Journal of AgriSearch, 11 (4): 254-261
An Open Access International Peer Reviewed Quarterly

ISSN : 2348-8808 (Print), 2348-8867 (Online)
https://doi.org/10.21921/jas.v11i04.15216

Assessing the Impact of Wastewater on Biochemical and Physiological Properties of Lentil (Lens esculentus Moench)

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ABSTRACT

An experiment was conducted to investigate how wastewater affects the growth and biochemical parameters of lentil plants. Thirty pots (20 cm high, 12 cm diameter) were selected for the study. In 15 pots, wastewater was applied, while the remaining 15 pots received fresh water. The results indicated that the wastewater had a negative impact on seedling growth of the lentil. Seedlings grown with wastewater had lower biomass and length than those grown with fresh water. The protein content in the seeds treated with fresh water was higher than those treated with wastewater, while the carbohydrate content was higher in the early stages but decreased later. The chlorophyll estimation showed higher concentrations in the seedlings grown with fresh water. In the initial stage, the root length and shoot length of the plants irrigated with the wastewater were lower than the fresh water. However, after the 20th day, the root length and shoot length of plants in wastewater treatment significantly increased and surpassed the fresh water treatment. At the 70th day, the root length and shoot length of the plants in wastewater treatment were 9.44±0.29 cm and 14.86±1.58 cm, respectively, indicating that plants in wastewater treatment were able to absorb more nutrients. Therefore, the study suggests that wastewater should be avoided during germination, and it can be used for irrigation after the 20th day of growth. Overall, the study highlights the potential benefits and drawbacks of wastewater as an irrigation source and provides guidance for its safe and effective use in lentil.

Keywords: Wastewater, seedling germination, irrigation and biochemical

ARTICLE INFO

Received on	:	14/08/2024
Accepted on	:	20/12/2024
Published online	:	31/12/2024

INTRODUCTION

Wastewater is a significant source of pollution in both urban and rural areas worldwide. It is generated from various sources, including households, commercial establishments, and institutions (Dash et al., 2023; Desai et al., 2023). Domestic wastewater, in particular, contains a variety of pollutants, such as heavy metals, organic compounds, and pathogens, that can have detrimental effects on both the environment and human health if not treated properly (Singh et al., 2020a, b). In addition to its role as a pollutant, domestic wastewater is increasingly considered a potential resource for crop irrigation, especially in regions where freshwater resources are scarce. However, the use of wastewater for irrigation is fraught with challenges, as it can adversely affect soil properties, crop yield, and quality (Singh et al., 2021a, b). The reuse of wastewater for irrigation purposes has been extensively studied, particularly its impact on seed germination and plant growth. Yadav et al. (2019) conducted a study on the effect of wastewater on wheat seed germination and growth and found that high concentrations of wastewater inhibited germination and growth of wheat. Similarly, study conducted by Rani et al. (2021) on the effect of wastewater on different crops reported that wastewater irrigation resulted in reduced germination, growth, and yield of crops. Despite these findings, the specific impact of domestic wastewater on the leguminous crop masoor (Lens esculentus Moench) remains underexplored (Karaer et al., 2021).

Masoor, also known as lentil, is a crucial leguminous crop valued for its protein-rich seeds. It is widely cultivated due to its high nutritional value and adaptability to diverse agro-climatic conditions (Choubey et al., 2013). Given the expanding cultivation of masoor, understanding the effects of domestic wastewater on its seed germination and biochemical parameters is essential for several reasons. Assessing the risks associated with using wastewater for irrigation is critical, particularly in areas with limited freshwater resources. The successful cultivation of masoor can provide farmers with a reliable source of income and meet the growing demand for protein-rich foods. Insights from such studies can inform policymakers and researchers in developing strategies to mitigate the potential harmful effects of wastewater while

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leveraging it as a valuable resource for sustainable agriculture. This research aims to investigate the effects of domestic wastewater on masoor seed germination and various biochemical parameters. The study will evaluate the impact of wastewater on germination rate, shoot and root length, and biochemical parameters such as total protein, proline, and chlorophyll content in masoor. By doing so, this study seeks to contribute valuable knowledge towards the safe and effective use of wastewater in agriculture, ensuring both environmental sustainability and agricultural productivity.

MATERIALS AND METHODS

Wastewater sampling and analysis

In this study, wastewater samples were obtained from the household drainage system in Srinagar, Garhwal, Uttarakhand. Pre-cleaned empty bottles were utilized for the collection of the wastewater that was later applied to the crops and transferred to the experimental site. The quality assessment of both the wastewater (ww) and fresh water (tw) used in the experiment is presented in Table 1. The physio-chemical parameters, including electrical conductivity (EC), pH, TDS, and alkalinity, were evaluated based on the guidelines provided by the US Salinity Lab Staff (1954).

Table 1: Quality parameters of fresh water and wastewater

Parameter	Fresh water	Wastewater
pH	7.56±0.28	8.92±0.21
TDS (mg L -1)	140±24.12	625±57.45
Na+ (mg L -1)	2.51±0.17	24.52±5.89
EC (dS m-1)	0.15±0.019	0.56±0.07
CO32- (mg L -1)	49.17±09.10	104.91±17.31

Experimental setup

An experiment was performed in the Department of Biochemistry, Hemvati Nandan Bahuguna Garhwal University, Srinagar, Garhwal, Uttarakhand, India. Thirty pots of 20 cm in height and 12 cm in diameter were selected. 3.5 kg of soil was filled in each pot. L. esculentus seeds were selected for the study and irrigated with wastewater. The seeds irrigated with the fresh water was selected as control in the study. Control and wastewater applied seeds were allowed to germinate in moist filter paper (moisten with fresh and waste water) in petri dishes for seven days. L. esculentus seeds were sown in the month of September in pots using three replicates for each treatment. The wastewater and fresh water were used for irrigation in respective treatment groups.

Growth parameter measurements

To evaluate the impact of fresh water and wastewater on growth, several parameters were assessed. Three replicates were used for each treatment, and the mean value for each measurement was calculated. The parameters evaluated included germination and biochemical parameters.

Germination percentage is an essential measure of seed viability. In this study, 10 seeds from both the treated and control groups were placed in sterilized Petri dishes with moistened filter paper. The Petri dishes were regularly watered to compensate for any evaporation losses. Germination was recorded when a radicle emerged to at least 1 mm. Daily counts of germinated seeds were taken to calculate the germination percentage as described by Rusan et al. (2015). Germination speed, which measures seed quality and performance, was determined based on the number of germinated seeds over the number of days, following the method described by McDonald (1980). Seeds that germinated more quickly were considered to have higher quality and performance. Mean germination time, an index of seed germination speed, was calculated by averaging the time taken for seeds to germinate and establish seedlings, using the method described by Gairola et al. (2011). This measure provides insight into the velocity of germination. Mean daily germination, which assesses the daily germination speed and completeness, was estimated by averaging the number of seeds that germinated each day during the germination period. This index was calculated according to Gairola et al. (2011). Peak value, another germination index, was determined by identifying the highest number of seeds germinated over the number of days, as outlined by Gairola et al. (2011). This value indicates the peak germination period within the observation timeframe. Germination value, a combined measure of germination speed and peak value, was calculated to provide a comprehensive assessment of seed germination performance.

Seedling mortality was evaluated by counting the non-germinated seeds on the seventh day and calculating the percentage based on the method described by Gairola et al. (2011). This measure helps in understanding the survival rate of seeds during the germination process. To determine biomass, fresh seeds were weighed, and a portion was dried in an oven at temperatures ranging from 100 °C to 60 °C until a constant weight was achieved. The dry weight was calculated following the dry weight method outlined by the International Seed Testing Association (ISTA, 1985). This process involved recording the fresh weight of seeds and then drying them in a hot air oven at 100 °C for one hour, followed by drying at 60 °C until a constant weight was obtained. Moisture content was measured by taking seeds from each treatment and control group, bringing them to room temperature in a desiccator, and determining their fresh weight using an electronic balance. The seeds were then dried in a hot air oven at 100 °C for one hour and then at 60 °C until a constant weight was achieved. The moisture content was calculated based on the difference between fresh and dry weight. The length of the radicle and hypocotyl was measured in centimetres using a thread and scale. Seedling length was then determined by adding the radicle and hypocotyl lengths.

Vigour index was calculated by multiplying the germination percentage by the seedling length, following the method described by Sharma and Saran (1992). This index provides a comprehensive measure of seed vigour, combining both germination success and seedling growth.

Biochemical parameters

Estimation of carbohydrates

One gram of seeds was taken and seed coat of every seed was carefully removed. The cotyledons were grinded with 10 ml of 80% ethanol and then the grinded samples were placed in a 15ml centrifuge tubes and centrifuged, so as to sediment the particles if any. The supernatant was decanted to evaporating dishes and was kept in boiling water bath at 80 to 85 °C to make it dry. The extract was diluted and the final volume was made up to 10 ml. The extract was collected in sterilized and labelled test tubes, for the estimation of total sugar. The total sugar was estimated by Anthrone method by taking O.D. at 620 nm.

Estimation of soluble protein

One gram of seeds from each treatment was separately homogenized in 20 ml of pre-chilled 0.2 M Tris HCl Buffer (pH 7.2) containing 0.1 mM EDTA. The homogenates were filtered through double-layered cheesecloth and centrifuged at 16,000 rpm for 15 min at 4°C. One millilitre of the resulting extract was mixed with 1 ml of ice-cold 20% TCA, and the resulting pellet was washed with acetone and centrifuged at 8,000 rpm. The supernatant was discarded, and the pellet was dissolved in 5 mL of 0.1 N NaOH for protein estimation. Total protein levels were determined using the method of Lowry et al. (1951) with BSA as a standard.

Chlorophyll estimation

The chlorophyll a and b and total chlorophyll (a+b) contents were quantitatively estimated by Arnon's (1949) method. 250mg of fresh leaf material was ground into a fine paste using mortar and pestle. 10ml of 80% acetone was added in to it. A pinch of $CaCO_3$ powder was added. The extract filtered through Whatman no.1 filter paper. The extract was kept away from direct sunlight. The optical density of the extract was read at 470, 645, 652 and 663 wavelengths using spectrophotometer. Chlorophylls a, b and total chlorophyll (a+b) were measured spectrophotometrically and calculated according to the formulae-

$$Chl\ a = 12.70\ A_{\_633} - 2.69\ A_{\_645} \qquad 1$$

$$Chl\ b = 22.90\ A_{\_645} - 4.68\ A_{\_663} \qquad 2$$

$$Chl\ a{+}b = 20.21\ A_{\_645} - 8.02\ A_{\_663} \qquad 3$$

RESULTS AND DISCUSSION

Germination parameters

Impact of wastewater in seed germination

Results indicated that the germination percentage of seeds in fresh water was consistently higher than the germination percentage of seeds in wastewater throughout the 7-day period (Table 2 and Fig. 1). On day 2, the germination percentage of seeds in fresh water was 84%, while in wastewater it was 27%. By day 7, the germination percentage of seeds in fresh water remained constant at 95%, while in wastewater it increased to 62%. The data shown that the germination percentage of seeds in fresh water was increased from 27% on day 1 to 95% on day 5 and remain stable thereafter. In contrast, the germination percentage of seeds in wastewater increased from 0% on day 1 to 62% on day 7, with a gradual increase observed over time. The standard errors for each data point suggest that there was variability in the results across the three independent experiments. However, the variability was relatively small, with the standard error generally ranging from 1.67 to 2.88. Overall, the results suggest that wastewater can stimulate seed germination, although the effect is less pronounced than with fresh water.

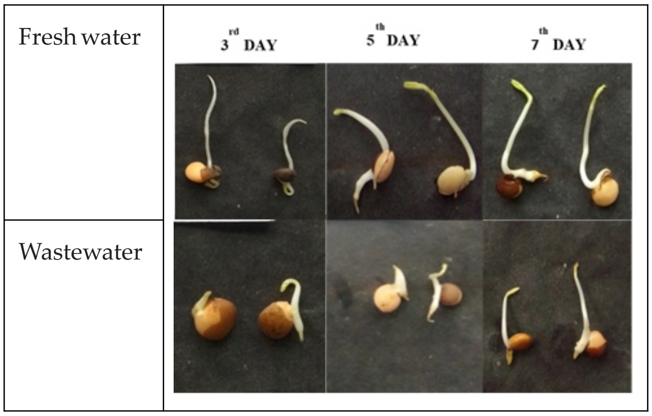

Fig 1: Growth of Seedling on day 3, 5 and Day 7 treated with fresh water and waste water

Table 2: Germination percentage of seed from day 1 to day 7. Data presented are means± standard error of three independent experiments.

Days	Fresh-water (%)	Wastewater (%)
1st	27 ±1.67	0
2nd	84 ±1.67	27±1.66
3rd	88 ±1.67	32±1.66
4th	93 ±1.67	48±1.66
5th	95± 0.00	50±2.88
6th	95± 0.00	52±1.67
7th	95± 0.00	62±1.67

Table 3 represents the effect of wastewater on different seed germination parameters. The results demonstrate that the germination parameters of seeds in fresh water were generally better than the germination parameters of seeds in wastewater. The germination speed (G.S.) of seeds in fresh water was 11.86 ± 0.59, which was almost three times faster than the germination speed of seeds in wastewater (4.26 ± 0.14). The mean daily germination (M.D.G.) of seeds in fresh water was 2.82 ± 0.08, which was also higher than the M.D.G. of seeds in wastewater (2.01 ± 0.14).

Moreover, the peak value (P.V.) and germination value (G.V.) of seeds in fresh water were significantly higher than the peak value and germination value of seeds in wastewater. The seed mortality (S.M.) of seeds in wastewater was much higher than in fresh water (99.9 ± 8.24 compared to 19.04 ± 4.76). Finally, the mean germination time (M.G.T.) of seeds in wastewater was 47.67 ± 1.34, which was higher than the M.G.T. of seeds in fresh water (35.8 ± 0.41). The data also suggest that the use of wastewater for seed germination may lead to reduced germination parameters and increased seed mortality compared to fresh water.

Table 3: Effect of wastewater on seed germination parameters.

Germination Parameters	Fresh-water	Wastewater
G.S	11.86±0.59	4.26±0.14
M.D.G	2.82±0.08	2.01±0.14
P.V	1.67±0.08	0.71±0.02
G.V	4.72±0.36	1.43±0.06
S.M	19.04±4.76	99.9±8.24
M.G.T	**35.8±0.41**	**47.67±1.34**

G.S. = Germination speed, M.D.G = Mean Daily Germination, P.V= Peak Value, G.V = Germination Value, S.M= Seed Mortality, M.G.T = Mean Germination Time. Data presented are means± standard error of three independent experiments.

Biomass

The fresh weight and dry weight of seedlings grown with fresh water and wastewater were measured at different time interval i-e- day 1, 3, 5 and 7 (Table 4). The results shown that the fresh weight of seedlings grown with fresh water was higher than the fresh weight of seedlings grown with wastewater.

On day 1, the mean fresh weight of seedlings grown with fresh water was higher (0.092 g) than the wastewater (0.080 g). Moreover, similar trends were also observed on day 7, the weights increased to 0.117 g for fresh water and 0.099 g for wastewater. But in case of dry weight, on day 1, seedlings grown in fresh water had a lower dry weight than those in wastewater, but by days 3, 5, and 7, seedlings in fresh water consistently had greater dry weights. Overall, seedlings germinated in fresh water showed significant increases in both dry and fresh weights compared to those in wastewater.

Table 4: Effect of wastewater on fresh weight and dry weight of seedlings as compared to fresh water. Data presented are means± standard error of three independent experiments.

Days	Fresh-water	Wastewater		
	Fresh weight	Dry weight	Fresh weight	Dry weight
1st	0.0923±0.004	0.031±0.001	0.080±0.002	0.038±0.002
3rd	0.087±0.002	0.038±0.001	0.076±0.005	0.036±0.001
5th	0.109±0.005	0.045±0.005	0.090±0.009	0.041±0.003
7th	0.117±0.004	0.056±0.003	0.099±0.011	0.044±0.002

Moisture content

The table 5 shows the moisture content of seedlings grown in fresh water and wastewater on different days. Decreased moisture content was noticed on each day. On 1st day, it was 0.061 in wastewater treated seeds while in fresh water treated seeds it was 0.041. On the 7th day, moisture content of seeds in wastewater was 0.054 while that of in fresh water seeds was 0.060. The results indicate that the moisture content of seedlings grown in wastewater is lower than those grown in fresh water, except for the third day where the moisture content of both groups is similar. Overall, the fresh water group has higher moisture content than the wastewater group. The results showed that the moisture content of the seedlings was generally higher in fresh water than in wastewater. For example, on the 1st day, the moisture content of seedlings grown in fresh water was 0.063 ± 0.001, while that of seedlings grown in wastewater was 0.040 ± 0.001. This result suggests that seedlings grown in wastewater may experience water stress, which can adversely affect their growth and development (Wortman and Lovell, 2013).

Table 5: Moisture content of seedlings

Days	Fresh water	Wastewater
1st	0.063±0.001	0.040±0.001
3rd	0.048±0.003	0.045±0.002
5th	0.064±0.001	0.048±0.001
7th	0.061±0.005	0.051±0.001

Seedling length

The seedling length was taken on 1st, 3rd, 5th and 7th day. Table 6 shows the length of seedlings (in cm) grown in fresh water and wastewater over four different time intervals (1st, 3rd, 5th, and 7th day). The results indicate that the seedlings grown in fresh water were longer than those grown in wastewater at each time point. On the 7th day, the seedlings grown in fresh water were 5.17±0.440 cm in length, while those grown in wastewater were only 2.24±0.145 cm in length. The difference in length between the two groups became more significant as the days passed. An increased Seedling length was observed in controlled one as compared to the wastewater treated seeds. This result suggests that wastewater may be detrimental to the growth of seedlings, possibly due to the presence of contaminants such as heavy metals and organic pollutants. The reduced growth of seedlings in wastewater was also reflected in the lower values of the vigour index. The findings of this study are consistent with those of previous study that have reported a decrease in plant vigour when using wastewater for irrigation (Li et al., 2017).

Table 6: Length of the seedlings (cm)

Days	Fresh water	Wastewater
1st	0.06±0.033	0.03±0.033
3rd	1.00±0.115	0.08±0.044
5th	1.60±0.230	0.17±0.033
7th	5.17±0.440	2.24±0.145

Vigour index

The seed vigour index of seedlings was measured on the 1st, 3rd, 5th, and 7th days, using fresh water and wastewater as sources. The vigour index accounts for both the length and fresh weight of the seedlings, with higher values indicating better performance. Seedlings watered with fresh water had higher vigour indices compared to those watered with wastewater. The vigour index increased over time, with the highest values recorded on the 7th day. On the 5th day, the vigour index for seedlings treated with fresh water (152.67) was greater than those treated with wastewater (9.16).

Table 7: Vigour index of seedlings on different days

Days	Fresh water	Wastewater
1st	1.60±0.05	0±0.00
3rd	84.34±2.33	3.35±0.42
5th	152.67±1.76	9.16±0.44
7th	488.67±4.48	141.00±2.08

Plant Parameters

Root and Shoot length

A pot study was carried out to analyse the effect of fresh water and wastewater on the growth of plant roots and shoots. The root length and shoot length of the plants were measured at different time interval (10th, 20th, 30th, 40th, and 70th day) of the experiment with both fresh water and wastewater (Table 8 and Fig. 2).

The results shown that, in general, wastewater has a positive effect on plant growth compared to fresh water. At the 10th day, the root length and shoot length were lower in wastewater treatment than in fresh water treatment. However, after the 20th day, the root length and shoot length in wastewater treatment increased significantly and surpassed the fresh water treatment. At the 70th day, the root length and shoot length of the plants in wastewater treatment were 9.44±0.29 cm and 14.86±1.58 cm, respectively, which were significantly higher than the fresh water treatment (root length: 8.24±0.32 cm; shoot length: 12.37±0.59 cm).

Table 8: Root and Shoot length of masoor plants

Days	Fresh water		Wastewater	
	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)
10th	3.90±0.37	4.78±0.33	3.10±0.86	3.00±0.87
20th	5.76±1.53	5.97±1.01	5.70±0.35	5.34±0.72
30th	5.77±0.99	8.40±1.01	6.44±0.61	9.93±0.47
40th	7.36±0.46	11.03±0.08	7.9±0.21	12.47±1.01
70th	8.24±0.32	12.37±0.59	9.44±0.29	14.86±1.58

Fig 2: Rot and shoot of the lentil plants (Fresh water and waste water)

Fresh weight and Dry weight of plant

The pot study also done to investigate the effect of fresh water and wastewater on the fresh weight and dry weight of plant tissues. The fresh weight and dry weight of plant tissues were measured at different time points (20th, 30th, and 70th day) of the experiment for both fresh water and wastewater treatments (Table 9).

The results show that the fresh weight and dry weight of the plant tissues were higher in wastewater treatment than in fresh water treatment. At the 20th day, the fresh weight and dry weight of plant tissues were similar in both fresh water and wastewater treatments. However, by the 30th day, the fresh weight and dry weight of plant tissues in wastewater treatment increased significantly and continued to increase by the 70th day. At the 70th day, the fresh weight and dry weight of plant tissues in wastewater treatment were 0.48±0.039 gm and 0.022±0.009 gm, respectively, which were significantly higher than the fresh water treatment (fresh weight: 0.45±00.037 gm; dry weight: 0.021±0. 003 gm). Moreover, initial stage, the root length and shoot length were lower in wastewater treatment than in fresh water treatment. Similar, trend was also recorded for fresh weight and dry weight in both the water. However, after the 20th day, the root length and shoot length in wastewater treatment increased significantly and surpassed the fresh water treatment. At the 70th day, the root length and shoot length of the plants in wastewater treatment were 9.44±0.29 cm and 14.86±1.58 cm. The plants in the wastewater treatment grew larger and had longer roots than those in the fresh water treatment, indicating that the plants in the wastewater treatment were able to absorb more nutrients from the water (Li et al., 2015). These findings suggest that wastewater may contain nutrients that are beneficial for plant growth.

Table 9: Fresh and Dry weight of plants

Days	Fresh water	Wastewater		
	Fresh weight (gm)	**Dry weight (gm)**	**Fresh weight (gm)**	**Dry weight (gm)**
20th	0.15±0.060	0.005±0.001	0.15±0.037	0.006±0.005
30th	0.18±0.060	0.007±0.001	0.21±0.037	0.010±0.005
70th	0.45±00.037	0.021±0. 003	0.48±0.039	0.022±0.009

Biochemical parameters

Protein estimation

Protein estimation was carried out on 1st, 3rd and 7th day of seeds (L. esculentus) germinated on petri dishes in both the waters. The results shown that the protein content is slightly higher in wastewater applied seeds as compared to fresh water seeds on the 1st and 7th day, whereas the concentration of protein is slightly lower in wastewater applied seeds on the 3rd day (Table 10). However, the differences between the two groups are not significant, as the standard errors overlap. Interestingly, the results showed that the protein concentration was higher in wastewater-treated seedlings than in fresh water-treated seedlings. For instance, on the 1st day, the protein concentration in wastewater-treated seedlings was 0.23 ± 0.20 µg/ml, while that of fresh water-treated seedlings was 0.21 ± 0.23 µg/ml. This result suggests that seedlings grown in wastewater may have a greater demand for protein synthesis to cope with the stress caused by the contaminants in the wastewater (Li et al., 2021).

Table 10: The protein concentration in wastewater applied seeds as compared to fresh water on different days.

Days	Concentration(µg/ml) Fresh water	Concentration(µg/ml) Wastewater
1st	0.21±0.23	0.23±0.20
3rd	0.16±0.28	0.13±0.18
7th	0.17±0.14	0.18±0.18

Carbohydrate (Total Sugar) estimation

Days 1st, 3rd and 7th were studied for the carbohydrate content of seedlings grown in fresh water and wastewater. On the 1st day, seedlings grown in fresh water had a slightly higher carbohydrate concentration than those in wastewater. By the 3rd day, there was no significant difference between the two. On the 7th day, fresh water seedlings had a significantly higher carbohydrate concentration than wastewater seedlings, although wastewater-treated seedlings showed a significant increase in sugar content overall. This result suggests that seedlings grown in wastewater may have lower energy reserves, which can limit their growth and development (Matsuda et al., 2014).

Table 11: Carbohydrate content of seedlings

Days	Concentration (µg/mL) Fresh water	Concentration (µg/mL) Wastewater
1st	0.14±0.12	0.12±0.24
3rd	0.13±0.68	0.12±0.24
7th	0.12±0.37	0.06±0.62

Chlorophyll estimation

The chlorophyll was estimated of the lentil seedling irrigated with fresh water and wastewater. The Table 12 shows the results of chlorophyll estimation in µg mL-1 for seedlings grown in fresh water and wastewater. The results indicate that seedlings grown in wastewater had higher levels of chlorophyll a, chlorophyll b, and total chlorophyll (a+b) compared to those grown in fresh water. The concentrations of chlorophyll a, chlorophyll b, and total chlorophyll (a+b) in wastewater-grown seedlings were 4.43±0.07, 2.84±0.04, and 7.27±0.09, µg mL-1, respectively. In comparison, the corresponding values for fresh water-grown seedlings were 2.73±0.30, 1.60±0.07, and 4.34±0.28 µg mL-1, respectively. It may also be opined that heavy metals like iron, manganese may contribute to the synthesis of chl a that is resulted into the stimulation of Chlorophyll under lower concentrations. This result suggests that seedlings grown in wastewater may have a greater demand for photosynthetic pigments to cope with the stress caused by the contaminants in the wastewater (Kamran et al., 2020).

Table 12: Chlorophyll a, chlorophyll b and total chlorophyll content (µg mL-1) in leaves on day 40 (µg mL-1)

	Chl a	Chl b	Chl (a+b)
Fresh water	2.73±0.30	1.60±0.07	4.34±0.28
Wastewater	4.43±0.07	2.84±0.04	7.27±0.09

CONCLUSION

This study examined the impact of wastewater on seed germination and seedling growth compared to fresh water over a 7-day period. Results showed that seed germination percentages in fresh water were consistently higher than those in wastewater, with fresh water achieving 95% by day 7 compared to 62% for wastewater. Fresh water seedlings exhibited superior germination parameters, including higher germination speed (11.86 ± 0.59 vs. 4.26 ± 0.14), mean daily germination, peak value, and germination value, while wastewater-treated seedlings had higher seed mortality (99.9 ± 8.24 vs. 19.04 ± 4.76) and longer mean germination time (47.67 ± 1.34 vs. 35.8 ± 0.41). Biomass measurements showed that fresh water-treated seedlings had higher fresh and dry weights. For instance, the fresh weight on day 1 was 0.092 g for fresh water versus 0.080 g for wastewater, with similar trends on day 7 (0.117 g vs. 0.099 g). Dry weights were consistently higher in fresh water-treated seedlings from day 3 onward. Fresh water also resulted in greater seedling lengths, with day 7 lengths being 5.17 ± 0.440 cm compared to 2.24 ± 0.145 cm for wastewater. Present study indicated that initial root and shoot lengths were lower in wastewater-treated plants but surpassed those of fresh water-treated plants after the 20th day. By day 70, root and shoot lengths were significantly higher in wastewater-treated plants. Biochemical analysis revealed that protein and chlorophyll levels were higher in wastewater-treated seedlings, suggesting an adaptive response to contaminants. However, overall seedling performance was better in fresh water, indicating its superior suitability for optimal seedling growth. Moreover, future studies could investigate the long-term impact of wastewater on plant growth and yield, as well as the potential risks associated with the use of wastewater in agriculture.

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Citation:
Gusai A, Singh N, Singh D, Singh H, Jeet P and Yadav D.2024. Assessing the impact of wastewater on biochemical and physiological properties of Lentil (Lens esculentus Moench). Journal of AgriSearch 11(4):254-261.