

## Bio Formulations of indigenous biocontrol agents - A Potential Alternative for Pests and Disease Management in Meghalaya

Pranab Dutta, Lipa Deb, R.K. Tombisana Devi, M. Pathak, R. K. Patidar and J K Chauhan

College of Post-Graduate Studies in Agricultural Sciences, Central Agricultural University (Imphal), Umiam, Meghalaya-793 103.

Corresponding Email ID: pranabdutta74@gmail.com

ARTICLE ID: 009

### Abstract

Bio formulation products consisting of native strains of beneficial microorganisms provide efficient strategies for eco-friendly management of agricultural pests and diseases in sustainable manner. These bioformulations are prepared by native/indigenous strains of *Bacillus subtilis*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces lilacinous*, *Pseudomonas fluorescens*, *Trichoderma harzianum* and *T. viride*. A series of *in vitro* studies showed potentiality of some strains of the mentioned biocontrol agents few soil borne plant pathogens as well as some insect pests of Meghalaya. Similarly *in vivo* study also showed positive results in pot condition with enhancing plant growth and development and significantly reduced the targeted diseases and pests of agricultural crops. Liquid bioformulation protocol has been standardized and tested in field conditions. Farmers field demonstration is in progress with positive feed back. Exploration of local strains into a low cost liquid bio formulation products not only paves way for niche management of biotic stresses but also provides scope for employment among local youths, women as well as farmers.

### Introduction

Bio formulations are defined as any biologically active substances derived from microbial biomass or product containing microbes and their metabolites or botanicals or plant incorporated products etc that could be used either in plant growth promotion, nutrient acquisition or pests and disease management without harming the environment. Bio formulated products offer green alternative to conventionally used chemical pesticides that

have degraded 25% of the productive agricultural lands into arid and non-fertile soils (Berger *et al.*, 2018). It also provides eco-friendly solutions to most hazardous impacts of agrochemicals such as loss of soil fertility, beneficial microbial populations as well as rapidly changing environmental scenario impacting agriculture *viz.* drought, temperature stress, soil salinity, depletion of mineral nutrients. Development of indigenous bio formulations involves efficient use of native agroecosystem that enhance agricultural production in sustainable manner with their immense potential to ameliorate the drastic effects of biotic stress.

### **Mechanisms of bioformulations:**

Mechanism of biocontrol agents of bioformulation is guided by the principles of host, pathogen, environment and microbes as biocontrol agents and their understandings is essential for improvement as well as wider use of biological methods. There are different types of direct and indirect mechanisms of biological suppression or control of plant pathogen or insect pests. Here the main mechanisms are discussed briefly as follows:

- **Mycoparasitism:** It is defined as a phenomenon under which a fungus parasitizes host fungus by invading the cell through coiling of hyphae or direct penetration *via* production of haustoria and lysis of hyphae (Baker and Cook, 1974). Eg. Mycoparasitism of *Rhizoctonia solani* and *Pythium ultimum* by *T. harzianum*.
- **Antibiosis:** Production of low molecular weight non-polar volatile compounds/ antibiotics which at low concentration are deleterious to the growth or other metabolic activities of other microorganisms (Handelsman and Parke, 1989). These metabolites include volatile organic compounds (VOCs), siderophore cell wall degrading enzymes *viz.* chitinases, cellulases, proteases, glucanases (Lo, 1998). Eg. 2,4-diacetylphloroglucinol (*P. fluorescens*), zwittermycin (*B. subtilis*), Harzianic acid (*T. harzianum*) *etc.*
- **Competition:** Intra- or Interspecific competition occurs when individuals attempt to obtain a resource that is inadequate to support all the individuals seeking it or even if resources competed for, such as nutrients and space to grow, nest and hide from predators (Lorito *et al.*, 1984). Eg. Competition of *T. harzianum* with *Sclerotinia sclerotiorum* and *Fusarium solani*.

### **Advantages of bio formulations:**

- Bio pesticides usually are inherently less harmful than conventional pesticides.
- It generally affects only the target pest and closely related organisms, in contrast to broad-spectrum conventional pesticides that may affect organism.

- They are effective in very small quantities and often decompose quickly, therefore resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides.
- When used as component of integrated pest management (IPM) programme, bio pesticides can greatly decrease the use of conventional pesticides, while crop yields remain high.

**Materials and Methodology:**

**Materials required:**

Media {potato 200g, dextrose 20g in 1000 ml distilled water}, beakers (1000 ml), spatula, electric heater, cotton plugs, autoclave or pressure cooker, glass bottle (250ml, 500ml, 1000 ml), muslin cloth, spirit lamp, dehydrating alcohol

**Techniques for mass production and bio formulation:**

**a) Media preparation- Potato dextrose broth (PDB) media:**

- Potato extract was prepared by boiling washed, peeled-off potato cubes (200g) for 10-15 minutes in 500 ml of water and squeezed through muslin cloth.
- Add dextrose (20g) and agar agar (20g) to the extract, stir to dissolve and make up volume to 1000 ml. Dispense the media into glass bottles filling up to 2/3<sup>rd</sup> of its capacity using plastic funnel and plug the bottles tightly with non-absorbent cotton.
- Cover the cotton plugs with brown paper or waste paper and tie with rubber band and sterilize the media in pressure cooker on a LPG and store for 20 minutes after first whistle.

**b) Inoculation process:**

- Sterilized media were inoculated with 10 ml of mother culture (Table 1) in sterilized inoculation chamber and incubated either at room temperature (25 ± 1 ° C) or in BOD incubator at (25 ± 1 ° C) at least for 7-10 days.
- **Organisms used for bio formulation:**

<b>Table 1: List of organisms used to prepare indigenous bio formulation</b>		
<b>Type</b>	<b>Bio agents</b>	<b>Source</b>
Insect pathogens	<i>Beauveria bassiana</i> (NCFT 9097.17)	School of Crop
	<i>Metarhizium anisopliae</i>	Protection, College of
	<i>Paecilomyces lilacinus</i> (NCFT 9094.17)	Post-Graduate Studies
Microbial	<i>Trichoderma harzianum</i>	in Agricultural

antagonists	<i>Trichoderma viride</i>	Sciences, Central Agricultural University (Imphal), Umiam, Meghalaya
Bacterial biocontrol agents	<i>Bacillus subtilis</i>	
	<i>Psuedomonas fluorescens</i>	

- The colour of media will be green for *Trichoderma*, white for *Beauveria*, dark pink for *Purpuroceocillium*, dark green for *Metarhizium*, grey for *Isaria* at later stage, forming thick mat spores and mycelium after 15-20 days and ready for production of bio formulations.

**c) Development of liquid bio formulations:**

- Grind the mycelial mat with suspended broth with the help of grinder and filter through a layer of muslin cloth.
- Mix the culture filtrate with appropriate carrier and adjuvants.
- Fill the bottles with prepared formulations, label it and store at room temperature (Plate 1; Table 2).

**Results:**

**Bio formulation produced:**

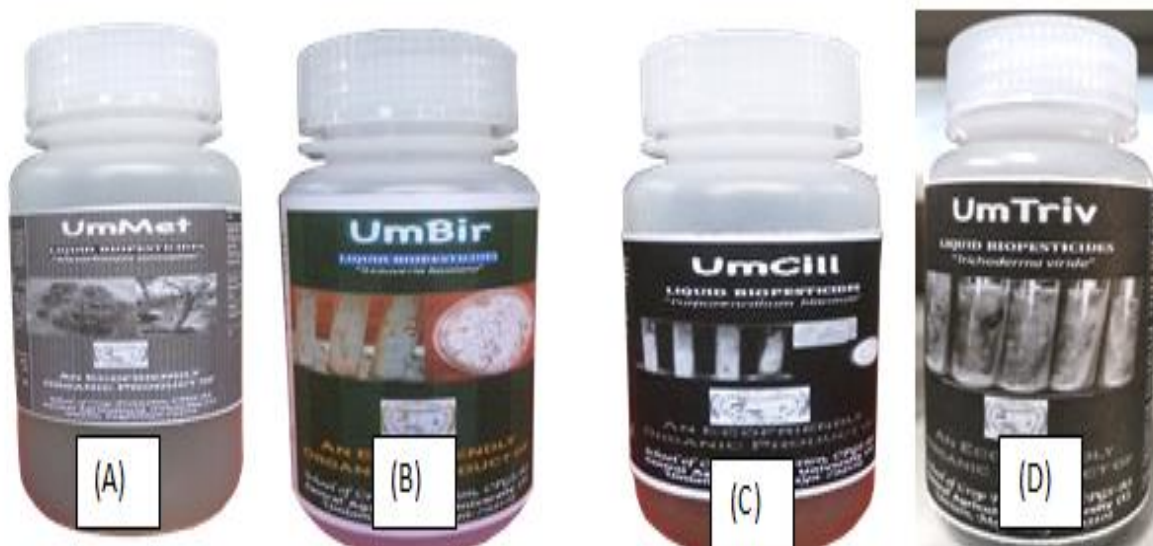


Plate 1: Indigenous bio formulations prepared from native strains of Meghalaya; (A) UmMet (*M. anisopliae*), (B) UmBir (*B. bassiana*), (C) UmCill (*P. lilacinus*) and (D) UmTriv (*T. viride*)



General view of mass production site of biopesticides at CPGSAS, CAU (Imphal), Umiam, Meghalaya

**Table 2: List of indigenous bio formulations prepared**

Product	Organisms	Target organisms
UmBir	<i>Beauveria bassiana</i>	Rice hispa, aphids, leaf hoppers, fruit scaring beetle
UmMet	<i>Metarrhizium anisopliae</i>	White grub, aphids, white fly
UmCill	<i>Paecilomyces lilacinous</i>	Root knot nematode
UmTricho	<i>Trichoderma harzianum</i>	Damping-off, root rot, sheath blight, wilt
UmTriv	<i>Trichoderma viride</i>	Root rot, white mold, wilt, damping-off
UmRaj	<i>Bacillus subtilis</i>	Bacterial wilt, sheath blight, damping-off
UmPsuedo	<i>Psudeomonas fluorescens</i>	Damping-off, sheath blight, Bacterial and fungal wilt

#### Method of application and dose:

##### a) Seed treatment:

- Mix 10 ml of bio formulation in 1000 ml of water and soak the seeds/ seedlings into it for 30 minutes.
- Shade dry treated seeds for 1 hour prior sowing.

##### b) Seedling root dip treatment:

- Mix 100 ml of bio formulation with 10 L of water.

- Dip the seedling root –zone for 10-15 minutes prior to transplanting.

**c) Drenching root zone:**

Mix 10ml of bio formulation in 1000 ml of water and drench the root zone depending upon the crop up to 1-2 feet from main stem.

**d) Soil application:**

- Mix 1 L of bio formulation with 1.5 kg of vermicompost and incubate for 7 days.
- Apply 100g of enrich compost in seed bed or in main field 3-4 days before sowing or transplanting of vegetables.
- Apply 100 g of bio formulation enriched compost in pit during transplanting and in soil around the 30 days old crops.
- Apply 5 kg of enriched compost per plant during February/ March and another during September/ October.

**e) Foliar spray:**

Mix 10 ml of bio formulation in 1000 ml of water and spray the foliar parts of plants twice at an interval of 30 days.

**Precautions**

- Store in cool, dry and well ventilated place, away from food stuff and animal feeds under lock and key.
- Keep away from direct sunlight and Spray preferably in the afternoon.
- Avoid inhalation and skin contact.
- Use sticker @ 0.02% during rainy days.

**Results in field activity:**

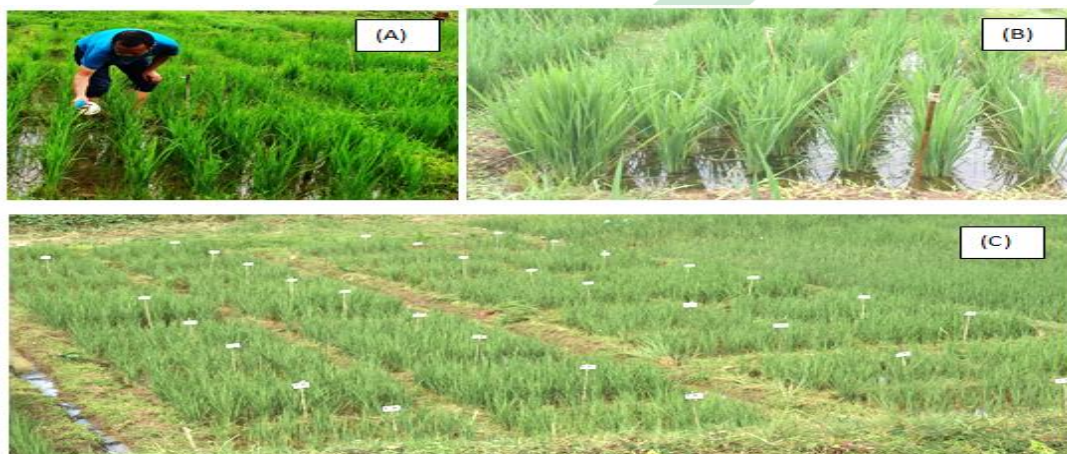


Plate 2: Foliar application of umBir (A) and site view of rice production (B, C)

**a) Disease and pest free production of rice in CPGSAS experimental farm, Umiam**

Successful production of rice was reported from rice experimental farm of CPGSAS, Umiam from Ri bhoi district of Meghalaya (Plate 2). It was done by solely limiting to organic agricultural practices that includes:

- Two application of 10 tonne/ha farm yard manure (FYM) during field preparation.
- Foliar spray of UmBir, four times from the days of transplanting at an interval of 15 days

**b) Management of brown spot and blast diseases of rice in CoA experimental farm, Krydamkulai, RiBhoi district, Meghalaya.**

Heavy occurrence of brown spot and blast disease incidence was recorded from rice experimental farms of College of agriculture, krydemkulai under rib hoi district of Meghalaya (Plate 3). The pathogenic agent responsible for the infection was identified as fungus viz. *Bipolaris oryzae* and *Pyricularia oryzae*. The remedial treatment for the management of target disease was:

- Combined application of UmBir and UmTricho as thrice foliar spray at 5 days interval.
- Application of cow dung suspension @ 1:2 in water 4-5 times initially at 3 days interval and later at 7 days interval.

Drastic reduction in disease incidence of brown spot and blast have been reported at 10<sup>th</sup> day after application along with improvement of growth of plants (Plate 3)

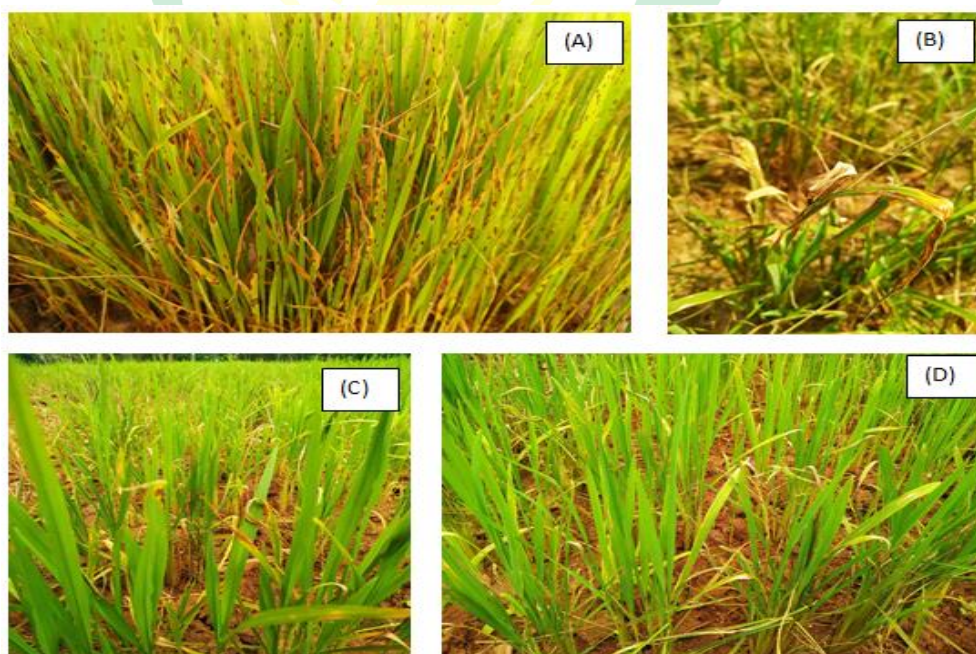


Plate 3: Heavy infection of brown spot (A) and blast of rice (B) and reduced disease incidence post bio formulation application (C, D)

### C) Management of fall army worm in maize field of (Kurba Mawlai)

In situ demonstration and application of UmMet in the farmer's field of Kurba Mawlai in Ri bhoi district of Meghalaya for the management of fall army worm of maize Plate 4a and 4b).



Plate 4(L-R). L: Demonstration on field application of UmMet for management of fall army worm, R: Distribution of UmMet to the maize grower

Training were also imparted to create awareness on biopesticides, mass culture technique and technical details of preparation of spray suspension, precautionary measures etc to the progressive farmers, faculties and graduate and post graduate students of Meghalaya, Assam, Mizoram, Tripura, Arunachal Pradesh etc.



Plate 5. Imparting training to farmers, faculties, graduate and PG students

### Conclusion

Indigenous bio formulation products are very effective in small scale trials against various pests and diseases. Trainings on mass production of bio formulation of indigenous biocontrol agents can be helpful in establishing small scale cottage industry of bio



formulation products as well as to increase bankability of small and marginal farmers as well as youths.

### References

- Baker, K. F., and Cook, R. J. 1974. Biological Control of Plant Pathogens. *Am. Phytopathol. Soc.*, St. Paul. MN.433 pp.
- Berger, B., Patz, S., Ruppel, S., Dietel, K., Faetke, S., Junge, H., Becker, M., 2018. Successful formulation and application of plant growth-promoting *Kosakonia radicincitans* in maize cultivation. *BioMed Res. Inter.* 6439481, 8.
- Handelsman Jo., and Parke, J. L. 1989. Mechanisms in biocontrol of soilborne plant pathogens. Pages 27-61. in: Plant-Microbe Interactions, Molecular and Genetic Perspectives, Vol. 3. T. Kosuge, and E. W. Nester, eds., McGraw-Hill, New York .
- Lo, C. T., Nelson, E. B., Hayes, C. K., and Harman, G. E. 1998. Ecological studies of transformed *Trichoderma harzianum* strain 1295-22 in the rhizosphere and on the phylloplane of creeping bentgrass. *Phytopathology* 88: 129 - 136 .
- Lorito, M., Harman, G. E., Hayes, C. K., Broadway, R. M., Tronsmo, A., Woo, S. L., and Di Pietro, A. 1993. Chitinolytic enzymes produced by *Trichoderma harzianum* : antifungal activity of purified endochitinase and chitobiosidase. *Phytopathology* 83:302-307.