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Influence of Untreated Pharmaceutical Effluent on the Germination and Growth of *Amaranthus Viridis* L. (African Spinach) Collected From Ilorin, Kwara State, Nigeria

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ABSTRACT: The swift advancement in the pharmaceutical industry has raised concerns regarding the impact of its wastewater on the environment, particularly on edible plants. It has also been observed that peasant communities close to pharmaceutical industries use waste effluents from the industries to grow crops. Hence, the objective of this paper was to investigate the influence of untreated pharmaceutical effluent on the germination and growth of *Amaranthus viridis* L. (African spinach) collected from Ilorin, Kwara State, Nigeria using appropriate standard methods. It was observed that low levels of pharmaceutical wastewater promoted growth, whereas higher levels negatively impacted plant development and decreased chlorophyll content, resulting in hindered growth and lower yields. The germination percentage was recorded at 100% in the control group. However, it decreased between 61.18% and 79.14% in spinach grown on soils polluted with pharmaceutical effluent. The spinach plants from 100% treatment soils had 2 branches, while the plants from 60% treatment soils had 4 branches. Total fresh weight in spinach from soil contaminated with 60% wastewater had the highest value of 65.82g, while those from soil containing 100% wastewater had the lowest value of 34.67g. The control group's total fresh weight was 51.69g. This study emphasizes how urgently the pharmaceutical industries need strict wastewater treatment procedures. Additionally, it offers useful information that helps farmers, environmental scientists, and legislators balance ecological health with agricultural output.

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Amaranthus viridis (commonly known as African Spinach) is a cosmopolitan species in the botanical family Amaranthaceae (Reyal-ul-Ferdous *et al*., 2015). The consumption of amaranth leaves has several health advantages. Antioxidants and vital phytonutrients are stored in the leaves. Because of its high water content and fiber content, it is excellent for the digestive system. Additionally, it lowers cholesterol, and people with cardiovascular disorders and high blood pressure can benefit from consuming it (Aderibigbe *et al*., 2020) An increasing volume of industrial effluent is discharged into the environment, often without adequate treatment. When contaminated crops are consumed, high amounts of toxins from this effluent, including heavy metals, organic pollutants, and excess nutrients, can accumulate in the soil and affect plant growth and human health (Maukeeb *et al.*, 2022). Because of their large surface area and root systems, which allow plants to absorb the metals from the soil, green crops like *Amaranthus viridis* have a tendency to accumulate heavy metals. (Ahmed *et al.*, 2022).

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Excessive amounts of organic waste, salts, and hazardous substances are frequently found in pharmaceutical industry wastewater, which has an impact on soil fertility and health. Industrial pollutants change the soil's microbial community, which lowers the amount of beneficial bacteria and fungi that are vital to plant health (Gaikwad *et al*., 2023). These alterations cause plants to grow more slowly, produce less food, and become more vulnerable to disease. Industrial wastewater irrigation can cause morphological and physiological alterations in vegetables. Crops from effluent-contaminated soils may show chloroplast necrosis and a decrease in leaf area due to high concentrations of heavy metals, which prevent photosynthesis and nutritional absorption. Chloroplast and other organelle formation can be hampered by heavy metals in pharmaceutical effluent, leading to abnormal plant growth and nutrient deficits (Rai *et al*., 2019). The bioaccumulation of heavy metals and other toxic compounds in edible plant components makes eating vegetables that have been irrigated with pharmaceutical effluent a major health danger (Koopaei and Abdollahi, 2017). Heavy metals including lead, cadmium, and chromium, sometimes found in pharmaceutical effluent drastically decrease germination rates in a variety of vegetable crops because they obstruct cellular functions necessary for the onset of growth (Hussain *et al*., 2021. Heavy metals in the effluent interfere with the synthesis of chlorophyll and enzyme function, which lowers photosynthetic rates (Angon *et al*., 2024). Plants irrigated with untreated pharmaceutical effluent exhibit abnormal root architecture, including shorter and less branching root systems, as a result of heavy metal toxicity (Mosharaf *et al*., 2024). Root cell division and elongation, which are essential for root growth, are inhibited by high concentrations of salts and toxic chemicals (Mumthas *et al*., 2010). A weak root system limits the plant's ability to absorb water and nutrients from the soil, making it more vulnerable to environmental stressors and ultimately impeding growth and productivity. Hence, the objective of this paper is to investigate the influence of untreated pharmaceutical effluent on the germination and growth of *Amaranthus viridis* L. (African spinach) collected from Ilorin, Kwara State, Nigeria.

MATERIALS AND METHODS

Collection of seeds and identification of plants: Seeds of *Amaranthus viridis* L. (African spinach) was purchased from Ministry of Agriculture, Ilorin and a sample of the plants that germinated from the purchased seeds was collected and taken to the Department of Plant Biology, University of Ilorin Herbarium for identification. The Herbarium voucher number is UILH/004/1776/2022.

Collection of effluent and preparation of different dilutions (concentrations) of the effluent used for soil irrigation: Effluent sample from a pharmaceutical industry in Nigeria was collected in plastic containers from its outlet at the point of effluent release before it went into the treatment plant. The effluent sample from the pharmaceutical industry was considered as 100% (effluent). Different dilutions (concentrations) (20, 40, 60, 80, 100% and control) of the effluent were prepared using treated water. They were used to irrigate the potted experiments every other day at 100 ml per 5 kg pot for the first week after planting and 200 ml for the second week after planting and subsequently. The control was irrigated with water, 20% dilution consisted of 20 ml of effluent + 80 ml of water, 40% dilution was prepared with 40 ml of effluent + 60 ml of water, 60% dilution consisted of 60 ml of effluent + 40 ml of water, 80% dilution consisted of 80 ml of effluent + 20 ml of water and 100% was prepared with undiluted effluent.

Preparation of experimental pots: Eight seeds of *Amaranthus viridis* were planted in plastic pots containing 5kg of loamy soil and this was replicated thrice. The seeds were sown after two weeks of irrigating the soils with the different concentrations of effluent from pharmaceutical industry. The experiment was Completely Randomized Design. The control replicates were irrigated daily with treated water while the other replicates were irrigated with different concentrations of the effluent and treated water on alternate days. Hand weeding was carried out weekly. The planting period took place over a period of twelve weeks.

Germination percentage: This was calculated using the equation 1.

$$\%\,Germination = \frac{TGS}{TSS} \times 100 \quad (1)$$

Where TGS = total number of germinated seed; TSS = total number of seed sown

Vigour Index of The Seedlings: The Vigour Index (VI) of the Seedlings was determined by multiplying the % germination by seedling length (SL) as in equation 2 (Ishiaku *et al*., 2017)

$$VI = \%\,Germination \times SL \quad (2)$$

Measurement of growth parameters: *Plant height*: The measurement was taken every seven days. The distance between the soil surface and the tip of the main axis. Meter rule was used for the measurement in centimeter (cm). This was carried out to know the rate of growth of the treated plants and the control (Lale *et al.,* 2014).

Number of leaves: This count was done every three weeks, comparing the treatments with the control. This was carried out to know the rate of increase in the number of leaves and was done by physical counting of the leaves attached to the stem (Ufoegbune *et al.,* 2016)

Leaf length, leaf width and leaf area: The measurement to know the length (L) and the width of the leaves was taken every three weeks and meter rule was used for the measurement in centimeter (cm). The length (L) and width (W) were measured with the use of meter rule and calculated by equation 3 (Lale *et al.,* 2014)

$$Leaf\ area = L \times W \times 0.75 \quad (3)$$

Number of branches: This was done by counting the number of branches on each plant under the different treatments (Oloniruha *et al*., 2021)

Total fresh weight and shoot fresh weight: The plants were carefully collected at the conclusion of each planting season, and the roots were thoroughly washed with water to remove any remaining soil. After draining the excess water from the roots, their weight was measured to determine their total fresh weight. After the roots of the plants were carefully cut off, the fresh shoot weight was promptly determined using a sensitive weighing balance with a precision of 0.01g. (Sumi Dash *et al.*, 2020).

Total dry weight and shoot dry weight: After the total and shoot fresh weights were measured, the shoot and roots were then put in an oven set at 80^{0}C until they reached a constant weight, and the dry weight was recorded to determine the total dry weight and shoot dry weight (Sumi Dash *et al.*, 2020).

Determination of chlorophyll concentration: Biochemical attributes were studied in term of photosynthetic pigments. The chlorophyll-a, chlorophyll-b and total chlorophyll (a + b) were determined spectrophotometrically. Leaves were cut into small pieces, mixed thoroughly and 0.25 g of leaves was be put into a mortar to grind them finely by pestle with 25 ml of 80% acetone for 5 minutes. The homogenate was filtered through filter paper and was made a volume of 25 ml with 80% acetone. After the extraction, chlorophyll contents were monitored by UV-Vis spectrophotometer. The optical density/absorbance of each solution was measured at 663 and 645 nm against 80% acetone blank in 1 cm quartz cuvette at room temperature. The Arnon's equation (equation 4, 5, 6) was used to calculate the amount of chlorophyll-a, chlorophyll-b and total chlorophyll (a + b):

$$Chla\ (mg.g^{-1}) = \frac{[(12.7 \times A663) - (2.69 \times A645)]}{M_{Lf}} * V_{Aceone} \quad (4)$$

$$Chlb\ (mg.g^{-1}) = \frac{[(22.9 \times A645) - (4.68 \times A663)]}{M_{Lf}} * V_{Aceone} \quad (5)$$

$$Total\ Chl = Chla + Chlb \quad (6)$$

Where M_{Lf} = mass (mg) of leaf tissue; $V_{Acetone}$ = volume (ml) of acetone

Data analysis: Data collected were subjected to descriptive statistics using SPSS version and Origin 7.0 was used to plot the graph. Means were separated using Least Significance Difference (LSD) at 0.05% level of significance.

RESULTS AND DISCUSSION

Table 1 shows how different concentrations of pharmaceutical effluent affect *Amaranthus viridis* seeds. At various sampling times, there were minor variations in the results. While *A. viridis* in the control group produced the highest value, plants receiving 80% and 100% treatment produced the lowest germination percentage in soil and petri dishes. In the control and 20% effluent-treated soils, *A. viridis* germinated first, but plants treated with 100% wastewater took the longest to germinate. The germination of *Amaranthus viridis* in a petri dish produced similar results. The impact of pharmaceutical company effluent on *Amaranthus viridis* plant height is depicted in Figure 1. Due to variations in effluent content, *A. viridis* plant height seasons exhibited notable variations at various sample times. From the time of seedling emergence to the end of the study, plant height increased linearly. In comparison to other treatments, pots treated with 60% effluent showed the tallest plants, while soil in the control and soil contaminated with 80% and 100% effluent produced the shortest plants in weeks 3, 6, and 9 of both planting seasons. The impact of effluent from pharmaceutical companies on the leaf area of *Amaranthus viridis* plants is depicted in Figure 2. Effluent concentrations had a substantial impact on the linear rise in *A. viridis* leaf area. The largest *A. viridis* leaves were found in soils that had 60% effluent contamination. On the other hand, the smallest leaves were generated by plants in the control and soils contaminated with 100% effluent concentration.

Table 1: Response of *Amaranthus viridis* seeds as affected by different concentrations of effluent from pharmaceutical industry.

Treatment	G.P (%) (soil)	dish)	Days to Germ. (soil)	Days to Germ. (Petri dish)
Control	100.00±0.00^{a}	100.00±0.00^{a}	5.00±0.00^{cd}	5.00±0.00^{c}
20%UT	79.14± 0.11^{b}	80.16± 0.30^{b}	5.00±0.19^{cd}	5.33±0.15^{c}
40%UT	73.14± 0.36^{b}	71.15± 0.82^{b}	5.33± 0.08^{c}	5.33±0.08^{c}
60%UT	70.87±0.81^{bc}	69.00±0.30^{bc}	5.33± 0.21^{c}	6.00±0.13^{bc}
80%UT	62.30±0.35^{bc}	70.81±0.19^{bc}	6.67± 0.22^{b}	7.33± 0.21^{b}
100%UT	61.18± 0.74^{c}	63.18±0.42^{bc}	8.33± 0.14^{a}	8.67± 0.04^{a}
Mean	75.84	75.18	5.67	5.74
LSD $_{(0.05)}$	18.33	17.44	0.61	0.86

Legend: GP Soil – Germination Percentage in Soil, GP Dish – Germination Percentage in Petri dish, D to G Soil – Days to Germination in Soil, D to G Dish – Days to Germination in Petri dish, 20UT= 20% untreated effluent, 40UT= 40% untreated effluent, 60%UT= 60% untreated effluent, 80UT= 80% untreated effluent, 100UT=100% untreated effluent.

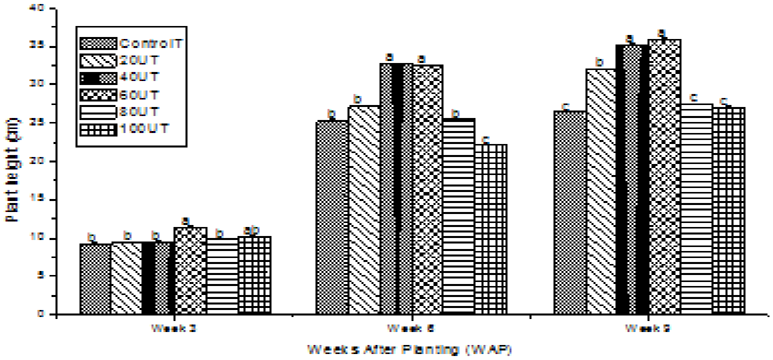

Fig 1: Plant height (in cm) of *Amaranthus viridis* plant as affected by different concentrations of effluent from pharmaceutical industry. *Legend: Control; T= Control, 20UT= 20% untreated effluent, 40UT= 40% untreated effluent, 60%UT= 60% untreated effluent, 80UT= 80% untreated effluent, 100UT=100% untreated effluent.*

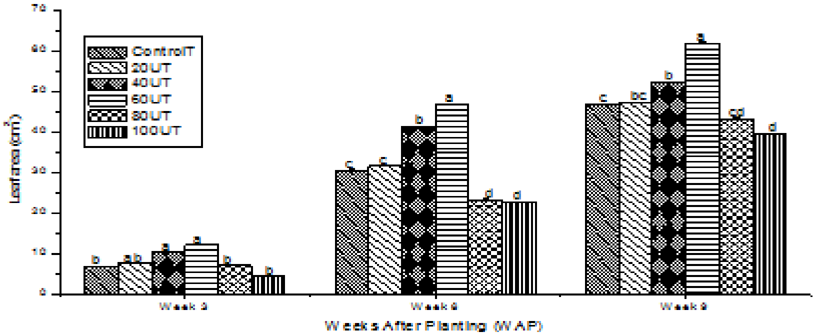

Fig 2: Leaf area (in cm^{2}) of *Amaranthus viridis* plant as affected by different concentrations of effluent from pharmaceutical industry. *Legend: Control; T= Control, 20UT= 20% untreated effluent, 40UT= 40% untreated effluent, 60%UT= 60% untreated effluent, 80UT= 80% untreated effluent, 100UT=100% untreated effluent.*

The number of leaves on *Amaranthus viridis* plants as impacted by effluent from the pharmaceutical industry is depicted in Figure 3. Effluent application had a considerable impact on *A. viridis* leaf output (at six and nine weeks after planting). The number of leaves increased linearly until the end of the sampling date, which was twelve weeks after planting, even though there weren't many when the seedlings emerged during the initial planting season. *A. viridis* treated with 60% effluent produced the most leaves by 6 and 9 weeks after planting, while plants treated with 80% and 100% effluent produced the fewest.

Figure 4 illustrates the impact of pharmaceutical company effluent on the concentration of chlorophyll (mg/g) in *Amaranthus viridis* plant leaves. Plants treated with 60% pharmaceutical industry effluent had the highest chlorophyll a value, while plants treated with 80% and 100% had the lowest value. Results for chlorophyll b and total chlorophyll content were similar. As a result, the photosynthetic capabilities of *A. viridis* from the 60% treatments were higher than those of those from the 80% and 100% treatments.

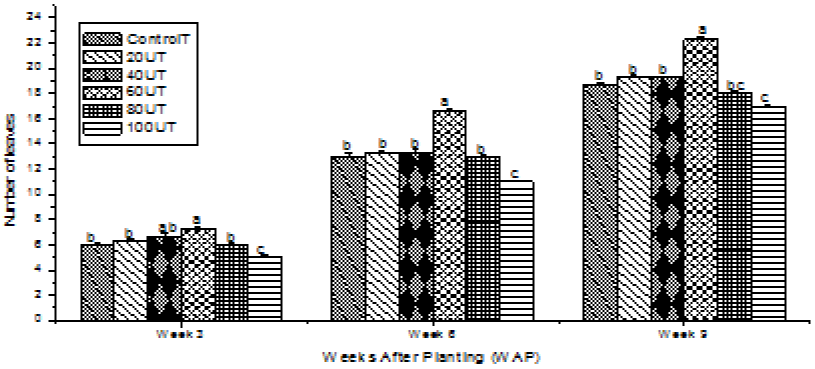

Fig 3: Number of leaves of *Amaranthus viridis* plant as affected by different concentrations of effluent from pharmaceutical industry.
Legend: Control; T= Control, 20UT= 20% untreated effluent, 40UT= 40% untreated effluent, 60%UT= 60% untreated effluent, 80UT= 80% untreated effluent, 100UT=100% untreated effluent.

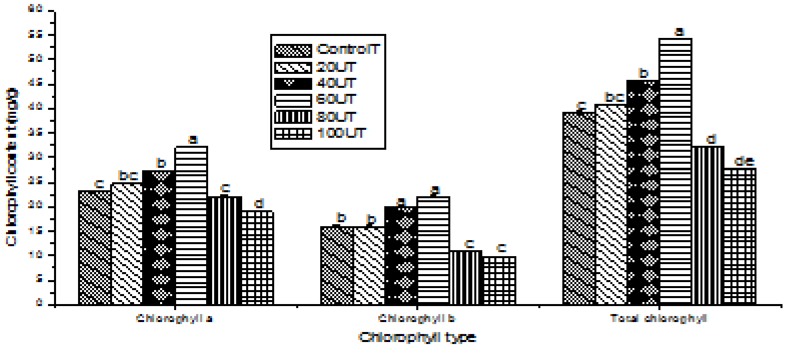

Fig 4: Chlorophyll content (in mg/g) of *Amaranthus viridis* plant leaves as affected by different concentrations of effluent from pharmaceutical industry
Legend: Control; T= Control, 20UT= 20% untreated effluent, 40UT= 40% untreated effluent, 60%UT= 60% untreated effluent, 80UT= 80% untreated effluent, 100UT=100% untreated effluent

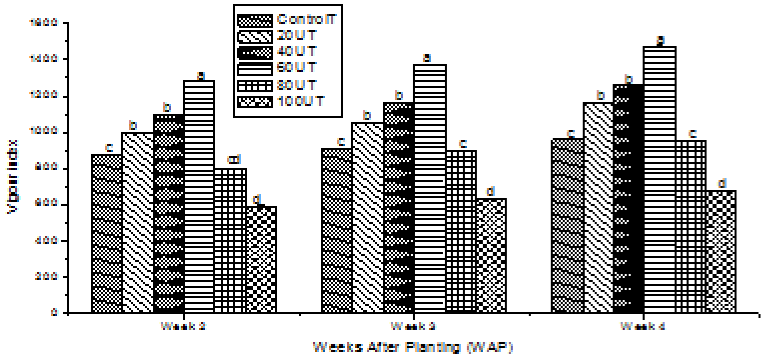

Fig 5: Vigour index of *Amaranthus viridis* plant as affected by different concentrations of effluent from pharmaceutical industry in the first and second planting seasons.
Legend: Control; T= Control, 20UT= 20% untreated effluent, 40UT= 40% untreated effluent, 60%UT= 60% untreated effluent, 80UT= 80% untreated effluent, 100UT=100% untreated effluent.

The vigour index of *Amaranthus viridis* plants as impacted by effluent from the pharmaceutical industry is displayed in Figure 5. The plants with the highest value at week two were those whose soil had been treated with 60% pharmaceutical effluent, whereas the plants with 100% treatment had the lowest value. The control and 100% effluent treatment plants had the lowest value at week three, while the treatments with 60% effluent from the pharmaceutical industry had the highest value as well. The similar pattern that was observed in week 3 was observed in week 4. This suggests that *A. viridis* has a higher potential for the quick and reliable emergence and establishment of seedlings in the 60% treatments, while this potential is reduced in the 100% effluent soil. The yield of *Amaranthus viridis* as influenced by varying concentrations of pharmaceutical effluent is displayed in Table 2. While

A. viridis from soils contaminated with the highest concentration of effluent had the lowest value in the aforementioned yield parameters, indicating less output per unit area, the results showed that *A. viridis* from soils contaminated with 60% wastewater produced the highest value of number of branches, total fresh weight, shoot fresh weight, total dry weight, and shoot dry weight, indicating more food production per unit area.

Table 2: Biomass yield parameters of *Amaranthus viridis* as affected by different concentrations of pharmaceutical industry effluent

Treatment	Number of branches	Total Fresh Weight (g) (TFW)	Shoot Fresh Weight(g) (SFW)	Total Dry Weight(g) (TDW)	Shoot Dry Weight (g) (SDW)
Control	3.33±0.51^{ab}	51.69±2.40^{b}	36.19±0.55^{b}	8.44±1.10^{b}	6.01± 0.72^{b}
20%UT	3.33±0.34^{ab}	52.86±1.16^{b}	40.18±0.57^{b}	8.93±0.09^{ab}	6.93±0.11^{ab}
40%UT	3.67±0.11^{a}	56.21±0.80^{ab}	47.35±0.38^{ab}	9.05±0.15^{a}	7.50±0.51^{a}
60%UT	4.00±0.86^{a}	65.82±3.12^{a}	54.25±0.60^{a}	10.27±0.06^{a}	7.98±0.80^{a}
80%UT	2.67±0.36^{b}	42.63±2.51^{bc}	31.11±2.45^{bc}	6.24±0.61^{bc}	4.94±0.93^{c}
100%UT	2.00±0.18^{bc}	34.67±0.58^{c}	25.21±0.84^{c}	5.06±1.22^{c}	4.02±0.36^{c}
Mean	3.18	51.15	37.94	8.02	6.05
LSD $_{(0.05)}$	0.89	13.11	13.68	2.15	1.81

Legend: 20UT= 20% untreated effluent, 40UT= 40% untreated effluent, 60%UT= 60% untreated effluent, 80UT= 80% ;untreated effluent, 100UT=100% untreated effluent

The findings showed that when the effluent concentration increased, the germination percentage of *Amaranthus viridis* decreased. Furthermore, as effluent concentration went up, the number of days to germination increased as well, indicating that a higher effluent concentration caused a delay in germination. In *A. viridis*, plant height, leaf area, number of leaves, and other yield parameters gained at lower effluent concentrations but decreased at the highest effluent concentrations. The vigor index and chlorophyll concentration showed a similar pattern. This suggests that the ability of plant seedlings to emerge quickly and reliably was higher in plants from soils with lower effluent concentrations but lower in plants from soil treated with the highest effluent concentration. Furthermore, the chlorophyll content data showed that vegetables from soils with lower effluent concentrations had greater photosynthetic capacities, whereas plants from soils with the highest wastewater concentrations had reduced photosynthetic capacities. This suggests that, in comparison to the control, pharmaceutical wastewater treatments increased plant height, leaf area, number of leaves, chlorophyll content, seedling vigor index, and other yield metrics at lower effluent concentrations. This may be explained by the fact that pharmaceutical effluent typically contains a variety of biological nutrients, salts, active components from various medications, bulk fillers, organic compounds, antibiotics, and solvents that may encourage the growth of plants.and this is supported by Hussain *et al*., (2021) and Akinola *et al*., (2016) who stated that at lower concentration, vegetable plant growth was stimulated by pharmaceutical effluent, but it was hindered at high concentrations. However, it has been shown that effluent from the pharmaceutical industry inhibits growth characteristics, vigor index, chlorophyll content, and other yield metrics at the greatest concentrations (80% and 100%). This might be due to the overwhelming impact of heavy metal stress and total solids, which may not be good for plant physiology at high concentrations (Akinola *et al.,* 2016). Because of the high concentration of pharmaceutical effluent, seedlings may not be able to absorb enough water by osmosis, which could explain the observed decrease in fresh and dry weight at high wastewater concentrations compared to the control (Balasubramaniam *et al*., 2023). Consumption of vegetables that have absorbed the effluent may cause significant changes in plant physiology (Mosharaf *et al*., 2024), which can lead to diseases in humans and animals (Agathokleous, *et al*., 2018) and should therefore be discouraged.

Conclusion: At lower concentrations, pharmaceutical effluent encouraged the germination and growth of the vegetable being studied, but at higher amounts, it inhibited the process. Pharmaceutical effluent is commonly used for household chores like farming in communities close to pharmaceutical firms. It is recommended that enterprises make it their responsibility to ensure that their waste is not disposed of carelessly, that is, without the necessary treatment. Pharmaceutical effluent can be kept out of the environment via regulatory agencies' constant monitoring and surveillance, community education to maintain a healthy environment, and more.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability: Data are available upon request from the first author or corresponding author or any of the other authors.

Contribution to knowledge: Concentrated, untreated wastewater from pharmaceutical industry is detrimental to vegetable germination, growth, development and yield although diluted concentrations of pharmaceutical wastewater may enhance germination, growth, development and yield of vegetables.

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