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Studies on influence of magnesium, calcium, ferrous and zinc nanomaterials on production and multiplication of *Beauveria bassiana* (Balsamo)

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Abstract
The entomopathogenic fungus, *Beauveria bassiana* displays a broad host range. More than 200 insect species from nine orders of insects, mainly Lepidoptera and Coleoptera have been recorded as hosts. The *B. bassiana* grown under nano based SDAY media recorded the highest number of conidia 9.1, 8.3, 7.5 and 6.7 per ml was recorded with MgO at 50ppm, CaO at 20 ppm, ZnO at 20 ppm, FeO 10 ppm. Similar trend was observed during 2017 also. The growth of the *B. bassiana* was increased upto certain concentrations of the nanoparticles, later the growth was decreased by increased concentrations. This might be due to disruption or disorganization of bacterial cell walls due to interaction of nanoparticles, induction of intracellular antibacterial effects, including interactions with DNA and proteins and accumulation of nanoparticles in the cytoplasm or on the outer membranes.

Keywords: Magnesium, calcium, ferrous and zinc nanomaterials, *Beauveria bassiana*

1. 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 [1] of which 40 are grown in India [2, 3]. It passes through 5-6 overlapping generations annually [4, 5] and if not controlled timely, it may causes in huge crop losses ranging from 25.8-100 percent in various parts of India [6].
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 population. 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. The entomopathogenic fungus, *Beauveria bassiana* displays a broad host range. More than 200 insect species from nine orders of insects, mainly Lepidoptera and Coleoptera have been recorded as hosts [7]. Nanoparticles demonstrate unique targeted characteristics with elevated strength, high conductance of electricity and extra chemical reactivity [8]. To enhance the activity of the *B. bassiana* by adding the growth promoting nutrients like calcium, magnesium, iron and zinc nanoparticles the present study was undertaken.

2. Materials and Methods
2.1 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 Sabouraud Dextrose Agar medeia (SDAY) media before sterilisation to study their activity on growth and multiplication of *B. bassiana*.

2.2 Evaluation of Nanomaterials on the Growth and Multiplication of *Beauveria bassiana*

Ingredients of SDAY Media

Composition of SDAY medium	
Ingredients	**Weight/Volume**
Agar	15 g
Peptone	10 g
Dextrose	40 g
Yeast	15 g
Distilled water	1000 ml
Chloramphenicol	80 mg l^{-1}

100 ml of nano solutions of Zn, Fe, Mg and Ca which were diluted to required strength of 10, 20, 50, 100 and 500 ppm were added to media before sterilization.

2.3 Procedure of SDAY Preparation

In a 1000 ml Borosil beaker, 500 ml of distilled water with 15 g agar was taken and boiled over gentle flame by constant stirring with a glass rod until the contents attain stickiness. In another beaker, again 500 ml of distilled water was taken and all the remaining above ingredients i.e. peptone, dextrose and yeast of given weight / volume were mixed thoroughly. These contents were added to the agar solution. The medium thus prepared was taken into 250 ml conical flasks @ 100 ml / flask, and plugged with sterilized non-absorbent cotton plugs. The conical flasks with medium were sterilized by autoclaving at 15psi pressure at 121 °C for 15 min. Then cooled and stored in the incubator at 22.°C. Antibiotic chloramphenicol @ 80 mg per litre was added to the sterilized media before pouring into media plates [9].

2.4 Inoculation of *B. bassiana* on SDAY Media

Seven days old culture of *B. bassiana* NR_2 isolate was chosen for the study. Culture discs of 10 mm was separated from and placed on freshly prepared SMAY media which was fortified with nanoparticulate solutions of Ca, Mg, Fe and Zn at the test doses. The plates were incubated in BOD incubator at 20±5 °C till the plates were filled with culture.

2.5 Conidial Count per unit Area

The circular disc of 10 mm diameter was cut randomly from the 10 days old uniformly grown culture plates. Each disc was placed in a test tube containing 10ml distilled water. The spores present in the disc were allowed to disperse uniformly in the water by rotating the test tube on a vortex for 1 minute. Proper care was taken to avoid spillage of the suspension during rotation. The suspension was serially diluted to 10^{-3} and the spores were counted with the help of an improved Neubauer haemocytometer under a compound microscope at 40x magnification and number of spores (Plate 1) present per ml was calculated by using following formula

$$\text{No. of spores per unit} = N \times 400 \times 1000 \times 10 \times D$$

Where,
D = Dilution factor
N = Mean number of spores per square of haemocytometer.
The diameter of the mycelial growth (in mm) was measured 5 days, 10 days and 15 days after inoculation (DAI) (Plate 2).
Substrates with maximum cell or spore load was selected and used for preparation of dust mixtures. This is further subjected for viability and virulence studies in Laboratory.

3. Results and discussion

3.1 Dosage standardization of nanoparticles with *B. bassiana* grown under nanomaterial based SDAY media

The circular disc of 10 mm diameter was cut randomly from the 10 days old uniformly grown culture plates. Each disc was placed in a test tube containing 10 ml distilled water and the spores were counted. The highest number of conidia 9.1 per ml was recorded with MgO at 50 ppm, followed by 6.9, 6.1 with 20 and 100 ppm, 4.8 with 10 ppm then the lowest number of conidia 3.2 resulted at 500 ppm concentration. In case of CaO the highest number of conidia per ml is 8.3 at 20 ppm, 6.2, 4.1, 3.0 and 2.3 at 50 ppm, 10 ppm, 100 ppm and 500 ppm respectively. With ZnO the highest number of 6.7 was recorded at 20 ppm, followed by 3.8, 2.6, 2.6 and 1.6 at 50 ppm, 100 ppm, 10 ppm and 500 ppm concentrations respectively.

The highest number of conidia 7.5 per ml was recorded with FeO at 10 ppm, followed by 4.5, 2.5, 2.3, 1.8 at 20 ppm, 50 ppm, 100 and 500 ppm respectively, where as in control it was recorded as 1.3 during 2016.

Similarly during 2017, the highest number of conidia 8.6 per ml was recorded with MgO at 50 ppm, followed by 6.6 with 100 ppm, 6.5 with 20 ppm, 4.6 with 10 ppm and 3.8 with 500 ppm concentrations. With CaO the highest conidia of 8.4 was recorded at 20 ppm, followed by 6.2 at 50 ppm, 4.1 at 10 ppm, 2.9 at 100 ppm and 2.2 at 500 ppm concentrations. While in ZnO the conidial count was 7.2, 3.5, 3.4, 2.5 and 2.1 with 20, 50, 10, 100 and 500 ppm concentrations respectively. Whereas in case of FeO 8.1, 3.7, 3.0, 2.5, and 2.0 number of conidia per ml was recorded at 10, 20, 50, 100 and 500 ppm concentrations respectively, while in control it was recorded as 1.3 (Table 1 & 2).

The size (diameter) of the fungal disc was measured 5, 10 and 15 days after inoculation. The maximum growth observed was 8.93 mm, 13.73 mm and 51.43 mm was with MgO at 50 ppm, which is on par with CaO 20 ppm, followed by 9.53 mm in 5 DAI, 13.63 mm in 10 DAI and 48.13 mm in 15 DAI with FeO 10 ppm and then followed by ZnO where as in control it was 7.2 mm, 10.93 mm and 28.50 mm during 2016. The similar trend was observed in 2017 also (Table 3 & 4).

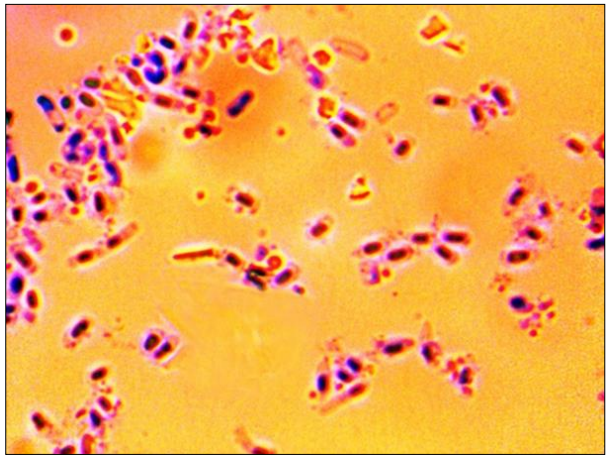

Plate 1: *B. bassiana* conidia

Table 1: Influence of different nanoparticles on growth conidial count of *B. bassiana* in SDAY media at different concentrations during the year 2016.

S. No.	Name of the Treatments	No. of conidia/ml)
		2016
1	Magnesium oxide (MgO) 10 ppm	$4.8x10^{5g}$
2	Magnesium oxide (MgO) 20 ppm	$6.9\ x10^{5\ i}$
3	Magnesium oxide (MgO) 50 ppm	$9.1\ x10^{5\ l}$
4	Magnesium oxide (MgO) 100 ppm	$6.1\ x10^{5\ h}$
5	Magnesium oxide (MgO) 500 ppm	$3.2\ x10^{5d}$
6	Calcium oxide (CaO) 10 ppm	$4.1\ x10^{ef}$
7	Calcium oxide (CaO) 20 ppm	$8.3\ x10^{5k}$
8	Calcium oxide (CaO) 50 ppm	$6.2\ x10^{5h}$
9	Calcium oxide (CaO) 100 ppm	$3.0\ x10^{5d}$
10	Calcium oxide (CaO) 500 ppm	$2.3\ x10^{5c}$
11	Zinc oxide (ZnO) 10 ppm	$2.6\ x10^{5c}$
12	Zinc oxide (ZnO) 20 ppm	$6.7\ x10^{5i}$
13	Zinc oxide (ZnO) 50 ppm	$3.8\ x10^{5e}$
14	Zinc oxide (ZnO) 100 ppm	$2.6\ x10^{5c}$
15	Zinc oxide (ZnO) 500 ppm	$1.6\ x10^{5ab}$
16	Ferrous oxide (Fe_2O_3) 10 ppm	$7.5\ x10^{5j}$
17	Ferrous oxide (Fe_2O_3) 20 ppm	$4.5\ x10^{5\ fg}$
18	Ferrous oxide (Fe_2O_3) 50 ppm	$2.5\ x10^{5c}$
19	Ferrous oxide (Fe_2O_3) 100 ppm	$2.3\ x10^{5c}$
20	Ferrous oxide (Fe_2O_3) 500 ppm	$1.8\ x10^{5b}$
21	Control	$1.3\ x10^{5a}$
	C.D.	2.46
	SE(m)	0.85
	SE(d)	1.21
	C.V	3.42

Alphabets indicating Duncan Multiple Range Test (DMRT)

Table 2: Influence of different nanoparticles on conidial count of *B.bassiana* in SDAY media at different concentrations during the year 2017.

S. No.	Name of the Treatments	No. of conidia/ml)
		2017
1	Magnesium oxide (MgO) 10 ppm	$4.6\ x10^{5g}$
2	Magnesium oxide (MgO) 20 ppm	$6.9\ x10^{5i}$
3	Magnesium oxide (MgO) 50 ppm	$8.6\ x10^{5l}$
4	Magnesium oxide (MgO) 100 ppm	$6.5\ x10^{5h}$
5	Magnesium oxide (MgO) 500 ppm	$3.8\ x10^{5d}$
6	Calcium oxide (CaO) 10 ppm	$4.1\ x10^{5e\ f}$
7	Calcium oxide (CaO) 20 ppm	$8.4\ x10^{5j}$
8	Calcium oxide (CaO) 50 ppm	$6.2\ x10^{5h}$
9	Calcium oxide (CaO) 100 ppm	$2.9\ x10^{5d}$
10	Calcium oxide (CaO) 500 ppm	$2.2\ x10^{5c}$
11	Zinc oxide (ZnO) 10 ppm	$3.4\ x10^{5c}$
12	Zinc oxide (ZnO) 20 ppm	$7.2\ x10^{5i}$
13	Zinc oxide (ZnO) 50 ppm	$3.5\ x10^{5c}$
14	Zinc oxide (ZnO) 100 ppm	$2.5\ x10^{5e}$
15	Zinc oxide (ZnO) 500 ppm	$2.1\ x10^{5ab}$
16	Ferrous oxide (Fe_2O_3) 10 ppm	$8.1\ x10^{5k}$
17	Ferrous oxide (Fe_2O_3) 20 ppm	$3.7\ x10^{5\ fg}$
18	Ferrous oxide (Fe_2O_3) 50 ppm	$3.0\ x10^{5c}$
19	Ferrous oxide (Fe_2O_3) 100 ppm	$2.5\ x10^{5c}$
20	Ferrous oxide (Fe_2O_3) 500 ppm	$2.0\ x10^{5b}$
21	Control	$1.3\ x10^{5a}$
	C.D.	1.94
	SE(m)	0.68
	SE(d)	0.95
	C.V	2.64

Alphabets indicating Duncan Multiple Range Test (DMRT)

Table 3: Influence of different nanoparticles on mycelial growth of *B. bassiana* in SDAY media at different concentrations during the year 2016.

S. No.	Name of the Treatment	*B. bassiana* colony size (diameter) in mm		
		5 DAI	10 DAI	15 DAI
1	Magnesium oxide (MgO) 10 ppm	8.57^{ab}	12.97^{abc}	37.67^{ef}
2	Magnesium oxide (MgO) 20 ppm	8.93^{ab}	13.03^{abc}	40.93^{gh}

3	Magnesium oxide (MgO) 50 ppm	9.93^{b}	13.73^{bc}	51.43^{k}
4	Magnesium oxide (MgO) 100 ppm	8.67^{ab}	12.37^{abc}	38.63^{ef}
5	Magnesium oxide (MgO) 500 ppm	7.90^{ab}	12.07^{ab}	32.67^{c}
6	Calcium oxide (CaO) 10 ppm	8.27^{ab}	13.07^{abc}	36.77^{de}
7	Calcium oxide (CaO) 20 ppm	9.53^{ab}	13.63^{bc}	48.13^{j}
8	Calcium oxide (CaO) 50 ppm	8.63^{ab}	13.03^{abc}	36.83^{i}
9	Calcium oxide (CaO) 100 ppm	7.87^{ab}	12.53^{abc}	32.50^{bc}
10	Calcium oxide (CaO) 500 ppm	7.97^{ab}	12.47^{abc}	30.27^{ab}
11	Zinc oxide (ZnO) 10 ppm	7.93^{ab}	13.03^{bc}	32.93^{c}
12	Zinc oxide (ZnO) 20 ppm	8.83^{ab}	13.33^{bc}	41.23^{ghi}
13	Zinc oxide (ZnO) 50 ppm	7.87^{ab}	12.43^{abc}	34.50^{cd}
14	Zinc oxide (ZnO) 100 ppm	7.87^{ab}	12.67^{abc}	32.33^{bc}
15	Zinc oxide (ZnO) 500 ppm	8.07^{ab}	12.97^{abc}	32.37^{bc}
16	Ferrous oxide (Fe_2O_3) 10 ppm	9.23^{ab}	13.13^{abc}	42.13^{ghi}
17	Ferrous oxide (Fe_2O_3) 20 ppm	8.83^{ab}	12.33^{bc}	43.43^{i}
18	Ferrous oxide (Fe_2O_3) 50 ppm	8.23^{ab}	13.10^{abc}	42.87^{hi}
19	Ferrous oxide (Fe_2O_3) 100 ppm	8.90^{ab}	13.07^{abc}	39.83^{fg}
20	Ferrous oxide (Fe_2O_3) 500 ppm	8.37^{ab}	14.63^{c}	39.60^{fg}
21	Control	7.20^{a}	10.93^{a}	28.50^{a}
	C.D.	1.28	2.25	1.20
	SE(m)	0.45	0.64	0.42
	SE(d)	0.63	0.62	0.59
	C.V	9.17	5.85	1.92

Alphabets indicating Duncan Multiple Range Test (DMRT)
DAI – Days after inoculation

Table 4: Influence of different nanoparticles on mycelial growth of *B. bassiana* in SDAY media at different concentrations during the year 2017.

S. No.	Name of the Treatment	*B. bassiana* colony size (diameter) in mm		
		5 DAI	10 DAI	15 DAI
1	Magnesium oxide (MgO) 10 ppm	8.53^{abc}	13.20^{bcdef}	38.03^{fg}
2	Magnesium oxide (MgO) 20 ppm	8.90^{abc}	13.97^{cdef}	40.87^{ghi}
3	Magnesium oxide (MgO) 50 ppm	10.07^{c}	19.57^{i}	51.57^{m}
4	Magnesium oxide (MgO) 100 ppm	8.97^{bc}	12.53^{abcdef}	39.13^{fgh}
5	Magnesium oxide (MgO) 500 ppm	8.33^{abc}	11.93^{ab}	33.10^{c}
6	Calcium oxide (CaO) 10 ppm	7.93^{ab}	13.80^{bcdef}	37.13^{ef}
7	Calcium oxide (CaO) 20 ppm	9.97^{c}	17.70^{hi}	48.63^{l}
8	Calcium oxide (CaO) 50 ppm	9.07^{bc}	14.07^{def}	37.33^{ef}
9	Calcium oxide (CaO) 100 ppm	8.27^{abc}	13.13^{bcdef}	32.43^{ab}
10	Calcium oxide (CaO) 500 ppm	8.23^{abc}	12.03^{abc}	30.60^{a}
11	Zinc oxide (ZnO) 10 ppm	7.63^{abc}	14.20^{ef}	33.27^{cd}
12	Zinc oxide (ZnO) 20 ppm	8.30^{abc}	16.23^{gh}	40.47^{ij}
13	Zinc oxide (ZnO) 50 ppm	7.57^{abc}	13.37^{bcdef}	35.33^{de}
14	Zinc oxide (ZnO) 100 ppm	7.17^{abc}	12.93^{abcdef}	33.23^{cd}
15	Zinc oxide (ZnO) 500 ppm	7.13^{abc}	12.33^{abcde}	32.30^{ab}
16	Ferrous oxide (Fe_2O_3) 10 ppm	8.90^{abc}	16.60^{h}	43.97^{k}
17	Ferrous oxide (Fe_2O_3) 20 ppm	8.77^{abc}	14.33^{fg}	43.23^{ijk}
18	Ferrous oxide (Fe_2O_3) 50 ppm	8.33^{abc}	13.07^{bcdef}	39.93^{ghi}
19	Ferroous oxide (Fe_2O_3) 100 ppm	8.17^{abc}	12.83^{abcdef}	39.47^{hij}
20	Ferrous oxide (Fe_2O_3) 500 ppm	7.23^{abc}	12.13^{abcd}	39.30^{fgh}
21	Control	7.23^{a}	10.97^{a}	27.43^{a}
	C.D.	1.05	2.06	2.46
	SE(m)	0.37	0.47	0.73
	SE(d)	0.52	1.92	1.32
	C.V	7.37	4.61	1.68

Alphabets indicating Duncan Multiple Range Test (DMRT)
DAI – Days after inoculation

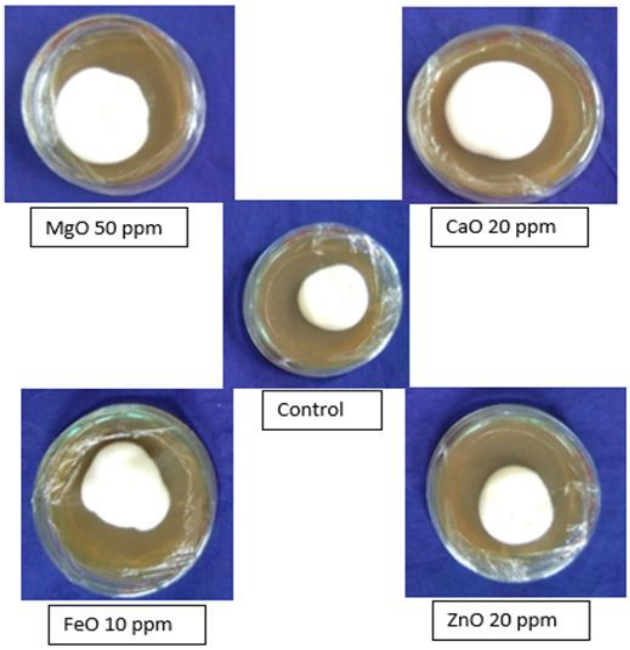

Plate 2: *B. bassiana* grown under different nanomaterial based SDAY media at 10 DAI

The CaO, MgO, ZnO and Fe_2O_3 nano based micro nutrients at 10, 20, 50, 100 and 500 ppm concentrations were added to the growth media of bio pesticides *viz*., *B. thuringiensis*, *N. rileyi* and *B. bassiana* for their growth and multiplication under laboratory conditions. Observations recorded at regular intervals indicated that all the four nanoparticles promoted the growth of all bio pesticides at certain concentrations. These results are on par with Joshi *et al*., (2015) [10] who observed the maximum growth of *Halophilic* bacteria by the supplementation of metal ions.

Calcium ions are involved in the maintenance of cell structure, motility, transport and cell differentiation processes such as sporulation, heterocyst formation and fruiting body development [11]. Magnesium ions are critical divalent cation for the stabilization of membranes and ribosomes, the neutralization of nucleic acids and as a cofactor in a variety of enzymatic reactions [12]. Ferrous participates in a large number of cellular processes, the most important of which are oxygen transport, ATP generation, cell growth and proliferation, and detoxification. Zinc is a metal ion, involved in many crucial biological processes exclusively as constituents of proteins, including enzymes, storage proteins and transcription factors [13]. The growth of biopesticide organisms was inhibited with the increase in concentrations of nanoparticles. These results are comparable with Schacht *et al*., (2012) [14] who tested the AgO nanoparticles for its antibacterial properties. They observed that Ag (o) concentrations above 80 µg ml^{-1} resulted in complete irreversible inhibition of microbial growth, whereas at 20-60 µg ml^{-1} concentrations maximum growth was observed. The production of reactive oxygen species (ROS), the disruption or disorganization of bacterial cell walls due to interaction of nanoparticles, induction of intracellular antibacterial effects, including interactions with DNA and proteins and accumulation of nanoparticles in the cytoplasm or on the outer membranes [15].

4. Conclusions

The results indicated that all the four nanoparticles promoted the growth of all bio pesticides at certain concentrations and later the bio pesticides growth was inhibited with increasing concentration of the nanoparticles. Further studies are needed deciphering pathways of nanoparticles in microorganisms and by adding the growth promoting nutrients at nano size, the dose of the bio pesticides may be decreased.

5. References

1. Mousa MA, Zaker MA, Koppy F. Abundance of cotton leaf worm, *Prodenia litura* (Fab.) in relation to host plants Host plants and their effect on biology. Bulletin of Entomological Society, Egypte. 1980.44:241-251.
2. Basu AC. Effect of different foods on the larval and post larval development of moth of *Prodenia litura* (Fab.). Journal of Bombay Natural History Society. 1981. 44:275-288.
3. Muthukrishnan N, Ganapathy N, Nalini R, Rajendran R. Pest Management in Horticultural Crops. New Madura Publishers, Madurai, 2005, 325.
4. Sasidharan KR, Varma RV. Laboratory evaluation of *Beauveria bassiana* (Balsamo) Vuillemin against *Indarbela quadrinotata* Walker (Lepidoptera: Metarbelidae) - a key pest of *Casuarina equisetifolia* L. in Tamil Nadu. Journal of Biological Control. 2005; 19:197-200.
5. Kumar K, Chapman RB. Sublethal effects of insecticides on the diamondback moth *Plutella xylostella* (L.). Pesticide Science. 2006.15:344-352.
6. Ahmad M, Saleem MA Ahmad M. Time oriented mortality in leaf army worm, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) by some new chemistry insecticides. Pakistan Entomology. 2005. 27(1):67-70.
7. De La Rosa W, Alatorre R, Barrera JF, Toriello C. Effect of *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycetes) upon the coffee berry borer (Coleoptera: Scolytidae) under field conditions. Journal of Economic Entomology. 2000; 93:1409-1414.
8. Nykypanchuk D, Maye M, Lelie VDD, Gang O. DNA-guided crystallization of colloidal nano particles. Nature. 2008; 451(7178):549-552.
9. Wang J, Zheng C. Characterisation of newly discovered Beauveria bassiana isolates to *Frankliniella occidentalis* Perganda a non-native invasive species in China. Microbiological Research. 2012; 167(2):116-120.
10. Joshi AP, Namrata S, Shekhawat DB. Effect of metal ions on growth and biosurfactant production by Halophilic bacteria. Advances in Applied Science Research. 2015; 6(4):152-156.
11. Dominguez CD. Calcium signalling in bacteria Calcium signalling in bacteria. Molecular microbiology. 2004; 54(2):291-297.
12. Groisman EA, Hollands K, Kriner MA, Lee1 EJ, Park SY, Mauricio H. Pontes. Bacterial Mg2+ Homeostasis, Transport, and Virulence. Annual Review of Genetics. 2013; 47:625-646.
13. Hood MI, Skaar EP. Nutritional immunity: transition metals at the pathogen-host interface. Nature Reviews Microbiology. 2012; 10:525-537.
14. Schacht VJ, Neumann SSK, Chen L, Henning T, Klar PJ, Theophel K *et al*. Effects of silver nanoparticles on microbial growth dynamics. Journal of Applied Microbiology. 2012; 114:25-35.
15. Vielkind M, Kampen I, Kwade A. Zinc Oxide Nanoparticles in Bacterial Growth Medium: Optimized Dispersion and Growth Inhibition of *Pseudomonas putida*. Advances in Nanoparticles. 2013; 2:287-293.