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Production and estimation of vermicompost from *Azolla* by using *Eisenia fetida*

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Abstract
The aim of the present investigation was to prepared from two different vermicompost. The experiment was conducted at the Vidya Pratishthan, Baramati campus in biofertilizer unit, during February to April in 2023. Vermicompost was prepared from cow dung and aquatic weeds *i.e. Azolla* by using *Eisenia fetida* species of earthworms. Vermiculture was carried out with two different types of sources such as animal excreta and plant biomass. Vermicompost was prepared conducting two different substances *i.e.* Cow dung and *Azolla.* These vermicompost were tested for moisture content, pH, nitrogen, carbon, potassium, phosphorus, ash, organic matter, Zinc, Iron, Manganese and Copper. The experimental results show significant variation between Physico-Chemical properties of both vermiculture bed. There was higher Physico - Chemical content observed in *Azolla* vermicompost. The study also confirmed that, the vermicomposting can produce high quality fertilizer which are better compared to other commercial fertilizer in the market.

Keywords: *Azolla*, *Eisenia fetida*, cow dung, vermicompost, organic

Introduction

In 1970 vermiculture was first introduced by Mary Appelhif. She was biology teacher and enlarges idea of using red wiggler worms for organic waste in indoor as well as outdoor system to convert kitchen waste to worm organic waste. Well known for its potential to increase not only organic matter but also trace mineral in soil. Vermiculture has been priority for Maharashtra Agricultural Biotech's in India; an organization has installed both commercial and educational progress to upgrade vermiculture. In 1955, Maharashtra Agricultural Biotech was structured habitual small plants to synthesize vermicompost per year.

Vermicompost uses are rapid and more efficient way to turn kitchen junk into nutrients soil accessory. The term vermiculture refers to the process of producing vermicompost from agricultural matter. The well-known organization nowadays produces 5000 tons of vermicompost through the practice of worms farming. These are useful as fertilizer to enhance the quality of the soil. Vermicomposting turns kitchen snip another green waste into lavish, dark soil that odour like earth and feels like magic. This compost is composed almost entirely of worm casting, making it highly nutrient-rich and superior variety of compost. It is rich in nutrients also loaded with microorganisms that produce and nourish healthy soil. Vermicompost fertilizer produced from earthworm digestion and aerobic malfunction at room temperature utilizing micro and macro organisms. Vermicomposting make rich organic soil modification, rich in plant nutrients and helpful bacteria. It increases the number of nutrients available to plants, allowing seeds to germinate quickly, grow faster, develop a strong root system, and yield more and More flowers or fresh fruits and vegetables are the consequence. Vermicompost does not have any unfavorable effect on soil as well as environment. It improves not only soil aeration but also texture by reducing soil concentration. It improves water retention capacity of soil due to its high organic waste. It also upgrades better root growth, (nutrients absorption also improves both macro and micro nutrients of soil) Vermicomposting is a process of manufacturing organic fertilizer from biodegradable material with earthworm. Composting with worms ignore the inessential disposal of vegetative unwanted food and have the benefit of high-quality compost. Vermicompost have more advanced level of applicable nutrients like potassium, calcium, magnesium, nitrogen, carbon etc. arises from the waste. Earthworms have been used for years to break down various types of waste, including plant debris, paper waste and cattle dung. As cattle dung, green waste is reach in carbon. In 60 days, these earthworms produce high-quality compost in a significant amount. Physical and chemical properties were examined during this period. The disposal of organic matter is a serious problem all over the world. Vermitechnology or vermicomposting is considered as a potential option in the ranking of unsegregated (combined) solid waste management. If anyone of these things is lacking composting becomes difficult.

Vermicompost consists of earthworm excrement, partially digested organic matter, earthworm eggs, and casts. Vermicomposting plays an important role in maintaining the balance of land, water and climate. Vermicompost contains most nutrients in plant- available forms such as Nitrates, Phosphates and exchangeable Calcium and soluble Potassium. By the use of vermitechniques in the presence of Oxygen organic waste turns into manure. Worm cast is known as Black gold. It is enriching soil quality by improve physicochemical and biological properties. Vermicomposting has obtained popularity in the pair commercial as well as private fixture because, as analyze with standard composting, it supplies a way to delicacy organic matter extra rapidly. In manure composting, it also achieves outcomes that have lower saltiness quantity.

Earthworms: Near about 350 species of earthworm were found in India with various food and also burrowing habits

Eisenia fetida (Red earthworm), *Eudrilus eugeniae* and *Perionyx excavatus* are few of the species that are reared to transform organic matter within manure. The efficient use of earthworms in organic matter control arrive at when a comprehensive grasp of biology of all certainly useful species. A mixture of epigeic species that appear no permanent burrows and alive on the surface anecic domestic that typically alive through the deeper layers may be examine. The lifetime of worms is about two years. Wholly grown worms may be separated and dried out in an oven to make 'worm meal' which is wealthy source for protein (70%) usually use in animal feed. Earthworms work for farmers day-night without any charge. These are made soil more nutritious. Earthworms are known as soil ecosystem modifiers. They are improving the soil nutrient profiles. Earthworms are the long, with cylindrical elongated body compressed out both the ends the body of earthworm is covered with a soft thin pellicle. Earthworms pellicles are transparent and temperature sensitive. Earthworms can be classified as geophagus and detritivores based on the feeding habit. Detritivores food on decaying organic waste like plant litters, the decaying vegetables, fruits, and plant, roots, cattle dung's at the soil surface and near the soil surface. The earthworms are the important drivers of the process by conditioning the substrate and altering the biological activity. Earthworms eat and excrete their own weight every day. Earthworms need three things to stay functional: 1) Moisture, 2) Dense shade, 3) Dung or partially. If anyone on these things is locking composting becomes difficult.

Fig 1: *Eisenia fetida* earthworm

Classification of *Eisenia fetida*
Kingdom- Animalia
Phylum- Annelida
Class- Clitellata
Order- Opisthopora
Family- Lumbricidae
Genus- Eisenia
Species- *E. fetida*

Red wigglers colour is reddish- brown. Small rings are present around their body. Have a yellowish tail. On each segment a number of groups of bristles are present, they move in and out to grip near by the surface. The worms are stretch and contract their muscles to push themselves forward and backward. *E. fetida* worms are native to Europe. Have been introduced to every other continent except Antarctica. *E. fetida* also possess individual natural protection system in their coelomic fluid cells called coelomocytes secrete a protein called lysenin, which is a pore- forming toxin, which is capable to permeabilize as well as lyse invading cells. It is most as targeting foreign cells whose membranes contain impact amount of sphingomyelin. *E. fetida* are used for Vermicomposting of both familiar as well as industrial organic matter. Vermicomposting septic systems have been used for decades as well as permit for decentralized on site preparing of backwater using *E. fetida*. Furthermore, red worms are really used in fishing, being one of the perfect baits for tench, bream as well as roach. When harshly control, a redworms exudes a pungent liquid, this particular name fetida meaning "foul- smelling". This is presumably an antipredator modification. *E. fetida* species is hermaphroditic as well as uniparental. Reproduction is conceivable, even if usually the reproduction is between mating personals. The two worms connect clitella, the big; lighter- colored rings which involve the worms' reproductive organs, and which are only prominent between the reproduction processes. The two worms transfer sperm. Both worms then produce cocoons which involve several eggs each. These cocoons are not only lemon shaped but also are pale yellow at first, becoming more brownish as the worms' inside turn into cultivated. Cocoons are clearly visible to the naked eye. At 25°C *E. fetida* hatches from its cocoon in about three weeks. Under controlled conditions life expectancy of *E. fetida* varies, according to different authors, between one as well as five years.

***Azolla*:** *Azolla* is a genus of aquatic ferns. That can be found on every continent except for Antarctica. The plants are small with leaves. That major between 0.5 and 2 cm in length. *Azolla* species are characterized by their mutual connection with the cyanobacterium and Anabaena *Azolla*e. These bacteria fix a nitrogen gas. This gas from the air, making it available to the plant provides the bacteria with *Azolla*e with a place to live and CO_2 for photosynthesis. *Azolla* plants grow rapidly, doubling their biomass in as small as four days. This makes them and charming options. Charming options for a farming and agriculture. *Azolla* contains high proteins, amino acids, Vitamins (vitamin A, vitamin B12, Beta Carotene) and minerals, so it is a superb nutrient feed for livestock (farm animal). Also, *Azolla* has little lignin content. So, animals smoothly digest.

Fig 2: *Azolla*

Azolla fix aerial nitrogen and stores it in leaves. Therefore, it is used as green compost. *Azolla* fixes nitrogen. It is a great source of nitrogen and has a great nutrient value for *Azolla*. Cultivation need less expenditure; hence it is a in inexpensive alternative for an excellent feed and excellent biofertilizer. Many farmers, due to small in amount resources, often try to produce enough feed for those animals although *Azolla* is the right choice in front of them. *Azolla* is used as animal feed and it is used as fertilizer. *Azolla* has a number of other uses. The plants can be used in bioremediation to remove heavy metals from water. They have studied as a potential source of biofuel. *Azolla* is high nutritious. They are also a very efficient fertilizer, providing up to ten times more nitrogen than other common fertilizers such as urea. *Azolla* can help to improve water quality by reducing level of ammonia and nitrates. They also reduce the amount of methane produced by livestock. *Azolla* is a type of water ferns that grow in many parts of the world. They can be found in ponds, lakes and other freshwater habitats. Both plants are very fast-growing plants and can double their biomass in as little as two days. This makes them an ideal plant for farming and cultivating.

Objectives –

1. To improve Physico- chemical properties – pH, carbon, organic matter, ash, nitrogen, phosphorus, potassium, iron, zinc, copper, manganese, copper.
2. To compost organic wastes for to produce excellent feature (quality) manure to feed our "chemical free matter hungry" soils.

Fig 3: Cow dung vermicompost

Materials and methods

Material: Generally, animal dung, mainly cow dung and dried chopped leaves residues, are the solution raw materials and *Azolla* for high nutrient value. *Eisenia fetida* is favored for the reason of its high multiplication value and thereby converts the organic waste into vermicompost within 45-50 days. Since it is a surface feeder, it changes organic waste into vermicompost top.

Methodology- Bed method (Vermicompost)

Vermicomposting is done by bed method. In- bed method composting is done on the pucca/ kaccha floor (6×2×2 feet size) by making a bed of organic mixture. Choose a suitable site for Vermicomposting; this should be cool, wet, water access, shady and availability of dung or matter. Vermibed is the layer of wet loamy soil availability of dung or waste and then handful lumps of fresh cattle dung are placed at randomly over the vermibed. The compost bed is then added and layered to about 5cm with dry leaves and grasses. Earthworms are introduced into the upper layer of the bed. Water is sprinkled immediately after the addition of the worm. For the next 45-50 days, the bed is kept moist by watering it whenever necessary.

Protect the compost from rainwater and direct sunlight. The compost will be ready in 40-50 days, and the material become moderately loose and crumbly with dark brown colour. And second *Azolla* vermicompost bed method is the same as above, but *Azolla* is, added before the earthworm adding time. Vermicompost processed is free from bad odour.

Methods for parameters

pH-

1. Take 10 gm of soil sample in a 50 ml beaker.
2. Add 25 ml distilled water.
3. Then mix it well (with a glass rod).
4. Take reading through pH meter.

Ash method

1. Take the weight 1g sample into high form porcelain crucible and place sample into a furnace and raise the temperature up to 500℃.
2. Then after reaching temperature- ash for three hours.
3. Wet with a little amount of dH2O and then add 5 to 10 ml of 6N HCL and conduct to close by dryness on hot plate.
4. Quantitatively shift dissolved ash into 100 ml volumetric flask after dissolve Ash by put in 10 ml 1N HCL to crucible.
5. Then wash sample with dH2O then dilute with dH2O to volume and shake well.
6. Place fractional into ICP test tube.

Kjeldahl Method

Since the evolution of Kjeldahl method about 100 years ago. The technique, as well as the apparatus have been alter and made greater. even with the new changes the main principles introduced by Johan Kjeldahl are still relevant and the main target of this method is the oxidation of organic compounds by using concentrated sulphuric acid. Generally, the Kjeldahl procedure is divided into 3 primary steps. The technique has to be realized out in proper sequence and the step includes- digestion, distillation and titration.

1. **Digestion:** In this procedure, a sure material is heated in the presence of sulphuric acid. The acid collapsing the organic sample via oxidation and reduced the nitrogen in the form of a Ammonium Sulphate is

liberated then potassium sulphate is usually added to increase the boiling point of the medium. Catalysts like Mercury, Copper, Selenium ions of Mercury or Copper are also applied in the digestion process.

2. **Distillation:** The distillation of the solution now takes place as well as a little amount of sodium hydroxide is added to turn the ammonium salt to ammonia and the distilled vapours are then bound in a particular trapping solution of hydrochloric acid and water.

3. **Titration:** The quantity of ammonia is the quantity of nitrogen present in the substance is then settle by back titration and as the ammonia dissolves in the acid trapping solution some hydrochloric acid is balance. The acid that is port behind can be back titrated with a standard mixture of a base such as NaOH or bases.

Vanadate- Molybdate method-

This technique is based on APHA Standard Method 4500-P C and description of the assay from the method is in a alter Ammonium Molybdate, Orthophosphate solution acts under acid conditions to form a molybdophosphoric acid, heteropoly acid. In strength of the yellow colour is corresponding to phosphate absorption.

Reagents and equipment

Cuvettes, colorimeter, 1 ml variable micropipette, Vanadate-molybdate reagent**.**

Colorimeter set-up

- Wavelength is 470nm and use BLUE led (colorimeter RGB board).
- Phosphate standard data- download a data file and prepare your own phosphate gauge data then before measuring substances, calibrate the colorimeter contra water or a distilled water sample expand with vanadate-molybdate reagent as define below.

Method

- Take 1 ml of sample water into a clean cuvette then0.25 ml of vanadate- molybdate reagent and mix by pipetting up and down certain times.
- Then wait at least 10 min for colour to grow.
- Place the cuvette with sample into the colorimeter and click measure then the plan will revert the phosphate concentration in ppm.

The Flame photometer

A traditional as well as easy technique for resolve sodium and potassium in biological fluids entail the method of emission flame photometry. This liberation on the principal that an alkali metal salt drawn into a non- luminous flame will ionize, consume energy from the flame and then exhale light of a characteristic wavelength as the stimulated atoms decay to the unexcited ground state. The depth of transmission is proportional to the concentration of the element in the solution and you are probably family with the reality that if you sprinkle table salt in a gas flame then it glows bright orange. This is the primary principle of flame photometry.

Atomic Absorption Spectrophotometer (AAS)

AAS Analyst 400 model used to determining the content of heavy metals in the previously digested soil samples. The nitrous oxide, acetylene gas and compressor were fixed and compressor turned on and the liquid trap blown to read of any liquid trapped. The extractor and the AAS control where were turned on. The cylinder tube and nebulizer piece were cleaned with purifying wire and opening of the burner cleaned with an arrangement card. fine was set in a 10 ml graduated chamber containing deionized water and yearning rate estimated. The various metal concentrations from the sample solution were determined a from the calibration, based on the absorbance obtained for the unknown sample.

Fig 4: Sample Tests

Result and discussion

Table No.1 shows the physical as well as chemical properties of different vermicompost. There were significant differences in each physical and chemical properties of the prepared aquatic weeds vermicompost's. In this project, a comparative study of cow dung vermicompost and *Azolla* vermicompost is very successful. The physical and chemical properties of *Azolla* vermicompost are high than cow dung vermicompost. *Azolla* is high nutritive. It is an excellent source of nitrogen and has an excellent nutrient value for *Azolla*. *E. fetida* earthworm species are used for vermicomposting of both domestic and industrial organic waste. The very important role of *E. fetida* in this vermicomposting method.

Fig 5: Compost

pH of cow dung vermicompost is 6.8 and pH of *Azolla* vermicompost is 7.2. The pH of AV is higher than CV. The total content of carbon in CV is 39.97, and in AV is 33.19. Organic matter in CV is 55, and in AV are 57.10. Ash content in CV is 45 and in AV is 42.90. Nitrogen is 0.45 in CV and 0.56 in AV. Phosphorus content in CV is 0.48, and in AV is 0.85. Potassium content in CV is 0.31, and in AV, is 0.58. Iron in CV is 21392.5, and in AV, is 35055. Zink content in CV is 84.30, and in AV is 120.10. Manganese in CV is 406.21, and in AV is 462.54. The copper content in CV is 97.43, and in AV, is 155.13.

There are different equipment and a variety of facilities available for vermicompost in KVK Baramati and types of sample testing are done by us with the help of KVK staff members. Also, in this format, we have done this vermicomposting of cow dung and *Azolla*.

Table 1: Physical and chemical properties of different vermicompost's.

Sr. No.	Parameters	CV	AV
1	pH (%)	6.8	7.2
2	Carbon (%)	39.97	33.19
3	Organic matter (%)	55	57.10
4	Ash (%)	45	42.90
5	Nitrogen (%)	0.45	0.56
6	Phosphorus (%)	0.48	0.85
7	Potassium (%)	0.31	0.58
8	Iron (ppm)	21392.5	35055.00
9	Zink (ppm)	84.30	120.10
10	Manganese (ppm)	406.21	462.54
11	Copper (ppm)	97.43	155.13

Data represents the mean value of two dimensions

CV- Cow dung vermicompost, **AV**- Azolla vermicompost.

Conclusion

From this comparative research study on we concluded that the nutritive quality of *Azolla* vermicompost is higher than cow dung vermicompost. We study different types of Physico -Chemical properties for better yield for farming. Fertility of soil is maintained by using vermicomposting in farming sector. *E. fetida* earthworm species play important role to enhance microbial processes. By using vermicomposting lifespan of crops and we can obtain easily better grain quality. This method is ecofriendly without any use of hazardous chemicals.

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