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Evaluation of the *Bacillus thuringiensis* Grown under Nanomaterial Enriched Media for Its Relative Efficacy against *S. litura* Under Laboratory Conditions

M. Gayathri[1], N. C. Venkateswarlu[2], T. Muralikrishna[3], R. Sarada Jayalakshmi Devi[4], T.N.V.K. Prasad[5] and G. Mohan Naidu[6]

[1]Ph.D Scholor, Entomology, S.V. Agril College, Tirupati,

[2]Professor & Head, Dept. of Entomology, Tirupathi

[4]Professor & Head, Dept. of Plant Pathology, Tirupathi

Acharya N.G. Ranga Agricultural University, Tirupathi

[3]Project Coordinator, KVK, Kalyanadurg

[5]Principal Scientist, Soil Science, IFT, Tirupati

[6]Professor & Head, Dept of Statistics & Computer Applications

*Corresponding Author E-mail: gayathrimungara@gmail.com

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ABSTRACT

The bioassay studies of S.litura treated with nano enriched Bt recorded the highest cumulative per cent mortality (96.67) with CaO based Btat 20 ppm which is on par with MgO based Bt (96.67) at 50 ppm, followed by FeO (90.01) 10 ppm and ZnO (86.67) at 20ppm. Where as in control it was 10.00 per cent.

Key words: *Bacteria, Viruses, Entomopathogens, Bacillus thuringiensis(Bt)*

INTRODUCTION

In India, it is cultivated in an area of 750,000 ha with production of 6048000 tonnes and productivity is 7462 kg/ ha. The total production of in shell groundnut in Andhra Pradesh was estimated at 603000 tonnes with an average yield of 595 kg/ha with an area of 1013000 ha. The tobacco caterpillar, *Spodoptera litura* (F.), passes through 5-6 overlapping generations annually[3,2]. and if not controlled timely, it may causes in huge crop losses ranging from 25.8-100 percent in various parts of India[1].The management of *S. litura* using insecticides has become difficult because of the development of resistance and effect to non-target organisms. 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.

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It has proven itself to be a valuable tool for the control of lepidopteran insects on vegetables, cotton, soybean, hardwood and coniferous forests. *Bt* toxins have been employed as topical pesticides against pests like *Helicoverpa armigera*, *Plutella xylostella*, *Ostrinia nubilalis*, *Agrotis ipsilon*, *Achaea janata*, *Spodoptera Exigua*, etc[7].The nanoparticles are of the size of 10^{-9} m in diameter with distinctive chemical, physical and biological properties[4].It is also supposed that efficiency of pesticides will be increased due to the development of nanostructured catalysts in coming years with reduced doses. To know the influence of the calcium, magnesium, iron and zinc (at nano scale) enriched *Bt*against *S. litura* the present investigations were carried out.

MATERIAL AND METHODS

Mass Multiplication of *S. litura*

For multiplication and maintenance of *S. litura* population in the laboratory equipment like wire cages for oviposition, plastic troughs for larval maintenance, plastic boxes for pupae were cleaned with ethanol and were dried under solar radiation. The mother culture of *S. litura* egg masses were collected from field and surface sterilized with 4 per cent formaldehyde, washed 3-4 times with distilled water and kept in plastic transparent covers tied with rubber band. Castor leaves were provided to freshly emerged spodoptera larvae. After three days, the larvae were segregated based on size into transparent plastic rearing troughs and covered with muslin cloth. The food material was changed twice in a day till pupation. Before pupation, larvae were transferred to another plastic container containing sterilized soil and allowed them for pupation. The emerged moths were transferred to oviposition cages. Cotton swab dipped in 10 per cent honey solution was kept in cage as food for the adults. For oviposition by the female moths fresh castor plant with tender twigs were arranged in conical flasks containing $3/4^{th}$ of water and kept in cages. Egg masses were collected every day and later sterilized. After hatching, the larvae were reared up to third instar for further laboratory bioassay studies.

3.3.2 Bioassay of *Bt* Grown on Nano based Media against *S. litura*

Bt (375 strain), which showed highest cfu count with Zn, Ca, Mg and Fe Nano based media at 10, 20, 50, 100 and 500 ppm concentrations were selected to conduct bioassay studies against *S. litura* along with reference *Bt* grown on without nano based media to ascertain their insecticidal activity. The individual treatment was streaked on Luria Bertani Agar plates and incubated overnight at 37°C. One loop of overnight cultures was inoculated in Luria broth and kept for sporulation under shaking condition at 28°C for 24 h.

The bioassay studies were conducted in the laboratory followed by leaf dip bioassay method developed by Shelton *et al.*[5]. Groundnut compound leaf containing four leaflets (quadrate leaf) was dipped for 10 minutes into *Bt* culture broth ($5x10^8$ CFU $1mL^{-1}$) containing 0.2 per cent Triton X-100, later leaflets were taken out and kept for air drying till leaf surface free from moisture . After drying, the petiole of leaf was swabbed with wet cotton to maintain leaf succulence and turgidity. Two compound leaves were used for one replication. which was placed in a Petri plate. Ten larvae were released per each treatment which was replicated thrice. In control treatment the leaflets (quadrat leaf) were dipped in distilled water and served as control. The larval mortality was assessed after 72 h at regular intervals.

RESULTS AND DISCUSSION

The *Bt*strain 375 was inoculated on Luria Bertani Agar plates added with different nano materials. After 18h of growth a single colony was inoculated into 1ml of Luria Bertanibroth and allowed to grow for 24h and later inoculated formulation was tested against third instar larvae of *S. litura* at $5 x10^8$ CFU ml^{-1}. A series of bioassays were conducted by providing groundnut leaves which were dipped into LB broth containing *Bt* and shade dried. Later inoculated leaves were kept in petridish and released ten *S. litura* larvae. The mortality was recorded daily up to pupation (4.11).

During 2016 the mortality per cent was recorded from 72 hours after treatment and it was ranged from 26.67 to 83.33 with MgO based *Bt*, 26.67 and 86.67 with CaO based *Bt*, 26.67 and 60.00 with ZnO based *Bt* and 36.67 and 66.67 per cent with FeO based *Bt*. The highest mortality per cent 86.67 was recorded with CaO at 20ppm, followed by 83.33 per cent with MgO at 50ppm,66.67 per cent with FeO 50ppm and 60.00 per cent with ZnO 10ppm.Where as in *Bt* without nano based it was recorded as 16.67 per cent and in control it was 3.33 per cent.

The mortality per cent 96 hours after treatment ranged from 10 to 33.33 per cent in different treatments. The highest per cent mortality 33.33 per cent was recorded with MgO at 100 and 500ppm. The per cent mortality 120 hours after treatment was ranged between 3.33 and 16.67 per cent. Later the mortality per cent was gradually decreased. The cumulative per cent mortality was ranged from 9.99 per cent to 96.67 per cent in different treatments. The highest per cent mortality (96.67) was recorded with CaO based *Bt* which is on par with MgO based *Bt* (96.67), followed by FeO (90.01) and ZnO (86.67). Whereas treatment *Bt* without nanoparticles the mortality was 46.67 per cent and in control it was 10.00 per cent.

Table 4.11. Influence of different nanoparticles on the efficacy of *B. thuringiensis* at different concentrations during the year 2016

S.No.	Treatment	Per cent mortality					
		72h	96h	120h	144h	168h	Cumulative
1	Magnesium oxide (MgO) 10ppm	26.67 (31.09)bc	16.67 (24.10)abcd	0 (0.00)	0 (0.00)	0 (0.00)	43.34 (41.17)b
2	Magnesium oxide (MgO) 20ppm	43.33 (41.17)def	23.33 (28.88)bcd	6.67 (14.97)ab	0 (0.00)	0 (0.00)	73.33 (58.91)def
3	Magnesium oxide (MgO) 50ppm	83.33 (65.90)i	13.33 (21.41)abc	0 (0.00)	0 (0.00)	0 (0.00)	96.66 (79.47)g
4	Magnesium oxide (MgO) 100ppm	43.33 (41.17)def	33.33 (35.26)d	10 (18.43)ab	0 (0.00)	0 (0.00)	86.66 (68.58)fg
5	Magnesium oxide (MgO) 500ppm	40.00 (39.23)cde	33.33 (35.26)d	0 (0.00)	0 (0.00)	3.33 (10.51)	76.66 (61.11)def
6	Calcium oxide (CaO) 10ppm	56.67 (48.83)fgh	16.67 (24.10)abcd	0 (0.00)	0 (0.00)	0 (0.00)	73.34 (58.91)def
7	Calcium oxide (CaO) 20ppm	86.67 (68.59)i	10 (18.43)ab	0 (0.00)	0 (0.00)	0 (0.00)	96.67 (79.49)]g
8	Calcium oxide (CaO) 50ppm	50 (45.00)defh	30 (33.21)cd	0 (0.00)	0 (0.00)	0 (0.00)	80 (63.43)efg
9	Calcium oxide (CaO) 100ppm	43.33 (41.17)def	30 (33.21)cd	0 (0.00)	3.33 (10.51)	0 (0.00)	76.66 (61.11)def
10	Calcium oxide (CaO) 500ppm	26.67 (31.09)bc	30 (33.21)cd	16.67 (24.10)b	0 (0.00)	0 (0.00)	73.34 (58.91)def
11	Zinc oxide (ZnO) 10ppm	60 (50.77)hgf	20 (26.57)abcd	6.67 (14.97)ab	0 (0.00)	0 (0.00)	86.67 (68.59)fg
12	Zinc oxide (ZnO) 20ppm	46.67 (43.09)dgef	23.33 (28.88)bcd	6.67 (14.97)ab	0 (0.00)	0 (0.00)	76.67 (61.12)def
13	Zinc oxide (ZnO) 50ppm	43.33 (41.17)def	16.67 (24.10)abcd	6.67 (14.97)ab	0 (0.00)	0 (0.00)	66.67 (54.74)cde
14	Zinc oxide (ZnO) 100ppm	33.33 (35.26)cd	16.67 (24.10)abcd	0 (0.00)	0 (0.00)	0 (0.00)	50.00 (45.00)bc
15	Zinc oxide (ZnO) 500ppm	26.67 (31.09)bc	16.67 (24.10)abcd	0 (0.00)	0 (0.00)	0 (0.00)	43.34 (41.17)b
16	Iron oxide (Fe_2O_3) 10ppm	36.67 (37.27)cde	20.00 (26.57)abcd	3.33 (10.51)ab	0 (0.00)	0 (0.00)	60.00 (50.77)bcd
17	Iron oxide (Fe_2O_3) 20ppm	40 (39.23)cde	20 (26.57)abcd	13.33 (21.41)ab	0 (0.00)	0 (0.00)	73.33 (58.91)def
18	Iron oxide (Fe_2O_3)50ppm	66.67 (54.74)h	16.67 (24.10)abc	6.67 (14.97)ab	0 (0.00)	0 (0.00)	90.01 (71.57)fg
19	Iron oxide (Fe_2O_3) 100ppm	43.33 (41.17)def	23.33 (28.88)bcd	0 (0.00)	0 (0.00)	0 (0.00)	66.66 (54.73)cde
20	Iron oxide (Fe_2O_3)500ppm	36.67 (37.27)cde	23.33 (28.88)bcd	3.33 (10.51)ab	3.33 (10.51)	0 (0.00)	66.66 (54.73)cde
21	*Bt* without nano	16.67 (24.10)ab	20 (26.57)abcd	10 (18.43)ab	0 (0.00)	0 (0.00)	46.67 (43.09)b
22	Control	3.33 (10.51)a	3.33 (10.51)a	3.33 (10.51)ab	0 (0.00)	0 (0.00)	9.99 9 (18.43)a
	C.D.	10.16	8.13	2.82	4.54	N/A	7.04
	SE(m)	3.55	2.84	3.98	1.59	1.01	2.46
	SE(d)	5.03	4.02	30.44	2.25	1.42	3.48
	C.V.	13.63	19.23	33.33	67.28	574.46	6.28

Figures in parentheses are arcsine transformed values
Alphabets indicating Duncan Multiple Range Test (DMRT)

The results revealed that, highest pet cent mortality was recorded with MgO @ 50ppm, CaO 20ppm fortified growth media for *Bt*. The results revealed that the significant highest per cent mortality was observed at 72h with *Bt* grown under nanoparticles fortified CaO at 20ppm, MgO at 50ppm, FeO at 10ppm and ZnO at 20ppm enrichedbiopesticides when compared with biopesticides without nanoparticles as well as control. The studies of Valicente *et al*.[6], on the influence of mineral salts of $FeSO_4$, $ZnSO_4$, $MnSO_4$ and $MgSO_4$ when added to LBA media at a concentration of 0.002g, 0.02g, 0.02g and 0.03g respectively, resulted an increased number of viable spores 2 x 10^8 cells/ml of *Bt* compared to control and as well as reported higher efficacy of 60 per cent mortality against first instar larvae of *S.frugiperda* under laboratory conditions.

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