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Effects of Greywater on Seed Germination, Survival, and Early Growth of *Capsicum annuum* (Bell Pepper)

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Abstract

Water scarcity has become one of the global issues due to climate change and utilizing greywater to irrigate plants may save a significant amount of water. This study aimed to determine the effects of greywater on seed germination, survival, and early growth parameters of *Capsicum annuum.* Seeds were germinated in a petri dish filled with moist filter paper and kept in a dark laboratory condition. Bath greywater, kitchen greywater, laundry greywater, and tap water, as control, were used to water the seeds. Germination, survival rate, and early growth parameters were observed within 21 days. *C. annuum* seeds germinated quickly in four days. At a 0.05 level of significance, treatments differed significantly in germination percentage, germination rate index, and early development characteristics. Bath greywater had the highest germination percentage (100%), and the laundry greywater had the lowest percentage (97.5%). Regarding germination rate index, *C. annuum* treated with bath greywater records the highest (9.21%), and the laundry greywater records the lowest (6.22%). In terms of fresh weight, root length, and shoot length, bath greywater treatment recorded the highest, while the lowest was recorded in laundry and kitchen greywater. Bath greywater demonstrated a promising possibility to be reused as irrigation water for *Capsicum annuum* compared to other greywaters. Studies on ground elements and heavy metal properties of greywater are further conducted to determine their effects on the plant.

Keywords: bath greywater, kitchen greywater, laundry greywater, root length, shoot length

Introduction

Water scarcity has been a worldwide issue nowadays. By 2025, three billion people are estimated to live in nations that are either water-stressed or water-scarce (Hanjra & Quereshi, 2010). According to a report published by the Asian Development Bank in 2016, 3.4 billion people in Asia could live in "water stressed areas" by 2050. Population growth, climate change, and increased water demand in the industrial sector are all contributing factors to water scarcity issue (Lubbe et al., 2016). In many developing countries, an insufficient supply of freshwater has become a major source of public health issues (WHO 2006; Katukiza et al., 2015) and a constraint for agricultural sectors in other nations in terms of food production (Hanjra & Quereshi, 2010). With this, innovative methods to water and food security, particularly in agriculture-dependent economies such as the Philippines, should be examined. According to Schumacher (2016), Philippines is one of 37 Asian countries with inadequate levels of water and water security.

Greywater reuse is one of the cutting-edge techniques to combating the global water shortage. According to Edwin et al. (2014), greywater is a wastewater from home uses and activities such as bathing, laundry, and dishwashing, excluding toilet waste. It accounts for 60–70% of home waste–water volume in the majority of the 29 developing countries (Friedler, 2004; Edwin et al., 2014). Its production is proportional to the amount of water consumed in a home. It is also influenced by factors such as service quality, pollution tolerance, and community awareness of health and environmental risks (Carden et al., 2006; Bakare et al., 2016). According to Ikhajiagbe et al. (2020), the reuse of untreated greywater in agriculture has become a prevalent practice in many parts of the world, particularly in areas where farmers have limited access to freshwater and fertilizer supplies due to scarcity of funds. Greywater contains plant nutrients like as organic matter from broken skin, nitrogen, and phosphorus compounds, hence, it has the potential to be used for irrigation (Carden et al., 2007). However, using it for irrigation has biological, chemical, and environmental implications.

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Laundry detergent is one of the most polluting products on the market. Synthetic detergents and laundry products contribute significantly to groundwater pollution and have negative impacts on vegetation (Ikhajiagbe et al. 2020). Detergents, unlike soaps, are neither soluble nor biodegradable, according to Ehilen et al. (2017). It persists in the water after being added, resisting conversion into less complicated and soluble molecules. People tend to handle or dispose of detergents recklessly due to a lack of information, education, and communication about the impacts of detergents on living beings. As a result, different laundry wastewater disposal methods must be implemented.

With the current economic uncertainties, it has been observed that people are growing crop plants near their homes to have a convenient supply of fresh veggies (Ehilen et al., 2017). *Capsicum annuum*, generally known as bell pepper, is one of those crops that is simple to grow, can be produced locally, and is available all year (Fatoba et al., 2011). Greywater reuse as a supplementary water source ensures the availability of water for home-based farming while also resolving environmental and sanitary issues associated with its disposal. Although greywater is used to meet the demands of various crops, little is known about the consequences of this, especially on germination, survival, and early growth stages of vegetable plants. Hence, this study was conducted to assess the effects of greywater qualities on *C. annuum* germination and survival rates, as well as early growth metrics. Shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weights (RDW), and the ratios of SL/RL, SFW/RFW, and SDW/RDW were used to determine early growth characteristics.

Materials and Methods

Plant Material and Greywater Collection

The *C. annuum* seeds were purchased on the market and tested for viability using the flotation method (Ehilen et al., 2017). Within 21 days, greywater from one residence was collected every other day for the experiment. The experimental variables were bath greywater (BGW), kitchen greywater (KGW), and laundry greywater (LGW), with tap water (TW) serving as the control. Bath greywater samples were collected from a bathtub. The laundry greywater samples were taken directly from the washing machine, whereas the kitchen greywater samples were taken from the sink. After each collection, the water quality checker was used to determine the physicochemical parameters of the greywaters and tapwater (Hana HI9829). The water quality parameters like pH, dissolved oxygen (DO), conductivity, total dissolved solids (TDS) and salinity were gauged in this study.

Experimental Design

The study employed a randomized complete block design (RCBD). The *C. annuum* seeds were grouped into blocks. Each block had six duplicates and was given a different greywater irrigation treatment, such as laundry greywater (LGW), kitchen greywater (KGW), bath greywater (BGW), and tap water (TW). The *C. annuum* seeds were germinated in Petri dishes using the top-of-paper method under controlled growth room conditions (Rao et al., 2006). Each greywater irrigation method was tested on six duplicates of 20 viable seeds (per Petri dish) (Figure 1). Two milliliter water irrigation treatments were applied to each block of the experimental unit every other day for 21 days (Shershen et al., 2014). Differences in germination and survival rates, as well as early growth metrics, were observed and assessed after being administered with greywater treatments.

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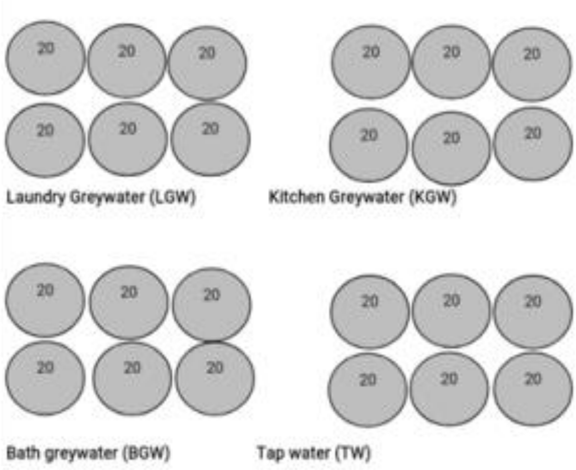

Figure 1. The Experimental Set-up

Data Collection and Analysis

Seed germination was carefully monitored and recorded on the 4th, 7th, 10th, 14th, 17th, and 21st days, according to the criteria of the International Seed Testing Association (ISTA 2004). After the 21st day of germination, the final count was made. The percentage of germination was computed using the formula:

$$\text{Germination (\%)} = \frac{\text{number of germinated seeds x 100\%}}{\text{total number of seeds sown}}$$

The germination rate index (GRI), as the speed of germination, was expressed following the formula (AOSA, 1983) below:

$$\text{GRI} = \frac{\text{number of germinated seeds}}{\text{days of 1st count}} + \ldots + \frac{\text{number of germinated seeds}}{\text{days of final count}}$$

The number of seedlings that sprouted was measured every day until 21 days. For both greywater treatments and controls, visual germination indicators were seen and documented. At the end of the 21-

day period, the survival rate was estimated using the formula (Seedling Emergence Guideline, 2018):

$$\% \text{ Survival} = \frac{\text{number of surviving plants at the end of the study} \times 100\%}{\text{number of seeds sown}}$$

Each seedling's early growth characteristics were measured. A graduated ruler was used to measure the length of the shoot (hypocotyl + cotyledon) and the root length (cm). To track the curve of the radicle, a tiny wire was used to compare and quantify its length (Nana et al., 2019). A digital electronic balance (KERN EW) was used to get the fresh and dry weights with 0.01g precision. After being dried individually in a 70°C oven for 24 hours, the dry weights of root and shoot lengths per treatment were determined. The shoot to root length ratio (SL/RL), shoot to root fresh weight ratio (SFW/RFW) and shoot to dry weight ratio (SDW/RDW) (Tadros et al., 2012) were also calculated.

Means were calculated and a normality test was performed using the collected data. The data were subjected to One-Way Analysis of Variance (ANOVA, $p<0.05$) with Tukey post hoc test after the test revealed that the data were in normal distribution and that the assumptions were met (Appendix A). The germination, survival rate, and early growth characteristics of *C. annuum* seedlings were also visualized using time series and whisker plots.

Results and Discussion

Physico-chemical characteristics of water treatments

With reference to the work of Edwin et al. (2014), the physico-chemical examination of greywater (Table 1) revealed that all the water treatment values are within the quantitative features of greywater. LGW had the highest pH of 10.06, TDS of 560 ppt, and salinity of 0.55 among the experimental water treatments. Due to the presence of alkaline

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compounds as surfactants in detergents, LGW had the highest pH value (Oteng-Peprah et al., 2018).

The KGW had the highest DO value of 0.85, indicating that this type of greywater came from dishwashing, vegetables, fruits, tubers, and other sources that may contain components that cause dissolved oxygen to rise. The fact that laundry detergents and soaps are made up of alkalis and various salts which may explain the increase in pH and conductivity values of the three water treatments (BGW, KGW, and LGW). These results conform to the observations recorded by Sawadogo *et al*. (2014), Saeed *et al*. (2015) and Ehilen *et al*. (2017).

Table 1. Mean variations and significant difference on the physico-chemical parameters of the greywater.

	Water Quality Parameters Mean (±SE)			
Parameters	TW	BGW	KGW	LGW
pH	8.21	8.01*	8.24	10.6*
DO (ppm)	0*	0.62	0.85	0.66
Conductivity(mS/cm)	118	1143	1443	1119.3*
TDS (ppt)	69	57*	71.7	560*
Salinity	0.06	0.45*	0.27	0.55

**The difference is significant when p-value is less than 0.05 significance level.*

The result of Tukey post hoc test revealed that the pH levels of KGW and TW did not differ substantially while that of BGW and LGW differed significantly. This could be due to the high sodium (Na) content of laundry detergents and powders used in washing machines, causing TDS and salinity in LGW to be statistically and significantly higher than in BGW and KGW (Friedler, 2004).

Effects of greywater on germination and survival rates of C. annuum

The Tukey post hoc test demonstrated a statistically significant difference ($p<0.05$ level) in *C. annuum* germination rate under various types of greywater treatments (Table 4). This means that the water irrigation treatments have a substantial impact on the germination percentage and rate index of *C. annuum.* TW, as the control treatment, has the highest germination percentage (100%). Table 2 shows that LGW has the lowest germination percentage of the three experimental water treatments, at 84%, followed by KGW and BGW, with 92% and 97%, respectively.

Table 2. Germination percentage (%) and germination rate index (GRI) of *C. annuum* after 4th, 7th, 10th, 14th, 17th and 21st days under four water treatments.

Treatment/ Day	Germination Percentage (%)						Germination Rate Index (GRI)
	4th	7th	10th	14th	17th	21st	
TW	11	69	93	99	100	100	9.21
LGW	3	26	82	82	84	84	5.62
BGW	9	58	89	95	97	97	8.17
KGW	8	51	87	90	92	92	6.96

Tapwater had the highest germination rate of all the treatments because it only includes trace levels of salts (Table 1) that the seedlings can easily absorb. Nana et al. (2019) found that the physicochemical properties of water treatments, particularly NaCl concentration and high conductivity, can be attributable to the variation in germination percentage in okra seeds. The osmotic effect of salty water on okra seeds caused for its poor germination. It was also recorded in the study of Benidire et al. (2015) that NaCl reduces the ability of beans to germinate. Moreover, increasing the conductivity of water treatments makes it more difficult for plants to absorb it. The findings of this work support the claims of Nana et al. (2019) and Benidire et al. (2015) that salinity might create a changeable osmotic impact and long-term harmful consequences during germination.

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As shown in Figure 2, *C. annuum* seeds started to germinate at day 4 in all four treatments. This observation was similar to the study of Nana *et al*. (2019) on okra seeds. All seeds germinated on Day 11 under all water irrigation treatments. TW, as the control, has the highest survival rate of *C. annuum*. However, on the greywater treatments, LGW had the lowest survival rate, while BGW had the highest.

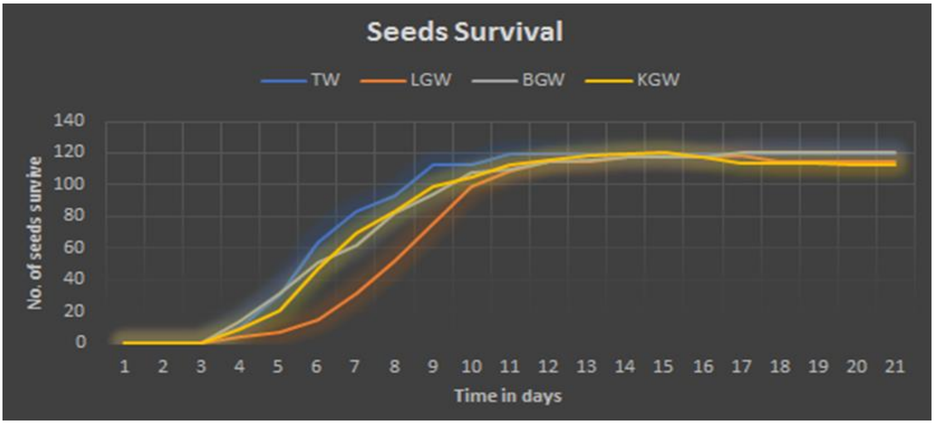

Figure 2. Time series plot on the number of C. annuum seeds survive up to 21 days.

LGW and KGW showed low survival rate due to the presence of detergents in the watering solutions which could result in a significant drop in seed germination (Ehilen et al., 2017). The result of this study is similar to the findings of Heidari (2013), who found out that high detergent dosages during seed germination can cause oxidative stress, lipid peroxidation, and increased cell membrane permeability to harmful ions, resulting in seedling mortality.

Table 3. Significant difference on the germination and survival rates of *Capsicum annuum* among the water treatment.

Treatment	Mean	F-test Statistic (3, 20)	P-value	Remark
On germination rate				
TW	14.8[a]			
LGW	11.4[b]	14.8	2.62E-05	Significant
BGW	14.5[a]			
KGW	13.4[a]			
On survival rate				
TW	99.2	0.26	1	Not significant
LGW	95.2			
BGW	100			
KGW	93			

The difference is significant when p-value is less than 0.05 significance level.
[a,b] Letter code for homogeneous grouping in the pairwise comparison

It can be gleaned in Table 3 that greywater treatments had a substantial impact on *C. annuum* seed germination but not on seedling survival. This finding suggests that the effects of greywater are visible during the germination stage. According to Ikhajiagbe et al. (2020), greywater has no immediate negative impact on plants, but the impact can be noticed over time. In a study on sunflower seeds, Heidari (2013) found out that seed germination was more vulnerable to polluted water than in early growth stages.

Effects of greywater on early growth parameters of C. annuum

The efficiency with which a plant employs photosynthetic resources is measured by early growth metrics (Hunt et al., 2003; Lubbe et al., 2016). *C. annuum* treated with BGW consistently has the highest mean root length, shoot length, and mean fresh weight, as shown in Figure 3a, b, and c, whereas those treated with KGW and LGW have the lowest. Lubbe et al. (2016) found that shoot height and root length of African

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leafy vegetables are significantly lower when compared to tapwater. Salinity stress, which is closely connected with an increase in conductivity, causes this reduction in plant height (Nana et al. 2019).

Plant growth is aided by a high shoot-to-root ratio, stomatal conductance, and photosynthetic rates (Lubbe et al., 2016). The usual pH for vegetable biological activity, according to Sawadogo et al. (2014), is between 5 and 9; if it exceeds 9, biological activity decreases. The effects of water treatment characteristics on the balance between ratios of early growth factors are well shown in this study (Figure 4). Early growth characteristics in Capsicum annuum treated with KGW exhibited balanced ratios. These results contrast with those of Al-tabbal & Ammary (2014), who found that TW had the lowest ratio but had a more balanced early growth feature in their study of barley and onion seeds.

Table 4. Significant difference on the early growth of *Capsicum annuum* among the water treatment.

Early growth parameters	Treatment	Mean	F-test Statistic (3, 400)	P-value	Remark
Fresh weight	TW	0.04[a]	38.27	9.16E-22	Significant
	LGW	0.03[a]			
	BGW	0.05[b]			
	KGW	0.03[a]			
Root Length	TW	60.49[a]	81.85	5.09E-42	Significant
	LGW	35.5[a]			
	BGW	69.98[b]			
	KGW	29.5[a]			
Shoot Length	TW	41.58[a]	16.13	5.81E-10	Significant
	LGW	38.28[a]			
	BGW	43.65[a]			
	KGW	31.2[b]			

The difference is significant when p-value is less than 0.05 significance level.
[a,b] Letter code for homogeneous grouping in the pairwise comparison.

Water irrigation treatments have a substantial effect on *C. annuum* early growth in terms of shoot length, root length, and fresh weight, as indicated in Table 4. According to the findings of Heidari (2013), detergent decreased shoot length, root length, seedling weight, and seed vigor. As plants grow, changes in these early growth characteristics can be observed.

Conclusions and Recommendations

Capsicum annuum seeds begin to germinate on the fourth day. The physicochemical parameters of the seedlings' water treatments are within greywater's quantitative features. Their effects on germination percentage, germination rate index, and early growth metrics differed significantly among treatments. BGW has the highest germination percentage rate, germination rate index, and survival rate among the greywaters, whereas LGW and KGW have the lowest. This is due to its high pH, conductivity, and salinity, but low DO, which adversely affects seedlings' early growth features, particularly shoot and root lengths. Only BGW shows potential as an alternate water irrigation option, especially in the early stages of plant growth. Studies on the properties of ground elements and heavy metals in greywater may be carried out in the future to determine their effects on plants.

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