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"Role of a Novel Microbial Consortium in Treatment of Human Waste and it's Effect on the Plant Growth and Yield-A Study on Tomato Crop"

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Abstract—*One of the major challenges faced by rapid growth of human population is accumulation and efficient disposal of human derived solid wastes. To address this problem we have devised a microbiological composition which can digest solid wastes in a more efficient manner and also give scope for improving the nutrient quality of the end product of microbial digestion. A group of soil and sludge borne microbial species such as Actinomycetes, Streptococcus, Bacillus, Pseudomonas were added at a concentration of $1.6x10^7$ and $8.5x10^6$cells/ml which involve both aerobic and anaerobic micro-organisms, respectively. A molasses derived substance has been used to supplement the nutrients required for the survival of microorganisms. In a time point study it was observed that the microbiological composition when added at 1-2% concentration (v/v) was able to digest an approximate 70 to 80% of human waste against the untreated control setup. Further, to find the role of nutrient quality of the microbiological composition and to evaluate the potential fertilizer value the total Nitrogen content has been estimated by Kjeldhal method and found to be 1606mg/L, which sheds a light on the role of microbial consortium in promoting the plant growth and agricultural productivity. Further the consortium has been applied on Tomato plants in a pilot level study. Whereas untreated tomato plants were taken as control, the microbial consortium treated plants have shown an increase in the height, number of leaves, early flowering and fruit formation. The results suggested that the microbial consortium and its treated wastes are facilitating plants to better adapt to the soil environmental conditions. Further research in this direction will help to understand the molecular mechanisms involved in improvement of nutrient quality. Taken together, our research work helped to create a microbial system which has a potential role in efficient treatment of human derived wastes, while improving the agriculture production through increased total nitrogen content required for plant growth.*

1. INTRODUCTION

Sustainable use of natural resources and reuse of wastes has become an important global concern which promotes the efficiency of ecological system and promotes health by decreasing disease transmission and can contribute to increase in agricultural yield. In underdeveloped and developing countries, mal nutrition constitutes approximately 14% of global burden of disease, which supersedes the sanitation related disease, which is only 3.4% (Lopez *et al.,* 2006). Human wastes contain millions of tons of fertilizer equivalents, which is roughly 20% to 30% of global industrial fertilizer production, annually (Winker *et al.,* 2009). To main agricultural yields at high levels over the years, the nutrients removed by crops have to be replaced. For example, urine is rich in nitrogen, which is most limiting nutrient for plant growth while feces are rich in phosphorous, potassium and recalcitrant organic matter which can give substantial yield, especially on poor soil (Jonsson *et al.,* 2004). This data reflects the urgent need of sustainable technologies of developing the agricultural practices towards improving the nutrient quality of foods. Hence, the developing nations like India are in great need of sustainable reuse oriented sanitational technologies, which prevent disease and promote health. Hence, the current research focusses on developing a sustainable technology which is microbial based conversion of human and animal excrements into agriculturally useful organic manure.

2. MATERIALS AND METHODS

Media Preparation & Cultivation of Microorganisms

Routine bacterial culture media such as Nutrient Agar, Nutrient Broth, Luria Broth, Luria-Bertani Agar (LB Agar) were prepared as per manufacturer (Hi Media) instructions. For cultivation of fungi, YPD broth or YPD agar media (1% Yeast Extract, 2% Peptone, 2% Dextrose & 1.5% Agar (HiMedia)) were used. Bacteria were cultivated at 37^OC while fungi were grown between 18 to 42^OC depending on strain type. All procedures of bacterial culturing performed as previously described. (VKN et al., 2014)

Isolation & staining of Soil borne fungi & microbes

Various microorganisms were isolated from wet soil by mixing soil with Starch Casein Agar medium and Mineral Salts Agar medium at a concentration of 2% (w/v) and plates were incubated at 37^{o}C to 42^{o}C for 7-10 days depending upon growth of the strain. Microbes were studied by Gram staining procedure and photographed under 1000X magnification using oil-immersion microscopy (Olympus).

Enumeration of Anaerobic microbes

To cultivate and enumerate anaerobic microorganisms, a candle jar method has been employed in which microbes under investigation were plated on selection media and incubated by using Candle Jar method (Dubey & Maheswari, 2010). The cells were enumerated after 24 to 48 hr incubation directly under colony counter.

Constitution of Microbial consortium

A microbial consortium has been prepared aseptically by taking log phase grown bacteria & fungal cells (10^6cells/ml) were collected by centrifuging at 5000rpm for 10 mins and briefly washed using sterile Phosphate Buffer Saline (PBS). This cell pellet was added to a 10% diluted molasses solution (Bengal chemicals) and thoroughly mixed. The solution has been further diluted (10:100) with double distilled autoclaved water and the molasses-cell solution was incubated at 37^{o}C for one week while periodically mixing the contents.

Application of microbial consortium on human waste

The microbial consortium made has been applied on human derived waste collected aseptically from Sulabh International, Gurgaon at 2-5% concentration and incubated at 30 to 37^{o}C and observed for different time periods. A time point study was performed by taking samples for analysis of total dry wastes present, against the untreated control setup.

Application of microbial consortium on duck weed

Microbial consortium at 2% was added to 1gm of 20 days old duck weed, aseptically and microscopy of roots, at 400x magnification was performed before and after addition.

Estimation of total Nitrogen by Kjeldahl method

The total Nitrogen present in the microbial consortium has been estimated by Kjeldhal method as described by Manivaskam, 1996.

Estimation of Potassium content

To estimate the potassium content in garden soil, microbial filtrate and toilet derived manure, we have used the Flame photometer (Labtroics, LT671), using standard operational procedure. Distilled water was taken as control.

Tomato Plant study

It was essential to take tomato plants of same age, variety and have similar or identical genetic constitution. For this, tomato plants were raised starting from seeds and plant seedlings were grown. Among them, 15 Tomato Plants of equal height and morphology were selected. They were made in to three groups of five plants in each group. One group (A) of tomato plants was transferred to pots containing sterilized soil, which was achieved using autoclaving. One group (B) of plants was used for supplying with raw water. Another group (C) was used for testing the effect of Microbial consortium on tomato plants. Only one month old tomato plants were considered for experimentation. Group A plants were given only filtered/distilled water, whereas Group B were given raw water and Group C were added with Microbial consortium at 5% (V/W) of soil weight of pots. Plants were added with respective solutions on regular basis. Changes in their morphology was monitored with an interval of 5 days and various parameters such as number of leafs, plant height, onset of flowering, number of fruits and fruit size was monitored. The comparison was made using Microsoft excel software and other statistical tools.

3. RESULTS & DISCUSSION

1. Microbial Consortium contains both aerobic and anaerobic bacteria

The microbial consortium prepared for the purpose of efficient disposal of human wastes contain microbes such as bacteria and fungi. Bacterial cultivation and enumeration under aerobic and anaerobic conditions suggested the presence of these cells at an approximate $1.6x10^7$ and $8.5x10^6$cells/ml, respectively. Further, Gram staining has been performed to understand the nature of prevalent bacterial species, which indicated the presence of several Gram positive microbes and few Gram negative bacterial species. The presence of diverse spectrum of microbes suggests a role in digestion of various complex macromolecular biochemical compounds present in human derived wastes and metabolites. However, the specific nature of the action of these microbes acting on biochemical compounds is under investigation.

2. Human wastes are digested efficiently in presence of microbial consortium

The digestion efficiency of microbial consortium has been evaluated *in-vitro* by adding it to human wastes which includes two pit derived solids and mixture of other bioorganic matter. The microbial consortium mixed wastes were incubated at variable temperatures for different time points as described in material and methods. A 30 day time point study under partial anaerobic conditions revealed digestion of an approximate 70-80% of solids against the control solids where 40-60% of solids were subjected to digestion. It was also conspicuous that the unadditized control sample has developed a green to brown lawn, indicating growth of unwanted nonspecific microorganisms. The experimental setup having microbial consortium has reduced solid contents and remained free of secondary contaminants.

3. The microbial consortium is rich in total Nitrogen content

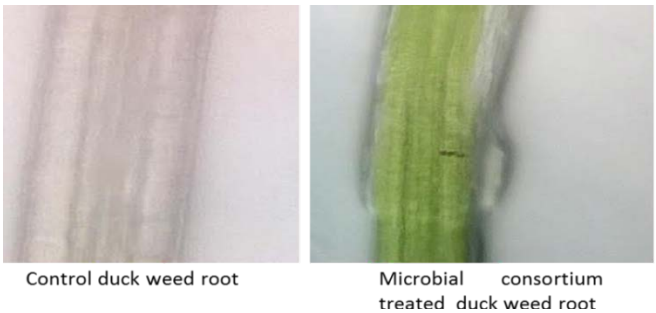
The observed promotion of plant root growth raised a possible presence of rich nitrogen content in the microbial consortium. It was known that microbes such as Rhizobium, Nitrobacter and Nitrosomonas can efficiently fix atmospheric nitrogen. The second source of Nitrogen is molasses, which carries trace amounts of nitrogen. Taken together, the promoted plant growth might be directly or indirectly related to presence of nitrogen in the microbial consortium. Hence, the nitrogen content of microbial consortium has been estimated by Kjeldahl method and found to be 1606mg/L, which is a reasonably high content of nitrogen, which might have promoted the plant root growth.

4. A comparative analysis of Potassium (K) content in microbial filtrate & garden soil

It is important to check the Potassium content in garden soil and microbial filtrate as it has an important role in plant growth and metabolism. The Potassium content was found as microbial filtrate: 94.8 mg/L of Potassium against Garden soil filtrate: 10.1mg/L of K.

5. Microbial consortium has a possible role in promotion of plant growth

A common observation that pit sites contain good vegetation prompted us to check the interaction of microbes with plant roots and their effect on plant growth. To verify the same, a tiny flowering plant, duckweed was taken and incubated with microbial consortium for 15 or 30 days in the presence of sunlight with proper aeration. A control experiment has been set up with distilled water. Microscopy of duckweed roots of both the experimental setups indicated presence of lush green portion and lengthy roots in microbial consortium treated plants than untreated plants. The observed phenomenon could have resulted from the interaction of microbes with the root of the plant and promoted the metabolic processes required for their growth.



6. Effect of microbial consortium vs. garden soil filtrate on tomato plant growth

To understand the effect of garden soil and microbial consortium on plant growth, we added garden soil filtrate and microbial consortium to tomato plant seedlings sowed in sterile soil. After 5 to 7 days of, it was observed that microbial consortium was able to provide habitat for other soil microorganism and acting as a good medium for plant growth. The garden soil filtrate also had a positive effect on growth enhancement of tomato plants. However the effect was over shadowed by the effect of Microbial consortium. As shown in the photographs there are three pots: test1, test2 and control. We added 50ml of Microbial consortium or garden soil filtrate in each pot and repeated the process with an interval of 6 days, excluding the control setup having sterile soil. It was clearly observed that, the average height of test plants added with Microbial consortium was higher than garden soil filtrate and control plants.

Day 1, Tomato plants. Arrows indicate the height of plant

Day 30, Tomato plants. Arrow indicates the height of plant

Tomato plants under study before fruit harvest (Control, Test-1, Test-2 are arrow marked)

4. CONCLUSION

With the rapid growth of human settlements and industrialization, the handling of waste generated has become an impossible task with the current technologies available. With the objective of addressing this problem, we have devised a microbial based disposal method for rapid digestion of solid wastes. The system involves selection and enrichment of simple and soil borne microbes, which otherwise are extensively present in soil environment. These microbes produce enzymes which can use varieties of biochemical compounds as their substrates. A plethora of microorganisms are available which perform such function. In our knowledge little or no microbial system has efficiently answered the question in a sustainable manner. A group of microbes such as Actinomycetes, Streptococcus, Pseudomonas and certain subspecies of Bacillus which can co-exist have been brought together and are maintained in a cheaply available molasses like medium. The use of a diluted molasses like solution helped us to minimize the cost and nourished microbes present apart from providing minerals, vitamins and nutrients required for microbial survival. This methodology when fully developed would facilitate the end user to easily follow the three R principle of bioremediation: “Reduce, Reuse and Recycle”. Taken together, our research work helped to create a microbial system with an enhanced fertilizer value which has a potential role in efficient treatment of human wastes and paves a way for improving the agriculture production through increased nitrogen content required for plant growth.

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