International congress - Compost and digestate: sustainability, benefits, impacts for the environment and for plant production CODIS-2008

"A novel vermicompost based formulation for bioinoculants with added plant and microbial growth promoting natural products"

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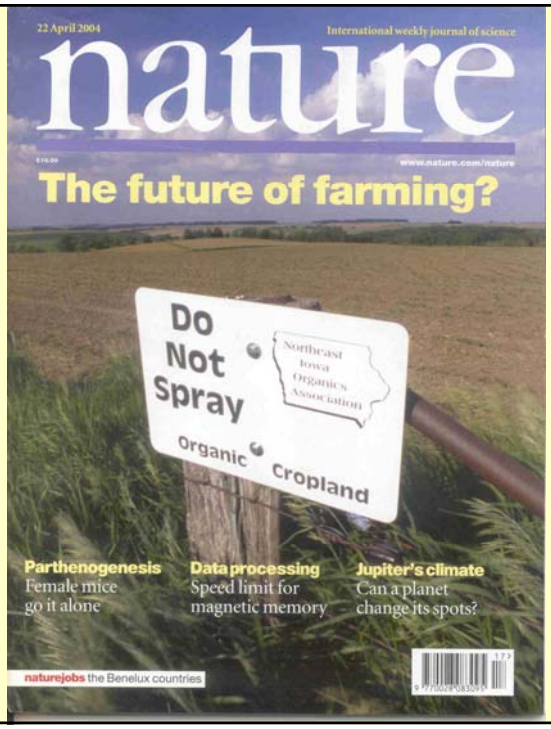


The Problem…and a Possible Solution

- **Intensive use of chemical fertilizers has caused ill effects on soil fertility;**
- **The social demand for safer agricultural products and food is increasing in developed countries;**
- **Beneficial microorganisms applied as biofertilizers play an important role in today's agriculture by improving soil fertility and crop productivity;**
- **Symbiotic N fixation by *Rhizobium* bacteria in legume roots is an important production aid for sustainable agriculture as inoculation with *Rhizobia* is long established and successful practice, especially with leguminous crops.**

The Problem…??

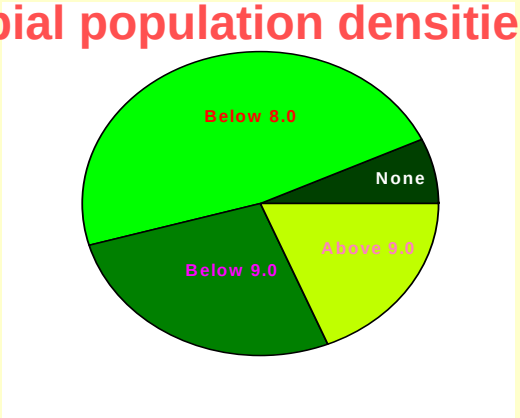
→Published data suggests that optimum nodulation and N2 fixation requires more than 1000 Rhizobia g-1 soil (1). The numbers can be readily established and maintained in soil through application of rhizobial inoculants. An effective seed/soil inoculant delivered in the field must have high population of *Rhizobia*.

Cell numbers does matter…

Current Minimum Standard vary with country, but ranges from $5x10^7$ to $1x10^9$ Rhizobium cells g-1 of freshly prepared inoculant

The most widely accepted standard for numbers of rhizobia delivered per seed are 10^3, 10^4 and 10^5 rhizobia for small, medium and large seeded species, respectively.

Proportion of inoculant products sampled from 12 developing countries with indicated ranges of rhizobial population densities



Solution..

- **Although *Rhizobia*, present in soil can fix nitrogen but a agricultural good practice and greater N fixation needs higher number of *Rhizobia* to be present in soil;**
- **Therefore the *Rhizobia* should be applied externally and which could be delivered in the form of inoculants.**

Carrier…a critical part of product formulation

- **a good water holding capacity and retention**
- **non-toxic to inoculant**
- **environmentally safe**
- **permit growth of introduced microbes**
- **acceptable pH**
- **release organisms upon use and**
- **available in abundance**
- **And most importantly should be capable of maintaining a standard population for 3 to 6 months**

Carriers studied

- Peat, charcoal, filter mud , lignite , coal , a coal-bentonite mixture , cellulose , bagasse , wheat straw , a compost of coir dust and soil ,manure, compost, powdered coconut shells, ground teak leaves, are the some carrier which have been studied.

The Indian Perspective…

Among them peat possesses a privileged position in the manufacturing of legume inoculants because of many advantages associated with it. However, there are no large scale deposits of peat in India and therefore any commercial exploitation of this as carrier seems to be a remote possibility

Efforts at CIMAP…

At CIMAP, efforts have been made for the development of technologies for Organic Agriculture especially related to medicinal and aromatic Plants.

Vermicultivation: Conventional vs. distillation waste

Plant/distillation waste	Number of worms harvested
Rice straw(**conventional**)	264
Distillation waste	
Cymbopogon winteranus	297*
C. flexuosus	333*
Mentha arvensis	297*
Pelargonium graveolens	248

* differ significantly from conventional(rice straw)

Intial population = 50 worms

Species used: *Eisinia fetida*
Perionyx excavatus

Practically feasible recycling of distillation waste and biogas slurry into vermicompost: System approach

US Patent granted

US Pat. No. 6488733

Shorter composting period, faster multiplication of worms, superior quality compost

Vermicompost based formulation…!

→Considering the usefulness of organic manure in today's world of organic and their easy availability the present study investigates the suitability of granular vermicompost as solid carrier material and its water extract for liquid carrier material using highly efficient strains of *Rhizobium meliloti* Rmd 201, for inoculant production is demonstrated in present investigation.

Plant growth promoting activities and isolation technology of calliterpenone : A 20 times better plant growth promoter than GA3

Effect of Calliterpenone(CT) on Bacterial growth

S. No.	Medium+ enhancer	Bacterial Strains	
		Bacillus thuringiensis	*Rhizobium meliloti* Rmd 201
1.	Medium + Calliterpenone (0.01 mM @ 50 µl/ml)	7.9×10^8	5.6×10^9
2.	Medium + Calliterpenone (0.01 mM @ 25 µl/ml)	1.44×10^9	6.62×10^9
3.	Medium + Calliterpenone (0.01 mM @ 12.5 µl/ml)	1.28×10^9	4.67×10^9
4.	Control (Only medium)	2.07×10^8	1.56×10^9

In case of *Bacillus thuringiensis* Nutrient Broth and incase *of Rhizobium meliloti* Rmd 201 Yeast Extract Mannitol Broth was used.

Effect of CIM 1865(CU) on Bacterial growth

S.No.	Medium+ enhancer	Bacterial Strains	
		Bacillus thuringiensis	*Rhizobium meliloti* Rmd 201
1.	Medium + CU (@ 50 µl/ml)	1.67×10^8	3.32×10^9
2.	Medium + CU (@ 25 µl/ml)	3.1×10^9	7.21×10^9
3.	Medium + CU(@ 12.5 µl/ml)	2.6×10^9	3.6×10^9
4.	Control (Only medium)	2.10×10^8	1.8×10^8

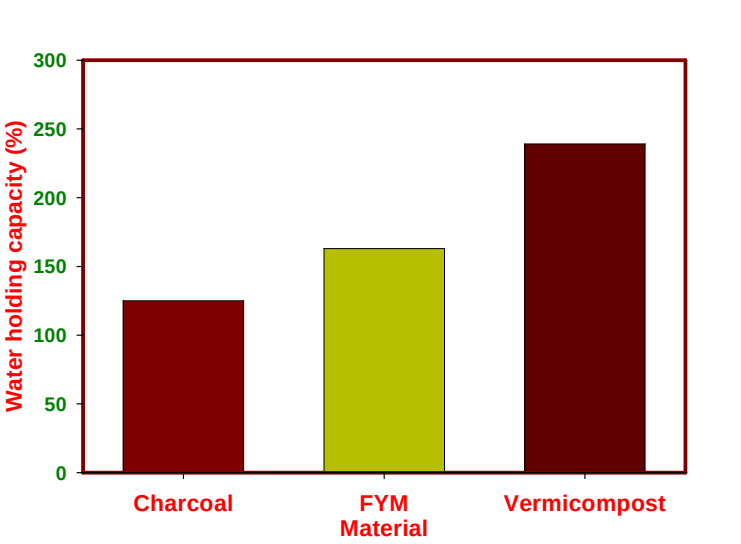
Rhizobium meliloti Rmd 201

- Wild Type: Spoteneous streptomycin resistant, colonies stain on congo red growth medium and remain unstained on aniline blue medium; grows on solid medium containing 20µg/mL NaN_3 0.5 M NaCl
- Nod^+, fix^+ $Strept^R$

Characteristics of vermicompost used in the study

Constituents	Quantity
Organic carbon (%)	5.60
Nitrogen (%)	1.59
Phosphorus (%)	0.85
Potassium (%)	1.05
Zinc (ppm)	46.80
Iron (ppm)	473.00

Vermicompost having higher water holding capacity



Methodology…

Three carrier materials, viz. granular vermicompost (GVC), farm yard manure (FYM) and charcoal (CHL) were used as carrier.

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sterilized in an autoclave (121^{0}C, 1 h) for three consecutive days.

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The broth culture (population size 2.6x10^9-5.6x10^9) of *R. meliloti* Rmd 201 was centrifuged at 10000 rpm for 10 min and suspended in sterile demineralized water and mixed with carriers, stored in high density polythene bags at 28^{0}C unless otherwise specified in the incubators up to 180 days. (For liquid carriers vermicompost aqueous extract was used).

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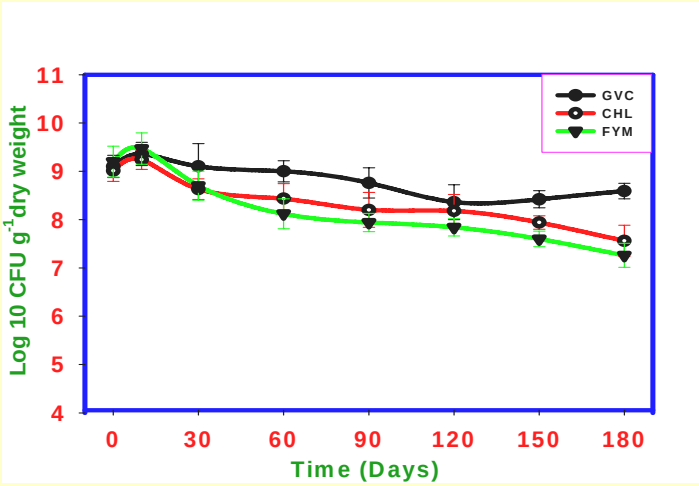
The viable count of *Rhizobium* was monitored periodically in all treatments by conventional viable plate count method using

YEMA (Yeast extract mannitol agar) medium.

Population of *Rhizobium* in solid carrier material



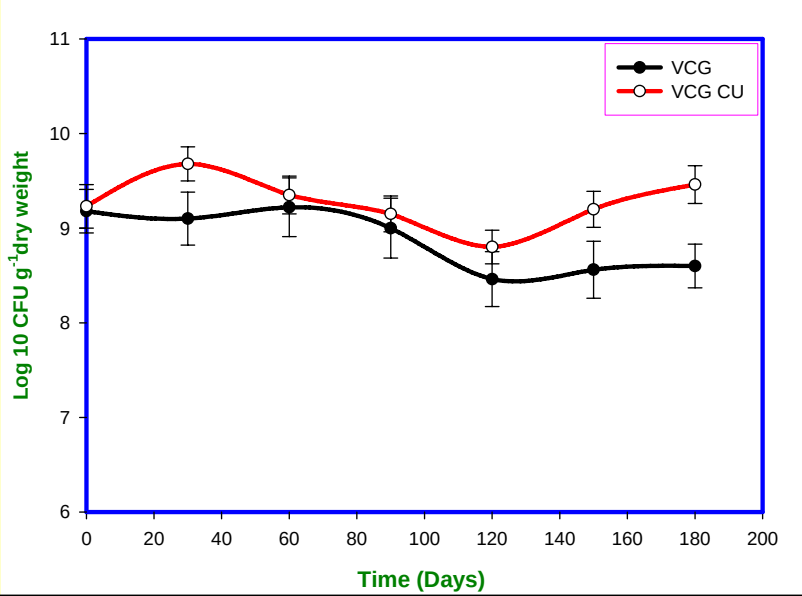
Output

- **Initially population in all test carriers was between 1.01 x 10^9 - 2.0 x 10^9 CFU g-1 which increased sharply (2.4 x 10^9 – 4.7 x 10^9 CFU g-1) in first 10 days and slowly declined thereafter.**
- **The rhizobia count again increased after a month and then showed a consistent decline except in GVC where an increase was again noticed after 4-5 months.**

Output …..

- **Maximum viable population of rhizobia could be recovered in GVC followed by charcoal and FYM after 6 months of incubation.**

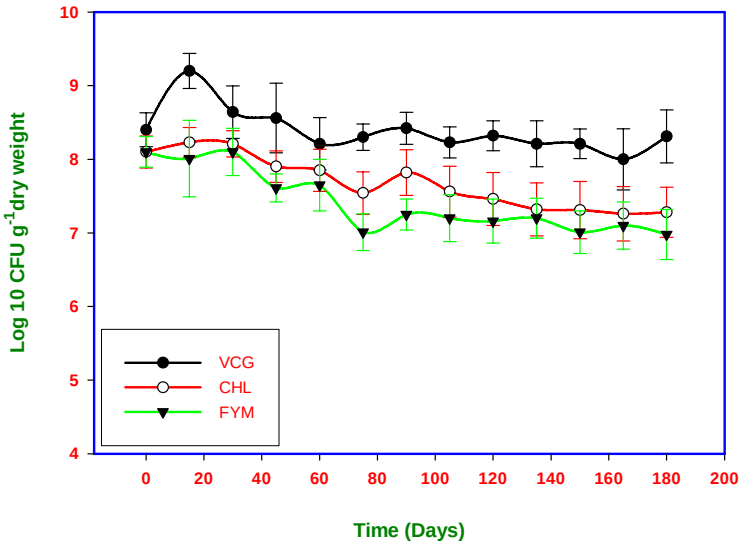
Population of *Rhizobium* in solid carrier material with and without addition of CU



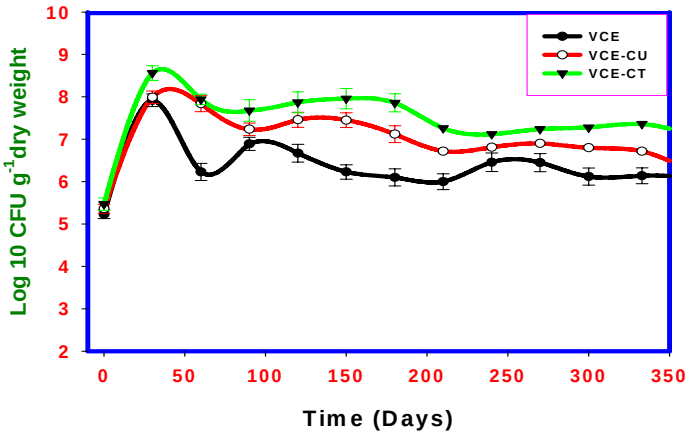
Output

- **Initially, upto 30 days this natural product enhanced the growth of rhizobia at faster pace and thereafter, population of rhizobia declined upto 120 days; however the population could be maintained between 1.23 x 10^9 to 2.28 x 10^9 for the remaining monitored period i.e., 180 days.**
- **Similar enhancements in rhizobial population were also noticed when CU was incorporated in liquid carrier material.**

Bacillus subtilis



Population of Rhizobium in liquid carrier material with and without addition of CU and CT



Output

- **Vermicompost extract (VCE) maintained the population of rhizobia for a longer period. In this carrier (vermicompost extract) population of rhizobia was monitored upto one year and it was observed that population of rhizobia $1.0x10^6$ – $2.1 x10^6$ could be maintained even after one year.**

Output..

- **Incorporation of CT increased population of rhizobia upto 100 fold after 5 days of inoculation in liquid carrier when compared to VCE alone Population size of 8.6x107- 9.6x107 CFU mL-1 could be maintained after 330 days when suspended with CT .**

Effect of vermicompost based formulation of *Bacillus subtilis* on the productivity of safed musli (*Chlorophytum borivillianum*)

Treatments	No. of fingers/plant		Fresh Finger yield/plant (g)
	Range	Average	
Charcoal based formulation	24-40	34.6	104.2
FYM based formulation	34-39	35.3	117.8
Vermicompost based formulation of *Bacillus subtilis*	31-51	40.0	137.5

Effect of vermicompost based formulation (Vc) on physical, chemical and biological characteristics of soil.

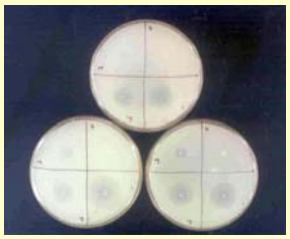
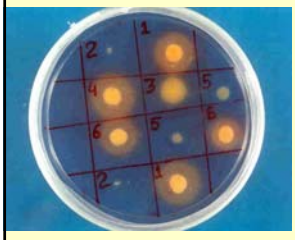
S. No.	Treatment	N (kg/ha)	P (kg/ha)	K (kg/ha)	OC (%)	pH (1:2)	Ec (m mhos)	d_b (g/cc)	dp (g/cc	WHC (%)	Porosity (%)
1	Control	157.23	16.0	91.84	0.35	8.3	0.202	1.68	2.89	45.70	41.87
2	10tVc/ha	165.33	19.8	113.2	0.46	7.10	0.200	1.44	2.76	53.64	47.83
3	15tVc/ha	179.20	37.0	120.96	0.49	7.20	0.200	1.42	2.73	65.31	48.00
4	30tVc/ha	207.47	41.6	137.76	0.52	7.03	0.201	1.40	2.70	72.64	48.15
5	Initial	160.01	17.4	101.92	0.36	8.2	0.200	1.67	2.80	51.13	40.36

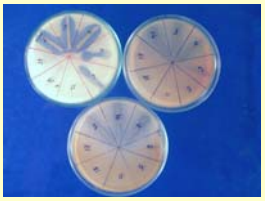
Observation recorded after 3 months of Vc application in pea grown pots:

The experiments conducted by us in our laboratory clearly indicate that vermicompost can be a carrier of choice for preparation of bioinoculant formulations of *Rhizobium*, *Bacillus*, *Pseudomonas* and many others for higher populations of inoculants and longer shelf life.

Value added compost with beneficial microbes useful in improving soil health and reducing chemical inputs.



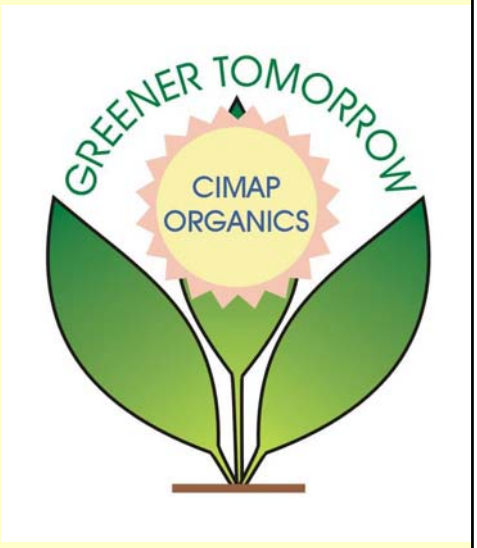


Advantages

- It has better potential than charcoal to support growth and survival of bioinoculants like diazotrophs, growth promoters and biopesticides.
- Such carriers also provides nutrients to the plant and helps improving soil structure
- Apart from seed treatment can be applied directly in fields.
- Especially suitable for standing perennial crops. plantation and orchards.
- Cheap and readily available material in large amounts.

- Environmental friendly.
- Formulation very useful in organic agriculture both the carrier as well inoculant are source of nutrient supplement (Organic and biological nutrient supplement).
- Carrier has high water holding capacity

With gratitude towards
CSIR
INSA
DST



The Team-CIMAP Efforts

Thank you