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EFFECT OF AGRO-INDUSTRIAL WASTEWATER TREATED WITH *Alcaligenes faecalis* BACTERIAL STRAIN ON THE GROWTH OF TWO CONDIMENTS *Capsicum annuum* L. Cv. Astra AND *Lycopersicum esculentum* L. Cv. Kristina

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Abstract

Water shortage has become global phenomenon including Pakistan which necessitates reuse of wastewater for agriculture. The objective of the study was to treat the agro-industrial wastewater with the bacterial strain *Alcaligenes faecalis* MT477813 so that it could be used for irrigation. Agro-industrial wastewater was examined for its physiochemical characteristics, including colour, odour, pH, temperature, electrical conductivity, turbidity, BOD, COD, TDS and decolourization %. *Lycopersicum esculentum* L. Cv. Kristina and *Capsicum annuum* L. Cv. Astra were used to test the influence of treated agro-industrial wastewater (TAIWW) and untreated agro-industrial wastewater (UAIWW) on seed germination. The germination parameters determined were based on the type of water treatments. Untreated agro-industrial wastewater germinated seedlings had an average fresh weight opposed to TAIWW seedlings. Untreated agro-industrial wastewater germinated seeds had a shorter average seedling length than seeds germinated with TAIWW. The average fresh weight of seedlings watered with UAIWW was lesser than the equivalent amount of TAIWW. These findings demonstrated that compared to seedlings irrigated with UAIWW, seedlings irrigated with TAIWW were longer and had higher FGP, MGT values, fresh and dry weights. This study supported the bio-treatment of wastewater prior to its usage for irrigation.

Key words: Water shortage, *Alcaligenes faecalis* MT477813, agro-industrial wastewater.

Introduction

Pakistan's changing climate, which includes increasing heat waves and floods is making the country's food insecurity problem worse (Ahmed *et al*., 2016). The lives and livelihood of Pakistan's underprivileged and impoverished groups are similarly impacted by these changing climatic circumstances (Qureshi *et al*., 2010). Reusing treated wastewater for irrigation has grown importance as a resource alternative to fulfil the expanding water needs of agriculture and to the environment and public health this reuse requires customized management. Untreated wastewater discharge into the environment can lead to contamination resources (Choudhary *et al*., 2020).

Several physiochemical processes, such as filtration, evaporation, chemical precipitation, oxidation, reduction and reverse osmosis, have been used to remove toxic metals from the environment in industrialized nations (Malik *et al*., 2019) but such projects need heavy investments. Micro-organisms such as bacteria and fungi from diverse sources including soil, water and sludge can be used to treat agro-industrial wastewater. The ability of isolated fungi and bacterial strains to break down particular pollutants can be determined (Mustafa *et al*., 2021). Thus, to minimize negative impacts, agro-industrial wastewater needs to be treated with the isolated bacterial isolates such as *A. faecales* MT477813 to make it suitable and safe for crop irrigation. The objective of the study was to evaluate the effects of treated and untreated wastewater on the growth of the selected varieties of tomato and sweet pepper.

Materials and Methods

Collection of wastewater samples: Agro-industrial wastewater sample was collected in sterilized water storage gallons from Bajwa Agro-industries Lahore, during the month of October from the point of discharge of drainage site and labelled as AIWW.

Physiochemical characterization of agro- industrial wastewater samples: The wastewater sample was first filtered at Plant Biotechnology Lab, Botany Department, GC University Lahore, to remove the impurities visible with naked eyes, after which the samples were stored in the sterilized jars or vessels and was headed for further physiochemical analysis. Following parameters of wastewater were studied before and after the treatment i.e., temperature, odour, pH, salinity, (COD), (OD), (BOD), (BI), (TDS), (TSS) by keeping in view the standards given by (APHA)

2005. The results obtained after physiochemical analyses were compared with the (NEQS).This process was repeated for the wastewater after its bio-treatment.

Decolorization Experiment: The potential of the bacterial isolate was determined with respect to the percentage of decolorization using a UV-Vis Spectrophotometer. An inoculation loop was used to remove the inoculum from the bacterial colony on the cultivated plate and add to 100 mL of distilled water. It needed to be 10% of the bacterial isolate (*A. faecalis*) concentration and 100 mL taken in a 250 mL conical flask for 48 hours at 37◦C, these flasks were placed in an incubator shaker at 120 rpm (Khan *et al*., 2021).The controls and treatments were all done in triplicate, and the treated wastewater was kept in 50 mL falcon tubes in the refrigerator at 4◦C.

Experimental design: This experiment included the seed germination and pot experiment. All the treatments were taken three times and complete randomized design was acquired.

Seed procurement: Two vegetables *Lycopersicum esculentum* (Tomato) Cv. Kristina and *Capsicum annuum* (Sweet pepper) Cv. Astra were selected for study and seeds were provided by Seed Certification Department, 4-Lyton Road Lahore.

***In vitro* seed germination:** Surface sterilized Tomato and sweet pepper seeds were added to Petri plates with double filter paper. Each plate contained 5 seeds, spaced equally apart. Three copies of each of the two plant seeds were created while they were being treated and under control. Autoclaved raw (filtered) of 25 ml agro-industrial wastewater were added to the first triplicate. Autoclaved agro- industrial wastewater with 25 mL of 50% (v/v) concentration were supplied to the second batch of triplicates, 25 mL of treated agro-industrial wastewater was administered to the third set of each variety of seed. As a control, a fourth set of each type of triplicate was given 25 mL of autoclaved pure water. The cling film was carefully wrapped around each Petri plate. They were put inside the growth room of the GC University in Lahore's botany department under regulated lighting settings with a constant 25°C temperature.

Observations of seed germination: Seed germination was monitored every day for 21 days, when their shoots began to emerge, they were considered to be germinating. The roots and branches of each type were measured. The shoot was measured in cm from the base to the tip of the longest leaf (Khan *et al*., 2016). From the base of the longest root shoot junction to the tip, the root length was measured in cm. When seedlings grew, the length of the seedling was calculated by summing the lengths of the roots and shoots in cm as well as the dry weights of the roots and shoots, which were dried in an oven at 65°C.

Pot experiments: Soil was collected from the Botanic Garden, GC University, Lahore. The seeds of Tomato and Sweet pepper were sown in the pots of medium size with the temperature of 26℃. The concentration of UAIWW was prepared with 50% distilled water to make 100 mL dilution. All the experiments were performed in triplicates. Each pot had twenty seeds. Distilled water was used as a control 250 ml. UAIWW filtered (autoclaved) was used in the comparison in addition to all the other treatments. The prepared treatments and control water was given to the plants on the daily basis to keep the moisture content on the soil. The length of the seedlings was observed for the next two weeks on daily basis. The seedling was considered to be germinating as the roots became visible and measurable. The length and weight of the germinated seedling was measured for each of the set of the experiment. From the base of the primary leaf to the base of the hypocotyl, the length of the shoot was measured in centimeters. From the tip of the primary root to the base of the hypocotyl, the root length was measured in centimeters. By combining the centimeter lengths of the root and shoot, the seedling's length was calculated. After drying the roots and shoots for 24 hours at 65°C in the oven, the dry weights were determined.

Statistical analysis: The data was statistically reviewed to assess the mean, SD and variance. The data was reported in tables and graphs and was stated as mean SD, variance and other statistics. The findings were shown as means SD. The concentrations of bio-treated agro-industrial wastewater were used as the factor in all statistical analyses of phyto-toxicity tests using one-way ANOVA, and the effects were the indices. Control was DW with each treatment, and the treated wastewater for each concentration level was compared with UAIWW, UAIWW50 and TAIWW.

RESULTS

4.1 Physiochemical analysis of wastewater sample: The sample of the wastewater collected had greenish grey colour, pungent smell. The values of most of the Physiochemical parameters were within the range of (NEQs, 2000). The wastewater became colourless and odourless after bio-treatment. There was a reduction in the actual values of the wastewater (Table 1).

Table 1. Physiochemical characterization of wastewater

Physiochemical parameters	NEQs	UAIWW	TAIWW
Temperature	<3°C	25°C	4°C
Colour	-	Greenish	Colourless
Odour	Tolerable	Pungent	Odourless
pH	6.6-8.5	7.90	7.70
EC (µs/cm)	-	756	433.3
Salinity (ppt)	-	0.3	0.50
Turbidity(NTU)	5	76.3	4.2
COD (mg/L)	155-1400	145	43
BOD (mg/L)	80-255	155	39
TDS (mg/L)	1000	735	640
TSS (mg/L)	<550	1200	290
BI	-	1.20	0.90

Note: Decolorization percentage of 90% has been noted after using the bacterial isolate.

UAIWW (Untreated Agro-industrial Wastewater), **TAIWW** (Treated Agro-industrial Wastewater)

4.2. Germination parameters observed for Sweet Pepper Cv. Astra and Tomato Cv. Kristina

The seeds of Sweet pepper Cv. Astra and Tomato Cv. Kristina were allowed to germinate for total 21 days (Aslam *et al.*, 2023). When the germination duration was completed following parameters of germination were observed. Speed of germination, final germination percentage and mean germination time were observed maximum with the Treated agro-industrial wastewater. DW: Distilled water UAIWW: Untreated agro-industrial wastewater TAIWW: Treated agro-industrialwastewater. Speed of germination, final germination percentage and mean germination time were observed maximum with the Treated agro-industrial wastewater (Dash *et al.*, 2012).

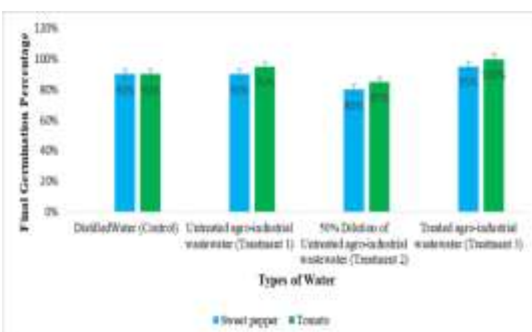

Fig. 1. Final germination percentage of Tomato Cv. Kristina and Sweet Pepper Cv. Astra against different wastewater treatments and control.

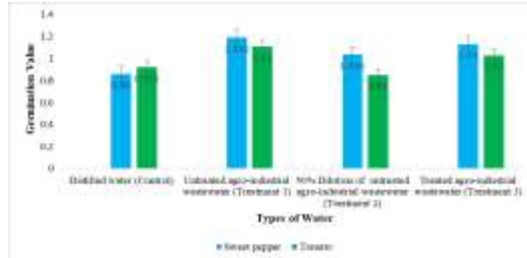

Fig. 2. Germination value of Tomato Cv. Kristina and Sweet Pepper Cv. Astra against agro-industrial wastewater treatments and control.

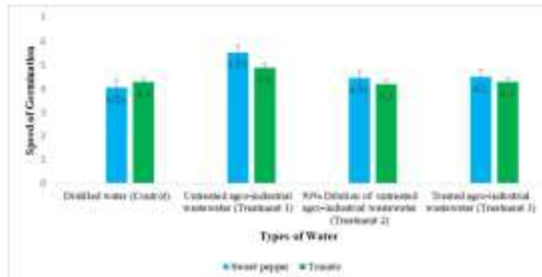

Fig. 3. Speed of germination of Tomato Cv. Kristina and Sweet Pepper Cv. Astra against different wastewater treatments and control.

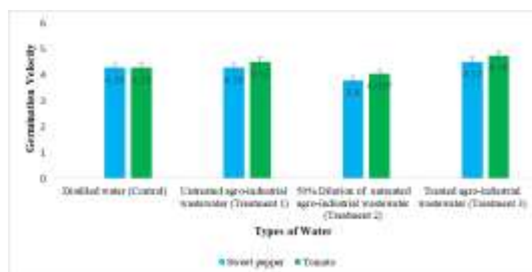

Fig. 4. Germination velocity of Tomato Cv. Kristina and Sweet Pepper Cv. Astra against different wastewater treatments and control.

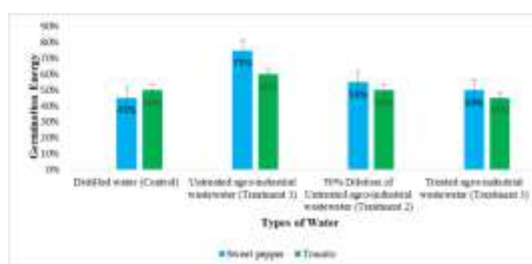

Fig. 5. Germination energy of Tomato Cv. Kristina and Sweet Pepper Cv. Astra against different wastewater treatments and control.

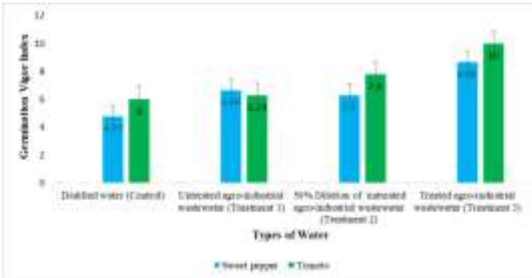

Fig. 6. Germination vigor index of Tomato Cv. Kristina and Sweet Pepper Cv. Astra against different wastewater treatments and control.

4.3. Morphological parameters of the two vegetables in different treatments

The morphological parameters of vegetable seedlings under different treatments and control were monitored for various types of water. For different water treatments the morphological parameters were clearly distinguishing from each other (Aslam *et al.*, 2023). The best outcomes were obtained from the bio-treated agro-industrial wastewater.

4.3.1. Morphological parameters observed for Sweet Pepper Cv. Astra

The seeds of Sweet Pepper Cv. Astra were allowed to grow for 21 days. There were three treatments and a control. The best outcomes were achieved through the bio-treated agro-industrial wastewater (Table 2).

Table 2. Morphological parameters observed for Sweet Pepper Cv. Astra.

Morphological parameters	**Types of water**			
	DW	**UAIWW**	**50% UAIWW**	**TAIWW**
Average seedling Length (cm)	5.423(±0.330, 0.109)	7.452(±0.010, 0.010)	7.68(±0.121, 0.0145)	9.114(±0.026, 0.00070)
Average shoot length (cm)	5.128(±0.540, 0.290)	6.296(±0.690, 0.480)	6.826(±0.431, 0.186)	7.134(±0.445, 0.198)
Average root length (cm)	0.714(±0.190, 0.036)	1.275(±0.390, 0.150)	1.064(±0.046, 0.020)	2.008 (±0.321, 0.102)
Average dry weight of seedling (g)	0.012(±0.009, 8.96E-7)	0.012(±0.0013 ,1.78E-6)	0.009(±0.00050, 2.59E-7)	0.022(±0.0019, 3.93E-6)
Average dry weight of shoot (g)	0.010(±0.008, 6.56E-7)	0.011(±0.0005, 2.49E-7)	0.008(±0.0020, 4.3E-6)	0.022(±0.0005, 3.25E-5)
Average dry weight of root (g)	0.0023(±0.07, 2.87E-6)	0.0012(±0.004, 1.42E-7)	0.0024(±0.005, 2.6E-57)	0.0027(±0.004, 2.14E-7)
Average fresh weight of seedling (g)	0.031(±0.012, 0.00015)	0.037(±0.0047, 2.26E-5)	0.057(±0.0074, 5.50E-5)	0.083(±0.0072, 5.208E-5)
Average fresh weight of shoot (g)	0.028(±0.053, 2.805E-5)	0.031(±0.008, 6.53E-5)	0.047(±0.0106, 0.00012)	0.07(±0.0174, 0.0003)
Average fresh weight of root (g)	0.017(±0.004, 1.55E-5)	0.012(±0.008, 7.12E-7)	0.018(±0.0108, 0.00012)	0.020(±0.0046, 2.113E-5)

4.3.2. Morphological parameters observed for Tomato Cv. Kristina

Tomato Cv. Kristina seeds were allowed to ripen for 21 days (Aslam *et al.*, 2023). Along with a control, there were three treatments. The bio-treated agro-industrial wastewater produced the best results (Table 3).

Table 3. Morphological parameters observed for Tomato Cv. Kristina.

Morphological Parameters	**Types of water**			
	DW	**UAIWW**	**50% UAIWW**	**TAIWW**
Average seedling length (cm)	5.46(±0.148, 0.022)	7.16(±0.613, 0.380)	8.32(±0.220, 0.048)	9.41(±0.590, 0.344)
Average shoot length (cm)	5.78(±0.417, 0.174)	4.76(±0.390, 0.152)	7.52(±0.604, 0.365)	7.86(±0.46, 0.208)
Average root Length (cm)	0.88(±0.037, 0.0013)	1.87(±0.065, 0.0041)	1.051(±0.106, 0.012)	2.130(±0.450, 0.202)

Average dry weight of seedling (g)	0.014(±0.0017, 2.86E-6)	0.011(±0.0010,1.19 E-6)	0.012(±0.00085,7.30 E-7)	0.015(±0.0022, 4.97E-6)
Average dry weight of shoot (g)	0.012(±0.0009, 9.12E-7)	0.010(±0.0008, 6.55E-7)	0.011(±0.0031, 9.06E-6)	0.012(±0.0015, 2.35E-6)
Average dry weight of root (g)	0.004(±0.0012,1.46)	0.002(±0.0015, 2.088E-6)	0.003(±0.0014, 1.94E-6)	0.005(±0.0034, 1.18E-5)
Average fresh weight of seedling	0.044(±0.006, 4.753E-5)	0.050(±0.00020, 3.90E-8)	0.040(±0.0058, 3.39E-5)	0.113(±0.0064, 4.13E-5)
Average fresh weight of shoot	0.047(±0.0070, 4.82E-5)	0.038(±0.089, 0.0080)	0.037(±0.0060, 3.591E-5)	0.092(±0.0071, 5.06E-5)
Average fresh weight of root	0.018(±0.0036, 1.3E-5)	0.024(±0.0053, 2.82E-5)	0.023(±0.0060, 3.54E-5)	0.035(±0.010, 0.00011)

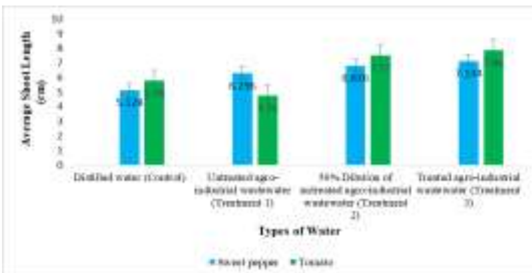

Fig. 7. Average shoot length of Tomato Cv. Kristina and Sweet Pepper Cv. Astra against different wastewater treatments and control.

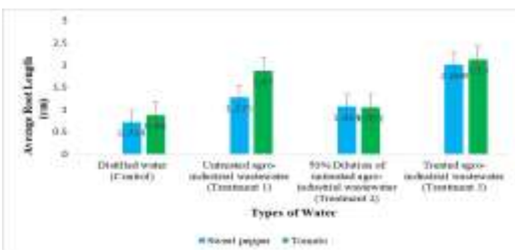

Fig. 8. Average root length of Tomato Cv. Kristina and Sweet Pepper Cv. Astra against different wastewater treatments and control.

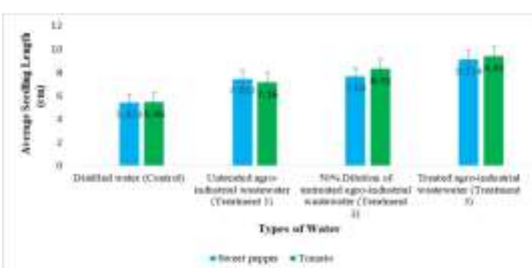

Fig. 9. Average seedling length of Tomato Cv. Kristina and Sweet Pepper Cv. Astra against different wastewater treatments and control.

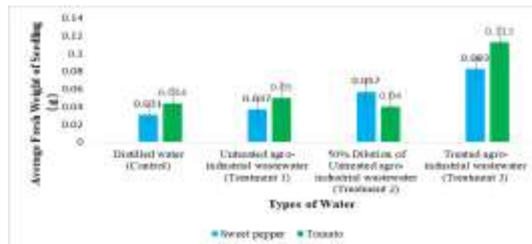

Fig. 10. Average fresh weight of Seedlings of Tomato Cv. Kristina and Sweet Pepper Cv. Astra against different wastewater treatments and control.

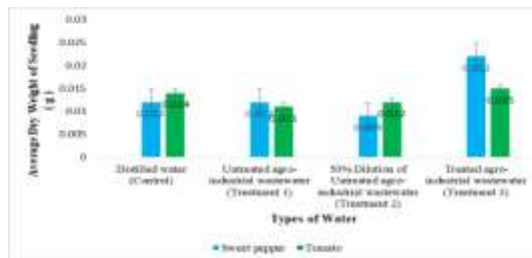

Fig. 11. Average dry weight of seedlings of Tomato Cv. Kristina and Sweet Pepper Cv. Astra against different treatments and control.

DISCUSSION

Pakistan is suffering a severe water shortage caused by a variety of issues such as changing climatic conditions, expanding population, a deficient irrigation system and rapid urbanization (Zhang *et al*., 2020). Roughly 32,500 acres of land is irrigated with wastewater, accounting for approximately 30% of total wastewater production (Ali *et al*., 2015). Direct use of wastewater causes health risks to animals. Present study was carried out to find out the effects of untreated agro-industrial wastewater and treated with the bacterial isolate for the seed germination and growth of the two selected vegetables.

According to previous studies, a BOD/COD ratio between 0.4 and 0.8 reflects a wastewater sample's biodegradability index and a range of 0.3 to 0.6 often suggests that these wastewaters are biodegradable (Rashid *et al*., 2020). A BOD/COD ratio more than 0.8 on the other hand, indicates that this wastewater sample requires biological treatment by bacterial isolate. In present work, treated agro-industrial wastewater received a significant reduction in pollutant concentrations after adequate treatment methods, making it safe for reuse (Xia *et al*., 2020) and the biodegradability index values of treated wastewater samples were within the range (Fito *et al*., 2019). This method is cost effective (Phugare, *et al.* 2011) as it does not require the investment of large water treatment plants that use excessive electricity, fuel and also chemical treatments.

The germination experiment of two vegetables *i.e.*, Tomato Cv. Kristina and Sweet pepper Cv. Astra were grown under controlled conditions for 21 days. The seeds of two vegetables *i.e.,* Tomato Cv. Kristina and Sweet Pepper Cv. Astra germinated best in the treated agro-industrial wastewater (Rashid, *et al*. 2020). The results for germination experiment and growth parameters were in the following order: Treated agro-industrial wastewater > Untreated agro-industrial wastewater > Distilled water > 50% Dilution of UAIWW (Shannag *et al*., 2021). Plant yields were predicted using biomass (the whole fresh weight of the plants). Treated agro-industrial wastewater irrigation produced significant changes in soil characteristics, according to a soil study. The study's noteworthy finding may be that TAIWW is a good candidate as a source of water and plant nutrients (El-Nahhal *et al*., 2013). The growth parameters of Tomato Cv. Kristina and Sweet Pepper Cv. Astra (Figs 1-11) were superior in TAIWW than the growth parameters of UAIWW (Dash *et al*., 2012). The relative SG, FGP and GVI values were greater in TAIWW than UAIWW. The average seedling lengths were longer and weight of both vegetables (Table 2 & 3) were heavier in TAIWW than the seedling lengths grown in UAIWW (Aslam *et al*., 2023).

CONCLUSION

The results of this study suggested that reuse of agro-industrial wastewater after treatment can help to reduce the shortage of water. This approach might be used to boost irrigation water supply. Furthermore, this practice would enhance soil nutrients and boost output without compromising the soil's structure. The treated agro-industrial wastewater could be a good source to overcome the irrigation water depletion problem. This method is cost effective as it does not require the investment of large water treatment plants that use excessive electricity, fuel and also chemical treatments.

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