

**Review Paper (NS-2)****BIO PESTICIDES AND FERTILIZERS : NOVEL  
SUBSTITUTES OF THEIR CHEMICAL  
ALTERNATES****Subrata Datta**

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*Received December 25, 2011**Accepted March 25, 2012***ABSTRACT**

Application of chemical pesticides and fertilizers show a long term residual toxic and teratogenic effects on the different animals and thus causing human health hazards after being incorporated into the ecosystem<sup>1,2</sup>. Chemical fertilizers and pesticides have provided food for first green revolution which now switches to use of biofertilizers and biopesticides in current era of organic farming and ecofriendly management practices<sup>4</sup>. In order to develop a low-input, Eco-friendly Technology,-following recommendations involving use of-Azolla, Azotobacter, Azospirillum, blue green algae and phosphate culture in different crops have been released for farming community by Anand Agricultural University, India. International 3<sup>rd</sup> Eco Expo Asia, October conference and trade fair (2011) has already given emphasis on "Green Technologies Govts. of Environmental Bureau decided to minimize - a low carbon environment and introduction of Bio-Organic Fertilizers including other policies in 2011.<sup>13</sup> Thus, alternatively biofertilizers and particularly the biopesticides are now used including at least one chemical pesticide<sup>5</sup>. Here, in this paper, an attempt has been made to construct a biopesticide and biofertilizer, absolutely based on biological origin without any inclusion of chemical pesticide. These are especially related to paddy cultivation.

**Key Words :** Pesticidal effects, Terata, Azolla, Biopesticide liquid, Biofertilizer, Chemical Fertilizer**INTRODUCTION**

It is already reported that chemical fertilizers and chemical pesticides have tremendous harmful long - term residual effect not only on the soil - health and crop-productivity but also they contaminate the ground water level and ultimately they are incorporated into the food chain in the ecosystem causing human health hazards<sup>1,3</sup>. Previously, we have reported that 'Furadan' and 'Malathion' pesticides caused teratogenic effects both in the adult and embryonic stages including birds (as animal model) at the histomorphological, genetical and biochemical levels<sup>1,2,3</sup>. Some pesticides and related chemicals are persistent in the environment and are accumulated in the fatty tissues of organisms and increase in concentration as they move up through the food-web.<sup>5</sup> These chemicals which show biomagnification, after bioaccumulation, are substances that can cause effects by interfering in

someway with the body's hormones or chemical messenger. A recent study indicated that atrazine effected the sexual diseases and development of frogs, even at extremely low doses.<sup>5</sup>

Unlike chemical pesticides and fertilizers, biopesticides and bio-fertilizers contain viable population of the selected microbes which may be colonized in the soil ecosystem.<sup>2,4,7</sup>

Pest control by biological agents appears to be very 'useful tool' in recent years<sup>4</sup>. Though there are a few reports that biopesticides are now being introduced into the agricultural fields but unfortunately not absolutely as biofertilizer or pesticides but mixing with a little amount of chemical pesticides for enhancing their effectiveness<sup>8</sup>.

**AIMS AND OBJECTIVES**

- 1) To construct a biopesticide and a biofertilizer which is absolutely biological based, without

- any addition or, mixing of chemical pesticides and fertilizer.
- 2) To get a 'Bird's eye view' of the toxic effects of the chemical pesticides and fertilizers based on some of the bio chemical parameters.
  - 3) Then, to know the beneficial and side effects (if any ) of these newly constructed (formulated) biopesticide and biofertilizer which are still in the field trial as pilot samples, though they are almost in final stage.

## MATERIAL AND METHODS

### Pesticide - application' and incubation

Furadan -3G (carbofuran) and Malathion (organophosphorous) water soluble chemical pesticides were injected onto the 'Nescently fertilized' chick eggs at sublethal doses (20 and 30 µg/ml, respectively) and sealed and incubated on an incubator at 37°C.

### Histological and Biochemical Assay

After different hours of incubation up to hatching, 'chemical pesticide -treated eggs' were taken out and the developing embryos were 'EOSIN' stained along with the control set as per standard protocol<sup>1,2</sup>. For histological assay, they were finally embedded in molten paraffin (57°C) blocks and microtomed (5-6µm) and stained as per standard protocol<sup>1,2</sup>. Colorimetric estimation and biochemical analysis like RNA, protein, cholesterol etc from the different hour's of chick embryos of both the treated and control - sets were done and their data were plotted against standard curves in each case according to standard protocol<sup>9</sup>.

### Biopesticide preparation and processing

*Bacillus thuringiensis* (B.t.), a bacterium was chosen for preparing a biopesticide. However, these bacteria were cultured in our laboratory and the bacterial - inoculum was processed as below as per standard protocol<sup>4</sup>. Yeast extract- mannitol agar with polymixin B for selective isolation of B.t (*Bacillus thuringiensis*) was taken and after final dilution , 0.1ml 10<sup>-6</sup> diluted sample (containing - B.t) spreaded individually on the above mentioned selective media. The procedure was carried out in the laminar air flow chamber to avoid contamination<sup>4,5,11</sup>. The inoculated

petriplates were incubated in BOD incubator at 30<sup>0</sup> ± 2<sup>0</sup>C temperature for optimum growth. The growth characteristics of these bacterial colonies were recorded after 48 hrs of incubation. (the isolated bacterial spores were observed under phase contrast microscope for identification and confirmation). The major steps in fermentation and the production process can be described below:- since it is now available in the text books of organic framing in the form of detailed Protocols.<sup>4</sup> (e.g. - vora, et al, etc.) Inoculation (B.t.)-> Fermentor -> Harvesting -> Blending -> Drying and Packing<sup>4</sup>.

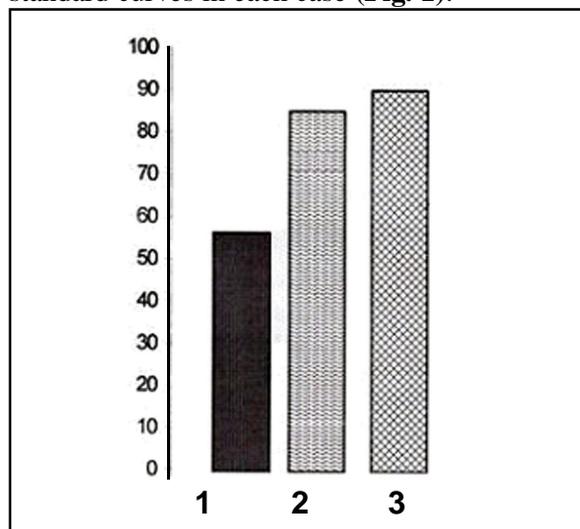
### Product process and formulation of the Biofertilizer in details

The chief nutrients of the plants including paddy are : Nitrogen, potassium and Phosphorous (N-P-P). we have prepared the 'LIQUID' Biofertilizer, mainly for paddy (rice) as per standard protocol according to conventional method<sup>4</sup> i.e., mother culture -> Flask culture -> Bottle culture -> Fermentor broth -> mixing in trays -> curing -> product (pilot sample)<sup>4</sup>. These steps are described in details in the text books of any organic framing in the form of Protocols.<sup>4</sup> Liquid-biofertilizer which is a new vistas is of 2 kinds -> N<sub>2</sub> fixing and phosphorous solubilizing. Among the 2 groups, *Azotobacter* inoculants are widely used compared to other biofertilizers<sup>4</sup>. we have added azolla (extract) which supplies 40 - 80 kg of N<sub>2</sub> per hectar (urea is not required). The new formulation is : 1) *Azotobacter* BF 1013 (ICBR - 1: 3 .45) ->30% 2) Azolla extract 30% (reared in tank, **Fig. 4 and Fig. 3**) Neem oil (containing azadirachin, salannin and bioactive terpinoids) - 20% 4) Ground nut and cashew nut shell oil (10:5) (contains anacardic acid and cardanol)15% plus 5) Toboacco extract (contains nicotin)- 5%. This formulated liquid biofertilizer must be stored in shade (protected from sunlight) and recommended to apply either in early morning or late afternoon to soil in the paddy field. It was named as- 'USHA - PUCHI' biofertilizer.

## RESULTS AND DISCUSSION

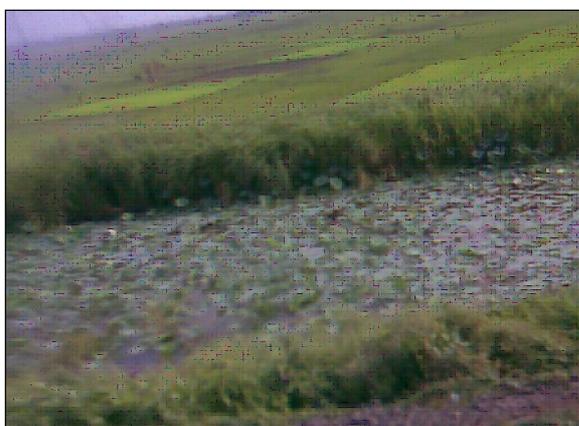
Both the 'Above - mentioned' newly formulated and synthesized biopesticides and biofertilizers described in this paper, are extremely in the final

stage of field-trials. Based on the colorimetric analysis like total RNA, cholesterol, protein etc and comparing their data with the corresponding standard curves in each case (**Fig. 1**).

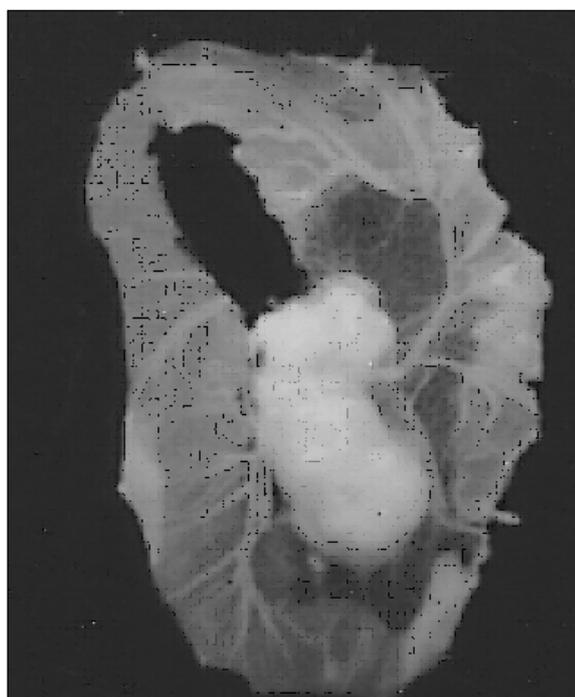


**Fig. 1 :** Total DNA of about 72hrs developing chick embryos. Lanes, 1. Pesticide(furan) treated, 2. Biopesticide treated, 3. Control (0-100µg/ml of pesticide & distilled water were used).

It was observed that chemical pesticide induced organs (like liver, brain etc) of the avian (chick) embryos showed maximum deviation from the controlled embryos at normal range in biochemical levels and in Histological levels too (**Fig. 2**). This is definitely due to the toxic and teratogenic effects of the chemical pesticides as observed at least in the animal model (chick)<sup>1,2</sup>. On the other hand, *Bacillus thuringiensis* (B.t) based biopesticide showed very good efficacy and satisfactory potentiality to control the pests during paddy



**Fig. 5 :** Experimental "Paddy Plots" in the field



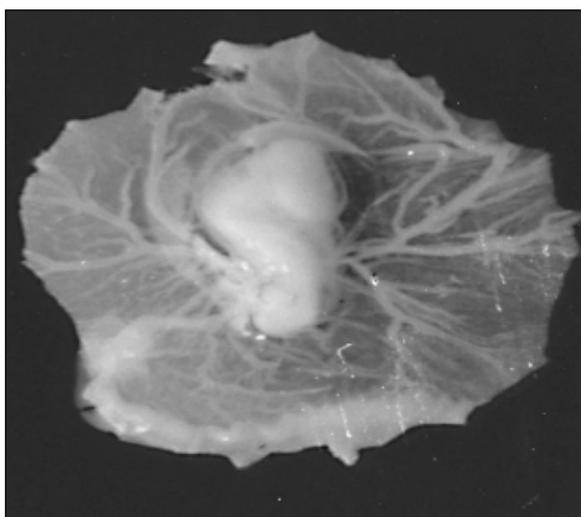
**Fig. 2 :** 72hrs. Furadan (chemical pesticide)-treated chick embryo showing Terata.

infection. Paddy or rice is the staple food of W.B and several other Indian states. There are reports that, Cadila pharmaceuticals Ltd, Ahmedabad, has launched 2 novel biofertilizers, VIZ: kalisena and josh based on *Aspergillus niger* and 'Glomus Vam', respectively<sup>4</sup>. Our formulated biofertilizer is a novel liquid one and named as "USHA - PUCHI" biofertilizer as a pilot sample and the trials were given in the paddy fields marked as 'plots' adjacent to our laboratory (**Fig. 5** and **Fig. 6**). Actually when this "USHA - PUCHI"- biofertilizer was applied to the different pest-infected "plots", it

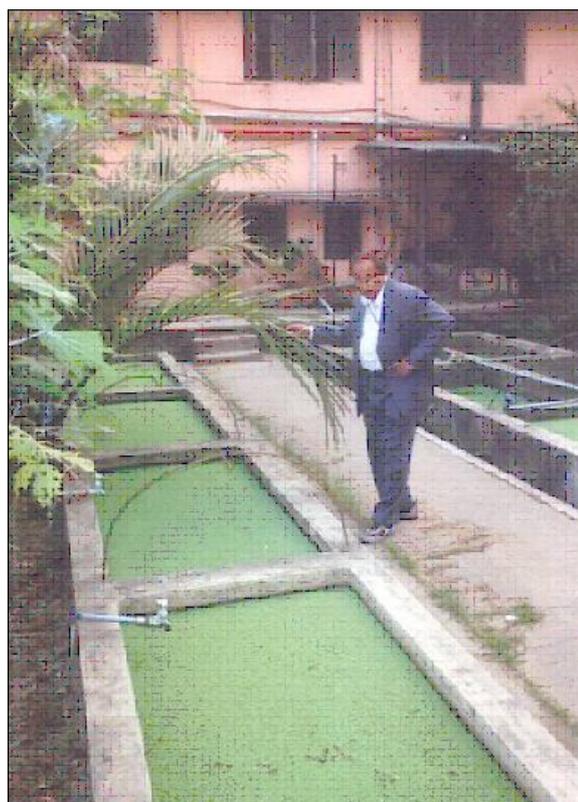


**Fig. 6 :** Paddy plots and farmer's training

showed that paddy seedlings grew well and yielded mature healthy paddy without any infection of fungi and bacteria under ideal condition with Protection and Precautions. Rice seedlings roots were dipped into the liquid biofertilizer suspension before transplanting. This observation was similar to the reports of Vivekananda institute of biotechnology, Sri Ramkrishna Ashram, Nimpith, West Bengal (2009). When the experimental soil sample was tested, it was observed that the soil fertility (tested in Soil-analytical Institute, in Kolkata) became increased after plantation of paddy seedlings using "USHA PUCHI" biofertilizers. Subsequent experimentation was performed with the genetically constructed 'B.t. based biopesticides to evaluate the toxic harmful effects of this newly constructed biopesticide in different organs of the developing avian (chick) embryos. In this case no terata was formed, normal growth and hatching was observed and even almost no biochemical changes was found.<sup>2</sup> (Fig. 3 and Fig. 4). There is evidence that birds are continued to be harmed by 'Chemical' pesticide use. Rachel Carson's Land mark book- 'silent spring' dealt with the topic of loss of bird's spp due to 'Bioaccumulation' of the chemical pesticides in their tissues<sup>7</sup>. Throughout Europe, 16 spp of birds are now threatened<sup>7,8,10</sup>. The herbicide 'Paraquat' causes growth abnormalities in the chick embryos and reduces the number of chicks that hatch.



**Fig. 3 :** About 72 hrs of biopesticide treated chick embryo- showing no side effects or Terata



**Fig. 4 :** Azolla culture and the author

Herbicides even endanger 'Bird- population'<sup>9</sup>. Fish and other aquatic biota may be harmed by chemical based pesticide contaminated water<sup>10</sup>. Many chemical pesticides are highly lethal to aquatic life, even killing all the fishes in a particular stream<sup>10</sup>. These are a few examples of different animals including chick which we have used as an animal model. Thus, chemical pesticides not only act as "SILENT KILLER" for the different animals including human beings but also causing extinction of many populations of global flora and fauna<sup>7,8,10</sup>. Not only that, use of chemical fertilizers and pesticides, has made the indian soils largely nonproductive, nonfertile, poor in organic matter and thus the growing nutrient imbalance possess a major threat to sustain soil health and crop-productivity<sup>4,7</sup>.

To minimize the use of these inputs without affecting the overall production and the ecosystem, it is at present scenario, is really becoming necessary to popularize the benefits of ecofriendly microbial inoculants in our Indian agriculture<sup>4,7</sup>.

Actually, biopesticides are potential alternatives

to chemical pesticides and widely recommended as a component in "Integrated Pest Management (IPM)"<sup>4</sup>. B.t. is a gram(+), aerobic, Sporulating bacterium that synthesizes crystalline proteins (cry), which is highly toxic to agriculturally important harmful-pests, especially caterpillars<sup>4,7</sup>. B.t. an endotoxin (cry proteins) has acquired acceptability as an ecofriendly biopesticide<sup>4,7</sup>. B.t. is now considered as a 'key biopesticide'<sup>4</sup>. Intact B.t. organisms (which contain pesticidal toxin) are used to control insects by farmers and home gardeners and for mosquito abatement in ponds and lakes.

Biological control of pests is one of the important means for checking Pest problems in agro ecology situations. Many scientists tested the toxicity of various organophosphate insecticide to IJS water suspension and obtained 90 - 100% mortality after 24h. Many other studied efficacy of steinernema sp against *S. litura* at Maduri<sup>4,10</sup>. The advantages of liquid biofertilizers are; longer shelf life (12 - 14 months) and high population can be maintained more than  $10^8$  cell/ml upto 1-2 years, no contamination, less effect of high temperature (upto 45°C.), better survival on seed and high commercial revenues<sup>4</sup>.

Farmers of Gujrat specially in South, which is marked as highly rainfall zone are advised to adopt the techniques of seedlings roots dipping for 15 minutes in one of the following. *Azotobacter* or *Azospirillum* culture suspension before transplanting for summer rice.<sup>4,10</sup>

In India, liquid formulations of biofertilizers are marketed by pune<sup>4</sup> and recently Anand Agricultural University, India has developed protocol for liquid formations. Very recently, B.t., neem based formulations and microbial pesticides are included in the schedule of Insecticide Act, 1968<sup>4</sup>. At present, Biopesticide registration is a must<sup>4</sup>. We have applied for registration of our 'B.t.based Biopesticide' to the secretary, Central Insecticides Board and Registration committee, Faridabad, U.P., India, ([http://cibrc.nic.in/reg\\_products.htm](http://cibrc.nic.in/reg_products.htm)). Field efficacy is another important factor. ICAR and state agriculture university have conducted field efficacy trials<sup>4</sup>.

In this regard, our newly constructed biopesticide is almost in final or Mature stage of field trial.

## CONCLUSION

Due to "chemical pesticide" problems in India, there is an urgent need to Promote environmental friendly 'Biopesticides' in the country. Moreover, recent Government policies also favour "Biopesticides and Biofertilizer."<sup>12,13</sup>

More over "Biofertilizers" also stimulate plant - growth through Production of "Plant growth promoting substances"<sup>3</sup>. Application of Biofertilizers and biopesticides has thus become an integral component of nutrient management system. Apart from these, they play a vital role in increasing the agricultural production, a need of the day<sup>4,7,13</sup>.

International 3<sup>rd</sup> Eco Expo Asia, October, conference and trade fair has already given emphasis on "Green Technologies". Govts. of Environmental Bureau decided to minimize - a low carbon environment and introduction of Bio-Organic Fertilizers including other policies in 2011.<sup>13</sup>

Finally, it can be concluded that our newly constructed " B.t. Based biopesticides" and the " Liquid USHA - PUCHI" Biofertilizer which seem to be very much efficient against the Paddy pest in the experimental paddy fields (plots) is really in the final trial stage. In spite of that it needs an " In depth" study and application before their marketing and commercialization, after fulfillment of all these requirements :- including patent, registration etc. Only then we now can say, "yes, these biopesticide and biofertilizer are definitely the substitute of chemical pesticide/fertilizer - atleast for paddy (rice) cultivation in India especially for Rice eaten states including West Bengal.

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