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M Gayathri
Ph.D Scholor, Dept of Entomology, S.V. Agricultural College, Tirupati, Andhra Pradesh, India

NC Venkateswarlu
Professor and Head, Dept of Entomology, S.V. Agricultural college, Tirupati, Andhra Pradesh, India

T Murali Krishn
Project coordinator, KVK, Kalyandurg, Ananthapur, Andhra Pradesh, India

TNVK Prasad
Principal Scientist, IFT, RARS, Tirupati, Andhra Pradesh, India

Correspondence
M Gayathri
Ph.D Scholor, Dept of Entomology, S.V. Agricultural College, Tirupati, Andhra Pradesh, India

Influence of magnesium, calcium, ferrous and zinc nanomaterials on production and multiplication of *Nomuraea rileyi* (Farlow) Samson

M Gayathri, NC Venkateswarlu, T MuraliKrishn and TNVK Prasad

Abstract
Nomuraea rileyi (Farlow) Samson is one of the cosmopolitan occurrence primarily infecting Lepidoptera and particularly the economically important, polyphagous noctuid pests. The *N. rileyi* grown under nano based SMAY media recorded the maximum number of conidia 8.0, 7.3, 7.2 and 6.9 per ml with MgO at 50 ppm, CaO at 20 ppm, ZnO at 20 ppm, FeO 10 ppm, whereas in control it was recorded as 1.5 per ml during the year 2016. Similarly during 2017 also the condial count was 8.6, 8.1, 7.9, 6.6 with MgO 50 ppm, CaO 20 ppm, ZnO 20 ppm and FeO 10 ppm treatments respectively. The inhibited growth with increasing nanoparticles concentration might be due to the production of reactive oxygen species (ROS), the disruption or disorganization of cell walls due to interaction of nanoparticles, including interactions with DNA and proteins and accumulation of nanoparticles in the cytoplasm or on the outer membranes.

Keywords: nanoparticles, production, multiplication, *N. rileyi*

1. Introduction
Groundnut (*Arachis hypogea*) is one of the principal oilseed crops grown in India, covering nearly half of the area under oilseeds. Though India ranks first in area under groundnut cultivation (4.72 M ha), the productivity is low (995 kg/ha) as compared other developing countries like China (2600 kg/ha), Argentina (2100 kg/ha) and Indonesia (1550 kg/ha) [1]. In Andhra Pradesh also the productivity is 595 kg/ha in an area of 1013000 ha [2]. 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 [3] of which 40 are grown in India [4]. 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. Vimaladevi *et al*., in 2005 [5] reported that *Nomuraea rileyi* (Farlow) Samson is one of the cosmopolitan occurrence primarily infecting Lepidoptera and particularly the economically important, polyphagous noctuid pests. Nanoparticles demonstrate unique targeted characteristics with elevated strength, high conductance of electricity and extra chemical reactivity [6]. To enhance the activity of the *N. rileyi* by adding the growth promoting nutrients like calcium, magnesium, iron and zinc at nano size the present study was undertaken.

2. Material and Methods
2.1 Preparation of Nan oparticulate 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.

2.2 Evaluation of Nanomaterials on the Growth and Multiplication of *Nomuraea rileyi*

2.2.1 Ingredients of SMAY Media

Composition of SMAY media	
Ingredients	**Weight/Volume**
Agar	20 g
Peptone	10 g
Maltose	40 g
Yeast	5 g
Distilled water	900 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 for Preparation of SMAY Media

In a 1000 ml Borosil beaker, 500 ml of distilled water with 20 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 (10 g), maltose (40 g) and yeast (5 g) 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 15 psi pressure for 15min at 121 °C. Then cooled and stored in the incubator at 22 ^{0}C. Antibiotic chloramphenicol @ 80 mg per litre was added to the sterilized media before pouring into media plates.

2.4 Inoculation of *N. rileyi* on SMAY Media

Four days old culture of *N. rileyi* NR_2 isolate was chosen for the study. Culture discs of 10mm 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 10 ml 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 present per ml was calculated by using following formula

No. of spores per unit = N × 400 × 1000 × 10 × 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).
The 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.

3. Results and discussion

3.1 Dosage standardization of nanoparticles with *N. rileyi* grown under nanomaterial based SMAY media

In order to standardise suitable dose of nanoparticles on growth and development of *N. rileyi*, the nanoparticles Mg, Ca, Fe and Zn were added to the SMAY media at 10, 20, 50, 100 and 500 ppm concentrations in 9:1 ratio (9 ml media : 1 ml nano solution). Later, 5 mm discs of 4-5 days old *N. rileyi* fungal culture was inoculated to the media. After one week of incubation the conidial count was taken with the help of Neubauer haemocytometer.
During the year 2016, the highest number of conidia 8.0 per ml was recorded with MgO at 50ppm, followed by 3.3 with 20 and 100ppm, 2.1 with 500 ppm then the lowest number of conidia 1.8 resulted at 10 ppm concentration. With CaO the highest number of conidia per ml was 7.3 at 20 ppm, 3.7, 2.6, 2.2 and 1.5 at 50 ppm, 100 ppm, 10 ppm and 500 ppm respectively. Whereas in ZnO the highest number 6.9 was recorded at 20 ppm, followed by 5.3, 3.0, 2.6 and 1.8 at 50 ppm, 100 ppm, 10 ppm and 500 ppm concentrations respectively.
The highest number of conidia 7.2 per ml was recorded with FeO at 10 ppm, followed by 5.0, 3.7, 2.1, 1.8 at 20 ppm, 50 ppm, 100 and 500 ppm concentrations respectively, whereas in control it was recorded as 1.5.

Table 3.1: Influence of different nanoparticles on conidial count of *N. rileyi* in SMAY media at different concentrations during the year 2016.

S. No.	Name of the Treatment	No. of conidia/ml
		2016
1	Magnesium oxide (MgO) 10 ppm	1.8 x10^{5b}
2	Magnesium oxide (MgO) 20 ppm	3.3 x10^{5ef}
3	Magnesium oxide (MgO) 50 ppm	8.0 x10^{5i}
4	Magnesium oxide (MgO) 100 ppm	3.3 x10^{5ef}
5	Magnesium oxide (MgO) 500 ppm	2.1 x10^{5bc}
6	Calcium oxide (CaO) 10 ppm	2.2 x10^{5bc}
7	Calcium oxide (CaO) 20 ppm	7.3 x10^{5h}
8	Calcium oxide (CaO) 50 ppm	3.7 x10^{5f}
9	Calcium oxide (CaO) 100 ppm	2.6 x10^{5cd}
10	Calcium oxide (CaO) 500 ppm	1.7 x10^{5ab}
11	Zinc oxide (ZnO) 10 ppm	2.6 x10^{5d}
12	Zinc oxide (ZnO) 20 ppm	6.9 x10^{5h}
13	Zinc oxide (ZnO) 50 ppm	5.3 x10^{5g}
14	Zinc oxide (ZnO) 100 ppm	3.0 x10^{5de}
15	Zinc oxide (ZnO) 500 ppm	1.8 x10^{5ab}

16	Ferrous oxide (Fe_2O_3) 10 ppm	7.2 x10^{5h}
17	Ferrous oxide (Fe_2O_3) 20 ppm	5.0 x10^{5g}
18	Ferrous oxide (Fe_2O_3) 50 ppm	3.7 x10^{5f}
19	Ferroous oxide (Fe_2O_3) 100 ppm	2.1 x10^{5bc}
20	Ferrous oxide (Fe_2O_3) 500 ppm	1.8 x10^{5ab}
21	Control	1.5 x10^{5a}
	C.D.	2.78
	SE(m)	0.97
	SE(d)	1.37
	C.V.	4.59

Alphabets indicating Duncan Multiple Range Test (DMRT)

Table 2: Influence of different nanoparticles on conidial count of *N. rileyi* in SMAY media at different concentrations during the year 2017.

S. No.	Treatment	No. of conidia/ml
		2017
1	Magnesium oxide (MgO) 10 ppm	2.2 x10^{5ab}
2	Magnesium oxide (MgO) 20 ppm	3.0 x10^{5c}
3	Magnesium oxide (MgO) 50 ppm	8.6 x10^{5h}
4	Magnesium oxide (MgO) 100 ppm	5.1 x10^{5f}
5	Magnesium oxide (MgO) 500 ppm	3.0 x10^{5c}
6	Calcium oxide (CaO) 10 ppm	3.2 x10^{5cd}
7	Calcium oxide (CaO) 20 ppm	8.1 x10^{5h}
8	Calcium oxide (CaO) 50 ppm	4.3 x10^{5e}
9	Calcium oxide (CaO) 100 ppm	2.6 x10^{5bc}
10	Calcium oxide (CaO) 500 ppm	1.8 x10^{5a}
11	Zinc oxide (ZnO) 10 ppm	2.9 x10^{5c}
12	Zinc oxide (ZnO) 20 ppm	6.6 x10^{5g}
13	Zinc oxide (ZnO) 50 ppm	3.7 x10^{5de}
14	Zinc oxide (ZnO) 100 ppm	3.8 x10^{5de}
15	Zinc oxide (ZnO) 500 ppm	1.8 x10^{5a}
16	Ferrous oxide (Fe_2O_3) 10 ppm	7.9 x10^{5h}
17	Ferrous oxide (Fe_2O_3) 20 ppm	5.1 x10^{5f}
18	Ferrous oxide (Fe_2O_3) 50 ppm	2.9 x10^{5c}
19	Ferrous oxide (Fe_2O_3) 100 ppm	2.2 x10^{5ab}
20	Ferrous oxide (Fe_2O_3) 500 ppm	1.9 x10^{5ab}
21	Control	1.7 x10^{5a}
	C.D.	3.58
	SE(m)	1.25
	SE(d)	1.76
	C.V.	5.50

Alphabets indicating Duncan Multiple Range Test (DMRT)

Table 3: Influence of different nanoparticles on mycelial growth of *N. rileyi* in SMAY media at different concentrations during the year 2016.

S. No.	Name of the Treatment	*N. rileyi* colony size (diameter) in mm		
		5 DAI	10 DAI	15 DAI
1	Magnesium oxide (MgO) 10 ppm	7.87^{ab}	12.38^{abc}	37.39^{abcde}
2	Magnesium oxide (MgO) 20 ppm	8.02^{ab}	12.72^{abc}	40.49^{ab}
3	Magnesium oxide (MgO) 50 ppm	8.95^{b}	18.80^{f}	51.02^{e}
4	Magnesium oxide (MgO) 100 ppm	8.07^{ab}	12.04^{ab}	38.79^{abcde}
5	Magnesium oxide (MgO) 500 ppm	7.67^{ab}	11.71^{ab}	32.33^{abc}
6	Calcium oxide (CaO) 10 ppm	7.50^{ab}	12.94^{abc}	36.47^{abcde}
7	Calcium oxide (CaO) 20 ppm	9.02^{b}	15.34^{de}	48.06^{de}
8	Calcium oxide (CaO) 50 ppm	7.96^{ab}	13.64^{abcd}	36.75^{abcde}
9	Calcium oxide (CaO) 100 ppm	7.33^{ab}	12.28^{ab}	32.14^{abc}
10	Calcium oxide (CaO) 500 ppm	7.25^{ab}	11.74^{abc}	30.02^{abc}
11	Zinc oxide (ZnO) 10 ppm	7.00^{ab}	13.76^{cde}	32.48^{abc}
12	Zinc oxide (ZnO) 20 ppm	8.23^{ab}	16.79^{ef}	40.84^{abcde}
13	Zinc oxide (ZnO) 50 ppm	7.58^{ab}	12.66^{f}	34.25^{abcd}
14	Zinc oxide (ZnO) 100 ppm	7.31^{ab}	12.36^{abc}	32.39^{abc}
15	Zinc oxide (ZnO) 500 ppm	7.38^{ab}	12.24^{ab}	32.03^{abc}
16	Ferrous oxide (Fe_2O_3) 10 ppm	8.63^{b}	15.79^{de}	42.00^{abcde}
17	Ferrous oxide (Fe_2O_3) 20 ppm	8.26^{b}	13.73^{bcd}	43.32^{cde}
18	Ferrous oxide (Fe_2O_3) 50 ppm	7.89^{ab}	12.66^{abc}	42.95^{bcde}
19	Ferroous oxide (Fe_2O_3) 100 ppm	7.35^{ab}	12.29^{ab}	39.54^{abcde}
20	Ferrous oxide (Fe_2O_3) 500 ppm	7.47^{ab}	11.87^{ab}	39.28^{bcde}
21	Control	6.12^{a}	11.44^{a}	27.76^{a}
	C.D.	1.31	1.26	1.24

	SE(m)	0.46	0.44	0.43
	SE(d)	0.64	0.72	0.81
	C.V	6.18	5.72	1.99

Alphabets indicating Duncan Multiple Range Test (DMRT)
DAI – Days After Inoculation

Table 4: Influence of different nanoparticles on mycelial growth of *N. rileyi* in SMAY media at different concentrations during the year 2017.

S. No.	Name of the Treatment	*N. rileyi* colony size (diameter) in mm		
		5 DAI	10 DAI	15 DAI
1	Magnesium oxide (MgO) 10 ppm	8.66^{ab}	12.96^{ab}	37.68^{ef}
2	Magnesium oxide (MgO) 20 ppm	8.96^{b}	13.22^{b}	40.71^{gh}
3	Magnesium oxide (MgO) 50 ppm	9.67^{b}	19.13^{e}	48.29^{j}
4	Magnesium oxide (MgO) 100 ppm	8.86^{b}	12.63^{ab}	39.08^{fg}
5	Magnesium oxide (MgO) 500 ppm	8.38^{ab}	12.23^{ab}	32.61^{cd}
6	Calcium oxide (CaO) 10 ppm	8.32^{ab}	13.50^{b}	36.71^{e}
7	Calcium oxide (CaO) 20 ppm	9.65^{b}	15.71^{cd}	41.12^{gh}
8	Calcium oxide (CaO) 50 ppm	8.75^{b}	14.11^{bc}	37.02^{e}
9	Calcium oxide (CaO) 100 ppm	8.17^{ab}	12.86^{ab}	32.46^{c}
10	Calcium oxide (CaO) 500 ppm	7.98^{ab}	12.26^{ab}	30.29^{b}
11	Zinc oxide (ZnO) 10 ppm	7.86^{ab}	14.17^{bc}	32.76^{cd}
12	Zinc oxide (ZnO) 20 ppm	9.00^{b}	17.25^{de}	42.27^{hi}
13	Zinc oxide (ZnO) 50 ppm	8.28^{ab}	13.16^{b}	34.52^{d}
14	Zinc oxide (ZnO) 100 ppm	8.15^{ab}	12.94^{ab}	32.64^{cd}
15	Zinc oxide (ZnO) 500 ppm	8.09^{ab}	12.68^{ab}	32.35^{c}
16	Ferrous oxide (Fe_2O_3) 10 ppm	9.38^{b}	16.22^{cd}	51.25^{k}
17	Ferrous oxide (Fe_2O_3) 20 ppm	8.82^{b}	14.27^{bc}	43.59^{i}
18	Ferrous oxide (Fe_2O_3) 50 ppm	8.57^{ab}	13.16^{b}	43.17^{i}
19	Ferroous oxide (Fe_2O_3) 100 ppm	8.19^{ab}	12.73^{ab}	39.79^{g}
20	Ferrous oxide (Fe_2O_3) 500 ppm	8.07^{ab}	12.46^{ab}	39.56^{fg}
21	Control	6.59^{a}	11.03^{a}	27.21^{a}
	C.D.	1.23	1.09	1.08
	SE(m)	0.39	0.33	0.32
	SE(d)	0.55	0.44	0.32
	C.V	7.90	4.76	1.70

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

Similarly during 2017, the highest number of conidia of 8.6 per ml was recorded with MgO at 50ppm, followed by 5.1 with 100 ppm, 3.0 with 20 ppm and 500 ppm and 2.2 at 10 ppm concentrations. While in CaO the highest number was 8.1 at 20 ppm, followed by 4.3 at 50 ppm, 3.2 at 10 ppm, 2.6 at 100 ppm and 1.8 at 500 ppm concentrations and in ZnO the conidial count was 6.6, 3.8, 3.7, 2.9 and 1.8 with 20, 100, 50, 10 and 500 ppm concentrations respectively. Whereas in FeO 7.9, 5.1, 2.9, 2.2 and 1.9 at 10, 20, 50, 100 and 500 ppm concentrations respectively, however in control it was recorded as 1.7 (Table 3.1 and Plate 1).

Similarly during 2017, the highest number of conidia of 8.6 per ml was recorded with MgO at 50ppm, followed by 5.1 with 100 ppm, 3.0 with 20 ppm and 500 ppm and 2.2 at 10 ppm concentrations. While in CaO the highest number was 8.1 at 20 ppm, followed by 4.3 at 50 ppm, 3.2 at 10 ppm, 2.6 at 100 ppm and 1.8 at 500 ppm concentrations and in ZnO the conidial count was 6.6, 3.8, 3.7, 2.9 and 1.8 with 20, 100, 50, 10 and 500 ppm concentrations respectively. Whereas in FeO 7.9, 5.1, 2.9, 2.2 and 1.9 at 10, 20, 50, 100 and 500 ppm concentrations respectively, however in control it was recorded as 1.7 (Table 3.2).

The size (diameter) of the fungal disc was measured at 5, 10 and 15 days after inoculation. The maximum growth observed was 8.95 mm, 18.80 mm and 51.02 mm with MgO at 50 ppm, followed by CaO 20 ppm, ZnO 20 ppm and FeO10 ppm, where as in control it was 6.12 mm, 11.44 mm and 27.76 mm diameter during the year 2016 (table 3.3). The similar trend was observed in 2017 also (table 3.4).

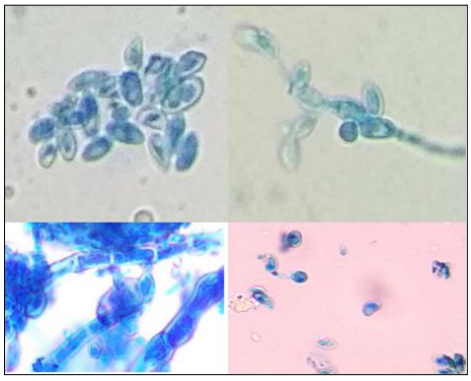

Plate 1: *N. rileyi* conidial cells and rachis

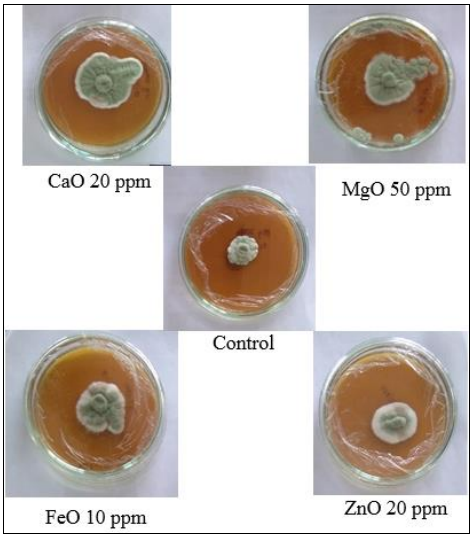

Plate 2: *N. rileyi* grown under different nano based SMAY media at 15 DAI

Influence of different nanoparticles on growth of biopesticides

The CaO, MgO, ZnO and Fe_3O_4 nanobased micro nutrients at 10, 20, 50, 100 and 500 ppm concentrations were added to the growth media of biopesticides *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 biopesticides at certain concentrations. These results are on par with Joshi *et al*., (2015) [7] who observed the maximum growth of *Halophilic* bacteria by the supplementation of metal ions. The reasons for the promoted growth might be

- 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 [8]
- 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 [9].
- 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 [10].

Observations on the growth of the nanobased biopesticides revealed that, the growth of biopesticide organisms was increased upto certain concentrations and later the growth was inhibited with the increase in concentrations of nanoparticles. These results are comparable with Schacht *et al*., (2012) [11] 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 results of present study are also in agreement with Vielkind *et al*. (2013) [12] who observed the inhibited growth in *Pseudomonas putida* with increasing ZnO nanoparticles concentration up to 500 mg/L and the reasons mentioned were 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.

4. Conclusions

The 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 and later with increasing concentration of the nanoparticles the growth of the biopesticides was inhibited. Further studies are needed by adding the growth promoting nutrients at nano size the dose of the biopesticides may be decreased.

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