

## Influence of Calcium, Magnesium, Iron and Zinc Nanomaterials on the Growth of Biopesticide *Bacillus thuringiensis*

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### ABSTRACT

The nanoparticles of calcium oxide, magnesium oxide, iron oxide and zinc oxide at 10 ppm, 20 ppm, 50 ppm, 100 ppm and 500 ppm were added to the Luria Bertani Agar media in 1:9 ratio to know the influence of nanoparticles on the growth of *Bacillus thuringiensis*. The maximum number of cfu/g 17.5, 17.4, 12.8 and 11.9 were recorded with MgO at 50ppm, CaO at 20 ppm, ZnO at 10 ppm, FeO 50 ppm, Where as in control it was 6.6 cfu/g.

**Key words:** Groundnut, Integrated Pest Management (IPM), *Bacillus thuringiensis* (Bt)

### INTRODUCTION

Groundnut (*Arachis hypogea*) is one of the principal oilseed crops grown in India, covering nearly half of the area under oilseeds. The tobacco caterpillar, *Spodoptera litura* (F.), has been reported as one of the major insect pest of groundnut and feed on 112 cultivated food plants all over the world<sup>1</sup>. of which 40 are grown in India<sup>2</sup>. The management of *S. litura* using insecticides has become difficult because of the development of resistance and effect to non-target organisms. Frequent use of these insecticides poses increasing problems

for human health and the environment. Biological control of insect pests is one of the most important components of Integrated Pest Management (IPM), wherein entomopathogens such as bacteria, viruses and fungi are exploited against insect pests. The insecticidal bacterium *Bacillus thuringiensis* (Bt) has been employed globally for insect pest management on several crops. Nano particles demonstrate unique targeted characteristics with elevated strength, high conductance of electricity and extra chemical reactivity<sup>3</sup>.

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To enhance the activity of the *Bt* by adding the growth promoting nutrients like calcium, magnesium, iron and zinc nanoparticles the present study was undertaken.

## MATERIAL AND METHODS

### Preparation of Nanoparticulate Solutions:

Oxide nanoparticles of Zn, Ca, Mg and Fe weighing 250 mg was added to 500 ml of distilled water (500 ppm) and from this solution different concentrations (100, 50, 20 and 10 ppm) of nanoparticulate solutions were prepared by adding the respective volumes of distilled water.

From the prepared nanoparticulate solutions Zn, Fe, Ca and Mg at 10 ppm, 20 ppm, 50 ppm, 100 ppm and 500 ppm in 1:9 ration (1ml of nanoparticulate solution to 9ml of LBA media) was added to the LBA media before sterilisation to study the catalytic activity of nanomaterials on the *Bt*. Similarly the nanoparticles of Zn, Fe, Ca & Mg.

### Evaluation of Nanomaterials on the Growth and Multiplication of *Bt*:

#### Preparation of LBA media:

Hiveg LBA media 60 g (Himedia make) with the composition of Agar, Tryptone, Yeast and Nacl was added to was added to 1000ml of

distilled water in a Borosil beaker. After stirring, the media was poured into 250ml conical flasks @ 100 ml / flask, and plugged with sterilized nonabsorbent cotton plugs. The conical flasks with medium were sterilized by autoclaving at 15 psi pressure for 15min at 121 °C. Then cooled and stored in the incubator at 22 °C.

#### Inoculation of *Bt* to the Nanobased Media:

From *Bt* strain 375 one loop full of bacteria was inoculated into 1 ml of LB broth and incubated at 25 °C over night. The culture was mixed into 9 ml of sterile water, from this 1ml was taken out and mixed into 10 ml of sterile distilled water, like wise 10<sup>-9</sup> serial dilutions were made. From this 0.1 ml was taken out by using micro pipette and dropped into petriplate and spread with L rod and wrapped with polythene film under Laminar Air Flow chamber. These plates were kept for incubation at 28±1 °C for 24 h.

#### Spore Assessment :

After 24 h incubation colony forming units (cfu) count was taken with the help of colony counter.

Colony forming units were calculated by using the following formula,

$$\text{Cfu/g} = \frac{\text{Average Number of Colonies} \times \text{Dilution Factor}}{\text{Volume Plated}}$$

## RESULTS AND DISCUSSION

In order to study the influence of Mg, Ca, Fe and Zn nanoparticles on *Bt* growth the nano solutions were added to Luria Bertani Agar media at 10, 20, 50, 100 and 500ppm concentrations. *Bt* strain 375 was inoculated to the media at 10<sup>-9</sup> serial dilution. The number of colony forming units were counted after 24h of inoculation and calculated per gram volume plated and the results were given in the table 1.

During the year 2016 the maximum number of cfu/g 17.5 were recorded with MgO at 50ppm concentration followed by 10.3 with 100ppm, 9.4 with 500ppm, 7.6 with 20ppm and 6.6 with 10ppm. Similarly 17.4 with CaO 20ppm, 8.4 with 50ppm, 8.1 with 100ppm, 7.7 with 10ppm and 7.5 with

500ppm. Simultaneously with ZnO the maximum number of colony forming units 12.8 recorded at 10ppm, followed by 7.4 with 20ppm, 7.1 with 50ppm, 7.2 with 100ppm and 6.8 with 500ppm. Whereas with FeO the highest number of cfu/g 11.9 recorded with 50ppm, followed by 8.1 with 100ppm, 7.6 with 10ppm, 7.5 with 500ppm, 7.4 with 20ppm. Where as in control it was 6.6 cfu/g.

Observations were recorded at regular intervals and indicated that the above results, all the four nanoparticles promoted the growth of all biopesticides at certain concentrations. These results are on par with Joshi *et al.*<sup>4</sup>, who observed the maximum growth of *Halophilic* bacteria by the supplementation of metal ions.

Observations on growth of the nanobased biopesticides revealed that, growth of biopesticide organisms was inhibited with the increasing concentrations of nanoparticles. These results are corroborated with Schacht *et al.*<sup>5</sup>, who tested the AgO nanoparticles for its

antibacterial properties. They observed that Ag(o) concentrations above 80  $\mu\text{g ml}^{-1}$  resulted in complete irreversible inhibition of microbial growth, whereas at 20-60  $\mu\text{g ml}^{-1}$  concentrations maximum growth was observed.

**Table 1. Influence of different nanoparticles on growth of *B. thuringiensis* (Cfu g<sup>-1</sup>) in LBA media at different concentrations during the year 2016**

S.No.	Treatment	No.of cfu g <sup>-1</sup>
		2016
1	Magnesium oxide (MgO) 10ppm	6.8 x 10 <sup>9gh</sup>
2	Magnesium oxide (MgO) 20ppm	7.6 x 10 <sup>9egh</sup>
3	Magnesium oxide (MgO) 50ppm	17.5 x 10 <sup>9a</sup>
4	Magnesium oxide (MgO) 100ppm	10.3 x 10 <sup>9bc</sup>
5	Magnesium oxide (MgO) 500ppm	9.4 x 10 <sup>9cd</sup>
6	Calcium oxide (CaO) 10ppm	7.7 x 10 <sup>9eg</sup>
7	Calcium oxide (CaO) 20ppm	17.4 x 10 <sup>9a</sup>
8	Calcium oxide (CaO) 50ppm	8.4 x 10 <sup>9e</sup>
9	Calcium oxide (CaO) 100ppm	8.1 x 10 <sup>9e</sup>
10	Calcium oxide (CaO) 500ppm	7.5 x 10 <sup>9egh</sup>
11	Zinc oxide (ZnO) 10ppm	12.8 x 10 <sup>9b</sup>
12	Zinc oxide (ZnO) 20ppm	7.4 x 10 <sup>9egh</sup>
13	Zinc oxide (ZnO) 50ppm	7.1 x 10 <sup>9egh</sup>
14	Zinc oxide (ZnO) 100ppm	7.2 x 10 <sup>9gh</sup>
15	Zinc oxide (ZnO) 500ppm	6.6 x 10 <sup>9h</sup>
16	Iron oxide (Fe <sub>2</sub> O <sub>3</sub> ) 10ppm	7.6 x 10 <sup>9eg</sup>
17	Iron oxide (Fe <sub>2</sub> O <sub>3</sub> ) 20ppm	7.4 x 10 <sup>9gh</sup>
18	Iron oxide (Fe <sub>2</sub> O <sub>3</sub> ) 50ppm	11.9 x 10 <sup>9b</sup>
19	Iron oxide (Fe <sub>2</sub> O <sub>3</sub> ) 100ppm	8.1 x 10 <sup>9e</sup>
20	Iron oxide (Fe <sub>2</sub> O <sub>3</sub> ) 500ppm	7.5 x 10 <sup>9egh</sup>
21	Control	6.6 x 10 <sup>9egh</sup>
	C.D.	10.17
	SE(m)	4.0
	SE(d)	5.7
	C.V.	3.8

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