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"ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF BACTERIA FROM VERMICOMPOST AND COW MANURE

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Abstract

Organic fertilizers are made up primarily or entirely of organic elements generated from plants and animals that have undergone a technical process. The fertilizers can be used to add organic matter to the soil as well as improve its physical, chemical, and biological characteristics. Organic waste disposal from home, agricultural, and industrial sources has produced increasing environmental and economic concerns in recent years, prompting the development of a variety of methods to solve the issue. Vermiculture is the cultivation of earthworms in organic wastes. In the present study total 10 samples were collected 5 samples of Cow manure and 5 samples of Vermicompost. The results can be explained by identifying bacteria we know their role in the plant growth and development and promote the growth of those bacteria which have beneficial role in vermicompost and cow manure to enhance the nutrient quality in vermicompost and cow manure

INDRODUCTION

Organic fertilizers are made up primarily or entirely of organic elements generated from plants and animals that have undergone a technical process. The fertilisers can be used to add organic matter to the soil as well as improve its physical, chemical, and biological characteristics. Because garbage contains both inorganic and organic components, organic fertilisers can be generated from it. Organic waste is made up of plants and animals that have been harvested from nature or created by farming, fishing, or other activities. These materials are easily characterised in natural processes.

The use of waste as an organic fertiliser for direct application to soil has a number of drawbacks, for example:

1) The waste contains inorganic material that is not biologically and easily weathered, such as glass, plastic, and metal.

2) The waste contains inorganic material that is not biologically and easily weathered, such as.

3) The composition of organic waste varies far too much, and dangerous compounds can occasionally be found.

4) Sewage pollution of the environment by heavy metals and chemicals causes other issues.

5) Toxic heavy metals such as arsenic, mercury, lead, and cadmium can pollute or harm the environment.

6) The heavy metals As, Cd, Pb, and Hg are extremely toxic and can accumulate in rice, grass, vegetables and other crops.

Earthworms are used in the bio-conversion of organic waste into vermicompost in a method known as vermitechnology. Vermicompost has a wide range of uses in organic waste management and has been demonstrated to be a cost-effective and efficient technique of managing organic waste materials. Vermicomposting is an organic waste-to-fertilizer conversion method. Given that the use of organic waste and compost in agriculture has numerous benefits, it is vital to do research on organic fertiliser using the vermicomposting method, which employs the performance of worms to process waste raw materials. As a result of the foregoing facts, a research of organic waste vermicomposting optimization was done, with the goal of producing vermicompost from urban waste materials, selecting one of the three options.

Organic waste disposal from home, agricultural, and industrial sources has produced increasing environmental and economic concerns in recent years, prompting the development of a variety of methods to solve the issue. Vermiculture is the cultivation of earthworms in organic wastes, while vermicomposting is the processing of organic wastes by earthworms (Edwards, 2004). For recycling and efficient utilisation of organic leftovers, there is a clear trend toward the adoption of innovative technologies, primarily based on biological processes. As a result, it is feasible to conserve available resources and recover natural products, as well as, in some situations, to address disposal issues and reduce pollution consequences. Vermicomposting has been arising as an innovative biotechnology for the conversion of agro-industrial wastes into value added products, which can be utilized for improving the soil structure and fertility in organic farming.

Reduced irrigation water use, reduced pest attack, reduced termite attack, reduced weed growth, faster seed germination and rapid seedling growth and development, more fruits per plant (in vegetable crops) and more seeds per year (in cereal crops) are just a few of the advantages of using vermicompost in agricultural production. Pure vermicompost is not so good for agricultural production, because it contains too much nutrients.

The benefits of using vermicompost-based substrates in agriculture (Olle, 2016b) include: it accelerates growth, increases crop yields; it creates a favourable environment for beneficial microorganisms; it improves soil structure permanently; it increases plant secretion; in the case of plants with longer growing seasons, additional fertilisation with biohumus or its leotion is sufficient; mineral fertilisers are not required in this case; it is 100 percent natural, ideal for use in organic farming and in artificial environments.

Vermicomposting

Vermicomposting is the solid phase breakdown of organic leftovers in an aerobic environment by taking use of earthworms' and microorganisms' optimal biological activity (Garg, Gupta, 2009). "Bioxidation and omogenizing of organic material involving the joint action of earthworms and mesophilic microorganisms" is how vermicomposting is defined. Vermicompost is high in macro and micronutrients, vitamins, growth hormones, enzymes such proteases, amylases, lipase, omogeniz, and chitinase, and omogenizin microflora created by earthworm activity. Even after the enzymes have been ejected from the worms, they continue to degrade organic substances (Barik *et al*., 2011).

Vermicomposting is the process of composting organic wastes using earthworms. It's been used to treat sewage sludge and solids from wastewater, brewers' materials, paper trash, urban leftovers, food and animal wastes, as well as horticultural residues from processed potatoes, dead plants, and the mushroom business (Dominguez, Edwars, 2004).

Vermicomposting is a decomposition process that involves earthworms and microorganisms working together. Although bacteria are responsible for the biochemical breakdown of organic materials, earthworms play an important role in the process by fragmenting and modifying the substrate and drastically changing its biological activity. Earthworms act as mechanical blenders, modifying the physical and chemical condition of organic matter by gradually lowering the C:N ratio, increasing the surface area exposed to microorganisms, and making the environment much more conducive to microbial activity and further decomposition. They move a lot of pieces and bacteria-rich excrements through the earthworm gut, omogenizing the organic material. Vermicompost is a finely divided peat-like material with great porosity and water holding ability that contains the majority of nutrients. In forms that are easily absorbed by plants These earthworm casts are high in organic matter and mineralization, implying that plant access of nutrients, particularly ammonium and nitrate, is considerably increased (Dominguez, Edwars, 2004).

The following are the different phases of the vermicomposting process (Garg, Gupta, 2009):
(1) Initial pre-composting phase: Before being fed to earthworms, the organic waste is pre-composted for around 15 days. During this phase, easily decomposable chemicals are decomposed, and potentially hazardous to earthworms volatile substances are removed.
(2) Mesophilic phase: During this phase, earthworms combine organic matter with soil particles, enhancing microbial activity and conditioning organic waste materials for the creation of organic manures, thanks to their unique role of breaking up organic matter.
(3) Phase of maturation and stabilization.

Fig. 1: Vermicompost

Roles of vermicompostq

(1) Red worm castings contain a high amount of humus, making them ideal for vermicomposting. Humus aids the formation of soil particle clusters, which produce air channels and increase the capacity of the soil to hold water.

(2) Humus is thought to help protect plants from dangerous diseases, fungus, nematodes, and bacteria.

(3) A worm casting (also called worm cast or vermicast) is a biologically active mound that contains thousands of bacteria, enzymes, and plant remains that the worms could not consume.

(4) Castings contain easily available nutrients for plants.

(5) The worm intestine functions as a small composting tube, mixing conditions and inoculating leftovers.

(6) Worm castings are the greatest potting soil available for greenhouse or houseplants, gardening, and farming.

(7) Plant Growth Regulating Activity: Some research hypothesised that the high quantities of nutrients, humic acids, and humates in vermicompost caused plants' growth responses to resemble "hormone driven activity."

(8) Plants' Ability to Develop Biological Resistance: Vermicompost contains antibiotics and actinomycetes that aid in improving crop plants' "power of biological resistance" against pests and illnesses. When earthworms and vermicompost were utilised in agriculture, sprays of chemical pesticides were reduced by more than 75%.

The beneficial impacts of vermicompost on soil

(1) Improve soil structure and reduce soil erosion by increasing 'Soil Organic Matter' (SOM).
(2) Increase the number of beneficial soil bacteria, as well as microbial activity and nutrient levels.
(3) Increase the capability of cation exchange.
(4) Soil bulk density is reduced, preventing compaction and erosion.
(5) Control of plant diseases that are transmitted through the soil.
(6) Increase the soil's water-holding capacity.
(7) Remove salt and sodicity from the soil.
(8) Keep the soil pH at a healthy level.

Vermicompost is ideal organic mature for better growth and yield of many plants due to the following reasons :

(1) Vermicompost contains more nutrients than ordinary composts.
(2) This is due to the action of earthworms, which has enhanced the pace of mineralization and the degree of humification.
(3) Porosity, aeration, drainage, and water-holding capacity are all high in vermicompost.
(4) It is suitable for plant growth because to the presence of microbiota, particularly fungus, bacteria, and actinomycetes. Vermicompost contains nutrients such as nitrates, phosphates, exchangeable calcium, and soluble potassium in plant-available forms.
(5) Vermicompost contains plant growth regulators and other plant growth affecting elements produced by microorganisms.
(6) The production of cytokinins and auxins was discovered in earthworm-processed organic wastes.
(7) Earthworms emit various metabolites into the soil, such as vitamin B, vitamin D, and other chemicals.
(8) In addition to increased N availability, P, K, Ca and Mg availability in the casts are found.

Microbes constitute the foundation of biodiversity and play a critical role in ecosystem functioning. Microbes such as bacteria, fungus, actimomycetes, and others are responsible for biochemical degration of organic materials and maintain ecological equilibrium (Emperor *et al.*, 2015). Vermicompost is a stable, non-thermophilic substance created by interactions between earthworms and microorganisms with high microbial activity. Vermicomposting is one of the simplest ways to convert agricultural waste into a high-quality compost product. Nutrients, growth-promoting chemicals, and beneficial bacteria abound in the compost. It is an important part of the organic agricultural system. The earthworm is a vital component of vermicompost, as it helps to aerate, change microbial activity, and speed up the decomposition process (Fracchia *et al.*, 2006).

Vermicompost promotes soil aggregation, fertility, plant nutrition, and beneficial microbial growth (Pereira et al.,2014). It enhances aeration and water retention in the soil. It helps to suppress plant diseases and improve plant growth by restoring the microbial population, which includes nitrogen fixers, phosphate solubilizers, and other microbes, as well as providing macro and micro nutrients to agricultural plants. It also helps to improve soil structural stability, which helps to minimise soil erosion (Zhu *et al.*, 2017) and, as a result, increases crop output (Khan *et al.*, 2011).

Furthermore, vermicomposting increases soil physiochemical and biological qualities, as well as the diversity and number of beneficial microbial communities (Pathma *et al*., 2012). Earthworms eat soil microbes along with organic leftovers from the soil, and their population may expand as they move through the intestinal tract. Ingested microorganisms and wastes enter the gut and contribute to earthworm nutrition by supplying microbial enzymes that aid in organic matter breakdown (Selvi *et al*., 2015). Thus, recognising the beneficial bacteria involved in the composting process and detecting the bacterial population in vermicompost

4

could provide insight into the quality of vermicompost produced Earthworm species suitable for vermicomposting are two types of species (1) Temperate species & (2) Tropical species. Temperate species includes Eisenia fetida , Dendrobaena rubida, Dendrobaena veneta, Lumbricus rubellus & Tropical species includes Eudrilus eugeniae, Perionyx excavatus, Pheretima elongate.

Rajendran Vijayabharathi, Arumugam Sathya & Subramaniam Gopalakrishnan in 2015 studied on Vermicomposting, a decomposition process of organic material through the use of earthworms and microbes, gets more attraction in sustainable agriculture because of its low cost technology, its utilization of a wide range of initial substrates, and because it's an eco-friendly process. Vermicompost has substantial quantities of macro- and microelements and plant

Cow Manure

Cow dung is emitted by herbivorous bovine animal species. It is made up of undigested remnants of eaten matter that have gone through the gastrointestinal system of a cow (Teo & Teoh, 2011). Cow dung is commonly used to make manure, which is then utilised as a bio-fertilizer. Cow dung manure improves soil mineral content while also strengthening plant tolerance to pests and diseases. It comprises around 80% water and some undigested plant material with a significant level of organic component.

Bacillus, such as lactobacillus as a central endospore creating bacillus, cocci, fungus, and yeast, such as Saccharomyces cerevisiae, are all found in the microflora of cow manure (Sharma & Singh, 2015). According to Ware et al., (1988), the presence of Lactobacillus plantarum, Lactobacillus casei, Lactobacillus acidophilus, Bacillus subtilis, Entrococcus diactylactis, Bifido bacteirum, and yeasts in the lower region of the cow's gut confers probiotic activity (Saccharomyces cerevisiae). There is evidence that fresh cow dung and cow urine are antifungal and antiseptic in nature, which could be owing to the release of antimicrobial compounds by the microflora in cow dung (Sharma & Singh, 2015).

Cattle rearing has long been a tradition in India, and it is closely linked to the agricultural economy. Various products made from cow milk, ghee, curd, urine, and dung are commonly employed in Ayurvedic formulations. For ages, cow excrement has been utilised as an organic fertiliser in Indian subcontinental agricultural. Cow dung improves soil mineral status, increases plant resilience to pests and diseases, and stimulates plant development as well as other positive actions such as sulphur oxidation and phosphorous solubilization. Cow dung is typically composed of around 80% water and contains a matrix of undigested plant material rich in nutrients, microorganisms, and their metabolites.

Cattle ranching is a tradition in India, and it is closely linked to the agricultural economy. Various products made from cow milk, ghee, curd, urine, and dung are commonly employed in ayurveda formulations. For ages, cow excrement has been utilised as an organic fertiliser in Indian subcontinental agricultural. Cow dung improves soil mineral status, boosts plant resilience to pests and diseases, and stimulates plant development as well as other positive actions such as sulpho oxidation and phosphorus solubilization.

According to the Hindu Vedas, the cow is sacred and should be worshipped. Cows are an important animal resource in India, and they are especially valuable in agriculture and the dairy sector. Cow's urine, milk, ghee, curd, and dung are among the five principal substances obtained from cows, and are referred to as panchagavya.

All five products have therapeutic characteristics that can help with a variety of ailments. Panchagavya therapy, also known as cowpaths, is a type of treatment. Cowpathy is a traditional Indian medical method known as Panchagavya Chikitsa in ancient Indian literature (Ayurveda). Panchagavya is used to manufacture ayurveda medications of animal origin, which stimulate the immune system and

make the body resistant to many ailments. Although some Indian literature claims that cow excrement has medicinal properties, only a handful have been proven. Researchers have also validated a number of valuable features of cow urine in their patent. However, there is no information on the antibacterial action of cow dung.

Cow dung is primarily the animal's rumen's rejections of herbivorous materials, which are digested by symbiotic bacteria. The resulting faeces matter is mineral-rich. Cow poo is th undigested plant stuff that has gone through the animal's digestive system. The resulting faeces matter is mineral-rich. Cow dung is made up of organic waste, including fibrous material that passed through the cow's digestive tract, as well as liquid digesta that was left over after fermentation, absorption, filtration, acidification, and re-absorption. The chemical makeup consists mostly of carbon, nitrogen, hydrogen, oxygen, phosphorus, and other elements, as well as salts, urea, mucus, cellulose, lignin, and hemicellulose, which are sloughed off as the digest passes through the digestive tract.

Therefore, the present study was carried out with an objective to find out the diversity of bacterial species involves in the vermicompost and cow manure.

Fig. 2: Cow Manure

MATERIALS AND METHODS

Collection of Vermicompost and Cow Manure Sample

Total 10 samples of Vermicompost and Cow Manure were collected from different villages of Nagpur District. The sample were collected in a sterile plastic bags. The samples were collected from villages Pimpalgaon, Bhishnoor, Thaturwada, Digras, Katol.

Fig. 3: Cow Manure Sample

Fig. 4: Vermicompost Sample

Isolation of bacteria from Vermicompost and Cow Manure .

For isolation of bacteria from vermicompost and cow manure sample was performed by serial dilution method. Each sample weighed 1gm and mixed in 9 ml sterile distilled water.

Nutrient agar media was used and Pour plate method was followed for isolation in which the serial diluted samples from test tube 10^{-7}, 10^{-8}, 10^{-9} 1ml diluted solution were poured on respective petriplates containing the nutrient agar media and then incubated at 37 °C for 24 hours in a incubator After incubation the appeared bacterial colonies on plates were subjected to characterization.

Characterization and identification of bacteria

The colonies on the nutrient agar plate were inoculated in nutrient broth. After this the selective media were prepared for the isolation. The media were Jensen's Medium, Mannitol Salt Agar, Pikovskaya's Agar, Cetrimide Agar, L-B Agar, Rhizobium Medium and petriplates are sterile at 15lbs pressure for 15 min after sterilization pour the media into petriplates and solidify the media. After solidifying the medium streaked the sample from nutrient broth by streak plate method and incubated at 37°C for 24 hours in a incubator. After incubation the bacteria were identified by identifying the colonies.

Gram's Characteristics:

The morphological appearance of the isolated pathogens was detected by performing gram staining & its gram nature was noted.

Flowchart of gram staining :

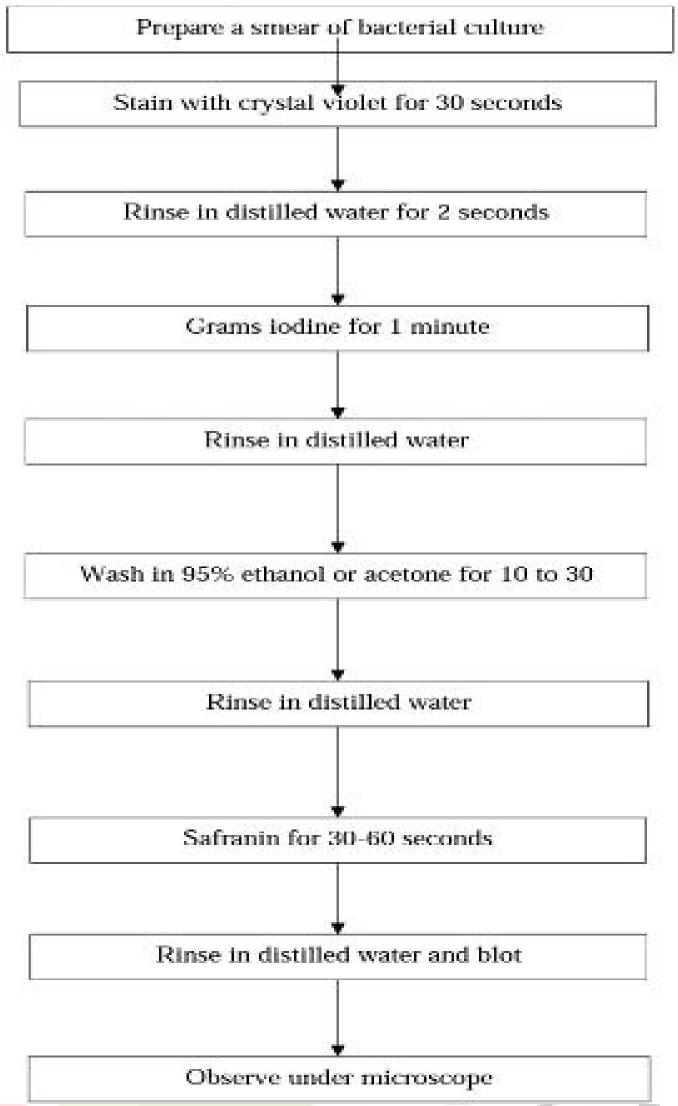

Fig. 5: Steps involved in Gram's Staining

Materials used

Materials used in the study works were selective media, reagent, samples, petriplates, distilled water, glassware, test tubes, burner, conical flask, inoculation loop, test tube stands etc.

Medium used : Nutrient agar, Nutrient broth, Jensen's medium, Mannitol salt agar, Pikovskaya's agar, Cetrimide agar, Rhizobium Medium, L-B agar

Results and Discussion

Samples of cow manure

The Cow Manure samples were collected from different villages of Nagpur district . Total 5 sample were collected and Serial dilution were perform to determine the colony forming unit (cfu) in 1 gm of sample.

- **Cow Manure**

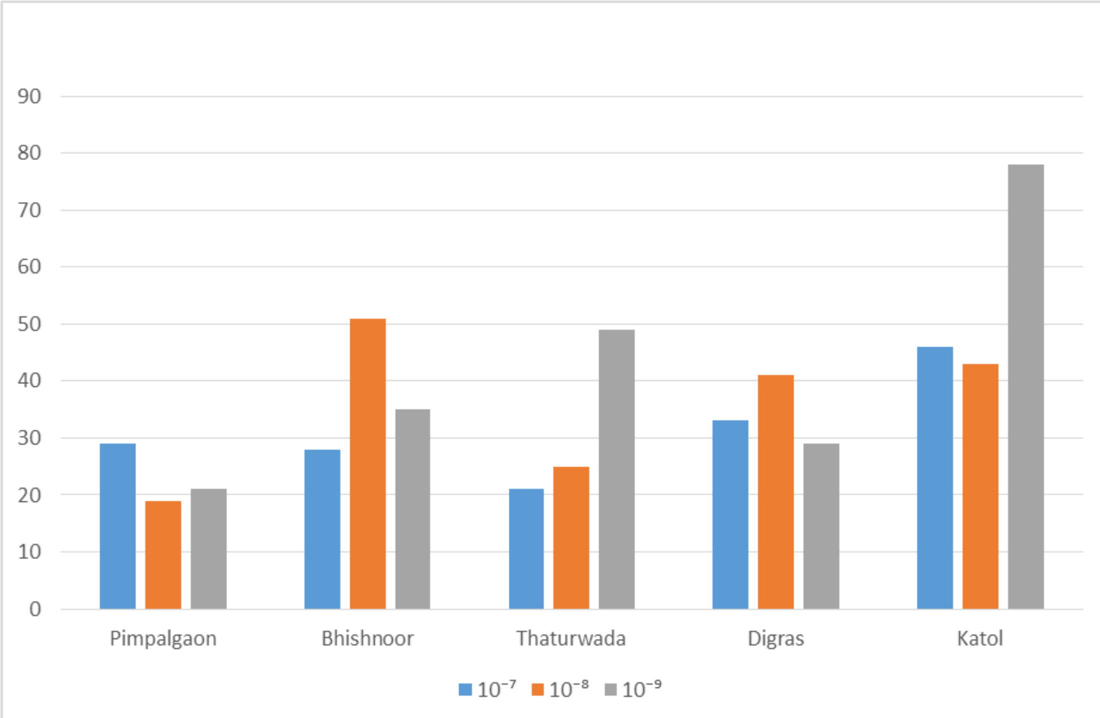

Fig. 6: Graphical presentation of colony forming unit shown in Cow Manure

The above figure shows that the isolates no from diluted 10^{-7},10^{-8},10^{-9} showed the colony forming unit (cfu) against the places. The highest colony forming unit (cfu) shown in the village katol and the lowest count is shown in the Pimpalgaon.

- **Vermicompost**

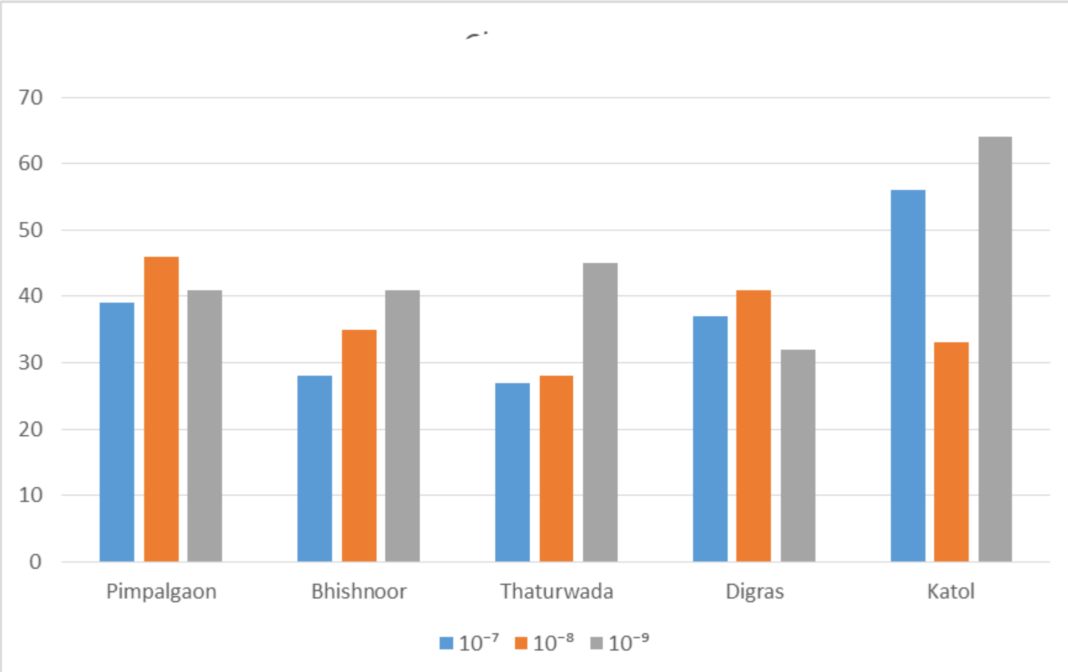

Fig. 7: Graphical presentation of colony forming unit shown in Vermicompost

The above figure shows that the isolates no from diluted 10^{-7},10^{-8},10^{-9} showed the colony forming unit (cfu) against the places. The highest colony forming unit (cfu) shown in the village katol and the lowest count is shown in the Thaturwada.

Results and Discussion

In the present study total 10 samples were collected 5 samples of Cow manure and 5 samples of Vermicompost. Serial dilution method was performed in which 1 gm of sample weighed in 9 ml distilled water 9 test tube were taken for serial dilution method. In serial dilution method from test tube 10^{-7},10^{-8},10^{-9} 1ml solution were poured on petriplates containing the nutrient agar media and then incubated at 37°C for 24 hours in a incubator. After that the colonies on the nutrient agar plates were inoculated in nutrient broth. The sample from nutrient broth were inoculate by streak plate method on the selective media plates.

Total 6 selective media were taken the media are Rhizobium medium for Rhizobium spp, Jensen's medium for Azotobacter spp, Mannitol salt agar for Micrococus luteus spp, L-B agar for Enterobacter spp, Pikovskaya's agar for Bacillus Subtillis spp, Cetrimide agar for Pseudomonas aeruginosa spp. Out of total 6 Rhizobium spp, Azotobacter spp, Micrococus luteus spp, Enterobacter spp, Bacillus Subtillis spp, Pseudomonas aeruginosa spp were isolates from vermicompost and total 5 Rhizobium spp, Azotobacter spp, Micrococus luteus spp, Enterobacter spp, Bacillus Subtillis spp were isolates from cow manure.

Therefore, In the present study the results obtained from the Isolation and Identification of bacteria from vermicompost and cow manure that enhancing soil fertility and also help in crop improvement.

The results can be explained by identifying bacteria we know their role in the plant growth and development and promote the growth of those bacteria which have beneficial role in vermicompost and cow manure to enhance the nutrient quality in vermicompost and cow manure. The organic biofertilizer is beneficial for agriculture used and vermicompost and cow manure both are organic biofertilizer which are essential in agriculture. In vermicompost 6 isolates were found and colony forming unit is also more as compare to cow manure. In cow manure 5 isolates were found and colony forming unit is less as compare to vermicompost. So the vermicompost is more beneficial as compare to cow manure for the used as organic biofertilizer.

Photo plate 1

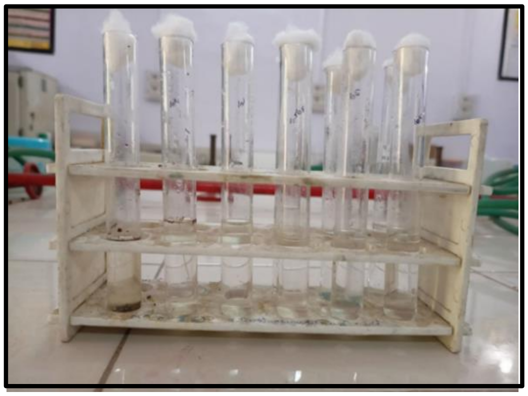

Fig. 8: Serial dilution of Vermicompost

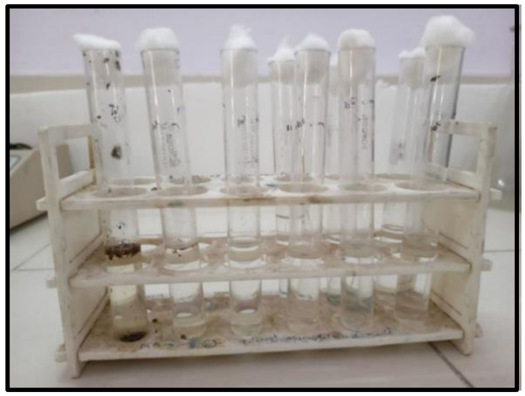

Fig. 9: Serial dilution of Cow Manure

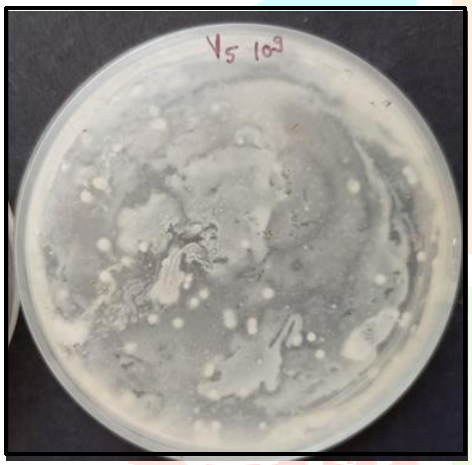

Fig. 10: Colonies of vermicompost on nutrient agar medium

Fig. 11:Colonies of cow manure on nutrient agar medium

CONCLUSION

From the study on the organic biofertilizer such as vermicompost and cow manure it can be conclude that the isolates present in vermicompost is more as compare to cow manure. In vermicompost 6 isolates were found and in cow manure 5 isolates were found. The isolates are *Azotobacter spp* , *Micrococcus luteus spp* , *Bacillus subtillis spp* , *Pseudomonas aeruginosa spp* , *Enterobacter spp, and Rhizobium spp*. The colony forming unit (cfu) in 1gm of vermicompost is 171.8×10^{-9} and in 1gm of cow manure is 149.6×10^{-9}. It can be conclude that the number of isolates and colony forming unit in vermicompost is more as compare to cow manure So, on that we can concluded that the vermicompost is more beneficial than cow manure for use as organic biofertilizer.

Vermicompost promotes soil aggregation, fertility, plant nutrition, and beneficial microbial growth .It enhances aeration and water retention in the soil. It helps to suppress plant diseases and improve plant growth by restoring the microbial population, which includes nitrogen fixers, phosphate solubilizers, and other microbes, as well as providing macro and micro nutrients to agricultural plants.

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