

E-ISSN: 2709-9369

P-ISSN: 2709-9350

www.multisubjectjournal.com

IJMT 2022; 4(1): 144-152

Received: 12-01-2022

Accepted: 06-03-2022

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Novel approach to overcome limitations of humanure usability for farming

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DOI: <https://doi.org/10.22271/multi.2022.v4.i1c.146>

Abstract

Disposal of humanure is a big challenge as well as has huge potential for farming. The major drawback of humanure is high levels of potassium, nitrogen, phosphorus, which enhance pathogenicity by degrading water quality which results in crop-causing cardiac problems. It also has a low level of organic contents and high levels of pathogenic fungus and bacteria affecting both crop and consumer. Trichoderma is a biocontrol agent known to have potential antimicrobial and antifungal activity against pathogens, which are also commonly present in humanure. Hence, the present study investigated treatment (8 days of incubation) of humanure with Trichoderma to find the effect on humanure's limitations for farming use. Afterward, germination of seeds was done in the 4 mediums: (i) Soil, (ii) Untreated Humanure, (iii) Treated Humanure, and (iv) Untreated Humanure with Soil, to see the effects of treatment of humanure on crops compared to other samples. The study interestingly found that Trichoderma treatment reduced high levels of potassium and phosphorus in humanure from 610.6 to 244.5 (kg/ hectare) and from 103 to 98.56 (kg/hectare) respectively, while increased low-level organic matter (2% to 10.28%) and sulfur (for immunity) (34.51 to 276.54 unit) indicating its potential. Moreover, Trichoderma treatment-mediated reduction of potassium and phosphorus shall also be reflected in the reduction of germination rate because high levels of potassium and nitrogen in humanure promote the rate of germination. The study showed that Trichoderma treatment reduced germination rate in humanure i.e. coefficient of the velocity of germination from 75.68 to 72.22 units, germination rate index from 21.79%/day to 17.78%/day and final germination percentage from 73.1% to 66.2%; though these values were higher compared to soil, yet indicating the potential of Trichoderma treatment. In addition, the increase in electric conductivity by Trichoderma treatment demands optimization of the approach for farming. Summarily, trichoderma treatment stands as the novel and potential approach to overcome the limitation of humanure to balance nutrient levels to that of ideal soil contents and to reduce pathogenicity of humanure.

Keywords: Trichoderma, humanure, decomposition, treatment, farming, potential

1. Introduction

Proper disposal of humanure will be a solution to two major problems – (i) Mass dumping of sewage into open and water bodies, and (ii) Lack of natural soil conditioner-cum-fertilizer. The best approach to overcome these is to use the humanure for such farming requirements. However, the use of unprocessed humanure, directly from STP plants, as fertilizer is not an optimum practice as it contains disease-causing pathogens and it has low nutrient content. Due to this, it should be given such a treatment that will reduce disease-causing pathogens in it and increase its nutrient content. The results of such a treatment to humanure, provided by using a bio fungicide named Trichoderma Viride, are studied. Different growth parameters are also examined to understand the difference in fertility of the mediums for crops if there arises any.

2. Background Research

2.1 Micro Limitation – Humanure has Imbalanced Chemical Composition

Feces are composed of water, protein, undigested fats, polysaccharides, bacterial biomass, ash, and undigested food residues. The major elements in feces as a percentage of wet weight are oxygen 74%, hydrogen 10%, carbon 5%, and nitrogen 0.7%, including the hydrogen and oxygen present in the water fraction of the feces (Synder W, 1975). The solid fraction organic material makes up between 84% and 93% in which carbon content is 44% to 55% in dry feces (Feachem, 1978)^[11], (Nwaneri C, 2008)^[29], (Bai F, 2011)^[29].

The organic solids fraction can be further broken down to the fractions of 25–54% bacterial biomass (Stephen 1980)^[37], (Guyton 2000)^[14], 2–25% protein, or nitrogenous matter (in addition 50% of bacterial biomass is protein) (Canfield 1963; Volk T, 1987)^[7, 39], 25% carbohydrate or any other non-nitrogenous undigested plant matter (Volk T, 1987)^[39], and

2–15% undigested lipids (Kien C, 1981) [21], (Chen HL 1998) [8], (Wierdsma NJ, 2011) [41]. These fractions are highly dependent on dietary intake and its biological availability.

The major challenge of using humanure in farming is its high levels of potassium, nitrogen, phosphorus, which enhance pathogenicity by degrading water quality which results in crop-causing cardiac problems (International Plant Nutrition Institute, No. 12). In addition, it also has a low level of organic contents which are not helpful for farming. Thus, it demands a certain biochemical composition of humanure which can help balance its composition to the level helpful for farming.

2.2 Macro Limitation - Presence of the Infectious Agents in Humanure

The second challenge for humanure in farming is the high levels of pathogenic fungus and bacteria affecting both crop and consumer. According to the study of srirashmi *et al.*, [37] Ecological sanitation can be used in waterscarce places, and this type of sanitation the end product obtained is humanure which can be applied to agricultural fields to improve the fertility of the soil. Groundwater contamination, soil pollution can be avoided by this type of sanitation but the use of such soil for agriculture can lead to several diseases in humans like skin infection, gastrointestinal problems, and also respiratory-related issues (Srirashmi 2021) [37]. The general bacteria responsible for skin infection, gastrointestinal problems, and also respiratory-related issues are *S. aureus*, *E. coli*, and pneumonia which are found in large amounts in humanure. Pathogens present in soil by mixing humanure may become resident in edible plants grown in said soil during their growth phase. If consumed, such products may have serious negative impacts on the health and wellbeing of those who consume the produce. According to a case study on An Assessment of the Viability and Sustainability of the use of Humanure for Household Agricultural Purposes the author suggests that the new technique of making humanure is required, to ensure that it is safe to grow food crops on it, and so that it will not lead to food-borne disease outbreaks. In their study, the presence of several pathogenic bacteria was found including salmonella, Coliform bacteria, *Escherichia coli* (*E. coli*), fecal *Streptococci*, *Pseudomonas Aeruginosa*, *Clostridium Perfringens*, *Legionella Pneumophila*, and Helminth ova in humanure (Vyasha Harilal).

2.3 Can Trichoderma Viride, as a Biocontrol Agent, Address Limitations of Humanure i.e. Infectious Status and Imbalanced Composition?

The use of *Trichoderma* species as a biocontrol agent is gaining a new dimension in their application to resist other pathogenic organisms. The antagonistic reactions that can lead to biological control include antibiosis, competition & hyper parasitism. Studies indicated that *Trichoderma* species is a source of ecofriendly biocontrol agents against pathogenic microorganisms (Leelavathi, 2014) [22]. *Trichoderma* species are also effective against various Gram-positive and Gram-negative bacterial species. They produce among 40 different metabolites of *Trichoderma* species and ciprofloxacin and norfloxacin in cultures of *Trichoderma viride* which are antibacterial in nature. Specific strains of fungi in the genus *Trichoderma* colonize

and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant, considered to be part of the plant defense response, which in the end leads to Induced Systemic Resistance (ISR) in the entire plant. The capability of *Trichoderma* species to promote increased growth response was verified both in greenhouse experiments and in the hydroponic system. *Trichoderma* species isolate showed strong antagonism against fungal species (*Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus candidus*, *Fusarium graminearum*, *Fusarium semitectum*, *Cladosporium*, *Rhizopus*, *Et al.*) (Jegathambigai, 2009) [18], (Nashwa, 2008) [27]. *Trichoderma* have antagonistic activity against the bacterial species (*Staphylococcus aureus*, *Proteus*, *E. coli*, *Klebsiella*) (Fethi, 2008; Parshikov 2002) [11, 30].

Thus, the anti-microbial and anti-fungal activity of *Trichoderma* is well studied. Also, *Trichoderma*'s capacity of producing secondary metabolites and as a biocontrol agent for xenobiotic degradation (Nur, 2020) helps to speculate that during this metabolism. It might also use nutrients of humanure and might affect its level to further balance or imbalance it to match that required for soil farming. Hence, the present study treated humanure with *Trichoderma* and plant growth-promoting enzyme in very little quantity and incubated them for 8 days and looked upon its chemical composition, and affirmed the changes through seed germination.

3. Hypothesis

Trichoderma treatment will decrease the pathogenetic nature of humanure significantly as well as balance the nutrient composition of humanure to make it a suitable medium for farming. Due to this, the medium of treated Humanure will perform the same (or better) in all the growth parameters (coefficient of velocity of germination, germination rate index, and germination percentage) and will have the same chemical composition as the soil.

The specific aim of the study:

1. To evaluate humanure chemical characteristics after *Trichoderma* treatment:
 - a. To perform treatment of humanure with *Trichoderma*.
 - b. To evaluate using sample soil analysis parameters.
2. To evaluate seed germination capacity of humanure after *Trichoderma* treatment:
 - a. To do the seed germination using 3 different varieties and evaluate.
 - b. To find the coefficient of the velocity of germination, germination rate index, and final germination percentage.

4. Materials and Methods

4.1 Sample collections and preparations

- **Humanure:** Raw humanure is the solid component of sludge, dried out in open after sewage treatment for a specific period (on average 2 weeks) for natural factors to work on it. Thus the Humanure was collected from Haridwar City's Sewage Treatment Plant.
- ***Trichoderma Viride*:** Bought from Government Agricultural Chemical Supplements store. The composition of *T. Viride* is as follows:

Trichoderma Viride

(2 * 10 ⁶ – 10 ⁸ CFU/gm)	1.5% W.P.
Moisture	6% - 8% w/W
Carboxy-Methy Cellulose	0.5% w/W
Carrier	Talk

- **Soil:** Soil was collected from two different fields in the ‘Haripur’ village in Haridwar. It was collected by removing the top layer of 10 centimeters from both sites. (Soil of 2 different fields was combined to get such a medium that portrays a net character of the soil of that surroundings).

4.2 Trichoderma Treatment to Humanure

For the experimental run, raw humanure was mixed with Trichoderma Viride to a dry weight ratio of Humanure/T. Viride as 21:2. The total wet weight of the mixture was 1 kg and 150 gm (Comprising of 1 kg 50 gm humanure and 100 gm of T. Viride). The batch was then kept between a temperature of 25o Celsius to 28o Celsius (298 K – 301 K), in an open container for ample ventilation and under shade. A total of 400 ml of water was given to the batch over a course of 8 days (50 ml/day) with a gentle mix each day for 1 to 2 minutes.

4.3 Preparation of Mediums for Seed Growth

Pots (with dimensions of height = 10 cm and top radius = 5 cm) were used with 350 grams of medium. Mediums were pretreated with 200 ml of water first. Total four groups of mediums were created: 1) Untreated (Raw) Humanure alone, 2) Humanure Treated with Trichoderma, 3) Untreated Humanure with Soil, 4) Soil alone, with 3 types of seed varieties - Methi (Trigonellafoenum-graecum), Moong (Vigna-radiate), Chana (Cicerarietinum) thus equating to a total of 12 pots.

4.4 Seed Growth Evaluation

Among the 12 mediums, each medium was evaluated for the number of seeds germinated for each day from day 2 to day 7. The cumulative numbers were plotted against the time of germination (days). The figure 12 top left graph indicated the number of seeds germinated and rate through line graphs.

4.5 Measurement of Height of Seedlings

Total seven days of seedlings were prepared. Length of seedling was measured at day 7. A maximum of 10 seedlings was chosen for height when available with all ranges of seedlings i.e., low, medium, and long height. The average (median) was plotted to measure the height (Figure 1-3 top right).

4.6 Chemical Analysis

Chemical analysis of all four mediums was tested with the help of the Government Soil Testing Laboratory, Dehradun.

5. Results and Discussions

5.1 Chemical Composition Analysis of Humanure treated with Trichoderma

Humanure obtained was treated with Trichoderma as described in method sections and incubated for 8 days at temperature of 25 to 28 C in the shade. After the reaction happened, the samples were analyzed as described. Humanure only, soil only, and soil plus humanure acted as controls to further understand the outcome. The results are shown in Table 1 below. The study interestingly found that Trichoderma treatment reduced high levels of potassium and phosphorus in humanure from 610.6 to 244.5 (kg/hectare) and from 103 to 98.56 (kg/ hectare) respectively, while increased low-level organic matter (2% to 10.28%) and sulfur (for immunity) (34.51 to 276.54 unit) indicating its potential. Changes in pH, zinc, iron, manganese were not clearly distinguished, while boron and copper levels were increased after Trichoderma treatment demanding further evaluation and effects in farming. Apart from the parameters discussed in table 1, the concentration of the treated humanure medium for specific contents was recorded to be: Amino Acid – 50 ppm, Phyco cyanins – 15%, Plant Extract – 15%, Xanthophyll – 0.1%, Vitamins (B1, B2, B6) – 20 ppm, and Chlorophyll – 0.85%. Organic matter also increased in soil plus humanure, which might be the outcome of the soil bacteria. The level of the potassium was also reduced to more than 50% after adding soil in humanure which might be due to a change in the ratio of humanure as well as due to bio decomposition due to soil happening. However, the bacterial level needs to be checked in this control (i.e. soil plus humanure) v/s Trichoderma treated humanure, which is out of the scope of the present study.

Table 1: Chemical composition of the different mediums used in the study i.e. humanure, humanure plus Trichoderma, humanure and soil, and only soil.

Parameter	Humanure	Humanure + Trichoderma	Humanure + Soil	Soil	Limit /Range
pH	6.30	5.48	6.05	5.94	Acidic < 6.5 Normal 6.5-8.2 Alkaline > 8.2
Electrical conductivity (dSm/m)	0.78	6.36	4.19	4.52	Normal < 1 Medium 1-3 High > 3
Organic matter (%)	2.00	10.28	12.52	2.81	Low < 0.5
Phosphorus (kg/hector)	103.04	98.56	80.64	89.6	Low < 28 Medium 28-56 High > 56
Potassium (kg/hector)	610.6	244.5	247.6	336.2	Low < 140 Medium 140-280 High > 280
Sulfur	34.51	276.54	286.93	259.78	Low < 10 Medium 10-20 High > 20

Boron	0.78	1.44	1.67	1.22	Low < 1 Medium 1-2 High > 20
Calcium	---	---	---	---	Low < 1.5 Medium 1.5-3.0 High > 3.0
Zinc	4.31	4.65	4.89	4.69	Low < 0.5 Medium 0.5-1.0 High > 1.0
Iron	23.42	36.30	34.98	31.90	Low < 5 Medium 5-10 High > 10

Table 1: Contd....

Parameter	Humanure	Humanure + Trichoderma	Humanure + Soil	Soil	Limit /Range
Manganese	7.47	11.67	24.74	9.36	Low < 5 Medium 5-10 High > 10
Copper	2.96	20.80	15.40	8.74	Low < 0.2 Medium 0.2-0.4 High > 0.4

Interestingly, Trichoderma treatment increased electric conductivity of humanure which demands optimization of the Trichoderma treatment approach for farming. As the previous study suggested the use of Trichoderma in soil results in higher electrical conductivity, the electrical conductivity plays a significant role in plant growth and seed germination. However, when electric conductivity is higher than 8 mS/cm, negative effects on soil microbial populations as well as on organic matter biotransformation are expected (Garcia, 1996) [12], (Banegas, 2007) [12]. A similar rise in electric conductivity through Trichoderma treatment is also observed, hence, it requires further study to optimize use.

5.2 Seed Germination Capacity of Humanure Reflecting the Change in Composition after Trichoderma treatment

Moreover, Trichoderma treatment-mediated reduction of potassium and phosphorus shall also be reflected in the reduction of germination rate because high levels of potassium and nitrogen in humanure promote the rate of germination. To test it out, three types of seeds i.e. Methi (*Trigonella foenum-graecum*), Moong (*Vigna radiate*), and Chana (*Cicer arietinum*), were germinated in humanure treated with Trichoderma. Humanure only, soil only, and soil plus humanure acted as controls to further understand the outcome.

Figure 1A-C showed the germination growth of Methi (*Trigonella foenum-graecum*), Moong (*Vigna radiate*), and Chana (*Cicer arietinum*) seeds after Trichoderma treatment in humanure along with other controls. It was clearly observed that the rate of germination (Figure 1A-C) was

distinguished from day 3 and the median of seedling height at day 7 (Figure 1D-F) (Table 3) was higher in humanure treatments than soil indicating the presence of higher potassium and nitrogen in humanure. The mean of all three seeds' germination rate and height is also reflected in Figure 2 and Table 4. In addition, the seed germination is higher in humanure and its variant (humanure plus Trichoderma and along with soil) than compared to soil only indicating the potential of humanure especially after treatment with Trichoderma.

The higher potassium and phosphorus content also reflected in seedling growth as the average of germinated seeds of the three species, were higher in humanure and humanure treated with Trichoderma i.e. 22 and 20 seeds germinated at day 7 than compared to soil and soil with humanure i.e. 11 and 14.33 respectively (Figure 2A). Similar to in this line, the mean height (mean + SD) of the seedlings of all three seed types at day 7 were also higher in humanure and humanure treated with Trichoderma i.e. 11.096 + 1.573666667 (n=11) and 11.296 + 1.023433333 (n=11), while the seedling height for soil medium was less i.e. 7.71 + 4.20(n=8) (Figure 2B).

Reflecting the reduction in the potassium and phosphorus and increase in organic matter after Trichoderma treatment in humanure, the study showed that Trichoderma treatment reduced overall germination rate and seedling height in humanure (Figure 2), though they were higher i.e. coefficient of the velocity of germination from 75.68 to 72.22 units (germination rate index from 21.79%/day to 17.78%/day and final germination percentage from 73.1% to 66.2%; though these values were higher compared to soil, yet indicating the potential of Trichoderma treatment.

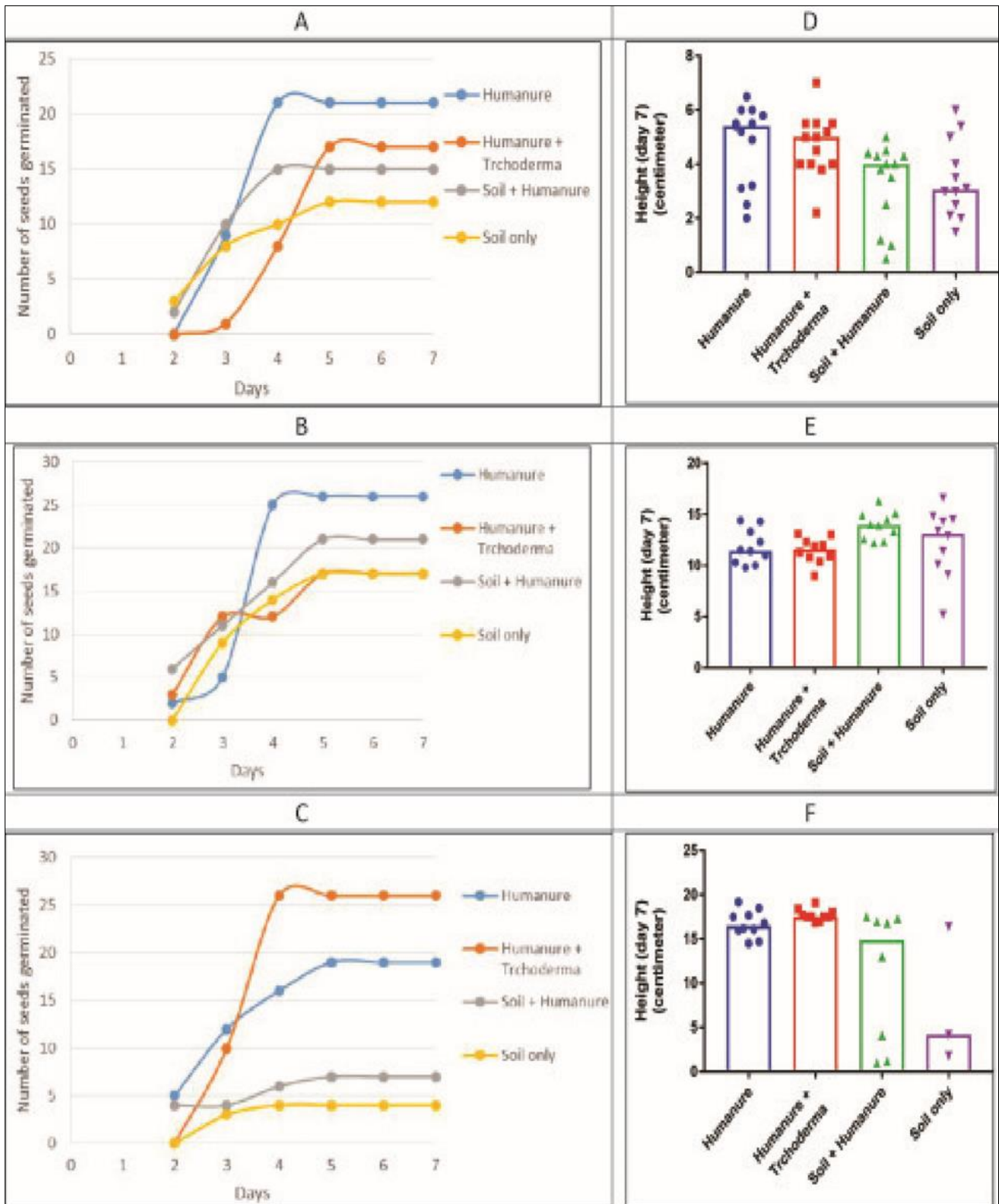


Fig 1: Germination rate and height of seeds Methi (*Trigonella foenum-graecum*) (A, D), Moong (*Vigna radiate*) (B, E), and Chana (*Cicer arietinum*) (C, F) when grown in different mediums i.e. humanure, humanure plus *Trichoderma*, humanure and soil and only soil. A-C: Line graph of germination rate and the number of seedlings grown in a different medium; D-F: median height of seedling at day 7 in all mediums (top right); A-C: humanure (blue color), humanure plus *Trichoderma* (red color), soil and humanure (grey color) and soil only (orange color).

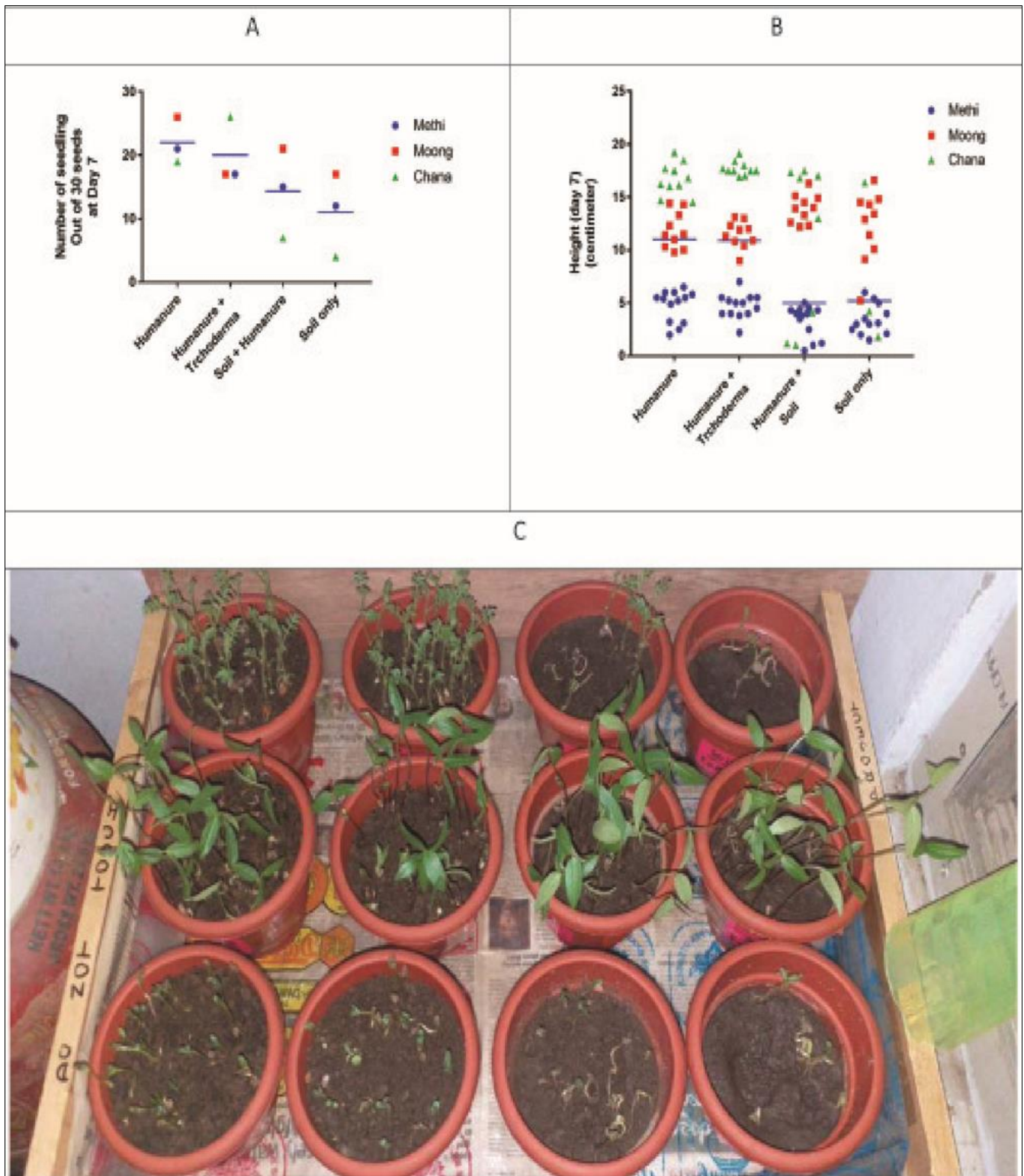


Fig 2: The total number of seeds germinated at day 7 out of 30 seeds in different mediums, B) the median height of seedlings at day 7 in different mediums of all three types of seeds i.e., Methi (*Trigonella foenum-graecum*), Moong (*Vigna radiate*), and Chana (*Cicer arietinum*), C) Image of seedlings at day 7. Left to Right: Mediums. Untreated Humanure, treated Humanure, Untreated Humanure with Soil and Soil. Top to bottom: Channa, Moong, Median height at day 7 (Number of seedlings)

Table 3: The median height of seedlings at day7 in different mediums grown.

	Humanure	Humanure + Trichoderma	Soil + Humanure	Soil only
Coefficient of velocity of germination (CVG) [units]	75.68	72.23	42.07	34.19
Germination rate index (GRI) [%/day]	21.79	17.78	16.12	11.28
Final germination percentage (FGP) [%]	73.1	66.2	47.77	36.43

Table 4: Average of all seeds of Coefficient of the velocity of germination (CVG), Germination rate index (GRI), and Final germination percentage (FGP) in humanure treated after Trichoderma and in other control mediums.

(cm)	Humanure	Humanure + Trichoderma	Humanure + Soil	Soil
Methi	5.4 (13)	5 (13)	4 (13)	3.05 (12)
Moong	11.45 (10)	11.6 (10)	13.95 (10)	13.15 (10)
Chana	16.5 (10)	17.5 (10)	14.9 (8)	4.2 (3)

6. Conclusions

The present study conducted the primary experiment of Trichoderma treatment to humanure with a hypothesis of change in the composition of humanure to probably balance it for farming use. The Trichoderma treatment has dual advantages i.e. it disrupts the cell membrane of the pathogens by producing its secondary metabolites and it also decomposes the complex organic matter through its xenobiotic degradation (Nur, 2020).

Based on the previous literature available on the anti-microbial and anti-fungal potential of Trichoderma and its known characteristics of xenobiotic degradation, the study specifically incubated humanure with Trichoderma and analyzed its composition. Interestingly study found that after treatment with Trichoderma, harmful levels of potassium and phosphorus in humanure reduced very remarkably and also distinguished increased organic content (Table 1), which was reflected in the decrease in mean germination index and coefficient of the velocity of germination of all 3 types of seedling grown (Figure 1-2, Table 3-4), and thus indicating that the potential of Trichoderma in treating humanure for utilizing farming purpose.

However, the present study is a preliminary attempt that there needs to have the optimization of the process because the treatment of Trichoderma increased the level of electric conductivity which is harmful to farming. In addition, soil also has natural bacteria which can help xenobiotic degradation of harmful complex matter, hence, further research can include other factors for complex study to optimize Trichoderma use in humanure treatment for farming purposes along with analysis of pathogen contents and how to make the production of humanure more accessible among the general public.

7. Acknowledgements

1. I would like to acknowledge Dr. Chinmay Pandya, Pro-Vice-Chancellor, University of Dev Sanskriti Vishwavidyalaya, Haridwar for allowing me to pay a visit to the school of biological sciences and sustainability, D.S.V.V.
2. I'd like to acknowledge Debashish Mahaptra, Faculty at Gayatri Vidyapeeth, who mentored me during my research work for this project.
3. I'd like to acknowledge Mr. Ajay Kumar, Executive Engineer, Maintenance Division, Ganga Jal Sansthan, Haridwar for allowing me to pay a visit to Haridwar's STP plant and take humanure samples for my research.
4. Mr. Pradeep Chauhan, Plant in charge, Jagjeetpur STP

Plant, Haridwar for helping me better understand the functioning of the STP plant.

5. I would also like to acknowledge Mr. Sitaram Sinha, Principal, Gayatri Vidyapeeth, for his support about my participation in the submitted project.
6. I would like to humbly thank Mrs. Praharsha Mehta, retired Science teacher from Surat, Gujarat for enlightening me about NCSC after looking at my work in this field of research.
7. I would like to portray my gratitude towards my beloved parents. Mrs. Padma Desai, and Mr. Kirtan Desai for their moral and emotional support throughout the process of research and writing this paper.

8. The objective of the study

The major drawback of humanure is high levels of potassium, nitrogen, phosphorus, which enhance pathogenicity by degrading water quality which results in crop-causing cardiac problems. It also has a low level of organic contents and high levels of pathogenic fungus and bacteria affecting both crop and consumer. Trichoderma is a bio-fungicide known to have potential antimicrobial and antifungal activity against pathogens, which are also commonly present in humanure. Hence, the present study investigated the treatment of humanure with Trichoderma to find the effect on humanure's limitations for farming use.

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