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Biosynthesis and effect of green silica nanoparticles on tobacco caterpillar, *Spodoptera litura* on cotton

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Abstract
Green silica nanoparticles were synthesised from Paddy husk. They were amorphous in shape and zetasizer revealed that average particle diameter of silica nanoparticles was 26.19 nm. Green silica nanoparticles were tested at various doses from 250 to 2000ppm and compared with metal nanoparticles and an insecticide check against second and fifth instar larva of *Spodoptera litura* larvae. Observations were also recorded on pupal and adult deformity. The cent per cent mortality of second instar larva of *Spodoptera litura* larvae was observed by green and metal silica nanoparticle at 1500 ppm at five days after treatment. The dead bodies became extremely dehydrated and shrunken in comparison with the live larva. Silica nanoparticles induced dehydration was the main reason behind their nanocidal property. At five days after treatment they registered 53.33 per cent mortality of fifth instar larva. The larval deformity was highest at green and metal silica nanoparticle at 2000 ppm which registered 23.33 and 30.00 per cent. The pupal deformity was noticed highest at green and metal silica naoparticle at 250 ppm with 23.33 and 16.67 per cent. In further observation adult deformity was highest with green and metal nanoparticle at lowest dosage of 250 ppm which recorded 16.67 and 13.33 per cent respectively. This might be due to the mortality of larvae at lower concentration was less compared to the higher concentration. The lower mortality in lower dosages resulted in deformed pupa and adults. Green silica nanoparticle can be used as an alternative to chemical pesticides.

Keywords: Silica, nanoparticle, deformity, *Spodoptera*, cotton

Introduction
Cotton an important commercial crop occupies 5% of the total cropped area distributed among three different agroclimatic zones in India, and consumes 55% pesticide share accounting for 40% of total production costs. This fact signifies the impact of insect pests and the increased agrochemical use in cotton production. Concern over human health and environmental consequences of agrochemicals besides pest resistance to pesticides has been a corner stone from the eighties. Nearly 130 species of insect pests occur on Indian cotton with a dozen of these arthropods requiring their management for realizing better cotton yields. The important foliage feeders are lepidopterans especially semilooper *Anomis flava* (Fabricius), and *Spodoptera litura* (Fabricius) and leaf roller *Syllepta derogate* (Fabricius. The tobacco caterpillar, *Spodoptera litura* is an economically important polyphagous pest in India and is considered as one of the major threats to the present day due to intensive agriculture and changing cropping patterns worldwide, it is next to *Helicoverpa armigera* (Hubner). *S. litura* is reported to feed on 150 species of plants [1] causing 26-100 per cent yield loss under field conditions [2].
Insecticide resistance to key insect pests remains a critical issue for producers and crop consultants. Among the several avenues to overcome the insecticidal resistance problem, replacement with new molecules of insecticide is one of the important considerations. The use of green nanoparticles is one such new avenue for pest management. Recently utilization of eco-friendly procedures have been developed for synthesis of nanoparticles to avoid use of toxic chemicals and to achieve biological compatibility. Application of biosynthesis methods through the use of microorganisms, yeasts, plants or plant extracts is known as green synthesis. Therefore, evaluation of green nanoparticles synthesised from plants having a novel mode of action for their toxicity and efficacy against *S. litura* was undertaken.

Materials and Methods

The green silica nanoparticles were synthesised from paddy husk, and evaluated in present study. The rice husk (variety, BPT-5204) was collected from the Shree Lakshmi Narayana Rice Mill, Manchalapur Road, Raichur, Karnataka, India. The metal nanoparticles were procured from M/s. High Media, Bangalore and M/s. Sigma Aldrich, Bangalore for conducting the experiment.

Preparation of Nano-silica

Silica nanoparticles were prepared by using refluxing technique from the extracted silica with 6 M of hydrochloric acid (HCl) at 85 °C for 4 h and washed repeatedly using distilled water to make it acid free. Then it was dissolved in 2.5 M sodium hydroxide (NaOH) by continuous stirring and sulphuric acid (H_2SO_4) was added until it reached to pH 8. The precipitated silica was washed repeatedly with warm distilled water to make it alkali free and then dried in the hot air oven at 50 °C for 48 h. Zetasizer (Malvern, ZETA Sizer, nano383 issue 5.0, England) was used (dynamic light scattering) to study the average particle diameter (nm) of biosynthesized silica nanoparticles. Biosynthesized silica nanoparticles were characterized by using UV- Visible spectrophotometer (Perkin Elmer, Lamda 35, Germany). X-ray diffraction (XRD) a rapid analytical technique primarily was used for phase identification of a crystalline material present in silica nanoparticles. The morphological features of biosynthesized silica nanoparticles were studied by using SEM (Carl Zeiss Microscopy, EVO 10, Germany).

Mass rearing of tobacco caterpillar

The rearing of tobacco caterpillar was carried out in the laboratory by collecting the egg masses from castor field. The larvae were fed with castor leaves till pupation. The pupae were collected and kept in an emergence cage (40 x 40 x40 cm) by providing 10 per cent honey solution as adult food. Four to five fresh castor leaves were collected from insecticide free plant, cleaned with water and shade dried. The petiole was wrapped in cotton, dipped in water and provided for egg laying. One to two egg masses along with the leaf bits was kept in each petri plate (10 x 1.5 cm) till hatching. Such larva was used for bioassay studies.

Bioassay studies on tobacco caterpillar

For bioassay studies the castor leaves were dipped in different concentrations (250, 500, 1000, 1500 and 2000 ppm) of nanoparticles and placed in plastic boxes as food. Later ten larvae were released on each plastic box and observations were recorded on mortality at 1, 3 and 5 days interval. It was compared with metal based nanoparticles and spinosad 45 SC at 0.12 ml/L as chemical check. It was replicated three times under lab conditions.

Per cent mortality of larvae was calculated by using the formula.

$$\text{Per cent larval mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \times 100$$

Larval deformities in the surviving treated individuals was observed and recorded. Per cent deformity was calculated by using the formula

$$\text{Per cent larval deformity} = \frac{\text{Number of deformed larvae}}{\text{Total number of larvae}} \times 100$$

Pupal deformity in the surviving treated individuals was observed and recorded. Per cent deformity was calculated by using the formula

$$\text{Per cent pupal deformity} = \frac{\text{Number of deformed pupae}}{\text{Total number of pupae}} \times 100$$

Adult deformity in the surviving treated individuals was observed and recorded. Per cent deformity was calculated by using the formula

$$\text{Per cent adult deformity} = \frac{\text{Number of deformed adults}}{\text{Total number of adults}} \times 100$$

Data collection and statistical analysis

Data was corrected by Abbot's formula and analyzed statistically using ANOVA 2 and MSTAT-C. The mean values were adjusted using the Duncan Multiple Range Test. Median lethal doses were calculated using probit analysis with log10 transformation of concentration of green nanoparticles. Data for a lethal time were corrected using Abbott formula and analyzed using the method of Finney.

Results and Discussion

Biosynthesis of silica nanoparticles from rice husk

In present study synthesized silica nanoparticles after ashing at 700 °C for 2 h showed amorphous form and similar result was recorded by Rafiee *et al.* [3]. The results of zetasizer revealed that average particle diameter of silica nanoparticles was 26.19 nm (Fig.1). The UV-Visible spectrum of SNPs recorded maximum absorption band edge of 310 nm in1.95 absorbance.

The X-ray diffraction pattern of silica nanoparticles which is characterized by a broad halo band of absorbance at about 2θ=15-25° region which confirms the amorphous structure of the biosynthesized silica nanoparticles. SEM analysis data showed that uniformly distributed silica nanoparticles were in the agglomerated form with spherical shape (Fig 4). Results are in accordance with findings of Vaccaro *et al.* [4].

Mortality of second instar *Spodoptera litura* larvae caused by silica nanoparticles

Effect of silica green nanoparticles against second instar *Spodoptera litura* larvae was studied in comparison with the metal form of the same compound at various dosages. The results revealed that as concentration of silica green nanoparticles increased from 250 ppm to 2000 ppm the mortality ranged from 36.67 to cent per cent (Table1). This situation was also found in the metal form of the same compound.

The green silica nanoparticle at 2000 ppm registered 60.00 per cent mortality and their metal form registered 63.33 per cent mortality at one day after treatment. This trend was similar at three days after treatment by recording 76.67 and 86.67 per cent mortality by green and metal silica nanoparticle at 2000 ppm respectively. The low toxicity in green nanoparticles may be attributed to impurities present in the silica nanoparticles synthesized from plant based which hinders its toxicity. The cent per cent mortality was observed by green and metal silica nanoparticle at 1500 ppm at five days after treatment (Fig. 2).

The bodies of dead third instar *Spodoptera litura* due to silica nanoparticles became dehydrated. The dead bodies became extremely dehydrated and shrunken in comparison with the live larva (Fig 5). Silica nanoparticles induced dehydration was the main reason behind their nanocidal property. Due to their ultra-small size, silica nanoparticles got impregnated in insect cuticle and damaged the cuticular water barrier. This caused insects to lose water from their body and ultimately they died because of desiccation [5].

In accordance with this study El-Helaly *et al.* [6] investigated that nanosilica at 500 ppm concentration is effective for suppression of *S. littoralis* on squash

Comparable to this study El-Samahy *et al.* [7] reported that nanosilica was the most effective treatment against *Tuta absoluta* at concentration of 300 ppm on tomato. The high efficacy of nanosilica against *T. absoluta* may be due to the absorbance of nanosilica into the cuticular lipids of the insect resulting in damage to the protective wax layer and induces death by desiccation

Table 1: Effect of silica nanoparticles against second instar larvae of *Spodoptera litura*

Treatment details	Dosage	Per cent mortality of *S. litura* at different intervals		
		1 DAT	3 DAT	5 DAT
T_1: Green Si nanoparticle	250 ppm	36.67 (37.27)^{h}*	53.33 (46.91)^{i}	76.67 (61.12)^{f}
T_2: Metal Si nanoparticle	250 ppm	43.33 (41.17)^{g}	56.67 (48.83)^{h}	83.33 (65.91)^{e}
T_3: Green Si nanoparticle	500 ppm	43.33 (41.17)^{g}	56.67 (48.83)^{h}	86.67 (68.58)^{d}
T_4: Metal Si nanoparticle	500 ppm	46.67 (43.09)^{f}	63.33 (52.73)^{g}	90.00 (71.57)^{c}
T_5: Green Si nanoparticle	1000 ppm	46.67 (43.09)^{f}	63.33 (52.73)^{g}	93.33 (75.04)^{b}
T_6: Metal Si nanoparticle	1000 ppm	53.33 (46.91)^{e}	73.33 (58.91)^{e}	93.33 (75.04)^{b}
T_7: Green Si nanoparticle	1500 ppm	53.33 (46.91)^{e}	70.00 (56.79)^{f}	100.00 (90.00)^{a}
T_8: Metal Si nanoparticle	1500 ppm	56.67 (48.83)^{d}	83.33 (65.91)^{c}	100.00 (90.00)^{a}
T_9: Green Si nanoparticle	2000 ppm	60.00 (50.77)^{c}	76.67 (61.12)^{d}	100.00 (90.00)^{a}
T_{10}: Metal Si nanoparticle	2000 ppm	63.33 (52.73)^{b}	86.67 (68.58)^{b}	100.00 (90.00)^{a}
T_{11}: Spinosad 45 SC	0.12 ml/l	76.67 (61.12)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}
T_{12}: Untreated control	--	0.00 (0.00)^{i}	3.33 (10.52)^{j}	3.33 (10.52)^{g}
S.Em±		0.13	0.23	0.16
CD @1%		0.52	0.90	0.65

n=30 second instar larvae DAT- Days after treatment

*Figures in the parentheses are “arcsine” transformed values

Means followed by same letters in a column are not significantly different (P=0.01) by DMRT

Mortality of fifth instar *Spodoptera litura* larvae caused by silica nanoparticles

Effects of silica green nanoparticles against fifth instar *Spodoptera litura* larvae were studied in comparison with the metal form of the same compound at various dosages. The investigations revealed that as concentration of silica green nanoparticles increased from 250 ppm to 2000 ppm the mortality ranged from 3.33 to 53.33 per cent at different days after treatment (Table 2).

The mortality of *Spodoptera litura* larvae at one day after treatment with green and metal silica nanopaticle at 2000 ppm was 16.67 and 26.67 per cent. Further observation at three days after treatment 40.00 per cent mortality was observed in green silica nanoparticle at 2000 ppm and metal silica nanoparticle at 1500 ppm. However, the similar fashion was observed at five days after treatment where they registered 53.33 per cent mortality. Thus silica green nanoparticles were moderately toxic to *Spodoptera* and have the potential to be used in alteration with chemical pesticides. This mortality may be attributed partly due to resistance imparted by silica to plants and partially to damage the cuticle of larva.

In support of these findings silica nanoparticles based insecticide is physically active i.e. these nanocide cause damage to the cuticle water barrier of the insects mostly by abrasion and to some extent due to adsorption and insect death occurs due to desiccation [8]. Silicon increases the crop resistance to pests and diseases [7]. Epstein [9] suggested that silicon deposited in the epidermal tissue may have several functions including support and protection as a mechanical barrier against pathogen and herbivore invasions.

The findings of the present investigation are in agreement with Chakravarthy *et al.* [10] who reported that Nano Ag caused maximum (56.89%) mortality of *S. litura* at 2400 ppm followed by 46.89 and 33.44 per cent mortality at 1200 and 600 ppm respectively, Bendary and Helaly [11] bioassayed and studied effects of the application of hydrophobic nano-silica against *S. littoralis* under field condition. It was applied in six doses 100, 150, 200, 250, 300 and 350 ppm at 50 ml/ plant, neonates of *S. littoralis* were exposed daily to tomato leaves. Results of treatment of hydrophobic nano-silica in larval test indicated high toxic action at all concentrations used parallel with concentrations. They concluded that nanosilica could be used in *S. littoralis* and among all 350 ppm of concentration has given the 98.24 per cent of mortality and it has affected the hatchability and fecundity of moth.

Table 2: Effect of silica nanoparticles against fifth instar larvae of *Spodoptera litura*

Treatment details	Dosage	Per cent mortality of *S. litura* at different intervals		
		1 DAT	3 DAT	5 DAT
T_1: Green Si nanoparticle	250 ppm	3.33 (10.52)^{g}*	16.67 (24.09)^{g}	26.67 (31.09)^{h}
T_2: Metal Si nanoparticle	250 ppm	6.67 (14.96)^{f}	16.67 (24.09)^{g}	23.33 (28.88)^{i}
T_3: Green Si nanoparticle	500 ppm	6.67 (14.96)^{f}	23.33 (28.88)^{f}	36.67 (37.27)^{f}
T_4: Metal Si nanoparticle	500 ppm	13.33 (21.42)^{d}	23.33 (28.88)^{f}	33.33 (35.26)^{g}
T_5: Green Si nanoparticle	1000 ppm	10.00 (18.43)^{e}	26.67 (31.09)^{e}	43.33 (41.17)^{e}
T_6: Metal Si nanoparticle	1000 ppm	16.67 (24.09)^{c}	36.67 (37.27)^{d}	46.67 (43.09)^{d}
T_7: Green Si nanoparticle	1500 ppm	13.33 (21.42)^{d}	36.67 (37.27)^{d}	46.67 (43.09)^{d}
T_8: Metal Si nanoparticle	1500 ppm	20.00 (26.57)^{b}	40.00 (39.23)^{c}	53.33 (46.91)^{c}
T_9: Green Si nanoparticle	2000 ppm	16.67 (24.09)^{c}	40.00 (39.23)^{c}	53.33 (46.91)^{c}
T_{10}: Metal Si nanoparticle	2000 ppm	26.67 (31.09)^{a}	46.67 (43.09)^{b}	63.33 (52.73)^{b}
T_{11}: Spinosad 45 SC	0.12 ml/l	16.67 (24.09)^{c}	56.67 (48.83)^{a}	83.33 (65.91)^{a}
T_{12}: Untreated control	--	0.00 (0.00)^{h}	0.00 (0.00)^{h}	3.33 (10.52)^{j}
S.Em±		0.29	0.24	0.25
CD @1%		1.15	0.94	1.00

n=30 fifth instar larvae DAT- Days after treatment
*Figures in the parentheses are "arcsine" transformed values
Means followed by same letters in a column are not significantly different (P=0.01) by DMRT

Deformity of fifth instar *Spodoptera litura* larvae caused by silica nanoparticles

Effects of silica green nanoparticles against fifth instar *Spodoptera litura* larvae were studied in comparison with the metal form of the same compound at various dosages. The results revealed that as concentration of silica green nanoparticles increased from 250 ppm to 2000 ppm the larval deformity increased from 10.00 to 23.33 per cent, pupal deformity decreased from 23.33 to 3.33 per cent, adult deformity also decreased from 16.67 to 6.67 per cent and total deformity ranged from 33.33 to 50.00 per cent (Table 3). This situation was also found in the metal form of the same compound. The larval deformity was highest at green and metal silica nanoparticle at 2000 ppm which registered 23.33 and 30.00 per cent. The pupal deformity was noticed highest at green and metal silica naoparticle at 250 ppm with 23.33 and 16.67 per cent. In further observation on adult deformity was highest at green and metal nanoparticle at lowest dosage of 250 ppm which recorded 16.67 and 13.33 per cent respectively. This might be due to the mortality of larvae at lower concentration was less compare to the higher concentration. The lower mortality in lower dosages resulted in deformed pupa and adults (Fig. 3).

In line with these findings Borei *et al.* [12] reported that silica nanoparticles at425 ppm cause 95.33 per cent mortality, pupal stage increased to16.20 days, adult longevity was increased 13.80 days, eggs/female were decreased to 115 and their per cent hatchability was decreased to 48.75. Thus they concluded that Nano-silica sprays affect the feeding preference of the *S. littoralis*, thus increasing the resistance of soybean. Concomitantly it affects biological parameters of the insect such as longevity and nymph production, thus reducing the reproductive potential of females on soybean and therefore reducing the insect population density, damages and yield losses to the crop.

Ayoub *et al.* [13] from their study on cotton leaf worm *Spodoptera littoralis* investigated the entomotoxic effect of the synthesized SiO_2 NPs is mainly dominated by particle size compared to surface characteristic. They suggested that silica nanostructures are more effective on adults than larvae. Therefore, the mortality is attributed to the impairment of the digestive tract and surface enlargement of the integument as a consequence of dehydration or blockage of spiracles and tracheas

Table 3: Effect of silica nanoparticles on deformity of *Spodoptera litura*

Treatment details	Dosage	Per cent deformity of *S. litura* at different stages			
		Larvae	Pupae	Adult	Total deformity
T_1: Green Si nanoparticle	250 ppm	10.00 (18.43)^{g}*	23.33 (28.88)^{a}	16.67 (24.09)^{a}	50.00 (45.00)^{a}
T_2: Metal Si nanoparticle	250 ppm	13.33 (21.42)^{f}	16.67 (24.09)^{b}	13.33 (21.42)^{b}	43.33 (41.17)^{b}
T_3: Green Si nanoparticle	500 ppm	13.33 (21.42)^{f}	13.33 (21.42)^{c}	13.33 (21.42)^{b}	40.00 (39.23)^{c}
T_4: Metal Si nanoparticle	500 ppm	16.67 (24.09)^{e}	10.00 (18.43)^{d}	10.00 (18.43)^{c}	36.67 (37.27)^{d}
T_5: Green Si nanoparticle	1000 ppm	16.67 (24.09)^{e}	10.00 (18.43)^{d}	10.00 (18.43)^{c}	36.67 (37.27)^{d}
T_6: Metal Si nanoparticle	1000 ppm	20.00 (26.57)^{d}	6.67 (14.96)^{e}	6.67 (14.96)^{d}	33.33 (35.26)^{e}
T_7: Green Si nanoparticle	1500 ppm	20.00 (26.57)^{d}	3.33 (10.52)^{f}	6.67 (14.96)^{d}	30.00 (33.21)^{f}
T_8: Metal Si nanoparticle	1500 ppm	26.67 (31.09)^{b}	6.67 (14.96)^{e}	3.33 (10.52)^{e}	36.67 (37.27)^{d}
T_9: Green Si nanoparticle	2000 ppm	23.33 (28.88)^{c}	3.33 (10.52)^{f}	6.67 (14.96)^{d}	33.33(35.26)^{e}
T_{10}: Metal Si nanoparticle	2000 ppm	30.00 (33.21)^{a}	3.33 (10.52)^{f}	0.00 (0.00)^{f}	33.33 (35.26)^{e}
T_{11}: Spinosad 45 SC	0.12 ml/l	16.67 (24.09)^{e}	0.00 (0.00)^{g}	0.00 (0.00)^{f}	16.67 (24.09)^{g}
T_{12}: Untreated control	--	0.00 (0.00)^{h}	0.00 (0.00)^{g}	0.00 (0.00)^{f}	0.00 (0.00)^{h}
S.Em±		0.26	0.22	0.18	0.21
CD @1%		1.04	0.86	0.72	0.83

n=30 fifth instar larvae
*Figures in the parentheses are "arcsine" transformed values
Means followed by same letters in a column are not significantly different (P=0.01) by DMRT

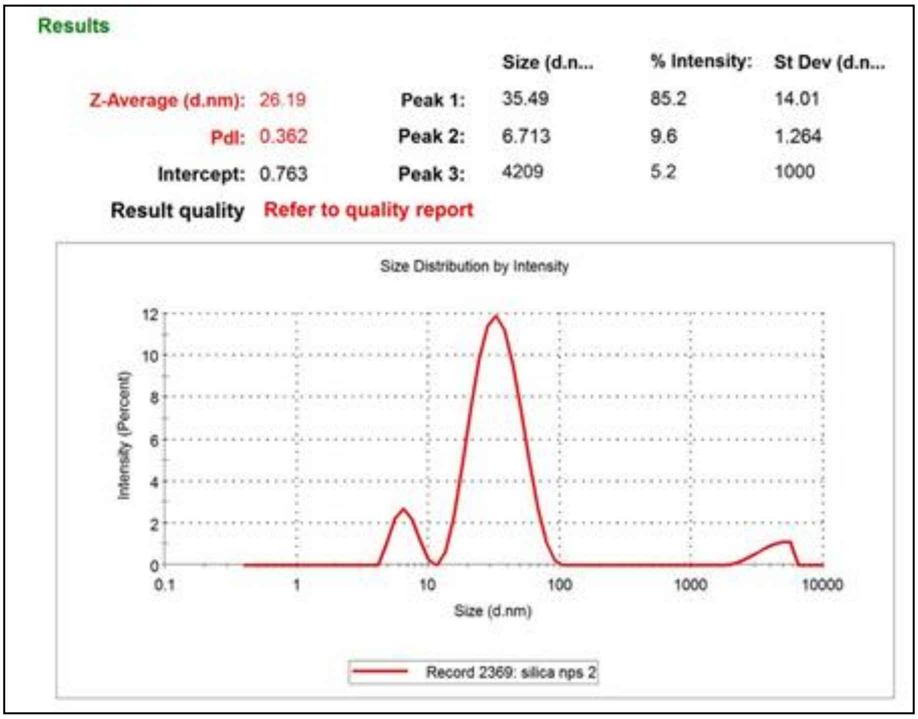

Fig 1: Average particle diameter of green silica nanoparticles

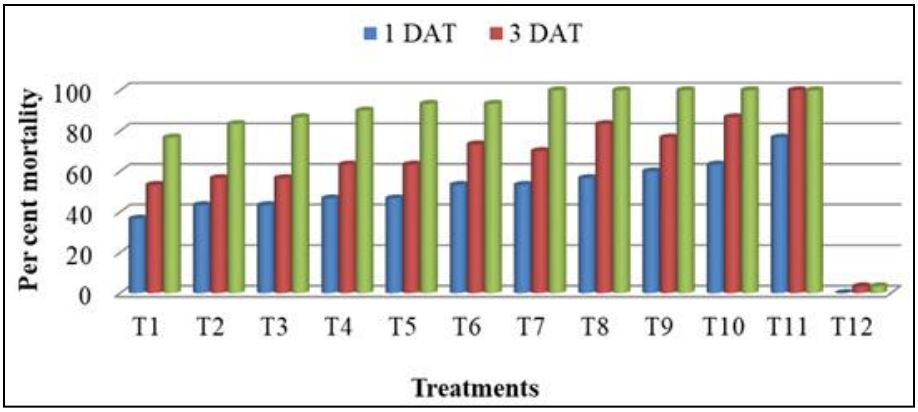

Fig 2: Mortality of second instar *Spodoptera litura* larvae caused by silica nanoparticles

T_1: Green Si nanoparticle @ 250 ppm T_2: Metal Si nanoparticle @ 250 ppm T_3: Green Si nanoparticle @ 500 ppm
T_4: Metal Si nanoparticle @ 500 ppm T_5: Green Si nanoparticle @ 1000 ppm T_6: Metal Si nanoparticle @ 1000 ppm
T_7: Green Si nanoparticle @ 1500 ppm T_8: Metal Si nanoparticle @ 1500 ppm T_9: Green Si nanoparticle @ 2000 ppm
T_{10}: Metal Si nanoparticle @ 2000 ppm T_{11}: Spinosad 45 SC @ 0.12 ml/l T_{12}: Untreated control

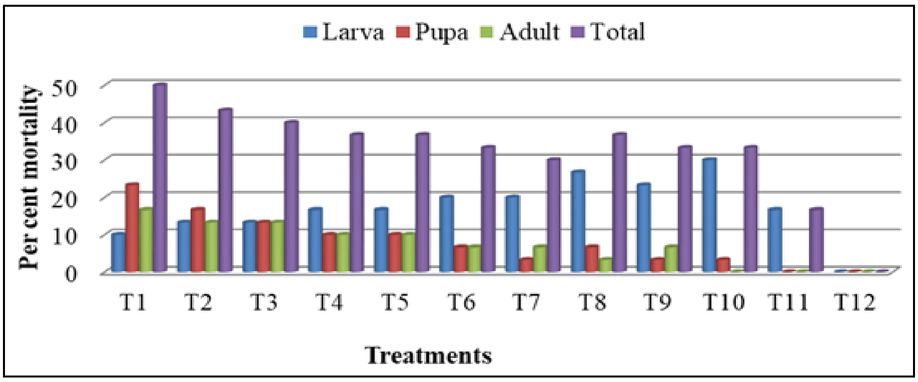

Fig 3: Deformity of fifth instar *Spodoptera litura* larvae caused by silica nanoparticles

T_1: Green Si nanoparticle @ 250 ppm T_2: Metal Si nanoparticle @ 250 ppm T_3: Green Si nanoparticle @ 500 ppm
T_4: Metal Si nanoparticle @ 500 ppm T_5: Green Si nanoparticle @ 1000 ppm T_6: Metal Si nanoparticle @ 1000 ppm
T_7: Green Si nanoparticle @ 1500 ppm T_8: Metal Si nanoparticle @ 1500 ppm T_9: Green Si nanoparticle @ 2000 ppm
T_{10}: Metal Si nanoparticle @ 2000 ppm T_{11}: Spinosad 45 SC @ 0.12 ml/l T_{12}: Untreated control

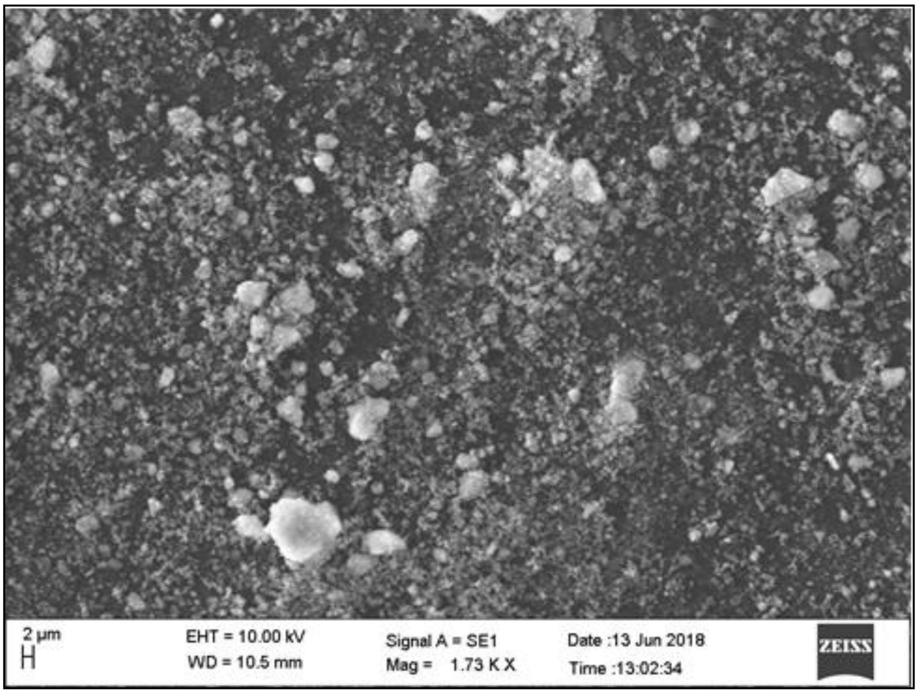

Fig 4: Scanning electron image of green silica nanoparticles

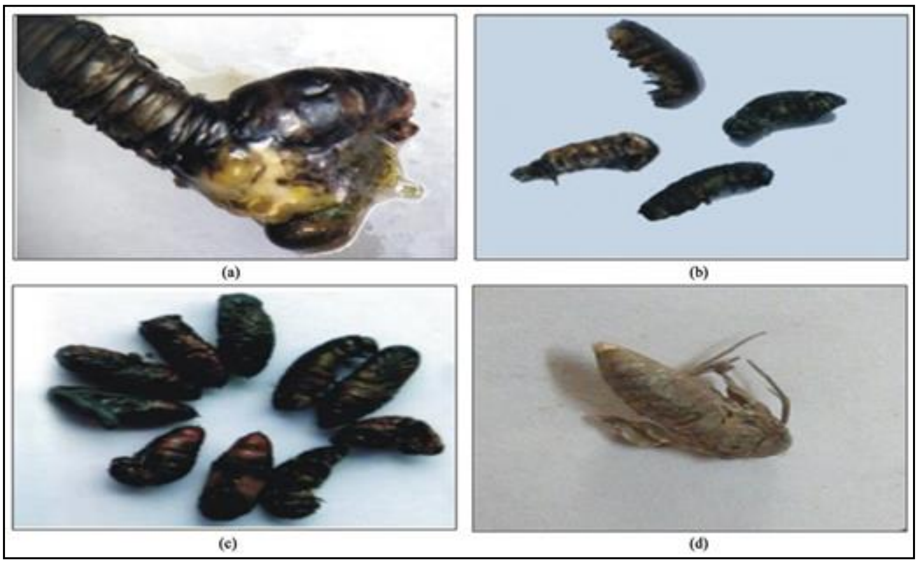

Fig 5: Mortality and deformity of *Spodoptera litura* larvae due to nanoparticles; a. Dead larvae b. Deformed Larvae c. Deformed Pupae d. Deformed adults

Conclusion

Silica nanoparticles synthesised from paddy husk can be used as an alternative to chemical pesticides for the management of defoliator *Spodoptera* Plant-mediated synthesis of NPs have a bright future and potential for developing safer and more effective chemical pesticide formulations for pest control, which potentially could result in revolutionary changes in this field.

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