

## Biochemical and Microbiological Analysis of Different Organic Manures: Their Effect on Germination of *Coriandrum sativum* (Cilantro) and *Solanum melongena* (Eggplant)

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### Abstract

Agriculture sector is observing a rise in the usage of organic fertilizers that are prepared from waste generated in farm, kitchen and other agro-industries. We studied the influence of 4 liquid organic manures (OM) on the germination of egg plant and coriander seeds. These organic fertilizers were prepared by using raw materials that are readily available in the fields and nearby sugar industry, thus providing the farmers with an option for preparation of cheap organic. Microbial analysis of these OM showed a rich microbial consortia comprising of nitrogen fixers, phosphate solubilizers, Lactobacilli, altogether providing enhancement in plant growth. In this study, we tested the efficacy of these OM on seed germination in red-, black- soil and coco peat. Application of OM resulted in improved seed germination for egg plant seeds. But we did not observe any significant effect for coriander germination in comparison to the controls, although it had positive effect on leaf coloration in field. To understand the biochemicals in these OM on positive effect on plant growth, we performed metabolomic studies using GC-MS and NMR spectroscopy. Analysis of biochemical cycles revealed that nitrogen metabolic pathways followed by carbohydrates and vitamins pathways were crucial. The need of the hour is to design OM that are suited for different crops along with few designed specifically for their growth in different soil conditions. We present importance of metabolomic approaches for understanding the efficacy of biochemicals in customizing preparations of OM.

**Keywords:** Organic manure; Nuclear magnetic resonance spectroscopy; Liquid chromatography; Gas chromatography; Mass spectroscopy; Metabolomics; Seed germination

### Introduction

The Indian traditional method of agriculture used organic manure formulations that were prepared using animal wastes, milk based products, dead animal carcass and plant based materials. These formulations were initially fermented for few days to weeks before their application to soil as manure. During fermentation, the microflora in animal dung degrade the complex biomolecules in the ingredients of organic manure into a myriad simple organic molecules that can be easily assimilated by the plants for their growth and crop yield. Some of the well-known traditional formulations include – Kunapajala, Panchagavya, and Jeevamruth to name a few [1-5].

With the advent of green revolution in the late 1960s the traditional Indian farming methods were replaced with modern equipments and chemical fertilizers. Initially there was an increase in food production but in the long run it proved to be unsustainable and harmful to the environment. Overuse of fertilizers rendered the soil unproductive due to decrease in organic content and microflora, increase in the salinity of soil, disturbance in soil pH etc [6,7]. To restore soil health, farmers in India are gradually resorting to organic manures and composts which have given good results in crop yield. Two very popular formulations in India are liquid organic manure (OM) viz. Panchagavya (*Pc*) and Jeevamruth (*Ja*) [1-3]. Recently our group, studied the metabolomics of *Pc* and *Ja* using non-destructive NMR spectroscopy method and it revealed the presence of vitamins, nucleobases, reducing sugars, amino acids, siderophores, organic acids and several plant growth hormones [2].

In the current study we formulated variations of *Pc* and *Ja* by using different carbon and protein sources that are readily available as industrial by-products. Herein, we performed a systematic analysis of the microbial flora in each preparation, identified the different

metabolites using a combination of GC-MS and NMR spectroscopy, reconstructed the different biochemical pathways and tested the effect of germination of the OM in different growth media. These formulations were fermented and tested for their efficacy in germination of Cilantro and Eggplant (also called Aubergine and Brinjal). India produces 80% of Cilantro in the world, making it the largest producer. Its leaves and seeds are commonly used in south Asian, Chinese, Thai and Mexican cuisines. It also has medicinal properties – rich source of vitamins, detoxification ability by removal of heavy metals from the body and the essential oil has antibacterial property [8-10]. Eggplant on the other hand is one of the most commonly grown vegetable crop in India which is grown throughout the year, with an average productivity of 16.3 MT/ha, contributing to 27% of the global production. Eggplant has medicinal properties and is a good source of polyphenols, antioxidants, minerals etc [11,12] and is used in various culinary delicacies.

India has 8 different kinds of soil - alluvial, black, laterite, red, mountain/forest, and desert, saline/alkaline and peaty/marshy. Out of these, red soil comprises the largest group among them. In order to comprehend the effect of various OM in different growth media, we

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studied the effect of germination of Eggplant seeds in coco peat, red soil and black soil. In order to understand the density and diversity of microbial flora, we enumerated them on selective media. Lastly, biophysical tools like NMR spectroscopy and GC-MS was used to study the different biochemicals in all the 4 OM formulations to understand how variations in organic composition reflect differently on seed germination. From these experiments we noticed that the OM formulation prepared from the by-products of sugar industry substantially promoted seed germination in egg plant.

## Materials and Methods

### Seeds and germination trays

Cilantro seeds were bought from Vikas Agro agencies, Pune, India and Eggplant seeds from Sarpan Hybrid Seeds Co. Pvt. Ltd., Dharwad, Karnataka. Fifty cup seed germination tray, coco peat, black soil and red soil were bought from local nursery.

### Preparation of different organic manures

Four different OM were formulated using waste from indigenous cow and plant based materials. These ingredients were mixed in different proportions and fermented before using them as OM. OM 1 was prepared using cow dung (10 Kg), cow urine (10 L), jaggery (2.5 Kg) and press mud (750 g, Sri Sri Sadguru sugar factory, Rajewadi, India). OM 2 consisted of cow dung (10 Kg), cow urine (10 L), gram flour (2.5 Kg), jaggery (2.5 Kg) and handful of top soil from nearby forest. OM 3 included cow dung (10 Kg), cow urine (10 L), Banana (1 dozen), milk (1 L), curd (1 L), clarified butter (0.5 Kg) and molasses (1 Kg, Sri Sri Sadguru sugar factory, Rajewadi, India) while OM 4 contains all ingredients same as OM 3 except molasses was substituted with jaggery (2.5 kg). The OM were prepared in separate plastic drums of capacity 20 L and the ingredients were homogenised by mixing it manually with a wooden stirrer. The OM were stirred daily for 20 minutes, three times a day for 15 days. The drums were covered with fine nylon cloth and maintained at room temperature (RT).

### Fertilization of cilantro and aubergine seeds

The OM were tested for their efficacy in germination of Cilantro and Eggplant seeds. The germination of Eggplant seeds were evaluated in coco peat, red soil and black soil while for Cilantro, in black soil. For this, the seed germination tray was filled to half its volume with soil followed by addition of seeds and then the seeds were covered with 2 mm layer of same soil. The OM were diluted to 9 different concentrations in tap water before applying it to the soil (0.10%, 0.30%, 0.50%, 0.75%, 1.00%, 1.25%, 1.50%, 1.75% and 2%). Gibberellic acid- $\text{GA}_3$  (0.01%) was used as a positive control and tap water was used as a negative control. For germination studies, 10 seeds/cup of Cilantro were sown in germination tray; for Eggplant, 2 seeds per cup in coco peat and red soil while for black soil it was 3 seeds per cup. The Cilantro and Eggplant seeds were fertilized with 10 ml of different concentration of OM, once a week, while the remaining days of the week same volume of tap water was used. Tray was incubated at RT. The experiment was repeated twice for Cilantro, while for Eggplant it was performed once. Observations were made on daily basis and the number of germinated Cilantro and Eggplant seeds, at different OM concentrations were expressed as a percentage of the number of seeds per cup. We performed ANOVA using the mathematical software Graphpad Prism 5.0 (GraphPad Software, San Diego, CA).

### Enumeration of bacteria from organic manures on different media

The diversity and density of bacteria in the OM were enumerated on selective medium and at different growth conditions. The diluted samples were spread plated on *Azotobacter* agar medium (Himedia, India), Pikovskaya's agar medium (Himedia, India) and *Lactobacillus* MRS agar medium (Himedia, India) to enumerate nitrogen fixing bacteria, phosphate solubilizing bacteria (PSB) and *Lactobacillus* respectively. To enumerate aerobic and anaerobic bacteria, different dilutions of OM were spread plated on Nutrient agar medium and incubated at aerobic and anaerobic conditions. The plates were incubated at 30°C and the bacterial counts were taken after 24 h and 48 h and expressed as log CFU/ml.

### Biophysical analysis - NMR Spectroscopy and GC-MS

Three ml of the OM samples were dried in a hot air oven at 55°C for 3 days. The dried preparation was suspended in 0.5 ml  $\text{D}_2\text{O}$  (deuterium oxide, 99.9% D, Board of Radiation and Isotope Technology) and  $\text{CDCl}_3$  (99.9% D Aldrich Chemicals Company) to characterize hydrophilic and lipophilic extracts of the sample, respectively. All the samples were measured at 25°C on a Bruker AVANCE III HD Ascend 500 MHz (Bruker Biospin Switzerland) with  $^1\text{H}$  frequency of 500 MHz coupled with a 5 mm probe  $\text{D}_2\text{O}$  and  $\text{CDCl}_3$  using as NMR lock signals. The 1D  $^1\text{H}$  NMR experiment was recorded at constant temperature of 298K (25°C) using the Carr-Purcell-Meiboom-Gill (CPMG) spin-echo pulse sequence [13]. The acquisition parameters included a 10.2 ms, 90° pulse, 128 transients collected into 32 K data points with a spectral width of 8389.26 Hz, an acquisition time of 1.95 s and a relaxation delay of 2 s. Prior to Fourier transformation, an exponential function corresponding to a line-broadening factor of 0.3 Hz was applied to the free induction decay (FID).  $^1\text{H}$  spectra were calibrated with respect to water and chloroform peaks at 4.8 and 7.11 ppm, respectively. All raw time domain FID were extracted to frequency domain spectrum using system supported TopSpin NMR software (version3.5, Bruker Biospin Ltd.).

One mg of each OM sample was dissolved in 1 ml of ethyl acetate and methanol (1:1 v/v) at RT for 15 minutes followed by centrifugation (3000 rpm, 10 min) at 4°C. One  $\mu\text{L}$  sample was injected by auto sampler into Rtx 5 MS column capillary column coated with 5% phenyl cross-linked 95% dimethylpolysiloxane (30 m  $\times$  0.25 mm Id., 0.25  $\mu\text{m}$  film thickness) in a Shimadzu GC MS QP 2010 Ultra Gas chromatograph Mass Spectrometer. Analyses were performed by a split mode with ratio 25 using helium gas as carrier at flow rate of 1.20 mL/minutes flow. Initially, column temperature was maintained at 40°C for 34 minutes, then raised to 280°C at 10°C/minutes, held at 280°C for 10 minutes. Mass spectra were recorded following electronic impact ionization at 70 eV. The mass range for data collection was 50-900 Da with 0.2 s scan interval.

### Metabolic profiling analysis

The GC-MS spectra were identified by Postrum Analysis software (Shimadzu) using the internal mass spectral databases of the instrument. Peak assignments were made using dual criteria of linear retention index match and mass spectral database. Retention indices were calculated on the basis of the standard alkane mixture (C11-C18). Assignments were derived by comparing and matching of the retention indices and unique mass spectra with the reference library and literature retention indices and only those compounds were considered that had a matching factor of more than 50%.

For NMR based identification, the 1D  $^1\text{H}$  NMR spectra includes information about the different metabolites present in the samples. Metabolites corresponding to these resonances were then identified using chemical shift of these available at Biological Magnetic Resonance Data Bank (BMRB - <http://www.bmr.b.wisc.edu/metabolomics/> [14]). BMRB analysis provides a list of organic compounds that may occur in the sample by indexing each molecule with a score. Higher score is reflective of greater peak match between recorded spectra and chemical shifts in database. Analysis of metabolites for pathway and visualization was carried out using Metabolic pathway tool in MetaboAnalyst webserver (<http://www.metaboanalyst.ca/> [15]). As we have used different agricultural raw materials coming from cattle waste and plant origin, we analysed the list of metabolites with the pathway database of *Bos taurus* and *Arabidopsis thaliana*. Furthermore, as the OM is prepared by microbial fermentation, we also chose three biochemical pathways for three different bacteria *Bacillus subtilis* (gram positive soil bacteria), (gram negative enteric coliform) and *Pseudomonas putida* (gram negative soil bacteria) as representative bacteria for gut microflora and soil dwellers.

## Results and Discussion

### Biochemical and microbial analysis of organic manures

To facilitate the growth of bacteria present in the microflora of the cow dung, we incorporated carbon (jaggery and molasses) and nitrogen (gram flour and milk) as ingredients in the OM. At first, we enumerated and identified the different types of bacteria in the OM by spread plating at different dilutions on selective bacteriological media. To identify nitrogen fixing bacteria, PSB and *Lactobacillus*; *Azotobacter* medium, Pikovskaya's medium and *Lactobacillus* MRS medium were used respectively. The aerobic and anaerobic bacteria were enumerated in nutrient agar medium under aerobic and anaerobic conditions respectively. The bacterial load was in the range of  $10^{10}$ - $10^{11}$  cells/ml (Figure 1).

We were particularly interested in nitrogen fixing bacteria [16,17] and PSB [18-20] because most of the biofertilizers that are currently available in the market belong to these two class of bacteria. *Per se*, the genera *Lactobacillus* is not a plant growth promoting rhizobia (PGPR) but few results have shown their presence in rhizosphere [21] and has plant-growth promoting activity [22] presumably due to its active secretions. We believe that it may be due to its antibacterial activities, secretion of vitamins and other co-factors due to which they display

beneficial properties in the mammalian gut. Furthermore, as the lactic-acid bacteria grow, they fend off various pathogens and increase the shelf life of the fermented product. Moreover, presence of *Lactobacillus* in large numbers is also an important criteria in the stability of the sample and in increasing its shelf-life.

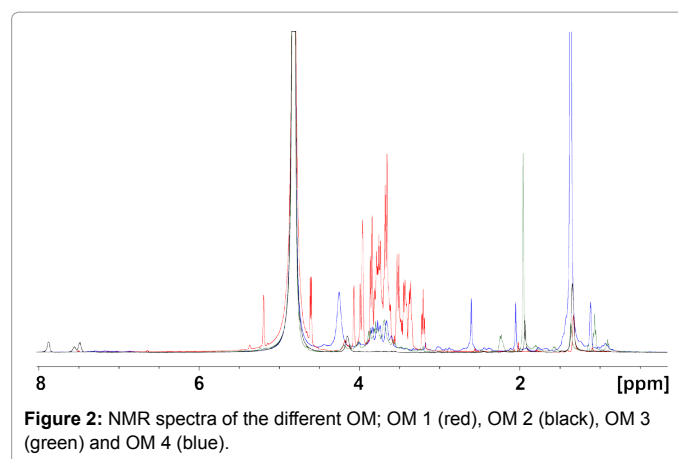
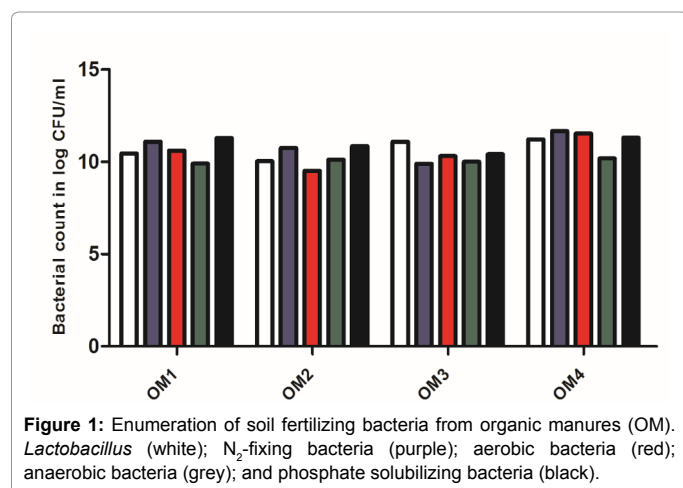
### Analysis of OM by biophysical techniques

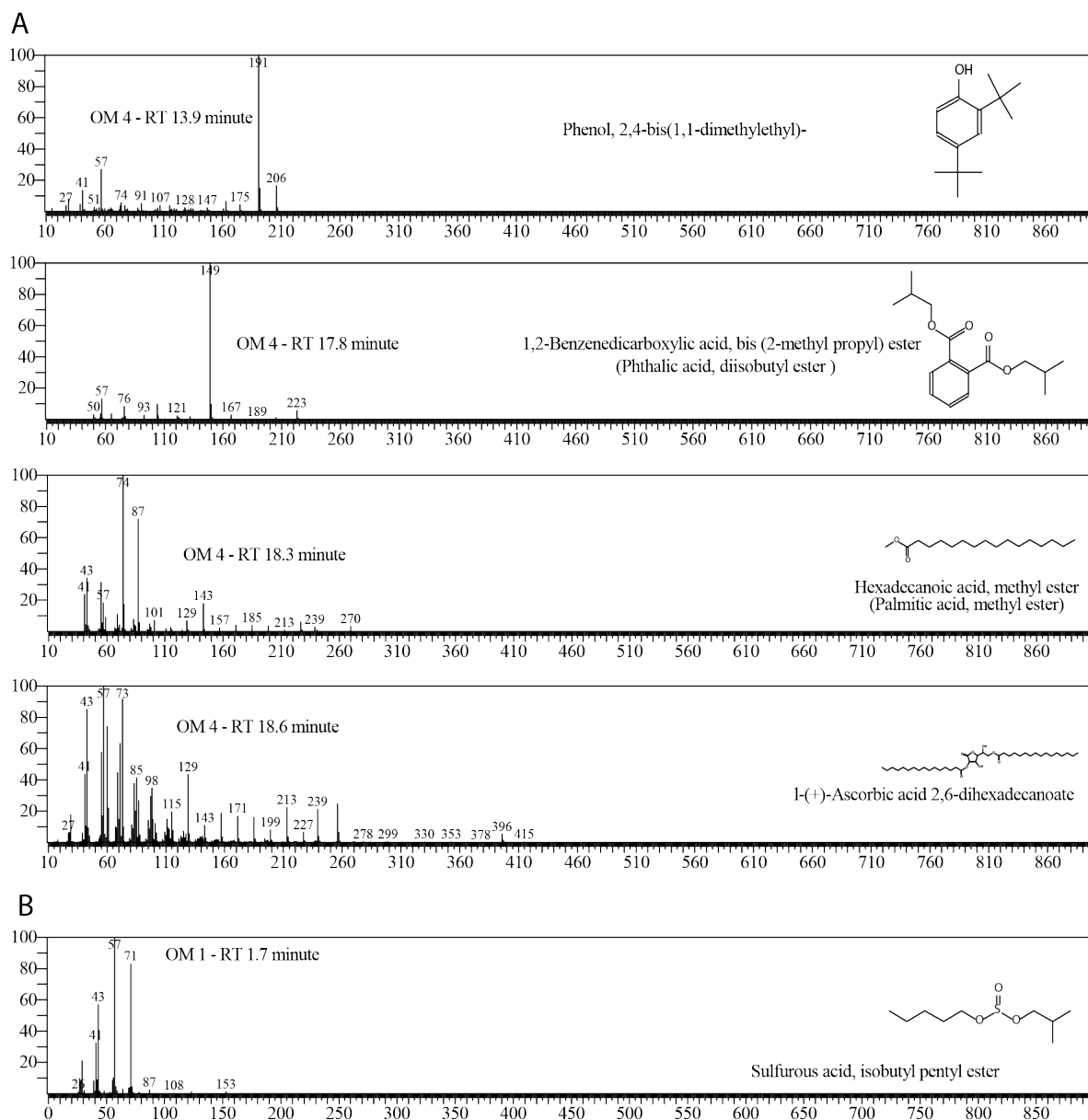
We analysed the aqueous and organic phase of the extracts. Most of the compounds identified by both NMR (Figure 2) and GC-MS (Figure 3) showed a rich profile of various metabolites such as organic acids, sugars, aliphats and aromats. This is best appreciated from the NMR spectra in Figure 2, where one can observe intense chemical shift resonances for polar molecules belonging to acids and sugars in the 3-4.5 ppm range of 1D  $^1\text{H}$  spectrum. Likewise, GC-MS based analysis also highlighted that all samples were rich in organic acids, long chained fatty acids and aromatic molecules.

Next, we compared the metabolomics results between the two techniques; firstly, there were very few absolute matching compounds between them but the identified metabolites belonged to similar functional groups. In GC-MS data, we observed long chained aliphats but did not notice the presence of molecules of basic building blocks for cellular metabolism. On the other hand, NMR experiments revealed the presence of several different metabolites from various different biochemical pathways. We speculate that the discrepancy may arise due to type of the database used for analysis and the number of molecules deposited in the database [23,24]. Perhaps, the slight differences may also reflect on the sampling method and sensitivity of the technique itself [23,25].

GC-MS analysis of aqueous and apolar fractions were rich in a variety of short chained aliphats (Table S1). In addition to it, the polar fraction contained esters, ketones, and alcohols. Apart from it, OM1 had the presence of sulphurous acid possibly due to the presence of press mud in the preparation, which is rich in Sulphur. The hydrophobic fraction on the other hand was rich in a variety of long chained fatty acids, vitamins, anti-oxidants, etc. Many of the organic molecules present in different OM showed presence of common organic compounds. Overall, these findings highlight that apart from various C- and N- rich molecules, OM preparations are rich in co-factors, anti-oxidants and surfactant like biomolecules, present in small doses that can help in enhancing the adhesion abilities of organic molecules and bacteria onto the plant.

The compounds that were identified by NMR metabolomics





**Figure 3:** Spectra of few metabolites from the GCMS chromatogram in (A) OM 4 and (B) OM 1.

revealed diverse set of biochemical pathways that were involved in the rich soup of biomolecules, in the fermented OM. To understand the finer details behind the source of various metabolites, we performed analysis of several pathway using the webserver <http://www.metaboanalyst.ca/> [15]. The pathway analysis tool contains metabolome pathways of 16 different organisms. As the raw material in our preparations include cow dung and urine; jaggery, molasses and press mud; dairy products; gram flour and microflora native to these samples, therefore we chose a metabolomes of representative species such as *Bos taurus*, *Arabidopsis thaliana*, *Bacillus subtilis*, *Pseudomonas putida* and *Escherichia coli*. We have tabulated few of the major biochemical pathways based on the identified metabolites (Table 1, Figure 4 - OM 4 and Figures S1-S3 for OM 1-3, respectively). In all cases, we observe that pathways involved in amino acid and nitrogen metabolism were predominate in all OM corroborating with previous finding [2], followed by carbohydrates,

vitamins/co-factor, bile salts and quinolone metabolic pathways. In addition to it, we also noticed predominance of pathways involving bile salts and xenobiotics.

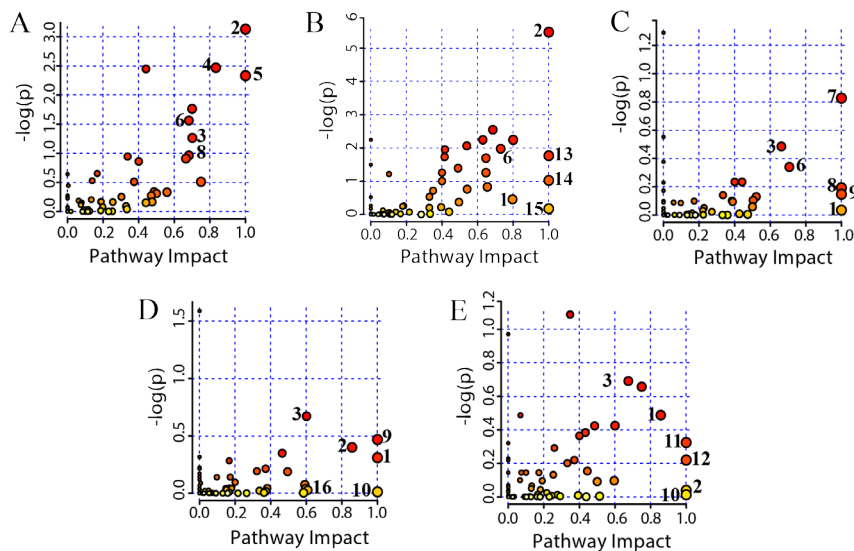
### Effect of organic manures on seed germination in black soil, red soil and coco peat

The Eggplant and the Cilantro seeds were fertilized with 4 different OM at concentrations ranging from 0.1%-2%. We observed that the germination was not dependent on the concentration but on the kind of OM. Good germination was observed over a range of concentrations for both coriander and Eggplant with no clear cut dependence on any specific concentration. Hence for all further analysis, we took the cumulative response of the different concentrations of each OM and their effect on seed germination. We observed an overwhelming response of all the OM on seed germination in black soil (Figure 5a)



OM 1	OM 2
Taurine and hypotaurine metabolism	beta-Alanine metabolism
beta-Alanine metabolism	Taurine and hypotaurine metabolism
Phenylalanine metabolism	Alanine, aspartate and glutamate metabolism
Isoquinoline alkaloid biosynthesis	Pyrimidine metabolism
Alanine, aspartate and glutamate metabolism	Phenylalanine metabolism
Glycine, serine and threonine metabolism	Glycine, serine and threonine metabolism
Nitrogen metabolism	Pantothenate and CoA biosynthesis
gamma-Hexachlorocyclohexane degradation	gamma-Hexachlorocyclohexane degradation
Alanine, aspartate and glutamate metabolism	1,2-Dichloroethane degradation
1,2-Dichloroethane degradation	Cyanoamino acid metabolism
Cyanoamino acid metabolism	Nitrogen metabolism
Biosynthesis of unsaturated fatty acids	C5-Branched dibasic acid metabolism
Naphthalene and anthracene degradation	
OM 3	OM 4
beta-Alanine metabolism	Taurine and hypotaurine metabolism
Phenylalanine metabolism	Phenylalanine metabolism
Alanine, aspartate and glutamate metabolism	Pyrimidine metabolism
Isoquinoline alkaloid biosynthesis	Isoquinoline alkaloid biosynthesis
Pyrimidine metabolism	Pentose and glucuronate interconversions
Taurine and hypotaurine metabolism	Glycine, serine and threonine metabolism
Glycine, serine and threonine metabolism	Nitrogen metabolism
Pantothenate and CoA biosynthesis	Ascorbate and aldarate metabolism
Nitrogen metabolism	gamma-Hexachlorocyclohexane degradation
Cyanoamino acid metabolism	1,2-Dichloroethane degradation
1- and 2-Methylnaphthalene degradation	1- and 2-Methylnaphthalene degradation
Biosynthesis of unsaturated fatty acids	Alanine, aspartate and glutamate metabolism
	Naphthalene and anthracene degradation

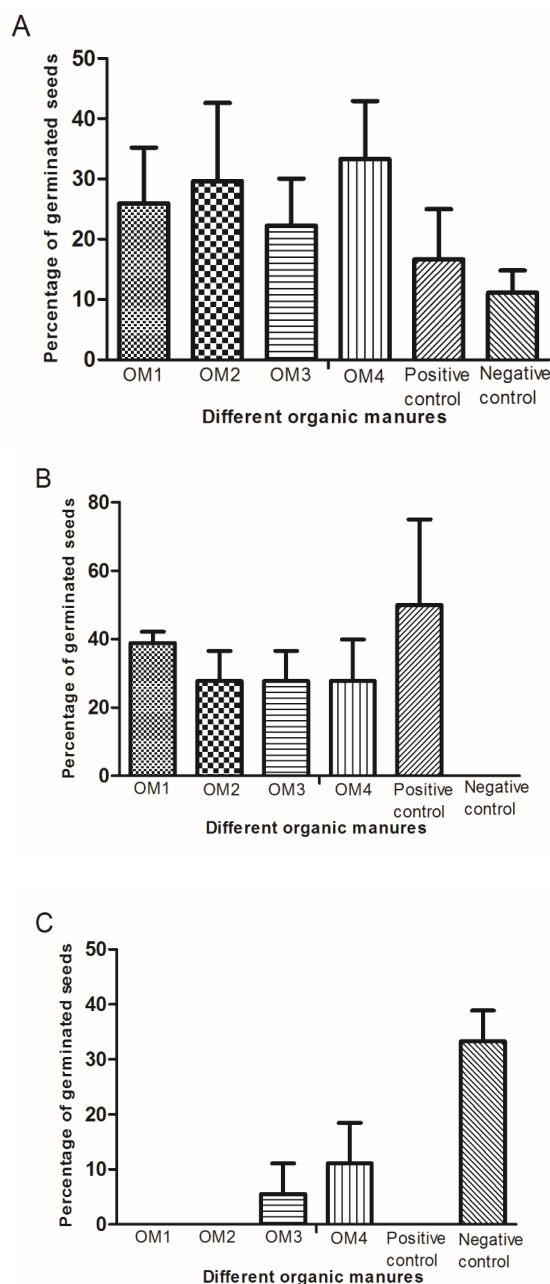
**Table 1:** NMR metabolic pathway analysis indicating the predominant biochemical pathways operational in the different OM.



**Figure 4:** Biochemical pathway analysis identified using NMR metabolomics for the organisms in OM 4. A- *Arabidopsis thaliana*; B- *Bos taurus*; C- *Bacillus subtilis*; D- *Pseudomonas putida*; and E- *Escherichia coli*. The numbers are indicative of the most prominent pathways such as 1-  $\beta$  Alanine metabolism, 2- Taurine and hypotaurine metabolism, 3- Alanine, aspartate and glutamate metabolism, 4- Phenylalanine metabolism, 5- Isoquinoline alkaloid biosynthesis, 6- Galactose metabolism, 7- 1,2-Dichloroethane degradation, 8- Tyrosine metabolism, 9- Naphthalene and anthracene degradation, 10- Inositol phosphate metabolism, 11- 2,4-Dichlorobenzoate degradation, 12- Ubiquinone and other terpenoid-quinone biosynthesis, 13- D-Glutamine and D-glutamate metabolism, 14- Phenylalanine, tyrosine and tryptophan biosynthesis, 15- Linoleic acid metabolism, 16- Glycerolipid metabolism.

especially OM 2 and OM 4 for Eggplant and only OM 4 for Cilantro (Figure 6). ANOVA analysis of the germination results highlighted a significantly positive response for the OM 2 and OM 4 over the other OM preparations. While we do not have a concrete evidence for all

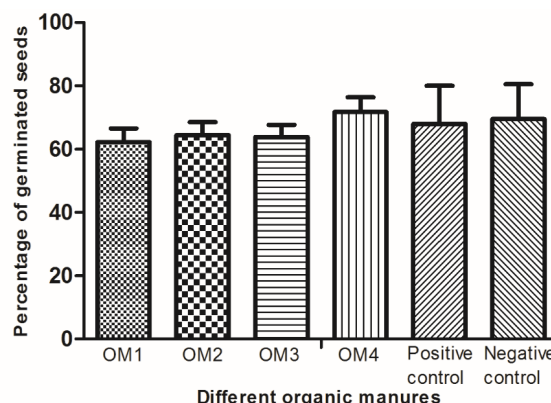
the results, we speculate that the germination results are dependent on the soil property and the constituent of the OM. For instance, black clay soil is slightly acidic and has a much higher water retention capacity. This also permits to hold the organic compounds from the



**Figure 5:** Germination of eggplant seeds expressed as percentage, fertilized with different organic manures (OM) in different support material. (A) black soil, (B) red soil and (C) coco peat. The vertical bars represent the SEM.

OM formulations. As the organic manure is rich in a variety of carbon and nitrogen nutrients; plant growth regulators and organic acids, it promotes seed germination and perhaps also PGPR activity of the mixed microbial consortia in the OM formulation.

For Eggplant, we tested seed germination against different OM as a function of supporting media, i.e., red mountain soil (Figure 5b), inert coco peat (Figure 5c) apart from black soil. In red soil for Eggplant, we noticed that the samples treated with the  $GA_3$  showed better response compared to all the other OM. We noticed that all the OM showed similar overlapping response, the germination of seeds treated with OM1 was comparable with  $GA_3$ . The red soil is rich in Fe and Al, and



**Figure 6:** Germination of coriander seeds in black soil, expressed as percentage when fertilized with different organic manures (OM). The vertical bars represent SEM.

	Black soil	Red soil	Cocopeat
OM 1	5	14	0
OM 2	5	13	0
OM 3	4	16	10
OM 4	4	10	14
$GA_3$	18	14	0
Water	18	0	13

**Table 2:** Days required for the germination of egg plant seeds in different substratum.

also has a medium water retention capacity. We believe that the organic molecules may be interacting with the soil constituents thereby making less availability of these to the plant and minimizing their effects.

Lastly, we did not observe any significant germination in coco peat between all the OM and  $GA_3$ , in fact the untreated control showed a greater response over the treated samples. This could be because coco peat has a very high water holding capacity and is devoid of any nutrients.

In addition to the germination rates, we noticed that all OM enhanced germination of egg plant seeds. We observed first set of seeds germinating in 4 days after sowing (DAS), while the seedlings in red soil and coco peat were first observed 10 DAS (Table 2). It would be noteworthy to test in the future is if higher dosage of organic manure or mixture of chelator in the sample or differently tailored OM for specific soil types can potentiate seed germinations.

## Conclusion

Animal wastes have been extensively used for farming purposes prior to green revolution. But extensive fertilization using chemicals has decreased soil fertility Hence we have to enrich soil by using organic rich manures that have ability to increasing crop yield, improve soil health and are also comparable to their synthetic counterparts. In one of our earlier publications, we had reported the metabolic profile and the pathways involved in Panchagavya and Jeevamrut [2]. Herein, we have presented data on OM that have been prepared by modifying industrial wastes and can be readily utilized for farming purposes. In India, farmers grow vegetables and herbs as main- and inter- crop between their produce in a variety of soil types. It is thus necessary to tailor OM formulations for different seeds and soil types.

We have presented our findings that the OM preparations work

efficiently in black soil for Brinjal and perhaps can be generalized for the Solanaceae family, as the previous report was tested on tomato [2]. The germination rate was efficient in red soil as well but one has to further tailor the formulation to enhance seed germination over GA<sub>3</sub>. We did not observe any significant effect of the OM in coco peat medium. Many farmers are turning to soil-less farming especially in metropolitans and protected farming. It represents an upcoming market that is ready to test newer and cleaner fertilization techniques. As coco peat represents a very favorable medium for soil-less farming techniques, who use only chemical means of farming, we also investigated the effects of seed germination in coco peat. The tested OM performed poorly in soil-less medium. On another note, we noticed that the OM did not have any significant effect on the germination of the coriander seeds. Meanwhile, we were also testing the effect of coriander growth in the fields of Nashik, India. We observed that OM treated coriander had rich green colour indicating a higher level of chlorophyll, therefore a better aesthetic value over their synthetic counterparts but there was no difference in the overall yield (Figure S4).

Our findings highlight that OM preparations can have different effects on seeds germination and plant growth depending on the growth substratum. Herein, we have used cattle wastes, dairy products and by products from industrial waste to identify the different microflora and biochemical that influence plant growth. A lot more understanding is required, for instance, we will have to devise newer formulations to enhance its effect in different media. In addition to it, a systematic set of experiments will be required to comprehend the effects of the different OM for various soil types. In this manner, one can customize and tailor OM preparations for different vegetable crops whilst growing in different soil- and soil-less media.

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