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Production of Biofertilizer from Vegetative Waste and Animal Excretory by Inoculating PGPR Consortium

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ABSTRACT
In recent decades, agricultural production has increased to meet the global demand for food, which has been fuelled by population growth. Sustainability research has increased its focus on other crop fertilization mechanisms, such as biofertilizers. The present work focuses on biofertilizer synthesis by mixing cow dung and household vegetative waste using PGPR consortia. This mixture was kept in 3 different composites: T-A (autoclaved carrier, no PGPR), T-W/A (without autoclaved carrier, with PGPR), and T-C (un-inoculated control), respectively. The method of composting was employed in earthen pots for lab-scale study. Aeration was accomplished by making holes in the earthen pots and turning the compost twice a week. pH, temperature, and moisture were determined at 7-day intervals up to 2.5 months. Determination of microbial load, phosphatase activity, and physicochemical characteristics of the soil and biofertilizer was done. The efficacy of the biofertilizer was analyzed by pot study on the legume crop Cicer arietinum. The experiment revealed that T-W/A composites have increased amounts of organic matter (3-fold), nitrogen (1.62-fold), carbon (1.55-fold), and macro (NPK) and micronutrients (Cu, Zn, Mn, Fe) as compared to un-inoculated control. After 2 months, plants were harvested, and vegetative growth parameters (shoot and root length, fresh and dry weight, and chlorophyll (a and b) content were measured. It was found that the consortium-treated soil improved not only the plant growth but also NPK values, thereby accelerating its fertility. Plant amendments containing biofertilizers were more promising approaches to sustainable agriculture in terms of reducing problems associated with chemical fertilizer use.
Keywords: *Biofertilizer, PGPR, Vegetative waste, Cow dung, Sustainable agriculture*

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INTRODUCTION

More than 58% of India's population is mainly dependent on agriculture, making the country one of the major players in the global agricultural market. About half of India's population works in agriculture in the country, which is the second-largest agricultural sector in the world [9]. In the Indian economy, agriculture plays an important role. Over the past six years, the agricultural sector in India has grown at a compound annual growth rate of 4.6%. It fell from 3.3% to 3.0% in 2021-2022. For a year, starting January 1, 2023, the government will provide free grain to nearly 81.4 million NFSA beneficiaries [1].

The threat to food security, national security, and human survival is posed by climate change and the growing demands of a rising population for food and feed. The most widely used agricultural practice to enhance soil nutrients and boost crop yield is the application of chemical fertilizers. However, the excessive use of chemical fertilizers leads to increased production costs, depletion of mineral sources, energy consumption, environmental hazards, soil structure damage, disruption of the soil's micro-ecosystem, as well as groundwater pollution [19]. The use of biological approaches, such as biofertilizers, is an essential alternative soil management technique to minimize the harmful consequences of chemical fertilizers. By enhancing soil fertility, soil bacterial diversity, water and nutrient uptake, and plant tolerance to stress, biofertilizers facilitate plant growth, which in turn increases crop production [22].

Vegetative waste is defined as residues from the growing and processing of raw agricultural commodities such as fruits, vegetables, meat, poultry, dairy, and crops. They are by-products of agricultural production and processing. The agricultural waste consists of crop residues from food processing such as corn stalks, sugar cane bagasse, fruit and vegetable drippings, etc. [6]. As such, agricultural waste is the most economical resource for the production of biofertilizers for farmers, which enhance soil fertility [9].

Cow dung is a common animal by-product with adverse environmental effects such as releasing the greenhouse gas methane, causing unpleasant odours, and becoming a vector for various diseases. It is estimated that it contributes 53% of the annual production of animal waste, amounting to 61 million tonnes. Currently, most of the cow manure produced in the world is used for renewable energy, while the production of biofertilizers from this source, which have a high nitrogen concentration for optimal plant growth, is largely ignored. Therefore, the goal of this study is to create a bio-fertilizer from composted cow manure and agricultural waste [16].

Plant growth-promoting rhizobacteria (PGPR) are a well-known category of biofertilizers. These strains of bacteria live in the rhizosphere of plants, interact with their roots, and have an impact on their growth and productivity [23]. When applied to soil, they easily colonize the roots, thus having a beneficial influence on various characteristics of the host plant. PGPR enhances plant growth through various mechanisms such as phytohormone production, water, and nutrient uptake, abiotic and biotic stress tolerance [25], nutrient solubilization, biological nitrogen fixation, protecting plants through different mechanisms [14] such as the production of siderophores, [21] secretion of antibiotic, enzymes, fungicidal compounds, etc., [22] and also helps in the elimination of toxic heavy metals from the ecosystem [26,24].

The main objective of this study was to develop a simple and cost-effective method to produce biofertilizer using several types of vegetative waste and animal excretory waste, i.e. cow dung. The solid-state fermentation method was used to produce biofertilizer, which was then applied to the legume crop ***Cicer arietinum***.

MATERIAL AND METHODS

All the experiments were carried out in the January – March 2023.

Preparation of fertilizer

Collection of PGPR cultures:

PGPR used in the preparation of biofertilizer was procured from the Department of Microbiology and Biotechnology, University School of Sciences, Gujarat University. The PGPRs are *Micrococcus luteus, Bacillus subtilis, Staphylococcus aureus, Klebsiella oxytica, Pseudomonas, Serratia marcescens, C5, and T15*. Collected isolates were enriched on enrichment media and set their optical density of 1.0 at 600 nm, which means the culture contains 1×10^8 cells/ml.

Collection of samples:

Various vegetative wastes, such as lady's finger, potato, cabbage, brinjal, peas, coriander, bitter gourd, lemon, tomatoes, corn, mint, banana, apples, etc., and organic waste, such as grass, coconut husk, weeds, stalks, stems, leaves, and fallen pruning dead branches, were collected from different households and vegetable vendors. Cow dung had been collected from various cattle farms. Earthen pots used in the preparation of fertilizer were collected from the Nehrunagar area of Ahmedabad, near Gujarat University.

Method:

a) Collected wastes were air dried under sunlight and properly crushed into granules, roughly below 40 mm in diameter, with the help of a mixer-grinder, and the granular form of waste was sterilized by autoclaving to remove contamination. The collected, air-dried, and autoclaved waste was used as a carrier for the preparation of biofertilizer.
b) The method of composting was employed in earthen pots for the lab-scale study. The prepared carrier was then transferred to methanol and acetone-sterilized earthen pots to form three different composites: T-A (autoclaved carrier, with PGPR consortium), T-W/A (without an autoclaved carrier, with PGPR consortium), and T-C (un-inoculated control), respectively.
c) All the cultures were mixed in equivolume and then each gram of dry material was inoculated with a tested microbial culture of PGPR (*Micrococcus luteus, Bacillus subtilis, Staphylococcus aureus, Klebsiella oxytica, Pseudomonas, Serratia marcescens, C5, T15),* containing about 1×10^8 cfu/ml, at an initial moisture content of 60–65 %, in earthen pots.
d) Aeration was accomplished by making holes in the earthen pots and turning the compost twice a week. Watering should be done every 15 to 20 days to keep it moist for up to 2-2.5 months.
e) pH, temperature, and moisture were analyzed at an interval of 5-7 days till 2.5 months.

Analysis of prepared fertilizer

Microbial load determination

Microbial load determination was done by a standard plate count or the total viable count. A quantitative bacteriological analysis enumerates the total viable population capable of growing under a given set of conditions. It is based on the assumption that each viable bacterium develops into a distinct colony. Microbial load determination was done by serial dilution of the fertilizer sample and plating a 0.1 ml sample from each dilution on N. agar plates, where colonies were observed [8].

Phosphatase activity (Acid and Alkaline)

Acid and Alkaline Phosphatase activity of the fertilizer and soil sample was determined by using p-nitro phenyl phosphate as a substrate and incubating it with MUB (Modified Universal Buffer) of pH 6.5 for acid or pH 11 for alkaline at 37°C for 1 hour, phosphatase enzyme releases p-nitro phenol, which is measured calorimetrically at 400–420 nm [28].

Physico-chemical characteristics of soil and fertilizer

Tests during composting

pH, temperature, and moisture were analyzed at an interval of 5-7 days until 2.5 months of incubation.

Tests after preparation of fertilizer

The estimation of electrical conductivity, organic loadings such as organic carbon content by the Walkley and Black method, various macronutrients such as nitrogen, available phosphorus by Olsen's method, available potassium, and various micronutrients such as sulphur, boron, copper, zinc, magnesium, and iron of fertilizer and soil were conducted in the soil testing laboratory, situated at IFFCO CORDET, Kalol.

Pot Study after Preparation of Biofertilizer

***Collection of samples*:**

The soil used for the pot study was collected from the Gujarat University area. The physico-chemical characteristics of soil were also conducted at IFFCO CORDET, kalol, to ascertain the number of various soil nutrients in the untreated soil. The soil was sterilized by autoclaving. The pots used for the pot study were collected from the Memco area of Ahmedabad.

Method:

Prepared biofertilizers were mixed with the soil in a ratio of 1:5. Healthy seeds of *Cicer arietinum* were taken, sterilized with mercuric chloride ($HgCl_2$), and sown in pots filled with a sterilized standard soil mix. Around 30 seeds were sown in each pot, which was watered regularly.

Analysis:After 2 months of growth, plants were harvested, and vegetative growth parameters of plants were recorded in treated and control plants, such as shoot and root length, fresh and dry weight of the plant, and chlorophyll content (a and b) in leaves. The yield was also observed. All pot experiments were done in triplicates under natural, uniform conditions.

RESULT AND DISCUSSION

Preparation of biofertilizer

After an incubation period of 2.5 months, compost is harvested when the finished product is rich, dark brown in colour, granular, nutrient-rich, smells like earth, crumbles in hand, and has no recognizable food material visible. This product is called a biofertilizer which is shown in Figure 1

Analysis of fertilizer

MICROBIAL LOAD DETERMINATION

Microbial load determination of the without autoclaved, autoclaved, and control sets were done to determine the viable number of microbes present in the biofertilizer, followed by observation of their cultural and morphological characteristics. The results of CFU/ml of without autoclaved set, autoclaved set, and the control set were shown in Figures 2,3,4. Cultural characteristics of the isolates obtained from the without autoclaved, autoclaved, and control sets of biofertilizer were shown in Tables 1,2,3. The morphological characteristics of some selected isolates obtained from without autoclaved, autoclaved, and control sets of biofertilizer were shown in Table 4. Gram staining of isolates obtained from without autoclaved, autoclaved, and control sets of biofertilizer were shown in Figures 5,6,7.

4.2.2 Phosphatase activity

The results of the alkaline and acid phosphatase activities of soil, control, autoclaved, and without autoclaved sets of biofertilizer were recorded. The without autoclaved set had the highest amount of alkaline phosphatase activity, with 1070.4 g of p-nitro phenol obtained per gram of biofertilizer, which is 15.46 folds higher than the soil, 2.56 folds higher than the control, and 0.63 folds higher than without autoclaved set. The without autoclaved set of biofertilizer had the highest amount of acid phosphatase activity, as 2154 µg of p-nitro phenol were obtained per gram of biofertilizer, which is 18.91 fold higher than the soil, 3.67 fold higher than the control, and 0.4 fold higher than the autoclaved set. The acid phosphatase activity of all the sets was higher than alkaline phosphatase. According to El-Sawah et al., 2021, mycorrhizal injection increased the dehydrogenase and phosphatase activities in the guar plant's rhizosphere. Additionally, they stated that in both growing seasons, the treatment with B. subtilis was followed by the treatment with Mycorrhiza, which produced the highest levels of phosphatase activity [7].

Physico-chemical characteristics of soil and fertilizer

1) Tests during composting :

The results of pH, temperature, and moisture content of without autoclaved, autoclaved, and control sets of biofertilizer were recorded at the 7-day interval up to 2.5 months during composting and are shown in Table 5. All the sets show similar results.

2) Tests after preparation of fertilizer

The results of the physico-chemical characteristics of the soil, original, without autoclaved, autoclaved, and control sets of biofertilizer were recorded after the preparation of the biofertilizer, which is described in brief below.

Out of all the sets without an autoclaved set of biofertilizer has a high amount of **electrical conductivity,** i.e. 4.5 microSiemens/cm, which is 17.75 folds higher than the soil, 0.73 folds higher than the control set, 0.55 folds higher than the original, and 0.12 folds higher than the autoclaved set. The high amount of electrical conductivity is seen due to the presence of a high amount of various macro- and micronutrients in the biofertilizer. According to Ding et al., (2018), the fresh weight, dry weight, and leaf size of Pak Choi plants gradually increased with the increase in EC, with the EC 4.8 treatment having the greatest values. The harmful effects of the extremely high nutrient solution concentration resulted in reduced productivity and leaf size in the highest EC 9.6 treatment [5].

Out of all the sets without an autoclaved set of biofertilizer has the highest amount of **organic carbon,** i.e., 1.53%, which is 1.55 fold higher than the soil, 0.61 fold higher than the control set, 0.35 fold higher than the original set, and 0.03 fold higher than the autoclaved set. High organic carbon content results from vegetative waste and various forest litter, such as dry leaves, grass, and woody materials. Hammad et al. (2020) reported that using organic manures considerably boosted soil NPK content and organic matter compared to using no fertilizer or inorganic fertilizers [10].

Out of all the sets without an autoclaved set of biofertilizer has a high amount of **nitrogen,** i.e., 0.13%, which is 1.6 fold higher than the soil, 0.62 fold higher than the control set, 0.44 fold higher than the original set, and 0.083 fold higher than the autoclaved set. Pindi et. al., (2012) reported that the best illustration of symbiotic nitrogen fixation is Rhizobium. In addition to non-legume crops, it can fix N2 in legumes. In several legume crops, Rhizobium has been demonstrated to fix up to 300 kg N/ha/year [13]. The C:N ratios calculated are shown in Table 6.

Out of all the sets without an autoclaved set of biofertilizer has a high amount of **phosphorous,** i.e., 20.6 µg per gram of biofertilizer, which is 1.41 fold higher than the soil, 0.48 fold higher than the control set, 0.32 fold higher than the original set, and 0.13 fold higher than the autoclaved set. Asoegwu et. al., 2020 reported that they have the capacity to solubilize/mobilize 30–50 kg of P_2O_5 per hectare under ideal circumstances, which could result in a 10–20% boost in crop output [3].

Out of all the sets without an autoclaved set of biofertilizer, has a high amount of potassium**,** i.e., 6780 µg per gram of biofertilizer, which is 30.53 fold higher than the soil, 0.53 fold higher than the control set, 0.2 fold higher than the original set, and 0.11 folds higher than the autoclaved set. Sugumaran p. and Janarthanam et. al. (2007) reported that *B.edaphicus* and *B.mucilaginosus* are known to improve solubilization as well as mobilization. *B. mucilaginous*, when inoculated in soil, improved the oil content and groundnut biomass by 35.4% and 25%, respectively, along with enhanced K and P availability [27]. According to Pramanik et al. (2019), a potassium-solubilizing strain of *Bacillus pseudomycoides* improved K uptake in tea plants in the soil treated with mica debris by boosting potassium availability [15].

Out of all the sets without an autoclaved set of biofertilizer has a high amount of sulphur i.e., 387.7 µg per gram of biofertilizer, which is 5.41 folds higher than soil, 0.98 folds higher than the control set, 0.46 folds higher than the original set, and 0.083 fold higher than the autoclaved set. Rana et. al., 2020 reported that sulfuric acid produced by the oxidation of sulfur by *Thiobacillus thiooxidans* decreases the pH of the soil, causing the dissolution of important plant nutrients such as phosphorous, which is required for healthy plant growth, thus increasing soil fertility. It can also be well utilized to treat saline and alkaline soil for better cultivation [17].

Out of all the sets, the without an autoclaved set of biofertilizer has a high amount of boron, i.e., 1.8 µg per gram of biofertilizer, which is 1.46 folds higher than soil, 1.36 folds higher than the control set, 0.63 folds higher than the original set, and 0.28 folds higher than the autoclaved set. Rasheed et. al., (2009) reported in their review that boron (B) is considered an essential element for plant growth and development. Sexual reproduction in the plant is more sensitive to low B than vegetative growth. It has also been reported that boron deficiency limits reproductive growth. In wheat, B deficiency causes poor anther and pollen development, low grain set, and stunted growth [18].

Out of all the sets without autoclaved set of biofertilizer has a high amount of copper**,** i.e., 210 µg per gram of biofertilizer, which is 2.81 folds higher than the soil, 2.5 folds higher than the control set, 2 fold higher

than the original set, and 1.33 fold higher than the autoclaved set. Nosheen et. al., 2021 in their review reported that VAM fungi could contribute to more than the twofold increased acquisition of less mobile nutrients like P, S, Ca, Mg, Zn, and Cu from the rhizosphere [12].

Out of all the sets without an autoclaved set of biofertilizer has a high amount of **zinc,** i.e., 1040 µg per gram of biofertilizer, which is 12 fold higher than the soil, 2.46 fold higher than the control set, 0.55 fold higher than the original set, and 0.31 fold higher than the autoclaved set. Vaid et. al., (2014) reported that rice plants inoculated with a suitable combination of Zn-solubilizing bacterial strains (*Burkholderia spp. and Acinetobacter spp.*) increased the growth attributes and rice yield and were found to be more efficient in acquiring Zn from the soil as compared to non-inoculated plants [29]. Hussain et. al., (2019) reported that biofertilizers containing Zn-solubilizing bacteria have been reported to boost maize production [11].

Out of all the sets without an autoclaved set of biofertilizer has a high amount of manganese**,** i.e. 1740 µg per gram of biofertilizer, which is 0.93 folds higher than soil, 0.54 folds higher than the control set, 0.26 folds higher than the original set, and 0.23 folds higher than the autoclaved set.

Out of all the sets without an autoclaved set of biofertilizer has a high amount of iron**,** i.e., 47.95 mg per gram of biofertilizer, which is 21.61 fold higher than the soil, 0.68 fold higher than the control set, 0.25 fold higher than the original set, and 0.12 fold higher than the autoclaved set. According to Rout et al. (2015), approximately 80% of the iron in photosynthetic cells is required for the manufacture of cytochromes and other heme molecules, including chlorophyll, the electron transport system, and the formation of Fe-S clusters [20].

The physico-chemical characteristics of soil and prepared biofertilizer were shown in Table 7.

Similar results of physicochemical analysis of soil and fertilizer were also obtained by Devi et. al., 2018 in their research on the production of biofertilizers from Agro-wastes. The values of pH, electrical conductivity, total organic carbon, nitrogen, phosphorous, potassium, boron, sulphate, copper, zinc, magnesium, and iron were significantly higher in the prepared biofertilizer as compared to the control [4].

Pot study after preparation of fertilizer

a) Pot study results

Biofertilizer prepared from a mixture of vegetative waste and cow dung by using PGPR isolates was tested on ***Cicer arietinum*** and its vegetative growth parameters were studied. The growth of *Cicer arietinum* after 2 months was shown in Figure 8.

b) Vegetative growth parameter study

Vegetative growth parameters were studied and recorded after 2 months of plant growth, and it was observed that plants treated with prepared biofertilizer have enhanced growth. Results compared with control biofertilizer show that pots inoculated with biofertilizer prepared from without autoclaved carrier give the best results in nearly all parameters such as shoot length, root length, wet weight, dry weight, and chlorophyll (a and b) contents of leaves of the *Cicer arietinum* plant. According to Ali et al. 2021, *Bacillus cereus* considerably boosted plant height, shoot dry weight, and branch number as compared to the control, by roughly 15%, 26%, and 27%, respectively [2].

The results of vegetative growth parameters of *Cicer arietinum* after treatment with prepared biofertilizer were shown in Table 8.

The root length of the plant inoculated with biofertilizer prepared from without autoclaved carrier was 20 cm, which is 81.81 % higher than the root length of the plant treated with control biofertilizer and 42 % higher than the root length of the plant treated with biofertilizer prepared with an autoclaved carrier.

The shoot length of the plant treated with biofertilizer prepared from without autoclaved carrier was 23.5 cm, which is 17.5 % higher than the shoot length of the plant treated with control biofertilizer and 6.8 % higher than the shoot length of the plant treated with biofertilizer prepared with an autoclaved carrier.

The fresh weight of the plant treated with biofertilizer prepared from without autoclaved carrier was 2.18 g, which is 172 % higher than the fresh weight of the plant treated with control biofertilizer and 17.9 % higher than the fresh weight of the plant treated with biofertilizer prepared using an autoclaved carrier.

The dry weight of the plant treated with biofertilizer prepared from without autoclaved carrier was 1.03 g, which is 171% higher than the dry weight of the plant treated with control biofertilizer and 5.1% higher than the dry weight of the plant treated with biofertilizer prepared from the autoclaved carrier.

The chlorophyll **a** content of the plant treated with biofertilizer prepared from without autoclaved carrier was 0.172 g, which is 207 % higher than the chlorophyll a content of the plant treated with control biofertilizer and 30.3 % higher than the chlorophyll a content of the plant treated with biofertilizer prepared with an autoclaved carrier.

The chlorophyll b content of the plant treated with biofertilizer prepared from without autoclaved carrier was 0.076 g which is 192.3% higher than the chlorophyll b content of the plant treated with control

biofertilizer and 76.7 % higher than the dry weight of the plant treated with biofertilizer prepared from the autoclaved carrier.

Similar results for vegetative growth parameters were also reported by Devi et. al., 2018 in their research on the production of biofertilizers from agro-wastes. The values of root length, shoot length, total height, and the number of seeds germinated were significantly higher in plants treated with biofertilizer as compared to control plants [4].

c) Yield

The yield was seen in *Cicer arietinum* after 2 months of plant growth after the treatment of the prepared biofertilizer as shown in Figure 9. Out of all three sets, a higher yield was seen in the plants treated with biofertilizer prepared from without an autoclaved set of carriers.

Figure 1: Harvested biofertilizer after 2.5 months

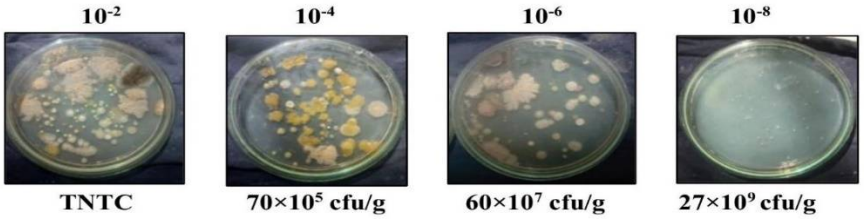


Figure 2: Microbial load determination of biofertilizer prepared from without autoclaved set of biofertilizer

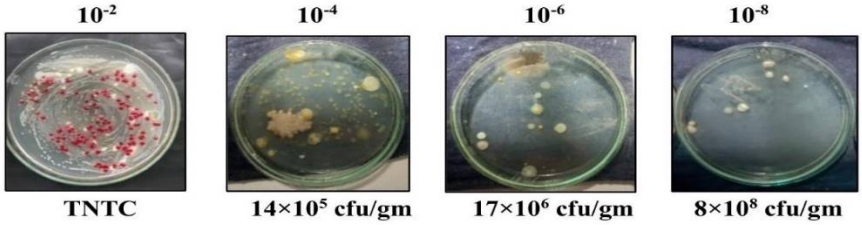


Figure 3: Microbial load determination of the autoclaved set of biofertilizer

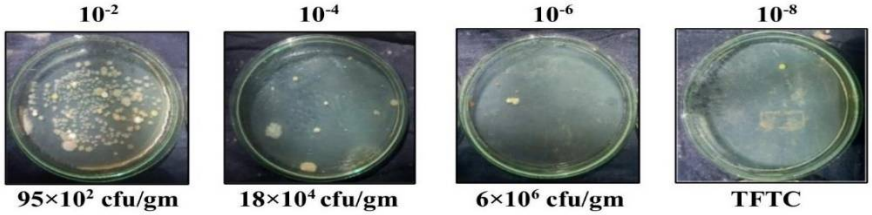


Figure 4: Microbial load determination of control set of biofertilizer

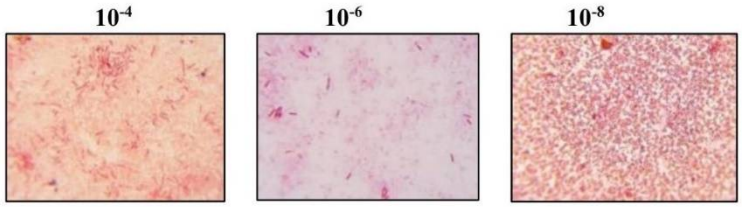


Figure 5: Gram staining of isolates obtained from without autoclaved set of biofertilizer

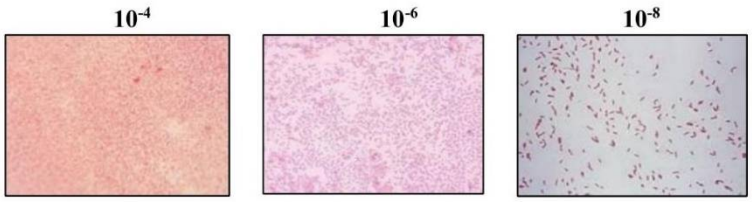


Figure 6: Gram staining of isolates obtained from an autoclaved set of biofertilizer

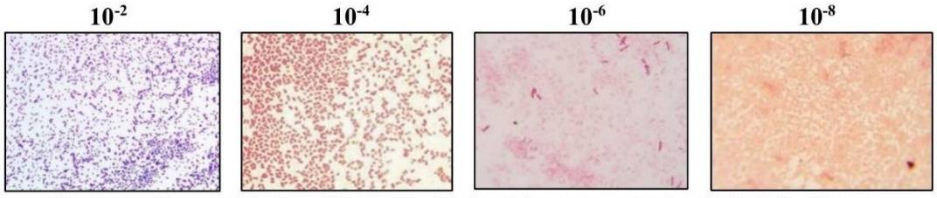


Figure 7: Gram staining of isolates obtained from a control set of biofertilizer

Figure 8: Growth of Cicer arietinum after 2 months

- C - Seeds inoculated with control fertilizer
- A - Seeds inoculated with fertilizer prepared from the autoclaved carrier
- W/A - Seeds inoculated with fertilizer prepared from without autoclaved carrier.

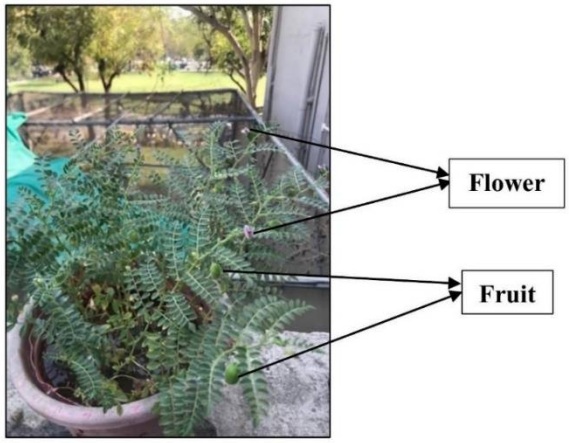


Figure 9: Yield observed after 2 months of treatment with prepared biofertilizer

Table 1: Cultural characteristics of the isolates obtained from the without autoclaved set of biofertilizer

Dilution	Size	Shape	Margin	Elevation	Pigmentation	Opacity	Consistency
10^{-2}	Small	Round	Entire	Convex	White	Opaque	Butyrous
	Small	Round	Entire	Convex	Yellow	Opaque	Butyrous
	Medium	Round	Entire	Convex	Dark yellow	Opaque	Butyrous
	Medium	Round	Entire	Flat	White	Translucent	Smooth
	Large	Irregular	Filamentous	Flat	Cream	Opaque	Rough
10^{-4}	Small	Round	Entire	Convex	white	Opaque	Butyrous
	Small	Round	Entire	Convex	Light yellow	Translucent	Viscous
	Large	Round	Entire	Flat	Cream	Opaque	Smooth
	Large	Irregular	Lobate	Flat	Yellow	Translucent	Smooth
	Large	Irregular	Filamentous	Flat	Cream	Opaque	Rough
10^{-6}	Small	Round	Entire	Convex	White	Translucent	Mucoid
	Medium	Round	Undulate	Slightly raised	White	Opaque	Brittle
	Medium	Round	Undulate	Raised	Light Purple	Opaque	Firm
	Rhizoid	Irregular	Irregular	Irregular	white	Opaque	Firm
10^{-8}	Small	Round	Entire	Convex	White	Translucent	Smooth
	Medium	Round	Undulated	Lobate	White	Opaque	Friable

Table 2: Cultural characteristics of the isolates obtained from the autoclaved set of biofertilizer

Dilution	Size	Shape	Margin	Elevation	Pigmentation	Opacity	Consistency
10^{-2}	Small	Round	Entire	Convex	Cream	Translucent	Butyrous
	Very small	Punctiform	Entire	Flat	white	Opaque	Butyrous
	Small	Round	Entire	Convex	White	Opaque	Butyrous
	Medium	Round	Entire	Convex	Red	Opaque	Butyrous
10^{-4}	Small	Round	Entire	Flat	Yellow	Translucent	Butyrous
	Medium	Round	Entire	Flat	Yellow	Translucent	Butyrous
	Large	Round	Undulate	Highly raised	Yellowish white	Opaque	smooth
	Large	Round	Umbonate	Raised	White	Opaque	Rough
	Large	Irregular	Filamentous	Flat	Cream	Opaque	Rough
10^{-6}	Small	Round	Entire	Convex	Light yellow	Translucent	Mucoid
	Medium	Round	Entire	Convex	Dark Yellow	Opaque	Butyrous
	Medium	Round	Undulate	Raised	Dark yellow	Opaque	Firm
	Large	Round	Entire	Flat	White	Opaque	Smooth
	Large	Round	Entire	Umbonate	Pinkish White	Slightly Translucent	Smooth
10^{-8}	Small	Round	Entire	Convex	White	Opaque	Smooth
	Medium	Irregular	Undulate	Raised	White	Opaque	Firm
	Large	Round	Undulate	Umbonate	White	Opaque	Firm
	Large	Round	Entire	Flat	White	Translucent	Smooth

Table 3: Cultural characteristics of the isolates obtained from the control set of biofertilizer

Dilution	Size	Shape	Margin	Elevation	Pigmentation	Opacity	Consistency
10^{-2}	Small	Round	Irregular	Flat	White	Translucent	Firm
	Small	Round	Entire	Convex	Yellow	Translucent	Butyrous
	Medium	Round	Entire	Flat	Yellow	Translucent	Butyrous
	Large	Round	Undulate	Raised	White	Opaque	Rough
10^{-4}	Small	Round	Entire	Convex	White	Opaque	Butyrous
	Medium	round	undulate	Raised	White	Opaque	Firm
	Large	Round	undulate	Slightly raised	White	Opaque	Firm
	Large	Oval	Entire	Slightly raised	White	Opaque	smooth
10^{-6}	Small	Round	Entire	Convex	Dark yellow	Opaque	Butyrous
	Medium	Round	Entire	Slightly raised	White	Opaque	Smooth
	Medium	Round	Entire	Flat	Dark yellow	Opaque	Smooth
10^{-8}	Small	Round	Entire	Convex	Yellow	Opaque	Butyrous

Table 4: Morphological characteristics of some selected isolates obtained from without autoclaved, autoclaved, and control set of biofertilizer

Set	Dilution	Size	Shape	Arrangement	Gram's reaction
Without Autoclaved	10^{-4}	Medium	Rod	Single, Chain	Gram-negative
	10^{-6}	Medium	Rods	Single, Pair	Gram-negative
	10^{-8}	Small	Cocci	Single, Cluster	Gram-positive, Gram-negative
Autoclaved	10^{-4}	Small	Cocci	Single, Cluster	Gram-negative
	10^{-6}	Small	Short Rod	Single, Pair	Gram-negative
	10^{-8}	Medium	Rod	Single, Pair	Gram-negative
Control	10^{-2}	Small	Cocci	Single, cluster	Gram-positive
	10^{-4}	Small	Short rod	Single	Gram-negative
	10^{-6}	Medium	Rod	Single, Pair	Gram-negative
	10^{-8}	Small	Cocci	Cluster	Gram-negative

Table 5: pH, Temperature, and Moisture during composting at 7-day interval

Week	pH	Temperature (C)	Moisture content (%)
1	7	25	62
2	7	25	61
3	7	24	61
4	6.9	23	60
5	7	22	59
6	6.8	24	60
7	7	25	60
8	7	24	60
9	7.1	25	59
10	7	25	59

Table 6: C: N ratio of prepared biofertilizer

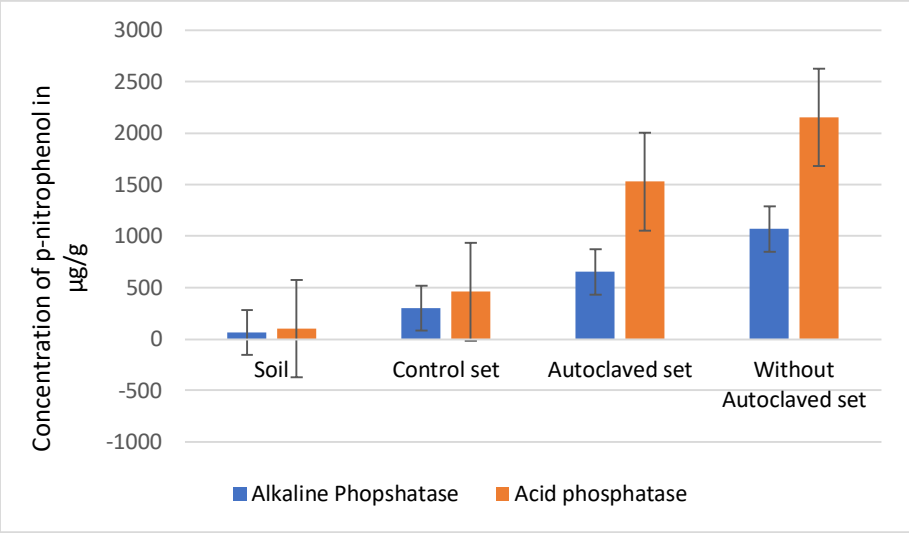
Sample	Organic carbon	Nitrogen	C: N ratio
Soil	0.6	0.05	12
Control set	0.95	0.08	11.9
Original set	1.1	0.09	12.5
Autoclaved set	1.5	0.12	12.3
Without autoclaved set	1.5	0.13	11.8

Table 7: Physico-chemical characteristics of soil and prepared biofertilizer

Nutrients	Soil	Control set	Original set	Autoclaved set	Without Autoclaved set
Electrical Conductivity (micro siemens/cm)	0.24	2.6	2.9	4	4.5
Total organic carbon (%)	0.6	0.95	1.13	1.48	1.53
Nitrogen (%)	0.05	0.08	0.09	0.12	0.13
C:N ratio	12	11.9	12.5	12.3	11.7
Phosphorous (µg/g)	8.5	13.9	15.6	18.1	20.6
Potassium (µg/g)	215	469	1023	2683	6780
Sulphur (µg/g)	60.4	195.2	265.2	357.7	387.7
Boron (µg/g)	0.73	0.76	1.1	1.4	1.8
Copper (µg/g)	55	60	70	90	210
Zinc (µg/g)	80	300	670	790	1040
Manganese (µg/g)	900	1103	1380	1410	1740
Iron (mg/kg)	2.1	28.4	38.1	42.6	47.9

Table 8: Vegetative growth parameters of *Cicer arietinum* after treatment with prepared of biofertilizer

Treatments	Root Length (cm)	Shoot Length (cm)	Fresh Weight (g)	Dry Weight (g)	Chlorophyll A (mg)	Chlorophyll B (mg)
Control	11	20	0.8	0.38	0.056	0.026
Autoclaved	14	22	1.8	0.98	0.132	0.043
Without autoclaved	20	23.5	2.1	1.03	0.172	0.076



Graph 1: Phosphatase activity of soil and prepared biofertilizer

CONCLUSION

According to the results of this investigation, the PGPR and carrier utilised to create the biofertilizer were quite effective. Results from the microbial load determination conclude that the PGPR may remain viable for up to 2.5 months in a closed system with an aeration facility. Out of all the sets of biofertilizers prepared from vegetative waste, without autoclaved set of biofertilizers shows a high amount of phosphatase activity, electrical conductivity, organic loading, and all the macro- (NPK) and micronutrients (Cu, Zn, Mn, Fe) essential for plant growth, which is also indicated by the pot study, in which the highest plant growth could be seen in pots treated with biofertilizer prepared from without autoclaved carrier. The reason behind the highest performance of biofertilizer produced from the without autoclaved set of the carrier was maybe because of the co-activity of both PGPR and the already present microbes within the carrier.

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CONFLICTS OF INTEREST

We declare that there are no conflicts of interest.

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