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RESEARCH ARTICLE

Effect of enriched humanure on soil properties and yield of Marigold (*Tagetes erecta* L) in Tropical India

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Abstract

Utilizing human excrement in agriculture shows great potential for meeting crop nutrient requirements, improving soil health and providing a sustainable solution for managing faecal waste. While a few studies have explored humanure as compost and human urine as a biofertilizer, no research has been conducted on enriched humanure, that is, humanure combined with biochar and urine. Enriched humanure is expected to enhance soil and crop benefits. To evaluate its efficacy, field trials were conducted during the winter and summer seasons in Vridhachalam on marigolds (Hybrid-Bens Tall) using a randomized block design with 11 treatments replicated three times. The treatments included: bioinputs alone, humanure + biochar + human urine at ratios of 1.0:1.0:1.0 and 1.0:1.0:1.5, enriched humanure (1.0:1.0:1.0 and 1.0:1.0:1.5, enriched for 30 days), 50 % enriched humanure + 50 % recommended dose of fertilizer (RDF), RDF + farmyard manure (FYM) and FYM alone. The results revealed that enriched humanure (1.0:1.0:1.0) significantly improved the soils' chemical and biological properties. Its application increased the soils' available nitrogen, phosphorus and potassium by 4.2 %, 18.59 % and 8.86 %, respectively. Moreover, the marigold yield reached 27650 kg ha^{-1}, a 42 % improvement compared to the control (FYM application alone).

Keywords

enriched humanure; enzymes; micronutrients; soil available nutrients; yield

Introduction

Sustainable agricultural practices are crucial for improving soil health, minimizing reliance on chemical inputs and promoting nutrient recycling in an environmentally sound manner. As global agriculture faces pressing issues like soil degradation, nutrient depletion and the high costs associated with synthetic fertilizers, alternative nutrient sources have gained attention for their potential to enrich soil and enhance crop productivity (1). Using human excreta for crop production and soil restoration represents a critical step toward creating a closed-loop nutrient cycle, gaining interest in the sanitation and agricultural sectors (2). Worldwide, on-site sanitation systems, such as pit latrines, septic tanks and container-based setups, are more common than traditional sewer networks, presenting vast potential for nutrient recovery from human waste (3). However, effectively managing this resource has been challenging, as improper treatment and use within the sanitation chain can lead to health risks from pathogens and system breakdowns. Issues like faecal sludge overflow during flooding (4), limited emptying services and inadequate disposal facilities are widespread obstacles (5-8).

Ecological or resource-oriented sanitation harnesses the nutrient potential of nearly 1 billion tons of faecal matter generated annually (3). Humanure (composted human waste) can serve as a valuable fertilizer alone or with human urine, which has shown benefits for crop yield and soil fertility in earlier studies (9-11). Combining biochar with human urine, further enhances this compost (12). Biochar, a carbon-rich material created through pyrolysis, can adsorb nutrients due to its high surface area and functional groups, making it a strong candidate for improving enriched humanure composts' nutrient profile and safety (12).

Marigold (*Tagetes* spp.) cultivation has high commercial demand in India, with annual production reaching 1.75 m MT (13, 14). Marigolds, especially *Tagetes erecta L.*, are primarily grown for non-edible uses, reducing concerns about human exposure to contaminants through consumption (15). Therefore, Marigold offers a sustainable approach to managing and converting human waste for productive use. However, limited research exists on the effects of humanure on marigold growth and yield. This study aims to evaluate how enriched humanure influences soil properties and marigold production in tropical India, offering insight into this approachs' potential benefits and viability.

Materials and Methods

Section-I

Section I described the materials and methods used for preparing enriched humanure. It covers the collection of bio-inputs, including human manure, urine and biochar and their initial characteristics. The section also detailed the process of preparing enriched humanure and the properties of the final product.

Material collection and preparation

The primary bio-inputs used for preparing enriched humanure were human manure, urine and biochar. The methods for separating, collecting and handling these bio-inputs are detailed below.

Separation of human manure and urine

Human manure (humanure) and urine used in the experiment were collected from Ecosan toilets in primary schools in the Cuddalore District. Samples were obtained from the toilets of Kummudimoolai Primary School and Nattham Meedu Primary School. The locations of these schools are shown in Fig. 1. Ecosan toilets were designed to separate urine and faeces at the source (Fig. 2). urine was collected in a separate container for three months to ensure proper sanitation. Faeces were deposited into a sealed chamber, where they underwent dehydration or composting over 6 to 12 months, resulting in nutrient-rich material (humanure). The treated faeces were used to prepare enriched humanure, which was applied to fields as compost.

Biochar preparation

The biochar used in the study was sourced locally from Cuddalore and was made from the wood of *Prosopis* trees. Prosopis wood was first cut into smaller pieces to prepare biochar and dried thoroughly. The slow pyrolysis method (400-600 °C) was used for production. The pyrolysis reactor had two chambers: one for combustion and the other for pyrolysis. Based on the bulk density of the biomass, a measured amount of dried Prosopis wood was placed in the pyrolysis chamber. Charcoal, used as a combustion fuel, was added to the combustion chamber. Once the pyrolysis process was complete, the reactor was sealed tightly to stop the reaction. The biochar was then cooled to room temperature and carefully collected (16).

Fig. 1. Ecosan toilets installed in the schools and village locations are marked in red in this image.

Fig. 2. Image of ecosan toilets fixed in primary schools.

Preparation of enriched humanure

The enriched humanure was prepared by mixing humanure, biochar and human urine in 1.0:1.0:1.0 and 1.0:1.0:1.5 ratios, respectively. The mixtures were incubated for 30 days before being used for crop cultivation. In contrast, the combinations of humanure, biochar and human urine in the same ratios were prepared by mixing the three bio-inputs in the specified proportions without storing them for 3 months after mixing. These were applied directly to the field.

Initial characteristics of bioinputs (manures)

***pH and Electrical conductivity (EC)*:** The pH and Electrical conductivity of the sample (humanure, urine and biochar) were determined in a 1:5 (manure: water) solution using a combined electrode pH meter and conductivity bridge, respectively (17).

Macronutrients

***Total nitrogen*:** The total nitrogen content in the manure sample was estimated using the Bremner method (18). One gram of the sample was digested with 15 mL of diacid (a mixture of concentrated sulfuric and perchloric acids in a 5:2 ratio). The digested mixture was distilled with an alkaline solution to release ammonia, which was then trapped in a boric acid solution. Finally, the ammonia content was determined by titration with a standard acid (0.02 N H_2SO_4) and the total nitrogen was calculated.

***Total phosphorous*:** Total phosphorous was estimated using the calorimetric (vanadomolybdate) method (19). A 1 g manure sample was digested with 15 mL of triacid (9:2:1 ratio of nitric acid, sulphuric acid and perchloric acid) until the content became colourless. The digested sample was then filtered through Whatman No.41. filter paper and makeup to 50 mL using distilled water. It was preserved for further use. A 5 mL extract was pipetted into a 25 mL volumetric flask and 5 mL of Bartons' reagent was added and made up to 25 mL using distilled water and kept for 30 min. for the yellow colour development. The intensity of the yellow colour was measured with the photoelectric calorimeter at 470 nm. The concentration of phosphorous in the sample was calculated from the standard curve.

***Total potassium*:** Total potassium was estimated using a flame photometer (17). The same tri-acid digestion method was applied for total phosphorus and the remaining digested content was preserved. A 50 mL aliquot of the extract was taken and directly fed into the flame photometer. The potassium readings were then recorded.

***Escherichia coli*:** The Most Probable Number (MPN) test was conducted to estimate the presence of *E. coli* in the sample (20). Serial dilutions of the sample were prepared and inoculated into lauryl tryptose broth (LTB) tubes, which were incubated at 37 °C for 24 to 48 hr. Tubes showing gas formation or turbidity were transferred to brilliant green bile broth (BGBB) for confirmation of coliforms and to EC broth for specific detection of *E. coli* at 44.5 °C. Positive tubes were recorded and the MPN value was calculated using standard MPN tables.

***Salmonella typhi*:** The presence of *Salmonella Typhi* was determined using standard microbiological methods (cultural method). The sample was pre-enriched in buffered peptone water and incubated at 37 °C for 24 hr. After pre-enrichment, 1 mL of the culture was transferred to Rappaport -Vassiliadis Soy Peptone (RVS) broth and incubated at 42 °C for 24 hr for selective enrichment. The enriched culture was streaked onto Xylose Lysine Deoxycholate (XLD) agar and incubated at 37 °C for 24-48 hours. Typical colonies of *Salmonella Typhi* (red colonies with black centres) were picked and confirmation was performed using biochemical tests (e.g., triple sugar iron, urea and citrate utilization) and serological tests with specific antisera (21).

Section-II

Section II provides details of the field study, including the study location, initial soil characterization and changes in soil nutrients during the crop period. Materials and methods used to measure plant and yield parameters of marigolds.

Field study

Study location: A field trial was conducted at Tamil Nadu Agricultural University's' Regional Research Station in Vridhachalam, Cuddalore district, India (11°31'46"N, 79°

21'31"E) shown in Fig. 3. During the summer and winter seasons of 2023-2024. After completing the initial trial, the same field was used for a Validation trial. The experimental site consists of red lateritic soil, classified within the Lalpettai series. Soil samples were collected from a 15 cm depth before planting to assess physical and chemical properties. These samples were shade-dried and sieved through a 2 mm mesh for testing.

Initial characteristics of soil samples

Soil pH and Electrical conductivity (EC): The soil pH was determined in a 1:2.5 (soil: water) solution after half an hour of equilibration with a glass electrode pH meter (17). The electrical conductivity of the supernatant suspension was measured using a conductivity bridge (17).

Organic carbon (OC)**:** The organic content of the soil was estimated by the wet digestion method (22). A 0.5 g of soil was taken in a 500 mL conical flask and 10 mL of 1 N $K_2Cr_2O_7$ and 20 mL of conc. H_2SO_4 were added. The contents were allowed to stand for 30 min. Then distilled water (200 mL), orthophosphoric acid (10 mL) and diphenylamine (1 mL) indicator were added. This was titrated against 0.5 N ferrous ammonium sulphates towards the end point of a bright green colour.

Macronutrients

Available nitrogen (AN)**:** The alkaline permanganate method estimates the available soil nitrogen (23). A 5 g of soil was taken in a distillation flask and 25 mL of each 0.32 % $KMnO_4$ and 2.5 % NaOH was added to the soil. Twenty ml of 2 % boric acid with a drop of the double indicator was taken in a beaker and kept near the delivery end. The distillation was carried out and the liberated NH_3 was collected and titrated against 0.02 N H_2SO_4. From the titre value, available nitrogen was calculated.

Available phosphorous (AP)**:** Available phosphorous was determined by a colorimetric method (24). A 5 g of soil sample was taken in a polycarbonate shaking bottle. Fifty mL of 0.5 M $NaHCO_3$ and a pinch of activated carbon were added to the soil and shaken for 30 min. The extract was filtered using Whatman No. 40 filter paper. A 5 mL filtrate was pipetted into a 25 mL volumetric flask and 4 ml of reagent B was added to make up to 25 mL. After 30 min, the absorbance value of the colour developed in the sample was read at 660 nm in a calorimeter and the available phosphorous was calculated from the standard curve.

Available potassium (AK)**:** A 10 g soil sample was transferred to a centrifuge tube and 25 mL of distilled water was added (25). The tube was shaken for 10 min, centrifuged and the clear supernatant liquid was filtered. The filtrate was collected in a 100 mL volumetric flask. Three additional extractions were made similarly and the combined extract was diluted to 100 mL with distilled water. The extract thus obtained was mixed well and potassium (K) was determined using a flame photometer.

Exchangeable calcium and magnesium

Exchangeable calcium and magnesium were estimated by the EDTA titrimetric method (17). A 5 g soil sample was extracted with 50 mL of 1 N ammonium acetate solution (pH 7.0) and filtered. An aliquot of the extract was titrated with a standard EDTA solution using ammonium purpurate as the indicator for calcium estimation. For magnesium, the total calcium and magnesium were first titrated with EDTA using Eriochrome Black T as the indicator and magnesium was calculated by subtracting calcium from the total.

Exchangeable sodium

Exchangeable sodium was determined using the ammonium acetate extraction method (26). A 10 g soil sample was extracted with 50 mL of 1 N ammonium acetate solution (pH 7.0) and filtered. The sodium concentration in the filtrate was measured using a flame photometer.

Exchangeable potassium

Exchangeable potassium was determined using the ammonium acetate extraction method (26). A 10 g soil sample was extracted with 50 mL of 1 N ammonium acetate solution (pH 7.0) and shaken for 30 min. The mixture was filtered and the potassium content in the filtrate was measured using a flame photometer.

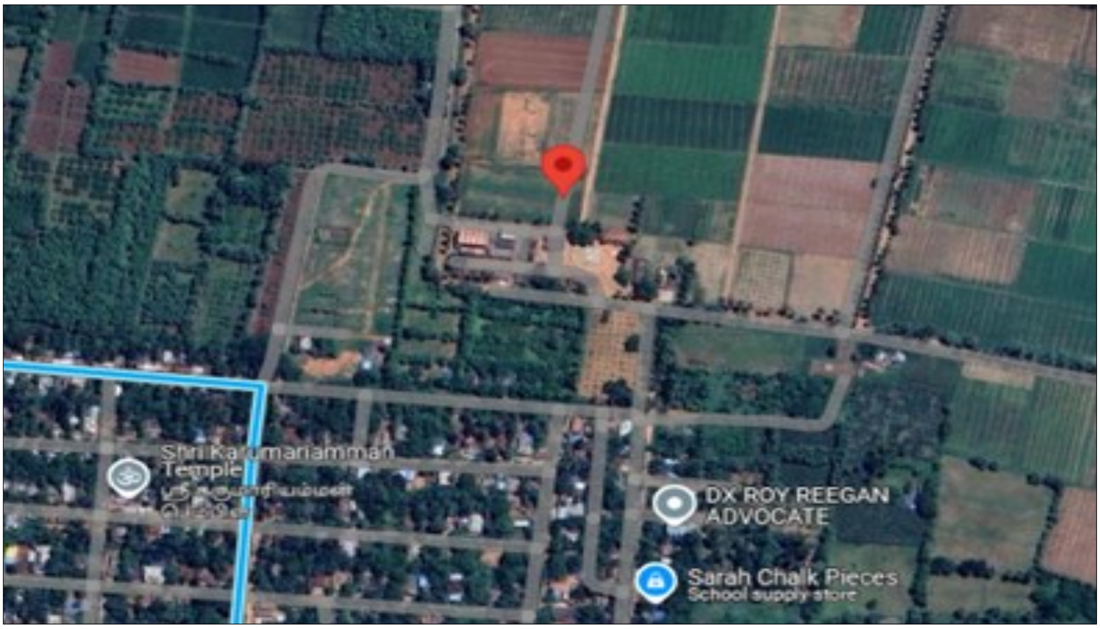

Fig. 3. Location of the experimental field marked in red colour.

Micronutrients

The micronutrients copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn) were estimated using the DTPA extraction method (27). A 10 g soil sample was shaken with 20 mL of DTPA extractant (0.005 M DTPA, 0.01 M $CaCl_2$ and 0.1 M TEA, adjusted to pH 7.3) for 2 hr. The mixture was filtered and the concentrations of Cu, Fe, Zn and Mn in the filtrate were determined using an atomic absorption spectrophotometer (AAS).

Heavy metals

Heavy metals such as lead (Pb), cadmium (Cd), chromium (Cr) and nickel (Ni) were analyzed using the Microwave Plasma-Atomic Emission Spectroscopy (MP-AES) method (28). The samples were prepared using acid digestion (USEPA Method 3051A). A 1 g soil sample was digested using a mixture of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a microwave digestion system. The digested solution was filtered, diluted to a known volume with distilled water and analyzed for heavy metal concentrations using MP-AES.

Enzyme activity

Dehydrogenase : Dehydrogenase activity in the soil was measured using the standard method (29). A 5 g soil sample was added to a 50 mL flask containing 1 mL of 0.1 M 2, 3, 5-triphenyl tetrazolium chloride (TTC) solution and 1 mL of glucose solution. The mixture was incubated at 37 °C for 24 hr to allow for enzymatic reduction of TTC. After incubation, 20 mL of methanol was added to extract the formed triphenyl formazan (TPF). The extract was filtered and the absorbance of the TPF was measured spectrophotometrically at 485 nm. The dehydrogenase activity was calculated as the amount of TPF produced per gram of soil per hour.

***Phosphatase*:** Phosphatase activity was measured using p-nitrophenyl phosphate (pNPP) as a substrate (30). A 1 g soil sample was added to a 50 Ml flask containing 10 mL of 0.1 M acetate buffer (pH 5.5) and incubated for 1 hr at 37 °C. After incubation, 1 mL of p-nitrophenyl phosphate solution (10 mM) was added to the flask and the mixture was incubated for an additional 30 min. The reaction was stopped by adding 1 mL of 0.5 M NaOH. The release of p-nitrophenol was measured spectrophotometrically at 410 nm. Phosphatase activity was calculated based on the amount of p-nitrophenol released and expressed as μg p-nitrophenol per gram of soil per hour.

Soil pathogens

***Salmonella typhi*:** The same cultural method used for manure samples explained in section I was followed (20).

***E.coli*:** *E. coli* was estimated using the MPN method (21). The same method was used for manure samples and applied to the soil samples. For the detailed procedure, please refer to Section I.

Field experimental detail

Marigold (Bens tall-hybrid) seedlings were purchased from a nearby nursery at Vridhachalam and transplanted. The experimental field consists of 11 treatments and was replicated thrice with the field layout made using a randomized block design. For the experiment, the marigold crop (Hybrid - Bens Tall) was spaced at 90 cm by 22.5 cm, the plot size was 21.6 m × 6 m and the recommended fertilizer dose was 90:90:75 NPK kg ha^{-1}. All manures are applied based on the crops' nitrogen requirement. The experimental field consists of 33 plots, each 6 × 21.6 m in size. The experiment tested various combinations of humanure, biochar and human urine, with treatments as follows:

- T_1: Humanure alone - 2571 kg ha^{-1}
- T_2: Biochar alone - 12,162 kg ha^{-1}
- T_3: Human urine alone - 7500 l ha^{-1}
- T_4: Humanure + Biochar + Human urine (1.0:1.0:1.0 ratio) - 2535 kg ha^{-1}
- T_5: Humanure + Biochar + Human urine (1.0:1.0:1.5 ratio) - 2785 kg ha^{-1}
- T_6: Enriched humanure (1.0:1.0:1.0 ratio)- 2267 kg ha^{-1}
- T_7: Enriched humanure (1.0:1.0:1.5 ratio) - 2222 kg ha^{-1}
- T_8: 50 % Enriched humanure (1.0:1.0:1.0 ratio) + 50 % RDF - 1134 kg ha^{-1} + 45:45:37.5 (N:P:K) kg ha^{-1}
- T_9: 50 % Enriched humanure (1.0:1.0:1.5 ratio) + 50 % RDF - 1111 kg ha^{-1} + 45:45:37.5 (N:P:K) kg ha^{-1}
- T_{10}: 100 % RDF + FYM - 90:90:75 (N:P:K) kg ha^{-1}
- T_{11}: FYM alone- 18000 kg ha^{-1}

Manure application

Other bio-inputs and enriched humanure were applied based on the nitrogen content of each type of manure. The manure was manually applied before transplanting and incorporated into the soil manually. Standard cultivation practices for marigolds were followed by the 2020 guidelines from Tamil Nadu Agricultural University (TNAU) (31).

Parameters recorded

Soil samples were collected and analyzed for soil pH, EC, available nitrogen (N), phosphorus (P), potassium (K), exchangeable calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) micronutrients such as iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn), as well as soil enzymes like dehydrogenase and phosphatase activity at the 30^{th}, 60^{th} and 110^{th} days after transplanting (DAT). Plant parameters such as the number of flowers per plant, the individual weight of a single flower and the marigold flower yield (recorded plot-wise and converted to kg per hectare) were also documented.

Plant parameters

***Number of flowers per plant*:** The total number of fully opened flowers per plant was counted manually. This was recorded for a randomly selected sample of plants in each plot at the peak flowering stage.

Single flower weight: Freshly harvested flowers were weighed individually using a digital weighing scale. The weight of single flowers was recorded in grams for randomly selected plants from each plot to determine the average weight.

***Flower* yield** : Flower yield was determined by weighing the total fresh weight of all flowers harvested from each plot. Yield was expressed in kilograms per hectare (kg ha^{-1}).

Statistical analysis

When the treatment effects were substantial, the randomized block design was used. SPSS statistical software was used for all statistical analyses (SPSS Inc, Chicago, IL). Differences at the $P < 0.05$ level were regarded as statistically significant.

Results and Discussion

Section 1

Humanure : The humanure exhibited a mildly alkaline pH of 8.01 and moderate salinity with an EC of 1.74 dS m^{-1}. Macronutrients such as total nitrogen (TN), phosphorus (TP) and potassium (TK) were recorded as 3.50 %, 1.72 % and 4.15 - 4.20 %, respectively. Additionally, no heavy metals or pathogens, such as *Salmonella typhi* and *Escherichia coli*, were detected. These results align with the study *"Human Waste Substitute Strategies Enhanced Crop Yield, Crop Quality and Soil Fertility in Vegetable Cultivation Soils in North China"* (32). In that study, human waste was stored in a sealed container for 12 months, composted like the current study and analyzed for its chemical and biological properties.

The stored humanure in the referenced study had a pH of 7.9, closely matching the pH value 8.01 reported here. The total N, P and K contents in the referenced study were 3.5, 5.1 and 3.2 g $kg^{-1,}$ respectively. Compared to these findings, TN values were similar, TP was lower and TK was higher. The referenced study also reported the absence of harmful pathogens, with faecal coliform levels at 0.04, an ascarid egg mortality rate of 100 % and no detectable *Salmonella* bacteria. These results comply with the Hygienic Requirements for Harmless Disposal of Night Soil (GB 7959-2012), confirming the safety and suitability of humanure as a fertilizer for agricultural use (32). The findings further support the use of human compost in agriculture. No harmful pathogens such as *Salmonella* or *E. coli* were detected and no heavy metals were observed in the humanure or soil following its application. This highlights that humanure poses no risk of heavy metal contamination when applied to fields. These results strongly support the safe handling and application of humanure as a sustainable and effective crop fertilizer.

***Biochar*:** Biochar had a neutral pH of 7.43 and a saline EC of 0.86 dS m^{-1} was recorded. The total N, P and K contents were 0.74 %, 0.20 % and 8.40 %, respectively. These results aligned with the "Production and characterization of biochar from different biological wastes" study by (33). In that study, different raw materials were used to produce biochar using the pyrolysis method and the pH, EC and macronutrients were characterized. Prosopis biochar recorded a pH of 7.57 and an EC of 1.3 dS·m^{-1} in that study. Compared to pH, it matches the current research, but in terms of EC, they also had a lower amount of salt. The study mentioned that Prosopis biochar had poor nutrient content but was high in sodium. These results are consistent with our findings in the current study, where 8.40 % total potassium (TK) was recorded. During the pyrolysis or oxidation process that generates biochar, heating causes some nutrients to volatilize, especially at the materials' surface, while others become concentrated in the remaining material. In general, biochars' carbon content is higher than nitrogen content. Biochar proves to be a stable and effective carbon sink. Due to microbial activity, the carbon locked in biochar does not release as CO_2. The pore spaces present in biochar help to support soil microbial colonization. Biochar cannot be used as crops' primary nutrient supplement, but it is an excellent carrier of nutrients. It is more important as a soil conditioner and plays a significant role in driving nutrient transformations in the soil rather than acting as a primary nutrient source (33).

***Human urine*:** Human urine exhibited a mildly alkaline pH of 7.89 and a high EC of 5.52 dS·m^{-1}. Macronutrients such as total nitrogen (TN), phosphorus (TP) and potassium (TK) were found to be 1.28 %, 1.56 % and 0.19 %, respectively. Additionally, no heavy metals or pathogens, such as *Salmonella typhi* and *Escherichia coli*, were detected. The results of the current study aligned with the findings of the study entitled *"Human Urine as an Efficient Fertilizer Product in Agriculture"* (34). That study reported that human urine was rich in nutrients, containing 80 % water, with the remainder comprising soluble nutrients. It was found to contain 15–19 % nitrogen (N), 2.5-5 % phosphorus (P_2O_5) and 3.0-4.5 % potassium (K_2O). Moreover, storing human urine before agricultural use is recommended to prevent harmful microbial growth. Storage for six months significantly reduced the risk of rotavirus and other viral infections and led to the total inactivation of *Ascaris* (parasitic worm). The study also emphasized using sealed containers during storage to minimize nitrogen loss, as nitrogen in urine was volatile and could evaporate, resulting in the loss of valuable nutrients. While urine contained microelements, including heavy metals, their quantities were typically negligible. In the current study, no heavy metals (Cd, Cr, Ni and Pb) were detected, which might have been because the urine was collected from primary school children, who were less exposed to heavy metals due to their stage of development and limited environmental exposure. The current study detected no pathogens because the urine was stored for three months before use (34).

Combinations of humanure + biochar + urine (1.0:1.0:1:0 and 1.0:1.0:1:5) and enriched humanure (1.0:1.0:1:0 and 1.0:1.0:1:5)

The combinations of humanure + biochar + urine at a 1.0:1.0:1.5 ratio recorded the highest pH, EC and leves of TN, TP and TK with values of 8.09, 1.99 ds·m-1 and 3.59 %, 1.89 % and 3.95 % respectively. At the same time, the lowest was recorded in the combinations of humanure + biochar + urine at 10:1.0:1.0 ratio, which recorded values of 8.01, 1.82

ds.m-1 and 3.55 %, 1.85 % and 3.89% respectively. This increase may be attributed to the high nitrogen content in the urine, along with other nutrients and nutrients in biochar. When mixed, the nutrient content of the combination increased. Additionally, biochar facilitated microbial activity and provided aeration for microbes. Research indicates the effectiveness of combining organic waste with nutrient-rich inputs like human urine to enhance the nutrient profile of compost (35). The study also emphasized that increasing the proportion of human urine improved nitrogen availability and overall nutrient concentration, which is consistent with the current findings.

Enriched humanure prepared. At a 1.0:1.0:1.5 ratio recorded the highest pH, EC and levels of TN, TP and TK, with values of 8.26, 2.08 ds.m-1 and 4.05 % , 2.15 % and 4.20 % respectively when compared to enriched humanure prepared at a 1.0:1.0:1.0 ratio, which recorded the values of 8.23, 1.93 ds.m-1 and 3.97 %, 2.12 % and 4.15 % respectively. This may be due to the enrichment process. During the enrichment process, human urine added soluble macronutrients (N, P, K), while biochar enhanced nutrient retention and provided a habitat for microbes. Microbial activity accelerates organic matter decomposition, releasing bound nutrients into bioavailable forms. Additionally, biochar reduced nutrient losses, especially nitrogen, through adsorption and its alkaline nature, combined with microbial action, contributed to stabilizing the mixture and reducing pathogens. Research indicates the increased nutrient content when humanure was combined with biochar and subjected to thermophilic composting (35).

Soil properties

Soil pH : In the present study, no significant difference in pH was observed between the treatments. It also indicated that adding faecal amendments did not alter the soil pH. However, it was noted that the pH increased until 60 DAT, followed by a decrease (Fig. 4). At the harvest stage (110 DAT), regardless of the treatments, all the soils exhibited a slightly neutral pH. Consistent with our findings, slightly acidic to neutral pH with the application of sewage sludge in marigold cultivation was reported (36, 37). The improvement in the soil pH was observed in the addition of rumen digesta and humanure amended plots due to the liming effect of agro-wastes reported by (38,39).

Soil EC: Similarly, Electrical conductivity (EC), an indicator of nutrient solubility, showed a gradual decline over time (Fig. 4). At 110 DAT, the application of enriched humanure (1.0:1.0:1.0 ratio -T_6) recorded the significantly higher EC of 0.63 dS m^{-1}, which was on par (0.61 dS m^{-1}) with the treatment of enriched humanure (1.0:1.0:1.5 ratio - T_7). This might be due to increased soil EC with enriched humanure supporting plant growth by enhancing nutrient availability. Higher EC indicates the presence of soluble ions like N, P and K, which are essential for photosynthesis and metabolic activities. Biochar in the mixture improved nutrient retention and slow release, while human urine provided readily available nutrients. Such amendments ensured continuous nutrient availability, prevented leaching and sustained plant growth, ultimately improving yield (32,35).

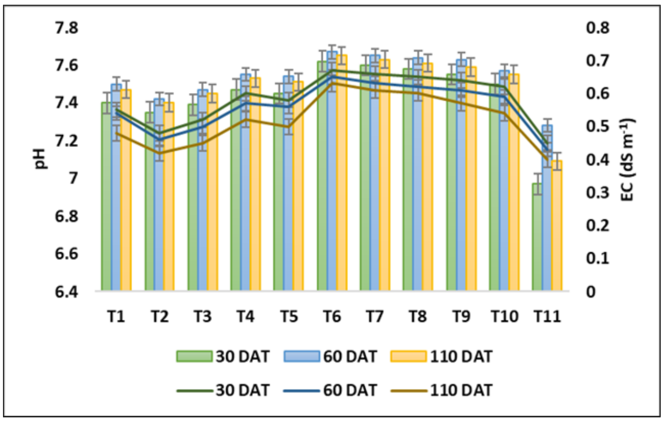

Fig. 4. Effect of enriched humanure on soil pH and EC (dS m^{-1}). Two seasons mean data. The bar graph represents the pH while the line graph represents the EC (dS m^{-1}). The error bars indicate the standard deviation values. T_1 - Humanure alone, T_2 - Biochar alone, T_3 - Human urine alone, T_4 - Humanure + Biochar + Human urine (1.0:1.0:1.0), T_5 - Humanure + Biochar + Human urine (1.0:1.0:1.5), T_6 - Enriched Humanure (1.0:1.0:1.0), T_7 - Enriched Humanure (1.0:1.0:1.5), T_8 - 50 % through Enriched Humanure (1.0:1.0:1.0) + 50 % through RDF, T_9 - 50 % through Enriched Humanure (1.0:1.0:1.5) + 50 % through RDF, T_{10} - 100 % RDF + FYM and T_{11} - FYM alone.

Soil organic carbon: Enriched humanure (T_6) significantly enhanced the organic carbon macronutrients (N, P and K - Fig. 5a-5b). In this study, organic carbon levels decreased consistently as the crop growth period. However, across all treatments and sampling periods, the highest organic carbon content of 0.55 %, 0.53 % and 0.48 % at 30, 60 and 110 DAT, respectively was noted (Fig. 5a) with the treatment received the application of enriched humanure at the ratio of 1.0:1.0:1.0 (T_6) followed by the treatment T_7 which received enriched humanure at the ratio of 1.0:1.0:1.5, While the lowest was recorded in the treatment T_{11}, which received the FYM application alone. An increase in soil organic carbon improved crop growth by enhancing soil structure, nutrient availability and microbial activity. Soil organic carbon promotes soil aggregation, increased water retention and enhanced aeration, which is essential for root development and nutrient uptake. It also served as a reservoir of macro and micronutrients, releasing them gradually through microbial decomposition to ensure a steady nutrient supply for plant growth. This elevation in enriched humanures' organic carbon content promotes marigold crop growth. Research indicates that increased organic carbon levels were noticed with humanures' application (39).

Soil available nitrogen (N), phosphorus (P) and potassium (K)

As with organic carbon, the available nitrogen, phosphorus and potassium levels were observed to decline as the crop grew (Table 1) gradually. During 30, 60 and 110 DAT, application of enriched humanure at the ratios of 1.0:1.0:1.0 (T_6) and 1.0:1.0:1.5 (T_7) registered significantly higher levels of available nitrogen (277, 265 and 248 kg ha^{-1}, respectively- Fig. 5a), available phosphorus (16.5, 14.5 and 12.5 kg ha^{-1}, respectively - Fig. 5b) and available potassium (193, 176 and 172 kg ha^{-1}, respectively - Fig. 5b) when compared to all other bio-compost treatments and the control. This might be due to the microbial population and enzyme activities present in the enriched humanure. These microbial populations promote crop growth and development through various

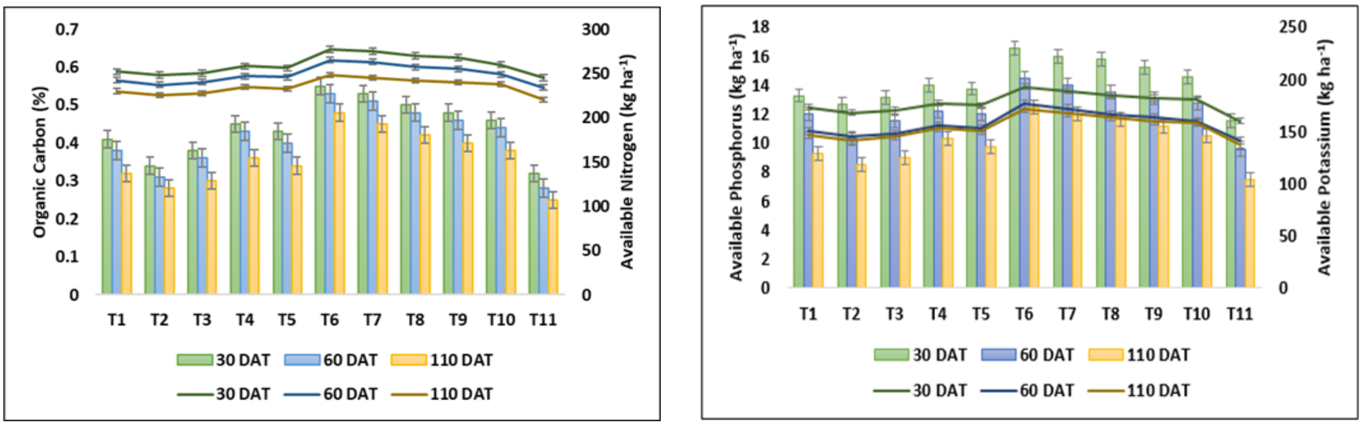

Fig. 5. Effect of enriched humanure on soil organic carbon and macronutrients. Two seasons mean data. a. Organic carbon (bar graph) and available nitrogen (line graph); b. Available phosphorus (bar graph) and available potassium (line graph). The error bars indicate the standard deviation values. T_1 - Humanure alone, T_2 - Biochar alone, T_3 - Human urine alone, T_4 - Humanure + Biochar + Human urine (1.0:1.0:1.0), T_5 - Humanure + Biochar + Human urine (1.0:1.0:1.5), T_6 - Enriched Humanure (1.0:1.0:1.0), T_7 - Enriched Humanure (1.0:1.0:1.5), T_8 - 50 % through Enriched Humanure (1.0:1.0:1.0) + 50 % through RDF, T_9 - 50 % through Enriched Humanure (1.0:1.0:1.5) + 50 % through RDF, T_{10} - 100 % RDF + FYM and T_{11} - FYM alone.

Table 1. Effect of enriched humanure on soil nutrient content under Marigold cultivation

Treatments	Available nitrogen (kg ha^{-1})				Available phosphorous (kg ha^{-1})				Available potassium (kg ha^{-1})			
	Days after planting											
	30	60	110	Mean	30	60	110	Mean	30	60	110	Mean
T_1	252	242	230	241.33	13.28	12.00	9.26	11.51	173	150	147	157
T_2	248	237	225	236.67	12.65	10.30	8.50	10.48	168	145	141	151
T_3	250	240	227	239.00	13.11	11.50	9.00	11.20	170	148	145	154
T_4	258	247	235	246.67	14.00	12.15	10.32	12.16	177	156	153	162
T_5	256	246	233	245.00	13.75	12.00	9.75	11.83	175	153	150	159
T_6	277	265	248	263.33	16.50	14.50	12.50	14.50	193	176	172	180
T_7	275	263	245	261.00	16.00	14.00	12.00	14.00	189	172	168	176
T_8	270	257	242	256.33	15.80	13.50	11.65	13.65	185	166	163	171
T_9	268	255	240	254.33	15.25	13.09	11.15	13.16	182	164	160	169
T_{10}	260	249	238	249.00	14.53	12.76	10.54	12.61	180	160	158	166
T_{11}	245	234	220	233.00	11.57	9.52	7.51	9.53	160	141	137	146
Mean	259.91	248.64	234.82	247.79	14.22	12.30	10.20	12.24	177	157	154	163
SE.d (0.05)	28.61	3.66	4.59	-	0.31	0.18	0.26	-	3.74	3.09	2.92	-
CD	NS	7.64	9.59	-	0.66	0.39	0.55	-	7.81	6.45	6.09	-

simple and complex mechanisms (40). In addition, the mineralization of organic wastes releases organic-bound nutrients into the soil, particularly NPK and organic matter. These findings are consistent with those of (38, 39). Furthermore, higher levels of macronutrients are bound by humic substances in enriched humanure, which is a key criterion for the utility of humanure in agriculture (41, 42). Additionally, adding faecal amendments significantly increased soil nitrogen, phosphorus and potassium levels by a median of 33 %, 26 % and 26 %, respectively, as reported by (37).

Soil-exchangeable calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K)

Similarly, the significant levels of exchangeable calcium (1.98 cmol (p+) kg^{-1}), magnesium (1.45 cmol (p+) kg^{-1}), sodium (0.64 cmol (p+) kg^{-1}) and potassium (0.56 cmol (p+) kg^{-1}) at 110 DAT (Fig. 6a and b) were recorded with the

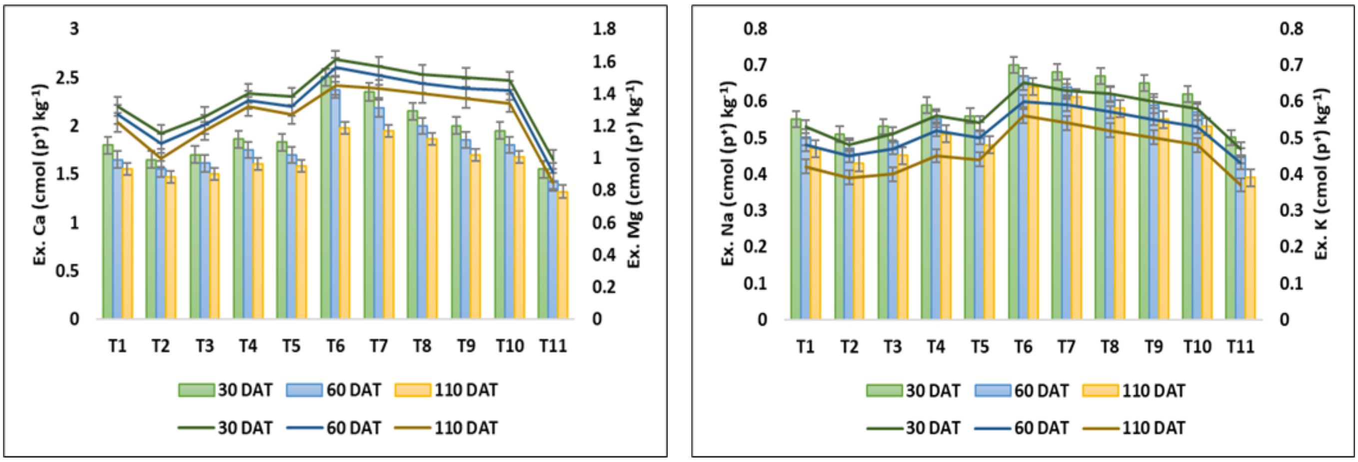

Fig. 6. Effect of enriched humanure on soil exchangeable cations**.** Two seasons mean data a. Exchangeable calcium (bar graph) and magnesium (line graph); b. exchangeable sodium (bar graph) and potassium (line graph). The error bars indicate the standard deviation values. T_1 - Humanure alone, T_2 - Biochar alone, T_3 - Human urine alone, T_4 - Humanure + Biochar + Human urine (1.0:1.0:1.0), T_5 - Humanure + Biochar + Human urine (1.0:1.0:1.5), T_6 - Enriched Humanure (1.0:1.0:1.0), T_7 - Enriched Humanure (1.0:1.0:1.5), T_8 - 50 % through Enriched Humanure (1.0:1.0:1.0) + 50 % through RDF, T_9 - 50 % through Enriched Humanure (1.0:1.0:1.5) + 50 % through RDF, T_{10} - 100 % RDF + FYM and T_{11} – FYM alone.

application of enriched humanure at the ratio of 1.0:1.0:1.0 (T_6) and 1.0:1.0:1.5 (T_7). The lowest exchangeable Ca, Mg, Na and K were recorded in the treatment T_{11}, which received FYM application alone. This notable increase in exchangeable cations might result from the application of enriched humanure. It can be attributed to the rise in soil pH from these organic amendments, which reduce soil acidity. When soil becomes less acidic, cations like Ca, Mg, Na and K are less likely to be tightly bound and more available in exchangeable forms, benefiting plants. Research indicates increased exchangeable Ca, Mg, Na and K in humanure application treatments (36, 39).

Biochar enhances the soils' cation exchange capacity (CEC) due to its porous structure and high surface area, which helps retain essential nutrients, making them more accessible to plants. The rich macronutrient content of humanure and urine, including Ca, Mg, Na and K, is released in plant-available forms during decomposition. The microbial decomposition of these organic materials boosts nutrient cycling and mineralization, increasing the availability of nutrients to plants. Combining humanure, biochar and urine synergizes soil, improving structure and nutrient availability. This combination enhances the release and retention of exchangeable cations by increasing organic matter and CEC (12, 36).

Soil micronutrients and heavy metals

Micronutrients such as iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) are crucial for crop growth. In this study, significant levels of micronutrients, including Fe (29.57 mg kg^{-1}), Cu (2.01 mg kg^{-1}), Mn (25.15 mg kg^{-1}) and Zn (5.39 mg kg^{-1}) (Fig. 7a-7b), were observed with the application of enriched humanure at the ratio of 1.0:1.0:1.0 (T_6) and 1.0:1.0:1.5 (T_7) respectively. Soil microbes make these essential micronutrients more available for plant uptake (39). Additionally, the biochar in enriched humanure likely helped retain nutrients and prevent their leaching due to its high cation exchange capacity and porosity (36).

Soil enzyme (phosphatase and dehydrogenase) activity

The beneficial impacts of enriched humanure on soil extended beyond enhancing crop yield and improving soil properties. They also stimulated soil biological activities in multiple ways. Soil enzymes, catalytic proteins crucial for numerous biochemical processes, are closely linked to soil fertility (42). In this study, the application of enriched humanure significantly enhanced enzymes involved in carbon (C) and phosphorus (P) cycles, specifically dehydrogenase and phosphatase activity (Table 2 and Fig. 8). These enzyme levels were observed to increase until 60 DAT, after which they declined. By 110 DAT, the maximum dehydrogenase (5.45 µg of TPF g^{-1} soil) and phosphatase activity (12.85 µg nitrophenyl g^{-1} soil h^{-1}) were recorded with the application of enriched humanure at the ratio of 1.0:1.0:1.0 (T_6) and 1.0:1.0:1.5 (T_7). The positive effects of these applications on soil enzyme activity are attributed to the high organic matter content of enriched humanure. Consistent with these findings, several studies have

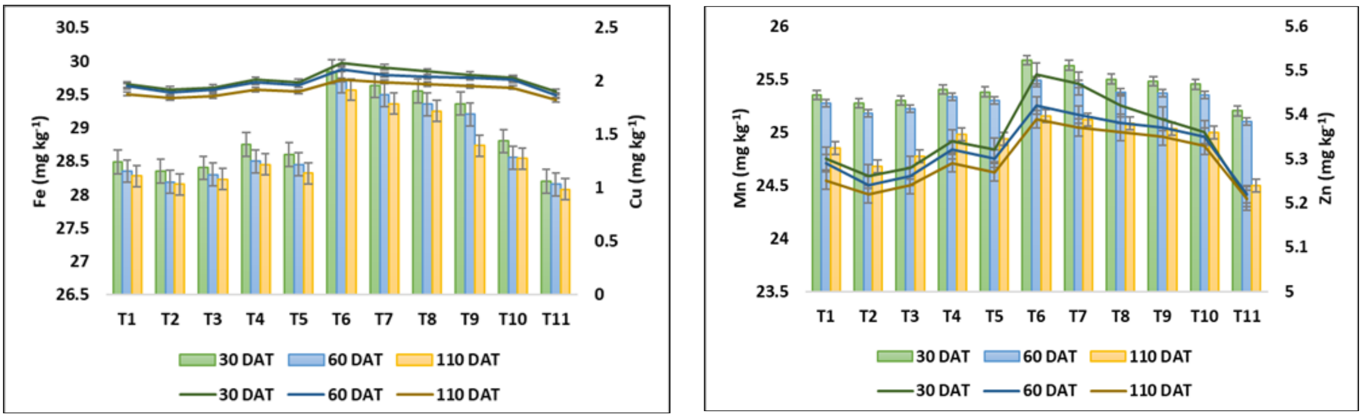

Fig. 7. Effect of enriched humanure on soil micronutrients. Two seasons mean data a. Iron (bar graph) and Copper (line graph); b. Manganese (bar graph) and zinc (line graph). The error bars indicate the standard deviation values. T_1 - Humanure alone, T_2 - Biochar alone, T_3 - Human urine alone, T_4 - Humanure + Biochar + Human urine (1.0:1.0:1.0), T_5 - Humanure + Biochar + Human urine (1.0:1.0:1.5), T_6 - Enriched Humanure (1.0:1.0:1.0), T_7 - Enriched Humanure (1.0:1.0:1.5), T_8 - 50 % through Enriched Humanure (1.0:1.0:1.0) + 50 % through RDF, T_9 - 50 % through Enriched Humanure (1.0:1.0:1.5) + 50 % through RDF, T_{10} - 100 % RDF + FYM and T_{11} - FYM alone.

Table 2. Effect of enriched humanure on soil enzyme activities under Marigold cultivation

Treatments	Phosphatase activity (µg nitro phenyl g^{-1} soil h^{-1})				Dehydrogenase (µg of TPF g^{-1} soil h^{-1})			
	Days after planting							
	30	60	110	Mean	30	60	110	Mean
T_1	8.93	10.18	9.24	9.70	3.12	4.52	3.00	3.55
T_2	6.57	7.75	6.11	6.81	2.45	3.55	2.15	2.72
T_3	8.35	9.80	8.15	8.76	2.85	3.75	2.45	3.02
T_4	10.45	11.80	10.75	11.08	3.83	4.87	3.55	4.08
T_5	10.00	11.46	10.50	10.80	3.36	4.72	3.00	3.69
T_6	14.52	15.65	12.85	14.34	5.50	6.75	5.45	5.90
T_7	14.00	15.14	12.67	13.93	5.35	6.33	5.25	5.64
T_8	13.63	14.56	12.10	13.43	5.00	6.11	4.96	5.36
T_9	13.00	14.11	12.00	13.03	4.75	5.89	4.37	5.00
T_{10}	11.75	12.86	11.26	11.95	4.11	5.35	4.00	4.49
T_{11}	6.00	7.16	5.75	6.30	2.15	3.10	1.65	2.30
Mean	10.65	11.86	10.13	10.92	3.86	4.99	3.62	4.16
SE.d	0.19	0.21	0.25	-	0.08	0.13	0.08	-
CD (0.05)	0.41	0.44	0.53	-	0.17	0.27	0.18	-

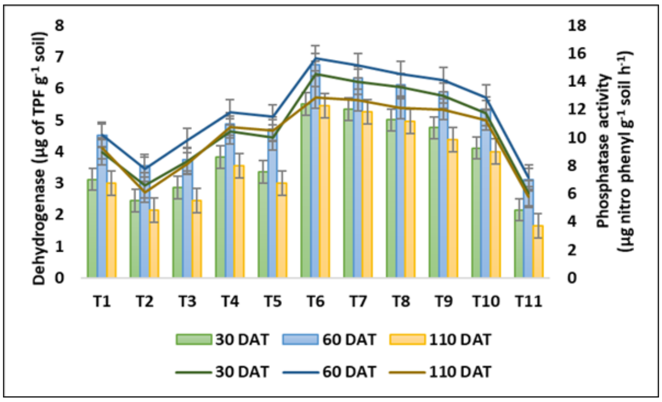

Fig. 8. Effect of enriched humanure on soil dehydrogenase (bar graph) and phosphatase activity (line graph). The error bars indicate the standard deviation values. Two seasons mean data. T_1 - Humanure alone, T_2 - Biochar alone, T_3 - Human urine alone, T_4 - Humanure + Biochar + Human urine (1.0:1.0:1.0), T_5 - Humanure + Biochar + Human urine (1.0:1.0:1.5), T_6- Enriched Humanure (1.0:1.0:1.0), T_7 -Enriched Humanure (1.0:1.0:1.5), T_8 - 50 % through Enriched Humanure (1.0:1.0:1.0) + 50 % through RDF, T_9 - 50 % through Enriched Humanure (1.0:1.0:1.5) + 50 % through RDF, T_{10} - 100 % RDF + FYM and T_{11} - FYM

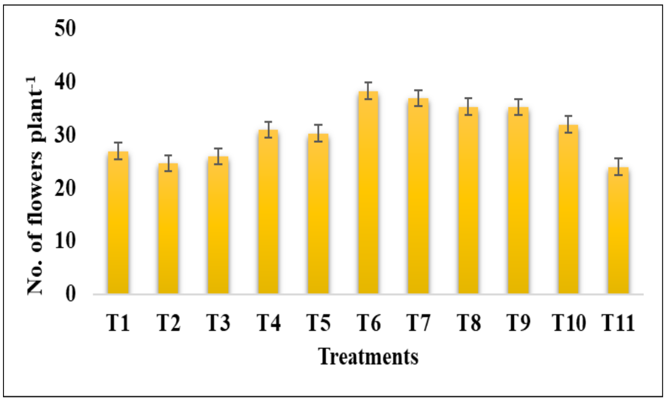

Fig. 9. Effect of enriched humanure on No. of flowers per plant. Two seasons mean data. The error bars indicate the standard deviation values. T_1 - Humanure alone, T_2 - Biochar alone, T_3 - Human urine alone, T_4 - Humanure + Biochar + Human urine (1.0:1.0:1.0), T_5 - Humanure + Biochar + Human urine (1.0:1.0:1.5), T_6 - Enriched Humanure (1.0:1.0:1.0), T_7 - Enriched Humanure (1.0:1.0:1.5), T_8 - 50 % through Enriched Humanure (1.0:1.0:1.0) + 50 % through RDF, T_9 - 50 % through Enriched Humanure (1.0:1.0:1.5) + 50 % through RDF, T_{10} - 100 % RDF + FYM and T_{11} – FYM alone.

demonstrated that sewage sludge significantly enhanced soil enzyme activities, promoting nutrient recycling for crops (42).

Number of flowers per plant

There is a significant difference in the number of flowers per plant between all the treatments (Fig. 9). Treatment T_6, which received the application of enriched humanure at the ratio of 1.0:1.0:1.0 recorded significantly more number of flowers per plant (38.33), followed by the treatment T_7 application of enriched humanure at the ratio of 1.0:1.0:1.5 (37.00) and the least number of flowers (24.00) was produced in application of farmyard manure alone (T_{11}) (Fig. 10). This increase in the number of flowers per plant might be due to the nutrients and enzyme activity of enriched humanure. Consistent with these findings, the increased yield and number of flowers per plant were also reported in French marigold plants when amended with sewage sludge (36).

Individual flower weight

Individual flower weight exhibited significant differences among the treatments with enriched humanure applications at the ratio of 1.0:1.0:1.0 and 1.0:1.0:1.5, respectively. Their combinations, such as 50 % enriched humanure + 50 % RDF (Fig. 11). The application of enriched humanure at 1.0:1.0:1.0 ratio (T_6) increased individual flower weight by 24.39 g, followed by the treatment T_7, which received the application of enriched humanure at 1.0:1.0:1.5 ratio (23.20 g), which were statistically on par. The lowest individual flower weight was recorded in the treatment T_{11}, which received FYM application alone. This increase in flower weight could be attributed to the enriched humanure improving soil fertility through the enhanced availability of nutrients. Additionally, the enriched humanure may have promoted the production of salicylic acid (SA), a key plant hormone involved in stress regulation and flower development. Previous studies have shown that applying enriched organic amendments in crops like marigolds and roses enhances SA production, leading to better growth and development (36, 43).

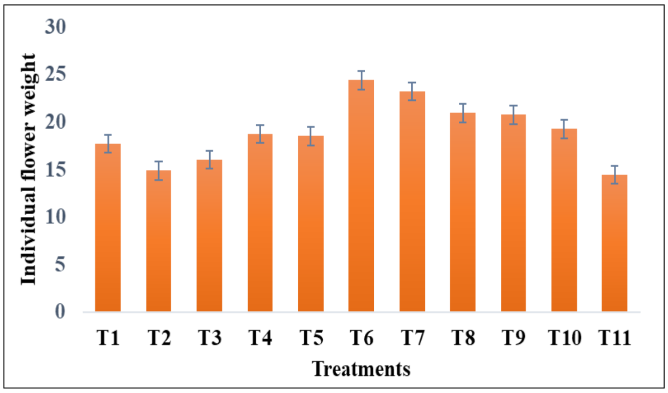

Fig. 11. Effect of enriched humanure on individual flower weight. The error bars indicate the standard deviation values. Two seasons mean data. T_1 - Humanure alone, T_2 - Biochar alone, T_3 - Human urine alone, T_4 - Humanure + Biochar + Human urine (1.0:1.0:1.0), T_5 - Humanure + Biochar + Human urine (1.0:1.0:1.5), T_6 - Enriched Humanure (1.0:1.0:1.0), T_7 - Enriched Humanure (1.0:1.0:1.5), T_8 - 50 % through Enriched Humanure (1.0:1.0:1.0) + 50 % through RDF, T_9 - 50 % through Enriched Humanure (1.0:1.0:1.5) + 50 % through RDF, T_{10} - 100 % RDF + FYM and T_{11} – FYM alone.

T_6 Enriched humanure (1.0:1.0:1.0) **T_7 Enriched humanure (1.0:1.0:1.5)** **T_{11} Farmyard manure alone**

Fig. 10. Image of highest and lowest number of flowers recording plots.

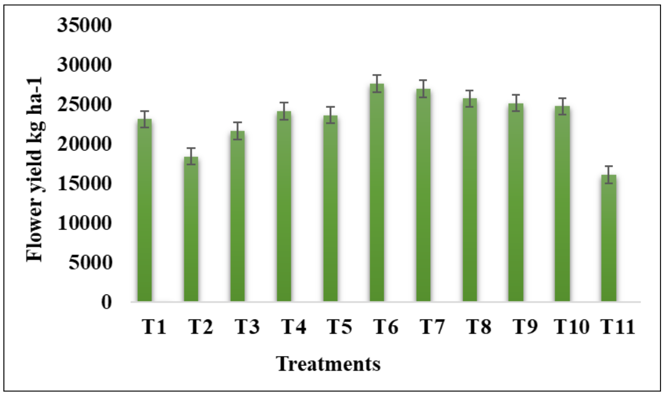

Fig. 12. Effect of enriched humanure on yield of marigold. The error bars indicate the standard deviation values. Two seasons mean data. T_1 - Humanure alone, T_2 - Biochar alone, T_3 - Human urine alone, T_4 - Humanure + Biochar + Human urine (1.0:1.0:1.0), T_5 - Humanure + Biochar + Human urine (1.0:1.0:1.5), T_6 - Enriched Humanure (1.0:1.0:1.0), T_7 - Enriched Humanure (1.0:1.0:1.5), T_8 - 50 % through Enriched Humanure (1.0:1.0:1.0) + 50 % through RDF, T_9 - 50 % through Enriched Humanure (1.0:1.0:1.5) + 50 % through RDF, T_{10} - 100 % RDF + FYM and T_{11} - FYM alone.

Yield recording

Flower harvesting

Marigold field view

Fig. 13. Flower harvesting and yield recording in marigold crop.

Flower yield

The significant difference between the treatments was noted in marigold yield (Fig. 12-13). The Marigold (Bens tall) yield was higher with the application of enriched humanure than all other treatments. The treatment that received application of enriched humanure at the ratio of 1.0:1.0:1.0 (T_6) recorded the highest yield of 27650 kg ha^{-1}, followed by the treatment that received enriched humanure at the ratio of 1.0:1.0:1.5 (T_7), which recorded 27000 kg ha^{-1}. The lowest yield of 16130 kg ha^{-1} was observed in the treatment, which received farmyard manure alone (T_{11}). It was observed that flower yield increased by 42 % in plots treated with enriched humanure when compared to those treated with farmyard manure alone. This improvement in yield can be attributed to the superior nutrient availability provided by enriched humanure, which combines the benefits of organic matter with enhanced nutrient release. Adding biochar and urine to prepare enriched humanure likely played a key role in improving soil fertility, enhancing microbial activity and providing essential nutrients in a readily available form for plant uptake. Research indicates that applying enriched organic amendments significantly improved crop yield and soil health by enhancing nutrient availability and soil structure (44, 45).

Conclusion

Enriched humanure represents a valuable reservoir of essential plant nutrients, predominantly nitrogen, phosphorus, potassium and various micronutrients. Its application to soil facilitates nutrient recycling and potentially reduces reliance on expensive fertilizers in agricultural fields. Its use in soil recycles these nutrients and offers a cost-effective alternative to chemical fertilizers in agricultural production. This study highlights that enriched humanure, particularly when combined with biochar and human urine in a 1.0:1.0:1.0 ratio, is highly beneficial for marigold cultivation. The treatment notably improved soil organic carbon, available macro- and micronutrients and soil enzyme activity, positively impacting marigold growth. Moreover, these soil quality and fertility improvements indicate the potential of enriched humanure for sustainable floriculture practices. Nonetheless, further research is warranted to assess the suitability of enriched humanure for cultivating other floricultural and horticultural crops. Its' also essential to study the long-term effects of this amendment to understand how long the released nutrients remain available in the soil after application

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Authors' contributions

PK conducted the experiment and wrote the original draft; PJ helped with data curation supervision and MM checked the data and supervision. NT helped with conceptualization, supervision & editing. MK and GS helped in editing the MS.

Compliance with ethical standards

Conflict of interest: Author's do not have any conflict of interest to declare

Ethical issues: None

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