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Research Article

ANALYSIS OF MICROBIAL POPULATION IN ORGANIC MANURE AND ITS AQUEOUS EXTRACTS ON SEED GERMINATION OF *TRIGONELLA FOENUM GRAECUM* L. (FENUGREEK)

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ABSTRACT

Biofertilizer are typically environmentally safe, cheaper and has the capability to recover the nutrient demands of crop. Cyanopith and jiwamrita used as a valuable biofertilizer for environment. The degraded coir pith by cyanobacterium (*Oscillatoria annae*) is called cyanopith. Jiwamrita is made up of naturally available farm wastes and it is rich in beneficial microbes. Preparation of organic manure from three different particle sizes of cyanopith with jiwamrita enriched the microbial population consequently the existing nutrient has been enhanced in organic manure. Hence, the present study was carried out for the production of organic manure from three different particle sizes of cyanopith with jiwamrita and to analyse the microbial population present in the organic manure. Conclude that the application of cyanopith with jiwamrita enhanced both the composting process as well as microbial population. Also the aqueous extracts of 3rd particle size organic manure enhanced the percentage of seed germination, radicle length and biochemical parameters of *Trigonella foenum graecum.*

Keywords: Biofertilizer, Cyanopith, Jiwamrita, Microbial population, Seed germination.

INTRODUCTION

Organic manure is derived from decaying material of plant or animal origin. It influences the available soil microbial populations which are capable of regulating the supply of nutrients to higher plant. Decaying organic matter acts as a food material for bacteria, fungi and other organisms. The microbial population of organic manure contains various groups, among these groups bacteria are the most abundant and the most important microbe for decomposing waste. Bacteria use wastes for their own metabolism and finally produce some simple and useful compounds which are important for soil health, plant growing and to keep a well balance natural ecosystem. Coir pith is a lignocellulosic waste material formed during extraction of coir fibre, causing environmental and disposal problems. It has been used as a valuable plant growth medium, plant nutrient source and conditions. Coir pith or coir dust is a major by-product of coir fiber extraction industries**.** It is contains 87% of organic matter, 6.28% organic carbon, 0.73% nitrogen (Reghuvaran and Ravindranath, 2010). Coir pith thus produced decomposes very slowly in the soil as its pentosan-lignin ratio is below 0.5 (Ghosh *et al*., 2007) and because of the chemical and structural complexity of its lignin-cellulose complex. Large amounts of coir pith (approximately 7.5 million tons annually in India) accumulate nearby coir processing units, causing severe disposal problems, fire hazards and ground water contamination due to the release of phenolic compounds (Namasivayam *et al*., 2001). Cyanobacteria used as a biofertilizer are typically environmentally safe, cheaper and has the capability to recover the nutrient demands of crop. Also cyanobacteria have an ability to degrade the coir pith, considered to be as effective and eco-friendly process. The degraded coir pith by cyanobacterium (*Oscillatoria annae*) is called cyanopith. Jiwamrita is made up of naturally available farm wastes and it is rich in beneficial microbes. These microbes convert the non-

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available nutrients in to available form. Generally, urea peril may also be used as a slow releasing fertilizer. It can be easily degraded by microorganisms before it can be incorporated by plants. Similarly, preparation of organic manure from three different particle sizes of cyanopith with jiwamrita enriched the microbial population consequently the existing nutrient has been enhanced in organic manure.

Germination is the growth of an embryonic plant contained within a seed. It results in the form of seedlings. *Trigonella foenum graecum* L. **(**Fenugreek**)** is a medicinally important plant possessing anti-diabetic, ant-cancerous, antimicrobial and hypocholesteramic properties. It is an annual legume crop and is extensively cultivated in most region of world for its medicinal value. Hence, the present study was carried out for the production of organic manure from three different particle sizes of cyanopith with jiwamrita and to analyse the microbial population present in the organic manure.

MATERIALS AND METHODS

Coir pith was collected from coir industries near Srirangam, Thiruchirappalli, Tamilnadu, India. Cyanobacterium (*Oscillatoria annae*) was grown in BG11 medium **(**Rippka *et al*., 1979), under white fluorescent light (10/14 hrs L/D cycle) of 1,500 lux at 25 ±2º C. The mass cultivation of cynopith fertilizer was prepared. After 30 days of incubation, pellet and supernatant were separated by filtration and dried under shadow. Thus, dried coir pith based cyanobacterial product was known as cyanopith and this was used as a basal fertilizer. *O. annae* treated coir pith was maintained under laboratory conditions at 25± 2˚C temperature and 1500 lux with 10/14 L/D cycle. After the degradation of coir pith by *O. annae*, the medium color was changed from colorless to dark-brown color. Additionally, the fiber trays and large pits were used for mass production of cyanopith. In outdoor conditions, the fertilizer production was carried out with 0.1% neem extracts and cow dung. The degraded coir pith by cyanobacterium has been named as cyanopith (solid). It is being used as basal fertilizer. Prepared Cyanopith was sieved into three different particle sizes (Figure 1 a and b) and it was revealed as I-1-2 cm; II- 0.1-1 mm; III- 0.01-0.1 mm. Then, it was mixed with jiwamrita (Palekar, 2006) in equal ratio. This was incubated under shadow for 30 days. Microbial populations of bacteria and fungi were analyzed at regular intervals. Seed germination studies were analyzed analysis of morphological parameters like Radicle length, Hypocotyl length, Germination Percentage. Biochemical parameters like total carbohydrate (Hedge and Hofreiter, 1962), protein (Lowry *et al.,* 1951), calcium using EDTA method and Magnesium (Jaoac, 1967) were estimated.

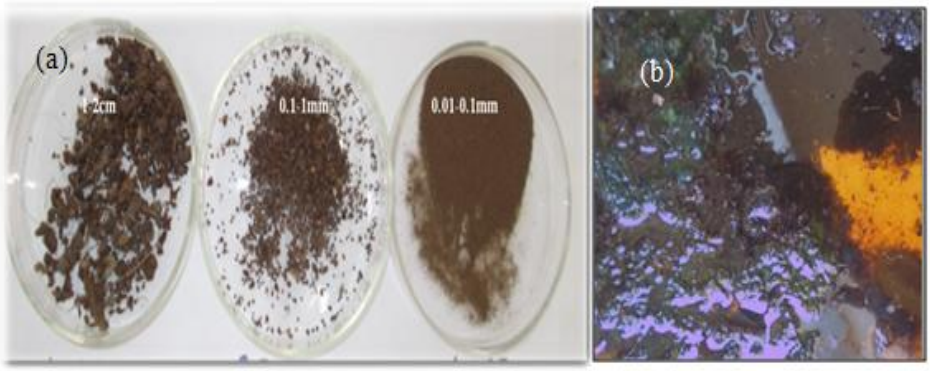

Figure 1. Three different particle sizes of organic manure (a), Cyanopith (b).

RESULTS AND DISCUSSION

Organism characteristics features

The selected cyanobacterial strain, *O.annae* has the ability to degrade coir pith. During the degradation, it releases some growth promoting substances and nutrients increase the attribute of the fertilizer. *O. annae* has the following characteristics features: (1) unbranched filamentous algae, occurring singly or in tangled mats; and (2) derives its name from its slow and rhythmic oscillating motion, which results from secretion of mucilage that pushes the filament away from the direction of excretion. Coir pith is very difficult to degrade in a natural environment and causes disposal problems. Hence, cyanobacterium was selected to degrade the coir pith, which converts it into fertilizers. *O.annae* treated coir pith was maintained under laboratory conditions. After the degradation of coir pith by *O. annae*, the medium color was changed from colorless to dark-brown color. Additionally, the fibre trays and large pits were used for mass production of cyanopith. In outdoor conditions, the fertilizer production was carried out with 0.1% neem extracts and cow dung. The degraded coir pith by cyanobacterium has been named as cyanopith (solid). It is being used as basal fertilizer.

Anandharaj (2007) and Chandrasekaran (2009) had cultivated *O. annae* with coir pith in water supplemented with 0.1g/L of urea. They also observed the luxuriant

growth (indicating oxygen bubbles) of cyanobacteria with coir pith by neem additives. Similarly, Bhuvaneshwari *et al.* (2011) cultivated coir pith based cyanobacterial biofertilizers with utilizing fresh water cyanobacterium *O. annae.*

Microbial analysis

Isolation of Bacteria and Fungi

The initial population of bacteria in three different particle sizes of organic manure was observed as 14x 10^5 CFU g^{-1}, 29 x 10^5 CFU g^{-1} and 70 x 10^5 CFU g^{-1} respectively. After 30 days of incubation, the bacterial population was increased from the initial level (Table-1). The bacteria population was 240x 10^5 CFU g^{-1}, 1910x 10^5 CFU g^{-1} and 2010x 10^5 CFU g^{-1} with respective to the fertilizer grade I (1-2 cm), II (0.1-1 mm) and III (0.1-0.01 mm) (Figure 2). These results revealed a remarkable diversity of bacteria in organic manure that enriched available nutrients. The reason might be jiwamrita nourished with three different particle sizes of cyanopith (coir pith based cyanobacterial fertilizer) provided an important habitat for microorganisms.

Obtained results were well supported by Bacterial population of various soils is closely corrected with their moisture content. The maximum bacterial density is found in regions of fairly high moisture content and the optimum level of the activities of aerobic bacteria often is a 50 to 75 % of the soil moisture holding capacity (Alexander, 1994). Chandrasekar (2009) who reported that application of cyanopith (50g) and cyanospray (0.3%) along with cow dung treated plots showed higher microbial (bacteria – 40x10^5 CFU g^{-1}; fungi - 9x10^4 CFU g^{-1}) population than the control (bacteria - 14x10^5 CFU g^{-1}; fungi - 1x10^4 CFU g^{-1}) and other treatments. Fouzia Ishaq and Amir Khan (2011) isolated higher bacterial (554) and fungal (93) colonies from organic field nourished with cow dung, ashes, mulches in comparison with inorganic field (309 bacterial and 36 fungal colonies). Similarly, Sreenivasa *et al.* (2009) observed that the population of bacteria in microbially enriched manures (compost, vermicompost and biogas slurry) significantly increased by 65 % as compared to the manure before enrichment.

Table 1. Bacterial populations in three different particle sizes of organic manure 1st day and after 30th day incubation.

S. No.	Samples	Number of colonies (CFU/g)	
		Bacterial population	
		1st day	30th day
1.	I (1-2cm)	14x10^5	240x10^5
2.	II (0.1-1mm)	29x10^5	1910x10^5
3.	III (0.01-0.1mm)	70x10^5	2010x10^5

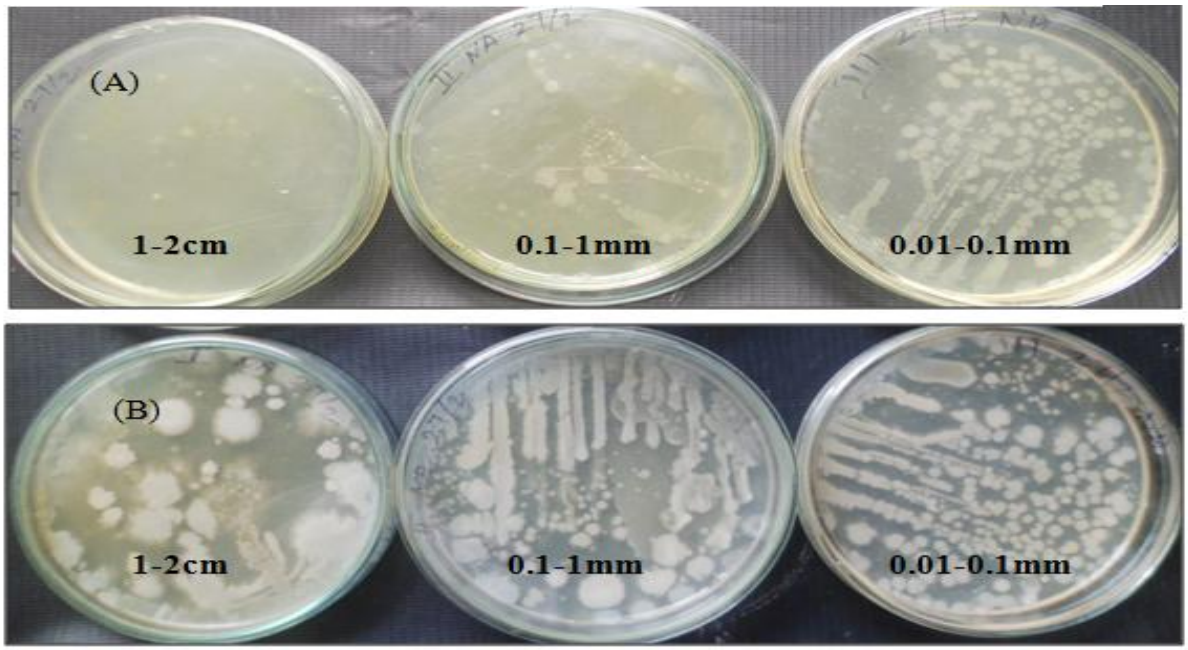

Figure 2. Isolation of Bacteria from organic manure on (a) 1st day (b) 30th day

The total fungal population initially in three different particle sizes of organic manure was found as 20x 10^3 CFU g^{-1}, 35x 10^3 CFU g^{-1} and 60x 10^3 CFU g^{-1}. Also, Table -2 showed that the number of fungal colonies were increased after 30 days of incubation and it was observed as 295x 10^3 CFU g^{-1}, 855x 10^3 CFU g^{-1} and 1445x 10^3 CFU g^{-1} (Figure 3). These results revealed that the essential nutrients present in the organic manure and the moisture provided for the enrichment which also nourishes the fungal growth after incubation. According to, Sreenivasa *et al.* (2009) among the manures, enriched biogas slurry had maximum fungal population. Among the manorial treatments, 50% FYM + 50% VC revealed increased rhizosphere fungal population in vegetative (34.93 x 10^3cfu g^{-1} of soil), flowering (39.55 x 10^3cfu g^{-1} of soil) and harvesting (43.79 x 10^3cfu g^{-1} of soil) stages.

Table 2. Fungal populations based on colony morphology in three different particle sizes of organic manure 0th day and after 30thday incubation.

S. No.	Samples	Number of colonies (CFU/g)	
		Fungal population	
		1st day	30th day
1.	I (1-2cm)	20x10^3	295x10^3
2.	II (0.1-1mm)	35x10^3	855x10^3
3.	III (0.01-0.1mm)	60x10^3	1445x10^3

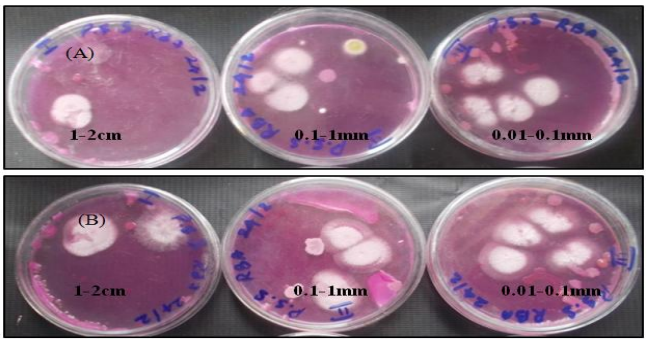

Figure 3. Isolation of Fungi from organic manure on (a) 0thday (b) 30thday incubation.

Seed germination Study

In this study, the effect of three different particle sizes of organic manure using petridish filter paper method were investigated on seeds of *T. foenum graecum*. The effects were analyzed based on the percentage of germination, morphological parameters such as radical length, hypocotyl length (Figure 4) and biochemical parameters such as carbohydrates, protein, calcium and magnesium. The experiments were carried out in triplicates.

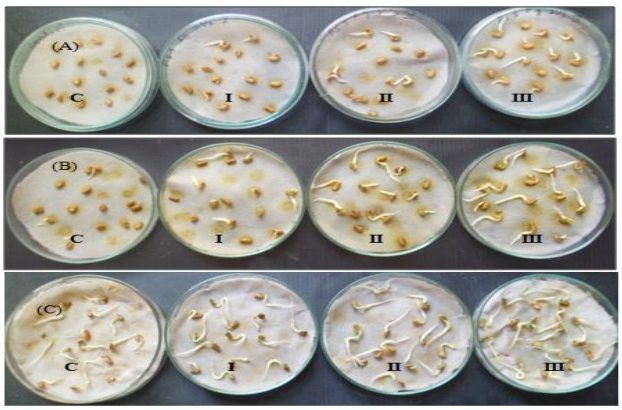

Figure 4. Seed germination of *Trigonella foenum graecum* using organic manure on (A)48 hrs, (B) 72 hrs and (C) 96 hrs.

Seed germination percentage

In individual applications, the seed germination percentage in grade III (0.01-1mm) treatment was significantly higher than the seed germination percentage of control, and other particle sizes of organic manure (grade I – 1-2 cm and grade II – 0.1-1 mm) (Figure 5) and the percentage of seed germination was calculated as 20%, 60%, 73.33%, 100% respectively on 72 hrs. This similar pattern was observed by Yadav and Lourduraj (2005a and b), Chandrasekar (2009), Yari and Sheidaie (2011) and Rajakumar (2013).

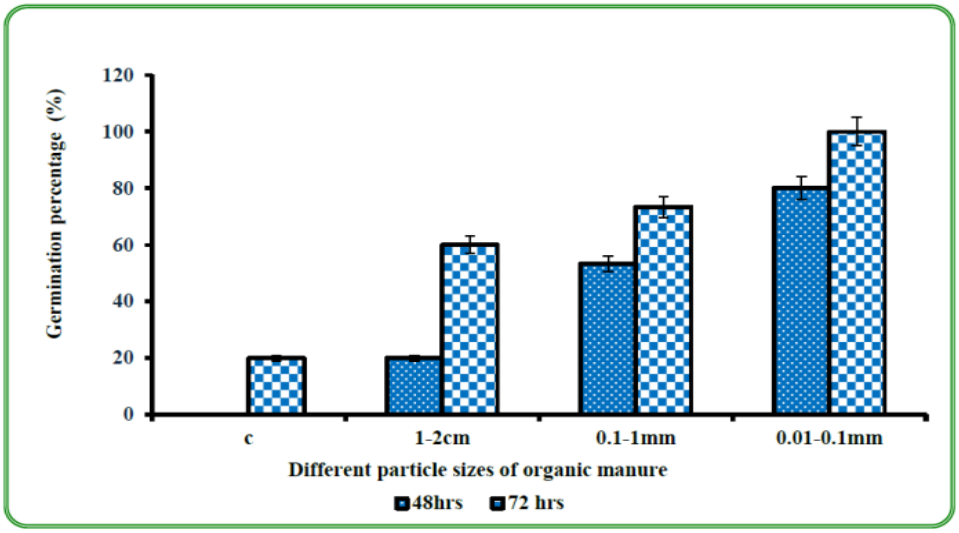

Figure 5. seed germination percentage in different particle sizes of organic manure.

Morphological parameters

The radicle length was significantly largest among all other three individual treatments than control at 48th and 72 hrs (Figure 6). Among these, the minimum (0.01-0.1 mm) particle size of organic manure applied seeds showed increased radical length (1.95 cm). This may be due to presence of micro and macro nutrients in organic manure that are responsible for the enhancement of morphological parameters and growth during germination.

Earlier study report indicated that Anandharaj (2007) reported that foliar applications of cyanospray showed significant growth of grain yield in *Oryza sativa*. Varalakshmi (2007) reported that the cyanobacterial extract enhanced hypocotyl and epicotyl growth in all the experimental plants. Subramaniyan (2013) reported that the highest number of radicals and maximum length of epicotyl and hypocotyls.

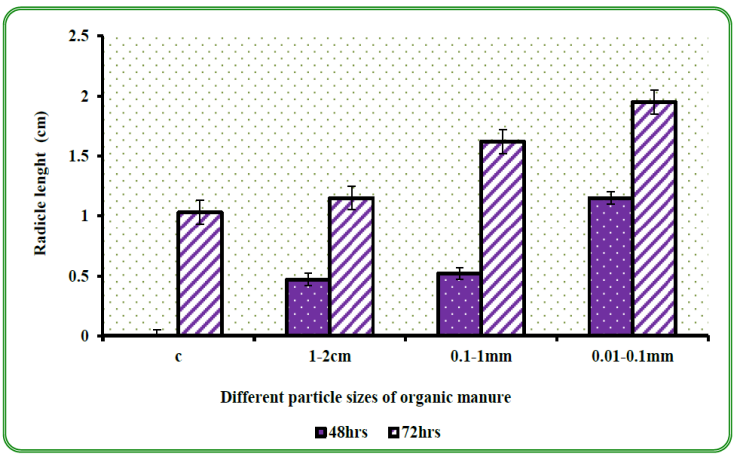

Figure 6. Radicle length of different particle sizes of organic manure

Biochemical parameters

The effects of three different particle sizes of organic manure on seed germination of fenugreek were determined by quantifications of biochemical contents such as carbohydrate, protein, calcium and magnesium.

Total Carbohydrate and Protein

The application of three different particle sizes of organic manure significantly enhanced the carbohydrate and protein level in *T. foenum graecum.* The carbohydrate (Figure 7) and protein (Figure 8) contents were increased in the minimum particle size (0.01-0.1 mm) of organic manure applied seeds when compared with control and other treatments. Early report by Groundnut seeds were reported to contain 9.5 to 19.0% of total carbohydrates in both soluble and insoluble form (Crocker and Barton, 1957; Rao *et al.*, 1965; Rahman, 1982 and Woodroof, 1983). Fernando (1995) who observed that the conversion efficiency of intercepted radiation to above-ground biomass was higher for maize, lower for soya bean and intermediate for sunflower. Sen and Bhattacharyya (2000) estimated the protein content in sunflower seed. It was also estimated by Chandrasekar (2009) that the pods of groundnut plants treated with cyanopith and cyanospray (B-50g + S-0.3%) showed rich carbohydrate content when compared to that of control and chemical fertilizer treated plant pods.

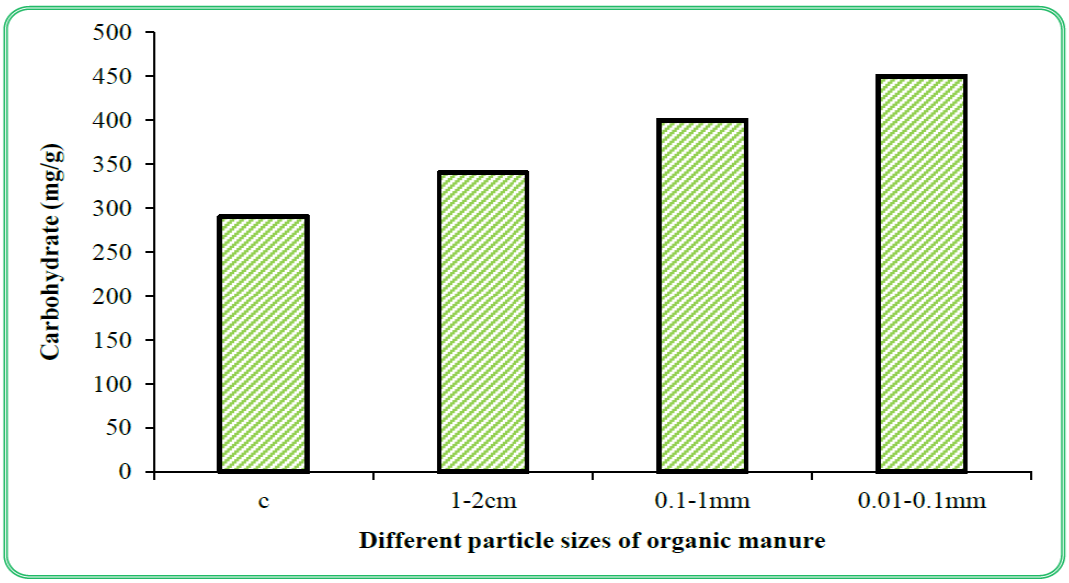

Figure 7. Estimation of carbohydrates of three different particle sizes of organic manure.

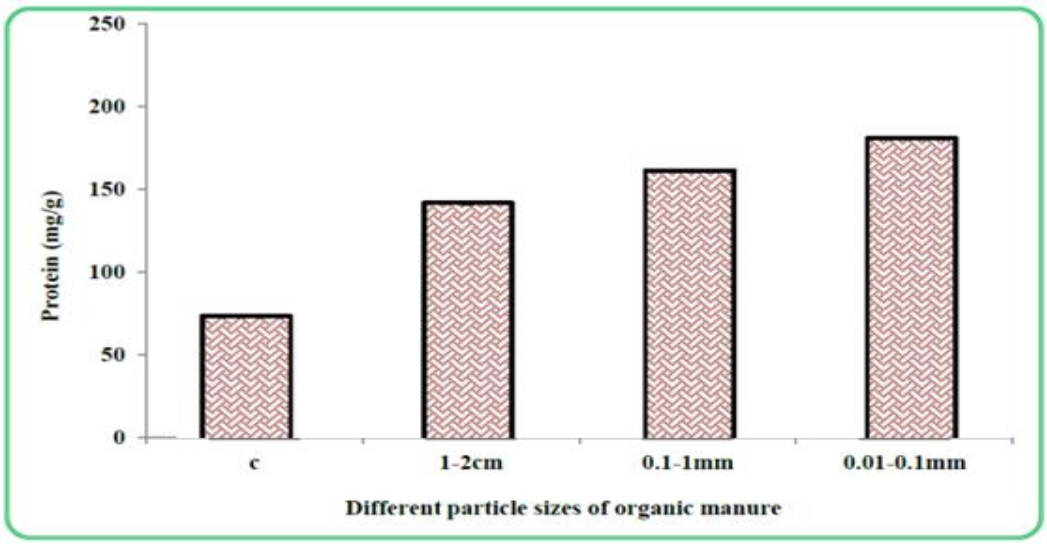

Figure 8. Estimation of protein of three different particle sizes of organic manure.

Calcium and magnesium

Calcium and magnesium content of seeds of *T. foenum graecum* were significantly improved by the applications of grade III (0.01-0.1 mm) when compared to the control and other treatments (Figure 9 and 10).

Biochemical analysis of *Manihot esculenta* (Crantz) showed effective variations and better results in the amount of calcium and magnesium contents was noted in organic fertilizers (Cyanobacterial fertilizer) treated field plants and tubers when compared with inorganic and combined fertilizers treatment tubers (Manoharan and Malliga, 2013). It was also estimated by Chandrasekar (2009) that the pods of groundnut plants treated with cyanopith and cyanospray (B 50 g + S 0.3%) showed rich calcium and magnesium contents when compared to that of control and chemical fertilizer treated plant pods.

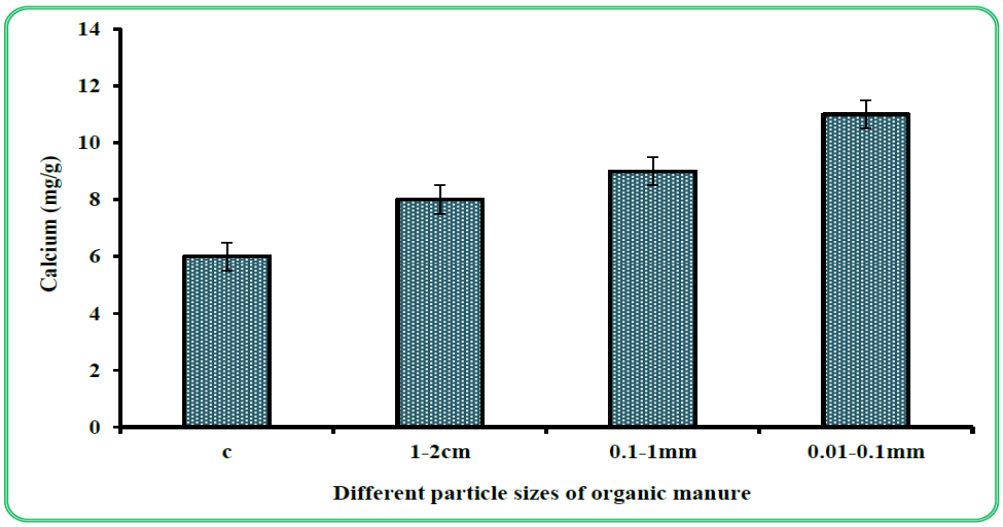

Figure 9. Level of calcium from *Trigonella foenum graecum*.

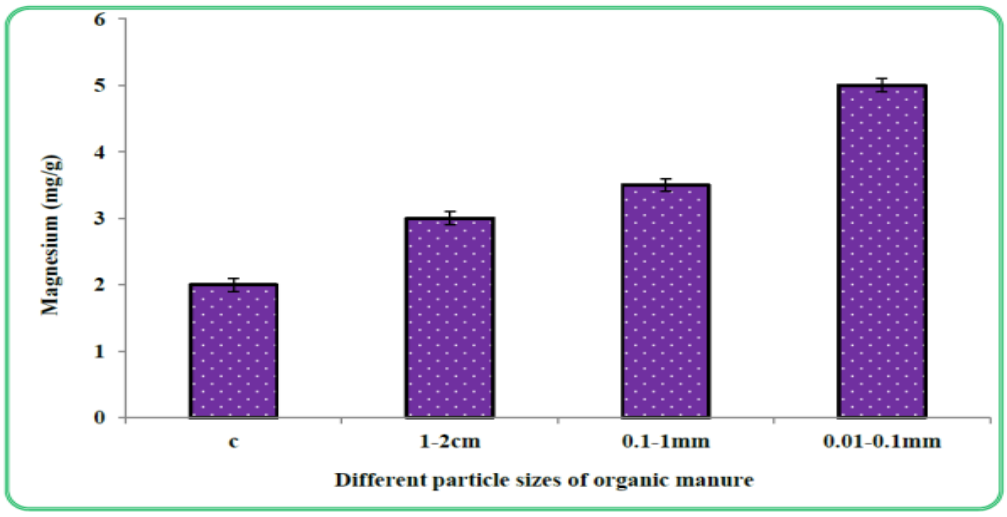

Figure 10. Level of magnesium from *Trigonella foenum graecum.*

CONCLUSION

The present study concludes that the application of cyanopith with jiwamrita enhanced both the composting process as well as microbial population. Also the aqueous extracts of 3rd particle size organic manure enhanced the percentage of seed germination, radicle length, and biochemical parameters of *T. foenum graecum*.

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