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Evaluation of the *Beauveria bassiana* Grown under Nanomaterial Enriched Media for its Relative Efficacy against *S. litura* under Laboratory Conditions

M. Gayathri[1*], N.C. Venkateswarlu[1], T. Murali Krishna[2] and K. Devaki[1]

[1]Department of Entomology, S.V. Agricultural College, Tirupati 517502, India
[2]KVK, Kalyandurg, Ananthapur district, 515761, India

**Corresponding author*

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A B S T R A C T

Beauveria bassiana (Balsamo) Vuillemin (Ascomycota: Hyphocreales) is a facultative pathogen with wide host range. The bioefficacy of entomopathogens in relation to number of conidia in *B.bassiana* were chance to increase the bioefficacy with respect to the mortality of lepidopteran larvae by adding minerals. These minerals are namely calcium, magnesium, iron and zinc were added to SDAY media at 10, 20, 50, 100 and 500 ppm. As a result the efficacy of entomopathogns will increase. The cumulative per cent mortality with nano enriched *B. bassiana* was ranged between 16.67 and 90.00 per cent in different treatments. The highest per cent mortality (90.00) was recorded with MgO at 50 ppm which is on par with CaO 20 ppm and FeO 10 ppm based *B. bassiana*, followed by ZnO at 10 ppm (83.33). Where as in control it was 16.67 per cent during 2016. Similarly during 2017 also the same treatments have given the highest per cent mortality compare to other treatments.

Introduction

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 (Mousa *et al.*, 1980) of which 40 are grown in India (Basu, 1981, Muthukrishnan *et al.*, 2005). It passes through 5-6 overlapping generations annually (Sasidharan and Varma, 2005; Kumar and Chapman, 2006) and if not controlled timely, it may causes in huge crop losses ranging from 25.8-100 percent in various parts of India (Ahmad *et al.*, 2005).

The management of *S. litura* using insecticides has become difficult because of the development of resistance and effect to non-target organisms *viz*., natural enemy population as well as frequent use of these insecticides increasing problems of human health and environmental pollution.

Biological control of insect pests is one of the most important component of Integrated Pest Management (IPM), wherein entomopathogens such as bacteria, viruses and fungi are exploited against insect pests.

Although 700 to 750 species of EPF have been reported as pathogenic to insects but only about a dozen have been exploited for insect control (Stark and Banks, 2003). Among these *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hyphocreales) is a facultative pathogen with wide host range (Armes *et al.,* 1997; Sahayaraj *et al.,* 2007). This fungus has potential to control over 70 insect pests belonging to different orders particularly lepidopteran pests, infesting various crops and appears to be innocuous to most non target organisms.

The bioefficacy of entomopathogens in relation to number of conidia in *B.bassiana* were chance to increase the bioefficacy with respect to the mortality of lepidopteran larvae by adding minerals. These minerals are namely calcium, magnesium, iron and zinc will enhance the conidial count in *B.bassiana.* As a result the efficacy of entomopathogens will increases. The nanoparticles of these minerals will increase the conidial count in *B.bassiana* and also chance to decrease the dose of biopesticides. Nanoparticles are atomic or molecular aggregates characterized by size less than 100 nm. These are actually modified form of basic elements derived by altering their atomic as well as molecular properties of elements (Suchea, *et al.,* 2006).

To enhance the biopesticides efficacy interms of increasing the number of conidial in *B.bassiana* the mineral salts *viz*., Calcium, magnesium, iron and zinc were added to the media before inoculation (Valicente *et al.,* 2010).

By considering all these issues, to know the impact of nanobased material on bio pesticides to enhance their efficacy for longer periods and their combined effect on *S.litura* management, the present study has been taken up.

Materials 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 ratio (1ml of nanoparticulate solution to 9ml of LBA media) was added to the Sabouraud Dextrose Agar media (SDAY) media before sterilisation to study the relative efficacy of *B.bassiana* against *S.litura*.

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 3/4th 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.

Bioassay of *B.bassiana* grownon Nanomaterial based media against *S. litura*

To know the efficiency of *B.bassiana* grown on nanomaterial based media, bioassay studies were conducted in laboratory against *S.litura*. The spore suspension was prepared from 15 days old culture of *B.bassiana* along with added nanomaterials *viz*., Zn, Ca, Mg and Fe at 10, 20, 50, 100 and 500 ppm concentration and without nanomaterials was evaluated against *S.litura* on 3rd instar larvae at 10 days interval. Sterilized plastic troughs (20 cm x 20 cm) were taken into which one groundnut compound leaf containing four leaflets (quadrate leaf) was dipped for 10 minutes into culture broth ($1x10^8$to $1x10^5$ spores $1mL^{-1}$), 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. One compound leaf was 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 120 h at regular intervals.

For each treatment ten third instar *S.litura* larvae were released to crawl on the treated leaf and daily observations were recorded on post treatment changes of larvae, larval mortality, pupal mortality, malformed pupae and adults until to death. Each treatment was replicated thrice along with an untreated control (Hafez *et al.*, 1994). The per cent larval mortality was expressed by using the following formula.

The larval mortality was converted to percentage before subjecting to statistical analysis by using the formula.

Per cent larval mortality =

$$\frac{\text{No. of larvae dead due to infection}}{\text{Total number of larvae treated}} \times 100$$

The per cent mortality was analysed statistically after transforming into angular values.

Results and Discussion

Evaluation of *B. bassiana* grown in nanomaterial enriched media against *S. litura*

B. bassiana (SGB strain) culture was maintained in the laboratory and 5 mm discs were made from four days old culture and inoculated in to SDAY plates which were added with different nanomaterials at different concentrations. Then the culture was allowed to grow for 15 days. Then spore suspension was prepared by mixing with 10 ml of sterile distilled water. A series of bioassays were conducted by providing groundnut leaves which were dipped into spore suspensions containing *B.bassiana*. Then the mortality rate was recorded daily up to pupation and results are presented in the table 1 and 2.

During 2016, the mortality of *S.itura* after 120 hours of treatment was ranged from 6.67 to 76.67 per cent. The maximum mortality of 76.67 per cent was recorded with MgO based *B.bassiana* at 50 ppm and 73.33 with CaO based *B.bassiana*, at 20 ppm and 56.67 with ZnO based *B.bassiana* and 20.00 and 43.33 per cent with FeO based *B.bassiana*.

Table.1 Influence of different nanoparticles on the efficacy of *B.bassiana* against *S.litura* at different concentrations during the year 2016

S.No.	Name of the Treatment	*S.litura* Per cent mortality				
		120h	**144h**	**168h**	**192h**	**Cumulative**
1	Magnesium oxide (MgO) 10 ppm	36.67 (33.21)cdef	23.33 (28.88)bcde	6.67 (14.96)	0.00 (0.00)	66.67 (54.74)ab
2	Magnesium oxide (MgO) 20 ppm	33.33 (43.09)def	40.00 (3923)ab	10.00 (18.43)	0.00 (0.00)	83.33 (65.91)ab
3	Magnesium oxide (MgO) 50 ppm	76.67 (58.91)a	13.33 (21.42)de	0.00 (0.00)	0.00 (0.00)	90.00 (71.57)a
4	Magnesium oxide (MgO) 100 ppm	43.33 (41.17)cd	30.00 (33.21)abcd	6.67 (14.96)	0.00 (0.00)	80.00 (63.43)ab
5	Magnesium oxide (MgO) 500 ppm	33.33 (35.26)def	40.00 (39.22)ab	3.33 (10.52)	0.00 (0.00)	76.67 (61.12)ab
6	Calcium oxide (CaO) 10 ppm	23.33 (28.88)efg	46.67 (49.09)a	6.67 (14.96)	0.00 (0.00)	76.67 (61.12)ab
7	Calcium oxide (CaO) 20 ppm	73.33 (58.91)a	16.67 (24.09)cde	0.00 (0.00)	0.00 (0.00)	90.00 (71.57)a
8	Calcium oxide (CaO) 50 ppm	23.33 (28.88)efg	36.67 (37.27)ab	16.67 (24.09)	0.00 (0.00)	76.67 (61.12)ab
9	Calcium oxide (CaO) 100 ppm	30.00 (33.21)def	33.33 (35.26)abc	10.00 (18.43)	0.00 (0.00)	73.33 (58.91)ab
10	Calcium oxide (CaO) 500 ppm	26.67 (31.09)def	33.33 (35.26)abc	10.00 (18.43)	0.00 (0.00)	70.00 (56.79)ab
11	Zinc oxide (ZnO) 10 ppm	43.33 (41.17)cd	33.33 (35.26)abc	6.67 (14.96)	0.00 (0.00)	83.33 (65.91)ab
12	Zinc oxide (ZnO) 20 ppm	53.33 (46.91)bc	16.67 (24.09)cde	10.00 (18.43)	0.00 (0.00)	76.67 (61.12)ab
13	Zinc oxide (ZnO) 50 ppm	26.67 (31.09)def	36.67 (37.27)ab	6.67 (14.96)	0.00 (0.00)	70.00 (56.79)ab
14	Zinc oxide (ZnO) 100 ppm	30.00 (33.21)def	33.33 (35.26)abc	13.33 (21.42)	0.00 (0.00)	83.33 (65.91)ab
15	Zinc oxide (ZnO) 500 ppm	20.00 (26.57)fg	33.33 (35.26)abc	13.33 (21.42)	0.00 (0.00)	80.00 (63.43)ab
16	Ferrous oxide (Fe_2O_3) 10 ppm	63.33 (52.73)ab	23.33 (28.88)bcd	0.00 (0.00)	0.00 (0.00)	86.67 (68.58)a
17	Ferrous oxide (Fe_2O_3) 20 ppm	36.67 (37.27)cdef	33.33 (35.26)abc	10.00 (18.43)	0.00 (0.00)	80.00 (68.43)ab
18	Ferrous oxide (Fe_2O_3)50 ppm	33.33 (35.26)def	33.33 (35.26)abc	6.67 (14.96)	0.00 (0.00)	73.33 (58.91)ab
19	Ferroous oxide (Fe_2O_3) 100 ppm	40.00 (39.23)cde	16.67 (24.09)cde	10.00 (18.43)	0.00 (0.00)	73.33 (58.91)ab
20	Ferrous oxide (Fe_2O_3)500 ppm	30.00 (33.21)def	23.33 (28.88)bcde	13.33 (21.42)	0.00 (0.00)	73.33 (58.91)ab

21	*Bb* without nano	23.33 (28.88)efg	16.67 (24.09)cde	13.33 (21.42)	0.00 (0.00)	60.00 (50.77)b
22	Control	6.67 (14.96)g	6.67 (1496)e	3.33 (10.52)	0.00 (0.00)	16.67 (24.09)c
	C.D.	6.427	8.859	6.427		10.754
	SE(m)	2.247	3.098	2.247		3.761
	SE(d)	3.178	4.381	3.178		5.318
	C.V.	12.234	25.848	88.588		10.856

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

Table.2 Influence of different nanoparticles on the efficacy of *B.bassiana* against *S.litura* at different concentrations during the year 2017

S.No.	Name of the Treatment	*S.litura* Per cent mortality				
		120h	**144h**	**168h**	**192h**	**Cumulative**
1	Magnesium oxide (MgO) 10 ppm	43.33 (41.17)bcd	23.33 (28.88)bcdef	6.67 (14.96)abc	0.00 (0.00)	73.33 (58.91)bcde
2	Magnesium oxide (MgO) 20 ppm	43.33 (41.17)bcd	30.00 (33.21)abcde	6.67 (14.96)abc	0.00 (0.00)	80.00 (63.43)abcd
3	Magnesium oxide (MgO) 50 ppm	80.00 (63.43)a	13.33 (21.42)ef	0.00 (0.00)c	0.00 (0.00)	93.33 (75.04)a
4	Magnesium oxide (MgO) 100 ppm	46.67 (43.09)bc	30.00 (33.21)abcde	10.00 (18.43)abc	0.00 (0.00)	86.67 (68.58)ab
5	Magnesium oxide (MgO) 500 ppm	33.33 (35.26)cdef	30.00 (33.21)abcde	13.33 (21.42)abc	0.00 (0.00)	76.67 (61.12)abcd
6	Calcium oxide (CaO) 10 ppm	26.67 (31.09)def	43.33 (41.17)a	10.00 (18.43)abc	0.00 (0.00)	80.00 (63.43)abcd
7	Calcium oxide (CaO) 20 ppm	76.67 (61.12)a	16.67 (21.09)cdef	0.00 (0.00)c	0.00 (0.00)	93.33 (75.04)a
8	Calcium oxide (CaO) 50 ppm	33.33 (35.26)cdef	40.00 (39.23)ab	10.00 (18.43)abc	0.00 (0.00)	83.33 (65.91)abc
9	Calcium oxide (CaO) 100 ppm	30.00 (33.21)cdef	30.00 (33.21)abcde	16.67 (24.09)ab	0.00 (0.00)	76.67 (61.12)abcd
10	Calcium oxide (CaO) 500 ppm	33.33 (35.26)cdef	30.00 (33.21)abcde	0.00 (0.00)c	0.00 (0.00)	63.33 (52.73)def
11	Zinc oxide (ZnO) 10 ppm	36.67 (37.27)bcde	20.00 (26.57)cdef	10.00 (18.43)abc	0.00 (0.00)	83.33 (65.91)abc
12	Zinc oxide (ZnO) 20 ppm	36.67 (37.27)bcde	36.67 (37.27)abc	3.33 (10.52)bc	0.00 (0.00)	76.67 (61.12)abcd
13	Zinc oxide (ZnO) 50 ppm	40.00 (39.23)bcde	26.67 (31.09)abcde	10.00 (18.43)abc	0.00 (0.00)	76.67 (61.12)abcd
14	Zinc oxide (ZnO) 100 ppm	30.00 (33.21)cdef	26.67 (31.09)abcde	20.00 (26.57)a	0.00 (0.00)	76.67 (61.12)abcd
15	Zinc oxide (ZnO) 500 ppm	30.00 (33.21)cdef	23.33 (28.88)bcdef	10.00 (18.43)abc	0.00 (0.00)	63.33 (52.73)def
16	Ferrous oxide (Fe_2O_3) 10 ppm	53.33 (46.91)bd	33.33 (35.26)abcd	10.00 (18.43)abc	0.00 (0.00)	80.00 (63.43)abcd
17	Ferrous oxide (Fe_2O_3) 20 ppm	36.67 (37.27)bcde	20.00 (26.57)cdef	10.00 (18.43)abc	0.00 (0.00)	66.67 (54.74)cdef

18	Ferrous oxide (Fe_2O_3)50 ppm	36.67 37.27)bcde	23.33 (28.88)bcdef	6.67 (14.96)abc	0.00 (0.00)	66.67 (54.74)cdef
19	Ferroous oxide (Fe_2O_3) 100 ppm	23.33 (28.88)ef	33.33 (35.26)abcd	0.00 (0.00)c	0.00 (0.00)	56.67 (58.83)ef
20	Ferrous oxide (Fe_2O_3)500 ppm	16.67 (24.09)fg	33.33 (35.26)abcd	6.67 (14.96)abc	0.00 (0.00)	56.67 (58.83)ef
21	*Bb* without nano	23.33 (28.88)ef	20.00 (26.57)cdef	10.00 (18.43)abc	0.00 (0.00)	53.33 (46.91)f
22	Control	3.33 (10.52)g	6.67 (14.96)f	3.33 (10.52)bc	0.00 (0.00)	13.33 (21.42)g
	C.D.	9.089	8.859	7.604	-	9.532
	SE(m)	3.178	3.098	2.659	-	3.333
	SE(d)	4.495	4.381	3.761	-	4.714
	C.V.	14.89	20.007	58.456	-	8.056

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

The highest *S.itura* mortality of 76.67 per cent was recorded with MgO at 50 ppm, followed by 73.33 per cent with CaO at 20 ppm, 63.33 per cent with FeO 10 ppm and 56.67 per cent with ZnO 10 ppm. Where as in *B.bassiana* without nanomaterial based it was recorded as 43.33 per cent and in control it was 6.67 per cent.

The mortality per cent after 144 hours of the treatment ranged from 6.67 to 46.67 per cent in different treatments. The highest per cent mortality 46.67 per cent was recorded with CaO at 10 ppm. The per cent mortality 168 hours after treatment was ranged between 3.33 and 16.67 per cent, and the mortality per cent was gradually decreased.

The cumulative *S.itura* larval mortality was ranged between 16.67 and 90.00 per cent in different treatments. The highest per cent mortality (90.00) was recorded with MgO based *B.bassiana* at 50 ppm which is on par with CaO 20 ppm and FeO 10 ppm based *N.rileyi*, followed by ZnO at 10 ppm (83.33). Where as in control it was 16.67 per cent.

During 2017, the mortality of *S.itura* after 120 hours of the treatment recorded was ranged from 33.33 to 80.00 with MgO based *B.bassiana*, 26.67 to 76.67 with CaO based *B.bassiana*, 16.67 and 53.00 with FeO based *B.bassiana* and 30.00 and 40.00 per cent with ZnO based *B.bassiana*. The highest mortality per cent 80.00 was recorded with MgO at 50 ppm, followed by 76.67 per cent with CaO at 20 ppm, 53.33 per cent with FeO 10 ppm and 40.00 per cent with ZnO 50 ppm. Where as in *B.bassiana* without nanomaterial based it was recorded as 23.33 per cent and in control it was 3.33 per cent.

The mortality *S.itura* after 144 hours of the treatment ranged from 6.67 to 43.33 per cent in different treatments. The highest *S.itura* mortality of 43.33 per cent was recorded with CaO at 10 ppm. The *S.itura* mortality 168 hours after treatment was ranged between 0 and 20.00 per cent and later the mortality per cent was gradually decreased.

The cumulative *S.itura* mortality was ranged from 13.33 per cent to 93.33 per cent in different treatments. The highest *S.itura* mortality (93.33) was recorded with CaO based *B.bassiana* at 20 ppm and MgO 50 ppm, followed by (80.00) FeO 10 ppm based *B.bassiana* and followed by ZnO 10 ppm (83.33) based *B.bassiana*. Where as in *B.bassiana* without nanoparticles was 53.33 per cent and in control it was 13.33 per cent.

The results revealed that, highest *S.itura* mortality was recorded with MgO @ 50 ppm, CaO 20 ppm fortified growth media for *Bt* similarly MgO @ 50 ppm, CaO 20 ppm was effective for enhancing the activity of *B.bassiana* against *S.litura*. The *B.bassiana* grown in nanoparticles enriched media were tested against 3rd instar larvae of *S.litura* under laboratory conditions.

The results revealed that the significant highest per cent mortality was observed at 120h with *B.bassiana* grown under nanoparticles fortified CaO at 20 ppm, MgO at 50 ppm, FeO at 10 ppm and ZnO at 20 ppm enriched biopesticide when compared with biopesticid without nanoparticles as well as control. The studies of Valicente *et al.*, (2010) 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 and these resulted in 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. Similarly Namasivayam *et al.*, (2013) observed the nano sized copper coated chitosan showed the distinct effect on the growth of *N.rileyi* under field conditions.

Summary

The *B.bassiana* grown under nano enriched media was tested against 3rd instar larvae of *S.litura* under laboratory conditions. The results revealed that the significant highest per cent mortality of *S.litura* was observed at 120h after treatment with *B.bassiana* grown under CaO at 20 ppm, MgO at 50 ppm, FeO at 10 ppm and ZnO at 20 ppm nanomaterial enriched media when compared with biopesticides without nanoparticles as well as control.

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