

## Bio efficacy of inorganic nanoparticles CdS, Nano-Ag and Nano-TiO<sub>2</sub> against *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae)

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### ABSTRACT

The cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is a cosmopolitan and difficult-to-manage pest. Nanoparticles offer an alternative method to manage *S. litura*. A study was initiated to explore the potential of CdS, Nano-Ag and Nano-TiO<sub>2</sub> nanoparticles in causing adverse effects on *S. litura*. The dose-response data of second instar *S. litura* larvae indicated that the virulence of nanoparticles, the LC<sub>50</sub> of CdS was 508.84, of Nano-TiO<sub>2</sub> and Nano-Ag the LC<sub>50</sub> was 791.10 and 1403.14 ppm, respectively. CdS nanoparticle caused higher larval mortality of 21.41 to 93.79 per cent at 150 and 2400 ppm, respectively. The Nano-TiO<sub>2</sub> showed maximum of 73.79 per cent larval mortality at 2400 ppm and the least was 18.50 per cent at 150 ppm. Nano-Ag caused maximum 56.89 per cent mortality at 2400 ppm followed by 46.89 and 33.44 per cent mortality at 1200 and 600 ppm, respectively. The three nanoparticles tested proved effective against *S. litura* larvae and hence can be selectively used for suppression of the pest.

**KEY WORDS:** CdS, Nano-Ag, Nano-TiO<sub>2</sub>, nanocide, *Spodoptera litura*, Integrated pest management

### INTRODUCTION

The tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is a major pest with high mobility and reproductive capacity (Holloway, 1989). It is widely distributed throughout tropical and temperate Asia, Australasia and Pacific Islands (Mohammad Monobrullah and Uma Shankar, 2008). *S. litura* feeds on more than 120 host plants (Ramana *et al.*, 1988) and major ones include tobacco, cotton, groundnut, jute, lucerne, maize, rice, soybeans, tea, cauliflower, cabbage, capsicum, potato and castor (Sharma and Bisht, 2008). Several outbreaks of this pest on cotton, tobacco and chillies have been reported in Tamil Nadu (Rao *et al.*, 1983)

and the economic loss range between 25.8-100 % depending upon the crop stage and its infestation level (Dhir *et al.*, 1992). Its resistance against almost all the insecticide groups (Kranthi *et al.*, 2002) including the new insecticides like lufenuron (Sudhakaran, 2002) and the adverse effects due to synthetic pesticides on pests and their subsequent impacts to ecological imbalance (Zadoks and Waibel, 1999) demands sustainable alternatives (Parmar, 1993). The use of nanoparticle pesticides is one such new avenue.

Nanoparticles possess insecticidal property due to novel characteristics like extra ordinary strength, chemical reactivity

and electrical conductivity. They have a distinct physical, biological and chemical properties associated with their atomic strength (Leiderer and Dekorsy, 2008). Nanoparticles are agglomerated atom by atom, and their size/shape may be maintained specifically (Roy, 2009) and particles can be arranged into ordered layers (Ulrich *et al.*, 2006). Such self-assembly is due to forces such as hydrogen bonding, dipolar forces, hydrophilic and hydrophobic interactions, surface tension and gravity. Since, inorganic nanoparticles have unique properties owing to the quantum size effects and the large number of unsaturated atoms, polymeric films containing inorganic nanoparticles can exhibit novel catalytic, magnetic and optical properties.

Nanoparticles in natural ecosystems have different biological responses than those observed in laboratory cell-based toxicity assays. Properties of nanoparticles can be exploited in the production of new insecticides (Owolade *et al.*, 2008). These particles are released slowly but efficiently to a particular host plant against an insect pest (Scrini and Lyons, 2007). Seema Singh (2012) and a team of researchers from IIT-Madras have developed nanoparticles from gold, silver, copper and several other metallic oxides that have been found effective against insect pests. This paper aims at examining the potential adverse effects of CdS, Nano-Ag and Nano-TiO<sub>2</sub> nanoparticles on *S. litura* in laboratory.

## MATERIAL AND METHODS

### *Insect culture*

The test insects (*S. Litura*) were reared on leaves of castor (*Ricinus communis* (L.) in the laboratory (Department of Entomology, UAS, GKVK, Bangalore) at room temperature (25±1°C) and 70 % r.h. second instar larvae (6-day old) were used in

the study. The larvae were reared in insect cages (0.3 m<sup>3</sup>) of wood and wire-mesh.

### *Preparation of nanoparticles*

**CdS:** CdS was prepared with 1% of one ml of CTAB dissolved in 100 ml of doubled distilled water. The solution divided into two parts (A&B). 0.1M of cadmium acetate used as a cadmium precursor dissolved in Part A. 0.1M of thiourea used as sulphur precursors dissolved in part B. Part B was added drop-by-drop into part A under vigorous stirring at room temperature. Finally, 25% of ammonia solution was added drop-by-drop into mixture solution till the pH value of 9 is reached. The solution slowly turned to greenish yellow color. After completing reaction, this solution was centrifuged and filtered. The prepared sample was dried at 70°C for 2 h. The nano CdS sample was characterized by UV-Visible-absorption spectroscopy using Lambda 35. The size of the particle was about 60 nm, the size is confirmed by other several experiments. All chemicals were of analytical grade, purchased from Merck Ltd., India.

**Nano-Ag:** The nano silver powder was prepared from acid leaching tail solution. Different controlled parameters were performed, including pH, residence time and temperature, mixing time, and reducing reagents to metals molar ratio. A brown precipitate was appeared due to addition of ammonia. Using ammonia was stopped at pH 10.5. The resulted mother liquor was blue. Silver with zero oxidation valent was gained by using hydrazinium sulphate in a stoichiometrical ratio and after drying precipitate for removing impurities it was washed with sulphuric acid. Resulting deposit was washed with water and it was dried again. Double distilled water and demineralized water were used throughout the

experiments. By dissolving this sediment in nitric acid while stirring silver nitrate was achieved at which ammonia was added until pH= 10.5. In this condition the brown silver oxide disappeared and  $[\text{Ag}(\text{NH}_3)_2]$  was produced in the medium. By gradual adding, hydrazinium sulphate salt ammoniacal complex of silver with (molar ratio 3:4) 20% methanol at 45 °C in 5 min, the silver nano powder was prepared and these particles were investigated by UV-Vis spectroscopy and scanning electron microscopy (SEM)/EDAX. Silver nano powder was less than 90 nm size.

**Nano-TiO<sub>2</sub>:** The method was performed with 0.4 g Anatase (Aldrich) and equimolar amounts of amine (DDA and ODA Aldrich 98%) were treated with 5 ml of 10 M NaOH (Merck) aqueous solution. The resulting suspensions were heated at 120–150 °C. Solid products obtained after 12, 30, 50 and 72 h were separated from the suspension by centrifugation and treated with HCl 0.1 M for 24 h and washed with deionised water repeatedly until at pH 6. The X-ray diffraction analysis using SEM (Phillips XL-30), TEM (JEOL 100-SX) clearly denotes the size of TiO<sub>2</sub> nanoparticles with average particle sizes are greater than 50 nm.

### Bioassay

To determine the lethal concentration of nanoparticles (CdS - acetone soluble, Nano-Ag - acetone soluble and Nano-TiO<sub>2</sub> – water soluble), serial dilutions of the nanoparticles from 150 to 2400 ppm were prepared in the geometric order. Thereafter, the nanoparticle suspension was subjected to trademark ultrasonication (Hielscher Ultrasonics with UP400S (400 watts)) for 20 min for proper dispersion and activation of nanoparticles (pressure = >1000 atm, temperatures = >5000K and heating and cooling rates = >109Ks<sup>-1</sup>). Before fixing the doses, bracketing was done to ensure the

concentration - mortality response curve. Bracketing is a preliminary study to find out the dosage range of an insecticide giving different levels of mortality before conducting the bioassay (Finney, 1952). Ten second instar larvae were released on the castor leaf discs (4 × 4 cm) treated with desired concentrations of the solutions of nanoparticles (leaf dip method bioassay technique) (Venkateswari *et al.*, 2008). Each leaf disc served as replicate × 3 per treatment and leaf discs treated with water served as control. The larvae after release on the castor leaf discs were incubated at 25±1 °C with a specific 70 % r.h. in an BOD incubator. The numbers of larvae killed were recorded at 24 h intervals up to nine days and the per cent mortality was computed. The data sets were subjected to Analysis of Variance (ANOVA) (SPSS, Inv., 1999 version 10.00) and means were separated by Least Significance Difference (LSD). The data sets were also used for Probit Analysis (Finney, 1952) to determine the LC<sub>50</sub> of the above mentioned products of nanoparticles.

### RESULTS AND DISCUSSION

All three nanoparticles tested were significant at P < 0.01 level (Table 2, 3 and 4). Table 1 shows dose response or mortality data of second instar *S. litura* larvae to nanoparticles. The analysis of data revealed that variations in virulence to nanoparticles, CdS treatment caused mortality with LC<sub>50</sub> and LC<sub>95</sub> were 508.84 and 3768.34 ppm, respectively (Table 1). Where as in Nano-TiO<sub>2</sub> the LC<sub>50</sub> was 791.10 and LC<sub>95</sub> was 10754.24 ppm and in Nano-Ag the LC<sub>50</sub> was 1403.14 ppm and LC<sub>95</sub> was 9587.65 ppm. Among the three test products the CdS showed the lowest LC<sub>50</sub> and fiducial limits compared with Nano-TiO<sub>2</sub> and Nano-Ag. The dosage-mortality data clearly indicated that the response was higher in CdS on *S. litura* than two other products.

**Table 1: Probit analysis of nanoparticles for dosage - larval mortality in *S. litura***

Treatment	$\chi^2$ (n-4)	Regression equation	LC <sub>50</sub> (ppm)	Fiducial limits (Conc. at 95% CI)
CdS	0.73	Y= -5.12+ 1.89X	508.84	29.97 - 3768.34
Nano-Ag	0.18	Y= -4.04+ 1.28X	1403.14	21.62 - 10754.24
Nano-TiO <sub>2</sub>	0.21	Y= -3.68+ 1.29X	791.10	11.30 - 9587.65

The nanoparticle, CdS initially showed negligible larval mortality and as the days advanced, larval mortality also increased (Table 2). On 3<sup>rd</sup> day after treatment (DAT) the larval mortality ranged from 4.50 to 13.79 per cent and on 5<sup>th</sup> and 7<sup>th</sup> DAT cumulative mortality was ranged from 8.52 to 40.45 and 18.50 to 40.45 per cent, respectively. Ninth DAT, the highest mortality was recorded and cumulative larval mortality ranged from 21.41 to 93.79 per cent in 150 and 2400 ppm, respectively. Maximum mortality (93.79 %) was recorded in 2400 ppm and followed by 1200 and 600 ppm (73.79 and 51.72 %). In case of other treatments the larval mortality was the least (150 and 300 ppm recorded 24.41 and 38.40 % mortality, respectively). The dead larvae due to nanoparticles showed colloidal disruption of midgut epithelial cells and the inner contents was discharged out showed the peculiar symptom on treated larvae compared with control, which were normal (Figure 1).

Nano-Ag caused only 56.89 per cent mortality at 2400 ppm (Table 2) followed by 46.89 and 33.44 per cent mortality at 1200 and 600 ppm, respectively. Least mortality (22.50 and 15.45 %) was observed in 300 and 150 ppm. Further the nanoparticles broken-down the digestive system and

caused larval mortality, the dead larvae showed symptoms of oozing of inner gut contents.

The effect of Nano-TiO<sub>2</sub> showed in probit analysis was in consistence with ANOVA test on second instar larvae of *S. litura*. The effect was very low at 3 and 5<sup>th</sup> DAT, the mortality ranged from 2.50 to 13.79 per cent and 7.84 to 33.79 per ppm, respectively. After 7 days, the mortality further increased and maximum of 53.79 per cent larval mortality was recorded in 2400 ppm and the lowest was 13.50 per cent in 150 ppm. During 9<sup>th</sup> day after treatment, resulted 73.79 per cent of cumulative larval mortality in 2400 ppm and the least was 18.50 per cent in 150 ppm. The data was clear that the mortality increased as the days advanced and maximum mortality was recorded in 7<sup>th</sup> and 9<sup>th</sup> days after treatment. Another peculiar observation recorded was in this treatment discharge of larval inner contents due to ruptured midgut during 7<sup>th</sup> and 9<sup>th</sup> days after treatment.

The CdS, Nano-Ag and Nano-TiO<sub>2</sub> ceased active movement larvae, the skin and entire body became stiff and hard and oozing of the body contents (lysis) was observed. Further, the body became swollen, pulpy and fragile and body turned dark brown. The larvae showed premature

moulting and larvae attained pupal shape, all the internal contents oozed out, and eventually death occurred.

The uses of nanoparticles in insect pest management is new to science (Bhattacharyya *et al.*, 2010) and made out from hexane, chloroform, ethyl acetate, acetone, methanol, silver along with aqueous leaf extract have been tested for insecticidal properties (Santhoshkumar *et al.*, 2011). Nano silver particles possess insecticidal properties due to morphological and structural features brings about physiological changes (Nel *et al.*, 2006). The use of nanoparticles in agriculture is still at a rudimentary stage. Stadler *et al.* (2010) successfully tested nano alumina against two stored grain pests *Sitophilus oryzae* Linn. and *Rhyzopertha dominica* (F.) and Chakravarthy *et al.* (2012) tested the DNA tagged gold nanoparticles against *S. litura* Fab. (Lepidoptera: Noctuidae). Our study presents the entomotoxic potential of CdS, Nano-Ag and Nano-TiO<sub>2</sub> against *S. litura* and may be exploited as one of the possible new protective agents against *S. litura*.

Glutathione-coated CdS quantum dots (GSH-CdS) exhibited an absorption peak at 366 nm, indicative of 2.4 nm core size. It also interacts with more than one protein molecule and affinity of GSH-CdS for proteins was tested (Gabellieri *et al.*, 2011). This study demonstrated that CdS nanoparticle has adverse effects on *S. litura* larvae and could be a better alternative to synthetic insecticides, in addition to being a toxicant that inhibits biological and physiological systems of insects and also essential components of new biosensors and self-assembled nano devices (Simonianet *et al.*, 2005; Biju, 2007; Levy *et al.*, 2006; Hsing *et al.*, 2007; Zhu *et al.*, 2010).

The acute toxicity of silver is dependent on its chemistry and free ions. Research has shown that aqueous concentration of 1-5 mg/l was sensitive to aquatic insects, trout and flounder (Bryan and Langston 1992; Wood *et al.*, 1994). Eisler (1997) indicated that the accumulation of silver has lead to adverse effects on growth, because of their different physico-chemical properties and free ions released from nano-silver. Asharani *et al.* (2007) reported that silver nanoparticles have the potential to cause chromosomal aberrations and DNA damage and are capable of inducing cell proliferation in cell lines of zebrafish. Further it was shown that these particles have the capability to enter cells and cause cellular damage (Hussain *et al.*, 2005; Ji *et al.*, 2007). Indeed, several lines of evidence support the enhanced efficiency of silver nanoparticles on antimicrobial activity and are highly reactive as they generate Ag<sup>+</sup> ions while metallic silver is relatively unreactive (Morones *et al.*, 2005). It was also shown that the nanoparticles efficiently penetrate into microbial cells, which implies lower concentrations of nano-sized silver would be sufficient for microbial control. This would be efficient, especially for some organisms that are less sensitive to antibiotics due to the poor penetration of some antibiotics into cells (Samuel and Guggenbichler, 2004).

## CONCLUSION

The study demonstrated that the inorganic nanoparticles CdS, Nano-Ag and Nano-TiO<sub>2</sub> are effective against *S. litura* and further field test would therefore required to include it in integrated pest management strategy. However, cadmium, silver and titanium oxides are costly, hence have limited use. Entomologists should evaluate naturally occurring, readily available nanoparticles for IPM.

**Table 2 Effect of CdS, Nano-Ag and Nano-TiO<sub>2</sub> nanoparticles on *S. litura* larvae**

Conc. (ppm)	CdS				Nano-Ag				Nano-TiO <sub>2</sub>			
	*Cumulative Larval mortality (%)				*Cumulative Larval mortality (%)				*Cumulative Larval mortality (%)			
	3 DAT	5 DAT	7 DAT	9 DAT	3 DAT	5 DAT	7 DAT	9 DAT	3 DAT	5 DAT	7 DAT	9 DAT
150	4.5 (12.24) <sup>b</sup>	8.52 (16.97) <sup>c</sup>	18.5 (25.47) <sup>d</sup>	24.41 (29.60) <sup>d</sup>	1.5 (7.03) <sup>bc</sup>	6.45 (14.71) <sup>c</sup>	9.5 (17.95) <sup>bc</sup>	15.45 (23.14) <sup>cd</sup>	2.5 (9.09) <sup>c</sup>	7.84 (16.26) <sup>c</sup>	13.5 (21.55) <sup>d</sup>	18.5 (25.47) <sup>d</sup>
300	6.2 (14.41) <sup>b</sup>	18.5 (25.47) <sup>bc</sup>	25.64 (30.42) <sup>d</sup>	38.4 (38.29) <sup>cd</sup>	2.84 (9.70) <sup>b</sup>	8.5 (16.95) <sup>bc</sup>	16.54 (23.99) <sup>b</sup>	22.5 (28.31) <sup>c</sup>	3.25 (10.38) <sup>bc</sup>	15.5 (23.18) <sup>bc</sup>	23.64 (29.09) <sup>c</sup>	31.27 (34.00) <sup>c</sup>
600	10.344 (18.76) <sup>a</sup>	24.13 (29.42) <sup>b</sup>	37.93 (38.01) <sup>c</sup>	51.72 (45.98) <sup>c</sup>	3.44 (10.68) <sup>ab</sup>	13.44 (21.50) <sup>b</sup>	23.44 (28.95) <sup>ab</sup>	33.44 (35.32) <sup>b</sup>	6.89 (15.21) <sup>ab</sup>	20.22 (26.72) <sup>b</sup>	33.56 (35.42) <sup>b</sup>	46.89 (43.21) <sup>bc</sup>
1200	13.79 (21.79) <sup>a</sup>	33.79 (35.54) <sup>a</sup>	53.79 (47.17) <sup>b</sup>	73.79 (59.56) <sup>b</sup>	6.89 (15.21) <sup>a</sup>	20.22 (26.72) <sup>a</sup>	33.56 (35.40) <sup>a</sup>	46.89 (43.31) <sup>ab</sup>	10.34 (18.75) <sup>a</sup>	27.01 (31.31) <sup>a</sup>	43.67 (41.36) <sup>ab</sup>	60.34 (50.96) <sup>b</sup>
2400	13.79 (21.79) <sup>a</sup>	40.45 (39.54) <sup>a</sup>	67.12 (55.01) <sup>a</sup>	93.79 (75.56) <sup>a</sup>	6.89 (15.21) <sup>a</sup>	23.56 (29.03) <sup>a</sup>	40.22 (39.36) <sup>a</sup>	56.89 (48.96) <sup>a</sup>	13.79 (21.79) <sup>a</sup>	33.79 (35.54) <sup>a</sup>	53.79 (47.17) <sup>a</sup>	73.79 (59.20) <sup>a</sup>
SEm ±	0.3	0.73	0.97	1.42	0.24	0.43	0.34	0.64	0.33	0.94	0.76	1.16
CD at 5%	0.93	2.23	2.97	4.34	0.74	1.25	1.05	1.96	1.04	2.88	2.33	3.56

Mean of three replications containing 10 larvae per replication (n=30)

Figures in parentheses are angular transformed values

\*Corrected mortality

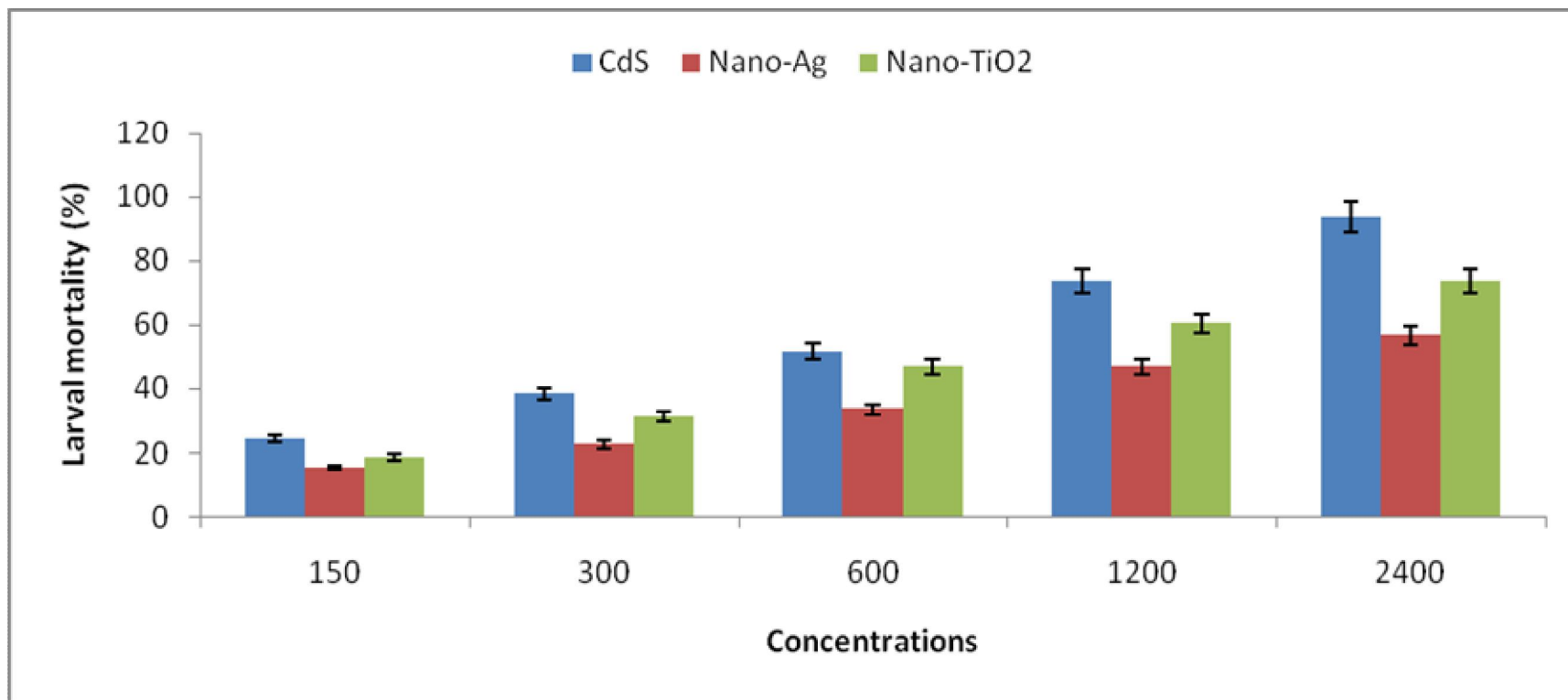


Fig. 1 killing effect of artificially synthesized CdS, Nano-Ag and Nano-TiO<sub>2</sub> nanoparticles on *S. litura*



Fig. 2 Malformed larvae of *S. litura* due to treatment of CdS (2), Nano-Ag (3), Nano-TiO<sub>2</sub> (4) and control (1)



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[MS received 14 October 2012;  
MS accepted 11 December 2012]

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