeds presented no significant variations according to irrigation water quality (ANOVA; $p$ = 0.8) and incubation conditions ($p$ = 0.4) factors. For MTG parameter, variations depending on irrigation water quality and incubation conditions were not significant ($p$ = 0.18 and $p$ = 0.09, respectively). Irrigation with TUW under light condition allowed obtaining the highest MTG (48.8 hours) while the triticale seeds irrigated with WW under light condition presented the lowest MTG (47.82 hours) (Fig.2). The moisture content (MC) did have not significant variations according to the considered factors irrigation water quality and incubation conditions. The maximum value of seeds MC (6.9 mg) was detected with TUW irrigation in light condition and the minimum (2.6 mg) when triticale seeds were irrigated with RUW in darkness (Fig.2). Germination index (GI) of triticale seeds showed significant variation according to irrigation water quality (ANOVA; $p < 0.05$) and no significant variations depending on incubation conditions ($p$ = 0.7). The highest GI value (186.2%) was detected after irrigation with TUW under light condition and the lowest (53.9%) with DW under darkness condition. Concerning seedling growth parameters, shoot length varied from a minimum of 2.25 cm obtained with DW under darkness condition and a maximum of 9.99 cm with TUW and WW under light conditions (Fig. 2). The maximum root length (11.8 cm) was attempt following irrigation with RUW under light conditions and the minimum (3.23 cm) with TUW under darkness conditions (Fig. 2). Triticale seeds developed lateral roots during the germination tests; their number varied from a minimum of 4 lateral roots when the seeds were irrigated with WW, RUW and TUW under darkness conditions (Fig. 2) and a maximum of 5 lateral roots with TUW in light condition. All seedling growth parameters had no significant variations (ANOVA, $p$ > 0.05) according to irrigation water quality and incubation conditions factors except for shoot length which varied significantly according to the incubation conditions ($p < 0.05$). In contrast, Shah el al. (2004 and 2005) considered that wastewater have better effect than ground water on triticale growth, leaf NPK, net assimilation rate, yield and quality[25, 26].

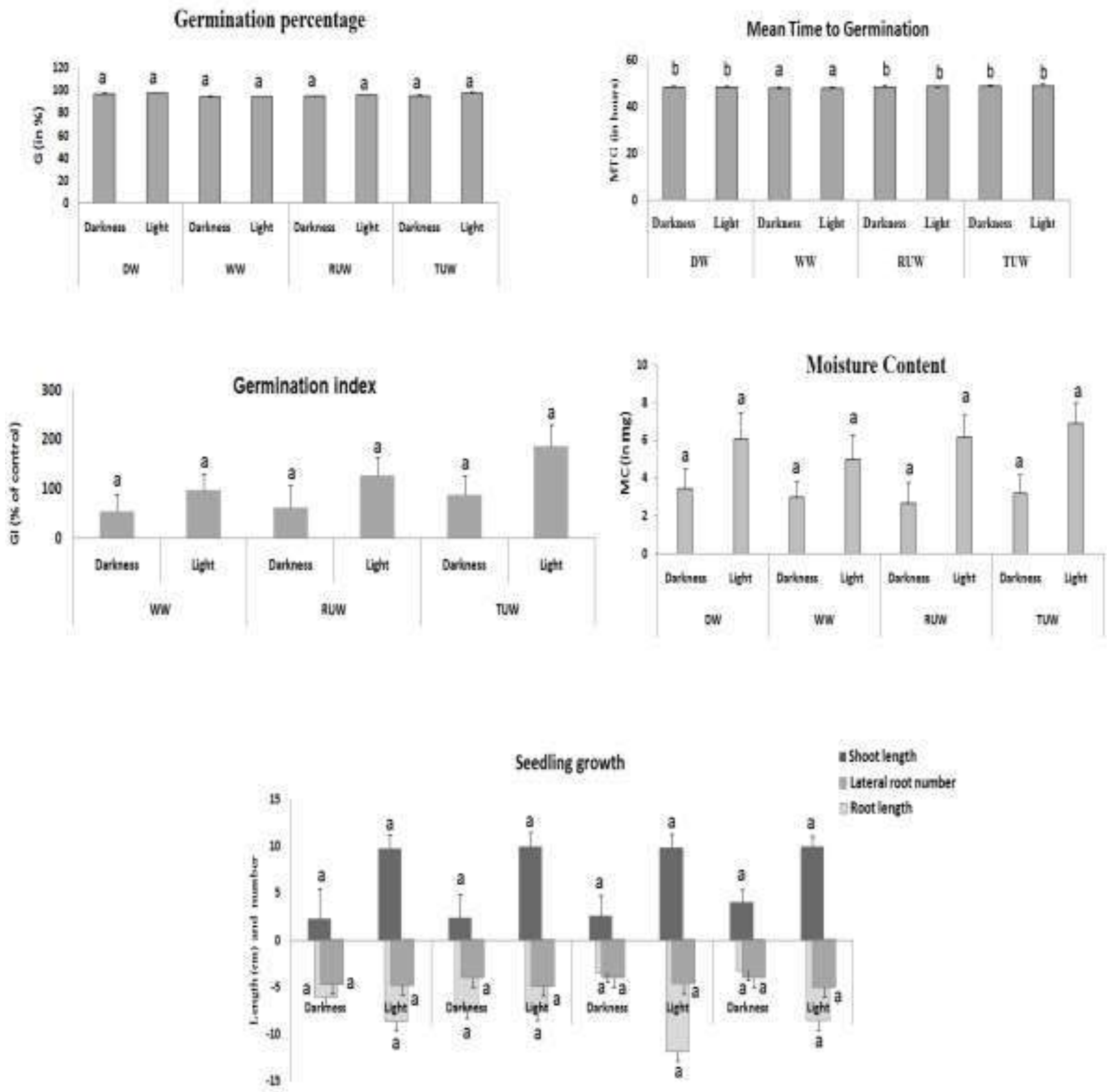


**Fig. 2 Variations of triticale Germination percentage, Mean time to germination, germination index, moisture content and seedling growth parameters according to irrigation water quality and incubation conditions.**

Seedling growth includes the parameters shoot and root lengths and lateral root number. DW: distilled water, WW: well water, RUW: raw urban wastewater, TUW: treated urban wastewater. Means (±S.E.) followed by the same letter are not significantly different at the $P < 0.05$ level, as determined by Tukey's HSD test.

In the case of our study, TUW used for seeds irrigation was in accordance with the standardized levels for treated wastewater quality found in Tunisian regulations NT 106.03 governing the agricultural reuse of treated wastewater[27]. In addition there are no great differences in the physico-chemical characteristics between the wastewaters and well water used in the germination tests (Table 1), that's why there were no significant variations according to irrigation water quality for most studied parameters.

Pearson correlation test presented significant correlations between germination percentage and root length, shoot length and root length, and finally between root length and lateral root number. The correlation between the different plant organs such as shoot and roots has been approved by several authors indicating that these organs are interdependent[28, 29] and adjustments in root and shoot growth are often assumed to be a fundamental facet of a plant's phenotypic plasticity in response to its environment[29].

## CONCLUSION

The crossing of different experimental conditions namely seeds incubation condition and water quality (distilled water used as control for comparison, well water, raw and treated urban wastewaters) used for irrigation, adopted to study the fenugreek and triticale germination and seedling growth, showed that fenugreek germination percentage and seeds moisture content varied significantly according to irrigation water quality but variations of the other fenugreek studied parameters were not significant depending on this factor. Triticale germination index showed significant variations according to water quality while variations of the other triticale parameters were not significant according to this factor. Incubation in light had no effect on the germination and seedling growth of the two studied forages, only triticale shoot length varied significantly according to the incubation condition. At this stage of early growth treated wastewater could be considered as potent water for fenugreek and triticale irrigation but this work should be completed by monitoring fenugreek and triticale behavior and their chemical compositions in the rest of growth stages.

### Conflict of Interest

The authors declare no conflicts of interest.

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