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Research Article

Sustainable Nutrient Recovery from Human Urine: a Soil–Plant–Microbe Interaction Study for Enhanced Agricultural Productivity of *Talinum fruticosum* L (Juss.)

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ABSTRACT
Sustainable nutrient recovery from human urine offers a desirable biotechnology approach for improving soil fertility, microbial activity, and crop productivity in low-input farming systems. This study evaluated the effects of 17-day stored human urine on soil–plant–microbe interactions and the reproductive performance of *Talinum fruticosum*. A completely randomized pot experiment was conducted using two urine application rates (360.10 mL and 620.10 mL) and an untreated control. Soil samples collected before urine application and after harvest were analyzed for bacterial and fungal populations, while reproductive parameters, including days to first flower bud initiation, number of buds, flowers, and fruits, were monitored for 30 days. The 360.10 mL treatment resulted in the earliest flower bud initiation (13 days) and produced the highest numbers of buds, flowers, and fruits, indicating optimal nutrient availability and microbial stimulation for reproductive development. Although the 620.10 mL treatment enhanced reproductive performance compared with the control, its reproductive output was lower than that of the 360.10 mL treatment, suggesting that excessive nutrient input may reduce reproductive efficiency. Microbial populations increased following urine application, with the highest bacterial and fungal counts recorded under the 620.10 mL treatment. Predominant bacterial genera included *Bacillus, Flavobacterium, Klebsiella,* and *Pseudomonas*, while *Aspergillus, Penicillium,* and *Rhizopus* were the dominant fungal genera. The findings demonstrate that moderate application of stored human urine can enhance soil microbial activity, flowering, and fruit production, highlighting its potential as an affordable and environmentally sustainable biofertilizer for nutrient-efficient agricultural production.

Keywords: Nutrient recycling; Reproductive growth; Soil microorganisms; Stored human urine; Sustainable agriculture

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INTRODUCTION

Human excreta reuse, particularly the application of stored or raw human urine has gained attention as a sustainable and affordable strategy for improving soil fertility. Although several studies have examined its nutrient contributions and agronomic effects, very few investigations have identified the specific bacterial or fungal species present in soil before urine application to determine whether stored human urine could enhance these microbial populations and

subsequently increase leafy fruit yields. Literature search found no peer-reviewed studies that simultaneously identified the microbial species listed in this research study in soil prior to urine application and assessed whether urine influences their abundance or activity. Consequently, some literature review synthesises the known ecological and plant-growth-promoting roles of the listed microorganisms based on existing microbiology and soil science.

Bacillus cereus enhances seedling establishment through enzyme secretion, nutrient mineralization, and stress-alleviating ting activities (Zhao *et al*., 2024). *Bacillus circulans* produces indole-3-acetic acid and metabolites that stimulate root development and improve disease tolerance (Qin *et al*., 2021). *Bacillus mycoides*, a rhizosphere colonizer, aids nutrient turnover through extracellular enzyme production. *Flavobacterium denitrificans* and *Flavobacterium vireti* contribute to carbon and nitrogen recycling by decomposing organic substrates. Nitrogen-fixing *Klebsiella oxytoca* and *Klebsiella pneumoniae* enhance cereal and legume growth through atmospheric nitrogen fixation and production of growth-promoting volatiles (Liu *et al*., 2018; Yu *et al*., 2025). *Micrococcus luteus* and related species participate in organic matter decomposition and help maintain microbial resilience in soil ecosystems. *Micrococcus denitrificans*, though less documented, likely contributes to similar nutrient-cycling processes. Among plant-growth-promoting rhizobacteria, *Pseudomonas fluorescens* is well recognised for siderophore production, phosphate solubilization, pathogen suppression, and overall biomass improvement (Garcia-Seco *et al*., 2015; Lally *et al*., 2017), *Pseudomonas putida* supports nutrient availability, degrades organic pollutants, and reduces plant stress, while nitrogen-transforming *Pseudomonas stutzeri* includes strains that enhance plant nutrition and resilience.

The fungal species identified also play important roles in soil productivity and plant health. *Aspergillus flavus*, although known for aflatoxin production, influences soil ecology and crop establishment (Shenge *et al*., 2019: Ngwenya *et al*., 2025). *Aspergillz nidulans* contributes to nutrient mineralization and soil enzyme pools (Agnihotri *et al.*, 1964). *A. niger, Penicillium* species (including *P. bissettii*, *P. chrysogenum*, and *P. expansum*, *P. chrysogenum*, *P. janthinellum, P. ortum*, *P. Radiatolobactum*) *Rhizopus oryza,* and *R. stolonifer* remain under-studied with respect to plant-yield enhancement in agricultural soils. *Trichoderma harzianum* is particularly well documented for phosphate solubilization, phytohormone production, biocontrol activity, and induction of systemic resistance, all of which contribute to stronger plant growth and yield (Altomare *et al*., 1999). Few previous studies had shown that bacterial species and *Rhizopus stolonifer* play central roles in nutrient cycling, decomposition, pathogen suppression, and root-stimulation processes that form the biological basis of soil fertility. Their populations and activities could theoretically be influenced by nutrient-rich inputs such as stored human urine. However, the current lack of studies identifying these specific microorganisms before urine application leaves a significant research gap. Addressing this gap will require experimental work that couples controlled urine-amendment trials with microbial identification and count to determine whether stored human urine can enhance beneficial microbial populations and improve crop productivity.

MATERIALS AND METHODS

Study Site

Field investigations were carried out in Alihame Community, Agbor, Delta State, Nigeria, Ika-South Local Government Area, Southern region of Nigeria as indicated in Fig. 1.

Plant Materials

Reproductive plant materials were obtained from *Talinum fruticosum* harvested from Dr.Akporobaro's U.A.'s garden in Alihame Community, Ika-South Local Government Area, Agbor, Delta State, Nigeria.

Materials and Equipment Used

Plant growth vessels of fixed capacity were employed alongside a mass-determination instrument, a volumetric apparatus for dispensing preserved biological fluid, and untreated supply liquid served as the reference condition.

Sampling and Handling of Growth Medium

Substrate material was obtained from the upper soil layer (0–10 cm depth) near cultivated land in the Alihame Community, Ika South Local Government Area, Agbor, Delta State, Nigeria. The soil was collected and placed into 50-kg and 20-kg bags.

Donor Origin and Processing of Liquid Amendment

A single volunteer supplied human waste (urine) gathered at dawn (between 5:30 and 6:00 a.m) over consecutive days using sealed polymer containers. The accumulated sample underwent seventeen days controlled retention before predefined aliquots were separated into marked vessels, while an additional unit containing untreated liquid functioned as a baseline. Acidity–alkalinity status

was evaluated using an indicator reagent, yielding a pale blue response.

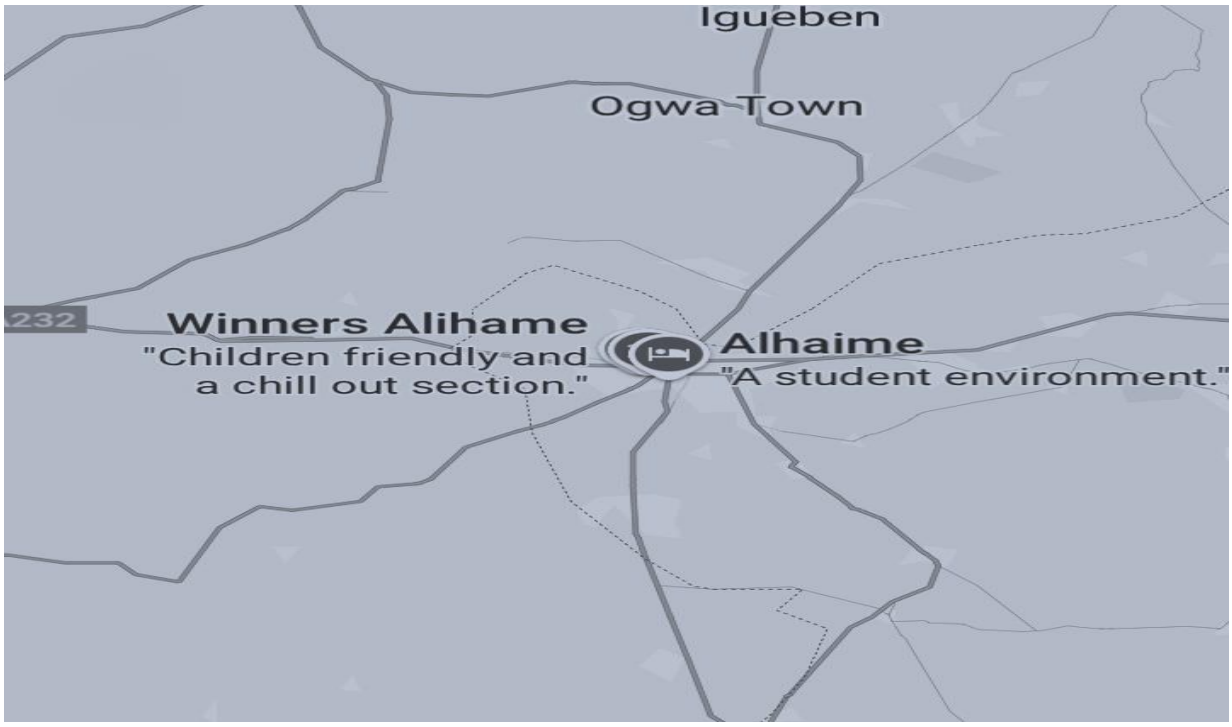

Fig. 1: Location of field work experiment

Filling and Arrangement of Cultivation Units

Cultivation units were loaded with a standardized mass of growth medium and arranged according to specified vertical depth and planar spacing measurements.

Amendment Delivery to Treatment Containers

Allocated treatments received defined volumes of the retained human waste (360.10 and 620.10 mL), whereas the control received no treatment (untreated siol(..

Source, Origin, and Identification of Seeds Material

Plant material (*Talinum fruticosum*) used in this study originated from a non-commercial single-family home garden. The origin of the seeds was traced to edible waterleaves purchased from Baleke Market, Agbor, Delta State, Nigeria. After consumption of the fresh stems and leaves, residual plant materials containing fruits with embedded seeds were discarded within the corresponding author's home garden, where natural germination occurred. The resulting plants matured, produced fruits, and seeds harvested from these fruits were used for the experiment.

Direct introduction of Propagules into Containers

Following moisture application, paired propagules extracted from mature reproductive structures were introduced into each container. Subsequent emergence was monitored, after which excess juvenile growth was removed to maintain a single individual per container.

Layout and Allocation Strategy

A randomised allocation framework governed the study, ensuring uniform exposure conditions across all growth units.

Climatic Conditions during Cultivation Period

The *Talinum fruticusom* seeds were planted on July 4, 2023, during the rainy season with a typical rainfall pattern. The plants were watered every 7 p.m., twice a week with 20 mL of tap water for each container for 4 weeks (8 times).

Evaluation of Reproductive Development

Developmental timing and reproductive output parameters, including initiation events and organ counts, were systematically monitored for all specimens.

Documentation of Structural Characteristics

Structural characteristics were visually captured and systematically recorded across treatments, with particular attention directed toward yield-related traits.

Isolation and Characterization of Heterotrophic Bacteria and Fungi from the Experimental Soil Samples

The soil microbial analysis was conducted in Quality Analytical Laboratory Services Ltd. in Benin City, Edo State, Nigeria. The steps below were followed:

Soil samples. were collected from experimental pots before the application of the 17-day stored human urine and after plant harvest to investigate the total bacteria and fungi species population present. The procedures were used to determined bacterial and fungal activities. All media were prepared according to manufacturers' instructions. The media used in the analysis included Nutrient agar (NA) and Potato Dextrose agar (PDA). Test tubes, Petri dishes and other equipment were washed, then sterilized using a hot-air oven.

Isolation and Identification of Soil Microorganisms

The microbial population of the soil sample was determined using the serial dilution and pour plate techniques. Ten grams of the homogenised soil sample was aseptically transferred into a sterile 250-mL Erlenmeyer flask containing 90 mL of sterile distilled water. The suspension was thoroughly mixed on a mechanical shaker for 10 minutes to obtain a 10^{-1} dilution.

Serial ten-fold dilutions were prepared by transferring 1.0 mL of the soil suspension into a sterile test tube containing 9.0 mL of sterile distilled water. The contents were mixed thoroughly before transferring 1.0 mL into the next tube containing 9.0 mL of sterile distilled water. This procedure was repeated sequentially until a dilution of 10^{-6} was obtained.

One milliliter aliquots from the 10^{-2}, 10^{-4}, and 10^{-6} dilutions were aseptically dispensed into sterile 90-mm Petri dishes. Approximately 15–20 mL of sterile molten Nutrient Agar (NA), cooled to 45–50°C, was poured into each Petri dish for bacterial isolation. The plates were gently swirled in a circular motion to ensure even distribution of the inoculum and allowed to solidify at room temperature. The inoculated plates were incubated in an inverted position at 37°C for 24–48 hours.

For fungal isolation, 1.0 mL aliquots from the same dilutions were aseptically transferred into separate sterile 90-mm Petri dishes. Approximately 15–20 mL of sterile molten Potato Dextrose Agar (PDA), cooled to 45–50°C, was added to each plate. The plates were gently swirled to mix the inoculum with the medium and allowed to solidify before incubation. The fungal plates were incubated in an inverted position at 28°C for 5 days.

Following incubation, plates containing 30–300 colonies were selected for enumeration. The number of colonies was counted manually, and the microbial population was expressed as colony-forming units per gram (CFU/g) of soil. Representative colonies exhibiting distinct morphological characteristics were selected and repeatedly subcultured on fresh media until pure cultures were obtained.

The purified bacterial isolates were characterized based on colony morphology, Gram staining reaction, and biochemical characteristics. Identification of the bacterial isolates was performed using the procedures and taxonomic criteria described in *Bergey's Manual of Systematic Bacteriology*. Fungal isolates were identified based on their macroscopic colony characteristics and microscopic features, including hyphal morphology, conidiophore structure, and spore characteristics, using standard taxonomic identification keys described by Samson *et al*. (2019).

Gram Staining and Biochemical Characterization of Bacterial Isolates

Gram Staining

Purified bacterial isolates were subjected to Gram staining according to standard microbiological procedures. A thin smear of each bacterial isolate was prepared on a clean, grease-free glass slide, air-dried, and heat-fixed. The smear was flooded with crystal violet for 1 minute and gently rinsed with distilled water. Gram's iodine was then applied for 1 minute, followed by rinsing with distilled water. The smear was decolourised with 95% ethanol for approximately 20–30 seconds and immediately rinsed with distilled water. The slide was counterstained with safranin for 1 minute, rinsed with distilled water, air-dried, and examined under a light microscope using the oil immersion objective (100×). The Gram reaction and cellular morphology of each isolate were recorded (Cappuccino and Welsh, 2020).

Catalase Test

The catalase activity of the bacterial isolates was determined using the slide method. A small portion of a fresh bacterial colony was aseptically transferred onto a clean, dry glass slide using a sterile inoculating loop. One drop of freshly prepared 3% hydrogen peroxide was added to the bacterial smear. The immediate production of visible oxygen bubbles indicated a positive catalase reaction, whereas the absence of bubble formation indicated a negative reaction (Cappuccino and Welsh, 2020).

Oxidase Test

The oxidase activity of the bacterial isolates was determined using the filter paper method. A piece of

sterile filter paper was moistened with freshly prepared 1% tetramethyl-p-phenylenediamine dihydrochloride oxidase reagent. A portion of a fresh bacterial colony grown on Nutrient Agar was transferred onto the moistened filter paper using a sterile wooden applicator stick. The development of a dark purple color within 10–30 seconds was recorded as a positive oxidase reaction, while the absence of a colour change within 30 seconds was recorded as a negative reaction (Cappuccino and Welsh, 2020).

Biochemical Characterization of Bacterial Isolates

The purified bacterial isolates were subjected to biochemical characterization using standard microbiological procedures described by Cappuccino and Welsh (2020). The biochemical tests performed included coagulase, urease, indole, citrate utilization, and carbohydrate fermentation tests. The coagulase test was performed to determine the ability of the bacterial isolates to produce the enzyme coagulase. The formation of visible clumping or agglutination following the addition of plasma was recorded as a positive reaction.The urease test was performed using urea agar slants to determine the ability of the isolates to hydrolyse urea. A change in the colour of the medium from yellow-orange to pink indicated a positive urease reaction. The indole test was carried out to determine the ability of the isolates to produce indole from the degradation of tryptophan. Following incubation, Kovac's reagent was added to the culture broth, and the development of a red ring at the surface of the medium was recorded as a positive reaction. The citrate utilization test was performed using Simmons citrate agar to determine the ability of the isolates to utilise citrate as the sole carbon source. Growth accompanied by a change in the medium from green to deep blue was recorded as a positive citrate utilisation reaction.

Carbohydrate fermentation tests were performed using peptone water supplemented with individual carbohydrates, phenol red indicator, and Durham tubes. Following incubation, a change in the color of the medium from red to yellow indicated acid production, while the presence of gas in the Durham tube indicated gas production. The fermentation patterns of the bacterial isolates were used as additional criteria for their identification. The results obtained from the biochemical tests were interpreted collectively and compared with the taxonomic characteristics described in *Bergey's Manual of Systematics of Archaea and Bacteria* (Whitman, 2023) for the identification of the bacterial isolates.

Identification of Fungi Isolates

The fungi isolates were identified microscopically using lactophenol cotton blue test. The identification was achieved by placing a drop of the stain on clean slide with the aid of a wire loop, where a small portion of the mycelium from the fungal cultures was removed and placed in a drop of lactophenol. The mycelium spread rapidly on the slide with the aid of the wire loop. A cover slip was gently applied with little pressure to eliminate air bubbles. The slide was then mounted and observed with 10x and 40x objective lenses respectively (Whitman, 2023)

Data Analysis

Data were presented as mean ± standard deviation based on three replicates. Statistical analyses were conducted using one-way analysis of variance (ANOVA) using IBM SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA). Where significant differences were detected, treatment means were compared using Duncan's Multiple Range Test (DMR) at the 5% level of significance. Differences among means were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Tables 1–4 present the microbial population and identification results for Samples 1–4. Sample 1 represents the experimental soil before application of 17-day stored human urine, whereas Samples 2, 3 and 4 represent the 0 mL, 360.10 mL and 620.10 mL treatments, respectively, after 30 days of *Talinum fruticosum* growth. The bacterial population increased from 15.04 ± 5.92 CFU/g × 10^n in Sample 1 to 20.61 ± 6.27, 48.41 ± 13.04 and 83.75 ± 17.33 CFU/g × 10^n in Samples 2, 3 and 4, respectively (Table 1). Similarly, the fungal population increased from 11.39 ± 4.26 CFU/g × 10^n in Sample 1 to 17.28 ± 3.46, 35.53 ± 8.59 and 60.16 ± 13.96 CFU/g × 10^n in Samples 2, 3 and 4, respectively (Table 3). Thus, the highest bacterial and fungal populations were associated with the 620.10 mL treatment.

The bacterial isolates identified included *Bacillus cereus, B. circulans, B. mycoides, Flavobacterium denitrificans, F. vireti, Klebsiella oxytoca, K. pneumoniae, Micrococcus denitrificans, M. luteus, Pseudomonas fluorescens, P. putida* and *P. stutzeri* (Table 2). The fungal isolates comprised *Aspergillus flavus, A. nidulans, A. niger, Penicillium bissetti, P. chrysogenum, P. expansum, P. janthinellum, P. ortum, P. radiatolobactum, Rhizopus oryzae, R. stolonifer* and *Trichoderma harzianum* (Table 4).

The increased microorganisms following urine application suggests that the nutrient-rich

amendment supported microbial proliferation. This agrees with Yu *et al*. (2025), Zhao *et al*. (2024), Qin *et al*. (2021), Liu *et al*. (2018) and Lally *et al*. (2017), who reported that soil microorganisms contribute to nutrient transformation, nutrient mobilization and rhizosphere functioning.

Despite the higher microbial populations under 620.10 mL, the reproductive response was superior under 360.10 mL. The first flower bud appeared at 18 days in the control, 13 days under 360.10 mL and 16 days under 620.10 mL (Table 5), indicating that 360.10 mL accelerated flower-bud initiation by 5 days relative to the control. The number of flower buds was also significantly higher under 360.10 mL, increasing from 18.67 ± 1.53 at 18 days to 42.33 ± 2.08 at 30 days, compared with 7.67 ± 3.21 in the control and 13.67 ± 1.53 under 620.10 mL at 30 days (Table 6).

Flower production followed the same pattern (Table 7). At 30 days, the 360.10 mL treatment produced 56.33 ± 2.08 flowers, compared with 0.33 ± 0.58 and 15.33 ± 1.53 flowers in the control and 620.10 mL treatments, respectively. Fruit formation was also greatest under 360.10 mL, with 54.33 ± 2.52 fruits at 30 days compared with 0.33 ± 0.58 in the control and 13.00 ± 2.65 under 620.10 mL (Table 8). The reproductive superiority of the 360.10 mL treatment was visually evident in Fig. 2, where plants receiving 360.10 mL showed greater reproductive development than the untreated and 620.10 mL plants.

The results indicate that the highest microbial population did not correspond to the highest reproductive performance. Although 620.10 mL supported greater bacterial and fungal proliferation, 360.10 mL produced earlier flowering, more flower buds, more flowers and substantially greater fruit formation. This suggests that the moderate stored human urine application provided a more favourable balance between nutrient availability, microbial activity and plant reproductive requirements. Bacterial and fungal activities associated with nutrient mineralisation, decomposition and nutrient mobilisation contributes to the improved reproductive development observed under stored human urine treatments.

This study demonstrates that 17-day stored human urine influenced both the culturable microbial population and reproductive development of *Talinum fruticosum*. The 620.10 mL treatment produced the highest microbial populations, whereas 360.10 mL was the optimum treatment for reproductive performance, reducing days to first flower-bud initiation and increasing flower-bud, flower and fruit production. These findings support the potential of appropriately applied stored human urine as a nutrient amendment while indicating that a higher application rate does not necessarily produce the best reproductive response.

Table 1: Mean population of bacterial isolates from experimental soils

Soil Sample	CFU/g × 10^n
Sample 1	15.04 ± 5.92
Sample 2	20.61 ± 6.27
Sample 3	48.41 ± 13.04
Sample 4	83.75 ± 17.33

Values: Arithmetic mean ± SD of the total viable bacterial plate count of identified isolates from each soil sample.

Table 2: Distribution of Bacterial species identified from experimental soil samples

Bacterial species Identified	Sample 1	Sample 2	Sample 3	Sample 4
Bacillus cereus	+	+	+	+
Bacillus circulans	+	+	+	+
Bacillus mycoides	+	+	+	+
Flavobacterium denitrificans	+	+	+	+
Flavobacterium vireti	+	+	+	+
Klebsiella oxytoca	+	+	+	+
Klebsiella pneumonia	+	+	+	+
Micrococcus denitrificans	+	+	+	+
Micrococcus luteus	+	+	+	+
Pseudomonas fluorescens	+	+	+	+
Pseudomonas putida	+	+	+	+
Pseudomonas stutzeri	+	+	+	+

Keys: + = organism detected/isolated; - = organism not detected/isolated. Identification was performed after isolation and purification of representative colonies obtained during the microbiological enumeration procedure.

Table 3: Mean population of Fungal isolates from experimental soils

Soil Sample	CFU/g × 10^n
Sample 1	11.39 ± 4.26
Sample 2	17.28 ± 3.46
Sample 3	35.53 ± 8.59
Sample 4	60.16 ± 13.96

Values: Arithmetic mean ± SD of the total viable fungal plate count of identified isolates from each soil sample.

Table 4: Distribution of Fungal species identified from experimental soil samples

Bacterial species Identified	Sample 1	Sample 2	Sample 3	Sample 4
Aspergillus flavus	+	+	+	+
Aspergillus nidulans	+	+	+	+
Aspergillus niger	+	+	+	+
Penicillium bissetti	+	+	+	+
Penicillium chrysogenum	+	+	+	+
Penicillium expansum	+	+	+	+
Penicillium janthinellum	+	+	+	+
Penicillium ortum	+	+	+	+
Penicillium radiatolobactum	+	+	+	+
Rhizopus oryzae	+	+	+	+
Rhizopus stolonifer	+	+	+	+
Trichoderma harzianum	+	+	+	+

Note: + = organism detected/isolated; - = organism not detected/isolated. Identification was performed after isolation and purification of representative colonies obtained during the microbiological enumeration procedure.

Table 5: Effect of 17-day stored human urine on days to first flower bud initiation in *Talinum fruticosum*

Concentration of urine	0 mL	360.10 mL	620.10 mL
Days to first flower bud	18-day	13-day	16-day

Table 6: Effect of 17-day stored human urine on number of flower buds in *Talinum fruticosum* at 18, 25, and 30 days after sowing

Days after sowing	0 mL	360.10 mL	620.10 mL
18	3.33 ± 1.15^c	18.67 ± 1.53^a	6.00 ± 0.00^b
5	6.67 ± 1.53^c	30.60 ± 1.06^a	9.00 ± 2.00^b
30	7.67 ± 3.21^c	42.33 ± 2.08^a	13.67 ± 1.53^b

Values are mean ± standard deviation; n = 3, mean with different superscript alphabets in a row are significantly different at $p \leq 0.05$.

Table 7: Effect of 17-day stored human urine on number of flowers in *Talinum fruticosum* at 25 and 30 days after sowing

Days after sowing	0 mL	360-10 mL	620.10 mL
25	0.33 ± 0.58^c	43.00 ± 1.00^a	11.67 ± 3.21^b

Values are mean ± standard deviation; n = 3. Mean with different superscript alphabets in a row are significantly different at $p \leq 0.05$.

Table 8: Effect of 17-day stored human urine on number of fruits (terreria) formed in *Talinum fruticosum* at 30 days after sowing

Days after sowing	0 mL	360.10 mL	620.10 Ml
30	0.0.33 ± 0.58^c	54.33 ± 2.52^a	13.00 ±2.65^b

Values are mean ± standard deviation; n = 3, mean with different superscript alphabets in a row are significantly different at $p \leq 0.05$.

Figure 2: Thirty-day reproductive growth of *Talinum fruticosum* grown in soil receiving 0 mL (A; control), 360.10 mL (B), and 620.10 mL (C) stored human urine treatments

CONCLUSION

The application of 17-day stored human urine significantly improved the reproductive performance of *Talinum fruticosum* and increased soil microbial populations. The 360.10 mL treatment produced the earliest flower bud initiation and the highest numbers of buds, flowers, and fruits, demonstrating that moderate nutrient enrichment provided the most favorable conditions for reproductive development. Although the 620.10 mL treatment generated the highest bacterial and fungal populations, reproductive performance was lower than that achieved with the moderate application rate. The study demonstrates that stored human urine can function as an effective, low-cost biofertilizer capable of enhancing soil biological activity and supporting sustainable crop production. Future studies should employ molecular approaches to verify microbial functions and further investigate the physiological mechanisms underlying plant responses to stored human urine.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Authors' Contributions

Uyoyou Agnes Akporobaro provided the 17-day-stored human urine and waterleaf (*Talinum fruticosum*) seeds used for planting, conceived and designed the study, and wrote the original draft of the manuscript. Uyoyou Agnes Akporobaro and Joy Anwuli Obiaigwe conducted the field experiment. Rita Nneka Nwaka performed the data analysis. Uyoyou Agnes Akporobaro, Odimegwu Agholor, Sandra Nkechi Iweriolor, and Evangeline Ogordinwa Chiedu conducted the microbial analyses in the laboratory. All authors provided financial support for the laboratory analyses, reviewed, and approved the final manuscript.

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