

Original Research Article

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Biosynthesis of Green Silica Nanoparticles and Its Effect on Cotton Aphid, *Aphis gossypii* Glover and Mealybug, *Phenacoccus solenopsis* Tinsley

G. Pavitra^{1*}, N. Sushila¹, A.G. Sreenivas¹, J. Ashok¹ and H. Sharanagouda²

¹Department of Agricultural Entomology, College of Agriculture, UAS, Raichur, Karnataka, India

²Department of Processing and Food Engineering, CAE, UAS, Raichur, Karnataka, India

*Corresponding author

ABSTRACT

Sucking pests have become a serious problem in *Bt* cotton. The cotton aphid and mealybug reduces its yield to the extent of 30-40 per cent and 40-50 per cent, respectively. In this context an investigation was carried out on synthesis of silica nanoparticles from rice husk and its impact against cotton aphid and mealybug during 2017-18 at centre for nanotechnology lab, UAS Raichur. For comparison purposes, metal silica nanoparticles and dinotefuran 20% SG was used as an insecticide. The biosynthesised nanoparticles were characterized by zetasizer (size: 26.19 nm), UV-visible spectroscopy (wavelength: 310 nm), X-ray diffraction (absorption at $2\theta=15-25^\circ$ region) and scanning electron microscope (agglomerated form with spherical shape). At five days after treatment green silica nanoparticle at 2000 ppm caused cent per cent mortality of both cotton aphid and mealybug. Pesticidal activity of silica nanoparticles revealed that at 2000 ppm concentration, maximum mortality of insects was recorded and hence can be selectively used for suppression of the pest.

Keywords

Rice husk, Silica nanoparticles, Cotton, *Aphis gossypii*, *Phenacoccus solenopsis*

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Introduction

Cotton 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. The low productivity of cotton is ascribed to many factors, but the most serious is the intensity of insect pest damage. The insect pest's spectrum of cotton is quite complex and as many as 1326 species of insect pests have been listed on this crop

throughout the world (Agarwal *et al.*, 1984). Cotton is subjected to damage by 162 species of pests right from germination to the final picking (Dhaliwal and Arora, 1998).

Cotton aphids are the most serious pests of cotton all over the world (Rummel *et al.*, 1995). Cotton aphids injure cotton plants by continually feeding on fluids in plant phloem tubes. This feeding can stimulate foliar alterations, delay of the plant growth, fewer fruit setting, lower fruit retention and reduced cotton lint weight (Raboudi *et al.*, 2002). In addition, virus transmission can lessen the

cotton yield up to 30-40%. Cotton aphids reduced leaf area by 58% and shoot biomass by 45% (Muhammad *et al.*, 2014).

During 2006, the mealy bug, *Phenacoccus solenopsis* caused economic damage, reducing yields by up to 40-50 percent in infested fields in several parts of Gujarat (Nagrare *et al.*, 2009). At around the same time, mealy bug infestations were found in all the nine cotton growing states. The mealy bug has become a major pest in almost all cotton growing states of India. Apart from yield losses, the cost of insecticide application has increased by US \$ 250-375 per acre in both India (Nagrare *et al.*, 2009).

Nanotechnology is an interdisciplinary science with a wide array of applications in various fields like medicine, information and communication, chemistry, environment, defence, security, consumer goods and agriculture.

Application of nanotechnology in crop protection holds a significant promise in management of insects and pathogens, by controlled and targeted delivery of agrochemicals and also by providing diagnostic tools for early detection. So the present investigation was carried out on synthesis of green silica nanoparticles and their effect on cotton aphid and mealybug.

Materials and Methods

The rice husk (variety, BPT-5204) was collected from the Shree Lakshmi Narayana Rice Mill, Manchalapur Road, Raichur, Karnataka, India. Cotton seeds were collected from Main Agricultural Research Station (MARS) UAS campus, Raichur. The chemicals and metal nanoparticles were procured from M/s. High Media, Bangalore and M/s. Sigma Aldrich, Bangalore for conducting the experiment.

Biosynthesis of silica nanoparticles from rice husk

Rice husk was washed thoroughly with potable water then dried in a hot air oven at 110 °C for 24 h. The dried rice husk was refluxed with an acidic solution of 1N HCl at 85 °C for 90 min. Then sample was cooled at room temperature and thoroughly washed with warm distilled water until the rinse became free from acid. The refluxed husk was dried in hot air oven at 110 °C for 24 h and then subjected to heat treatment in muffle furnace at 7000 °C for 2 h to obtain the ash. Later 20 g of ash was stirred in a 160 ml of 2.5 M NaOH solution and solution was heated in a covered beaker at 90 °C for 3 h by stirring constantly and filtered through filter paper (Whatman No.4). Then the solution was allowed to cool at room temperature and 10 M H₂SO₄ was then added under constant stirring at controlled conditions until it reached to pH 2, then NH₄OH was used to adjust pH level up to 8.5 and was allowed to stand at room temperature for 3 h. White silica precipitate was washed repeatedly with the distilled water until the filtrate was completely free from alkali. The silica was dried in hot air oven at 105°C temperature for 24 h.

Silica nanoparticles were prepared by using refluxing technique of the above 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 (H₂SO₄) 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 (Rafiee *et al.*, 2012) and finally obtained the silica powder. Later characterization was carried out by zetasizer, UV-visible spectroscopy, X-ray diffraction and scanning electron microscope.

Bioassay studies on aphid and mealybug

The aphid nymphs required for the experiment was sourced from the insect culture maintained in the laboratory of Department of Agricultural Entomology. For bioassay studies the cotton leaves were placed in petri plates as food. Then ten nymphs of aphids were released on each petri plate. Later different concentrations (250, 500, 1000, 1500 and 2000 ppm) of silica nanoparticles were sprayed using potters tower sprayer and observations were recorded on mortality at 1, 3 and 5 days interval. It was compared with metal based nanoparticles and dinotefuron 20% SG at 0.3 g/L as chemical check. It was replicated thrice.

Similarly, for mealybug, cotton leaves were treated with silica nanoparticles and nymphs were released. Later mortality was recorded at 1, 3 and 5 days interval. It was compared with metal based nanoparticles and buprofezin 25 SC at 1ml/L as chemical check. It was replicated thrice.

Turgidity of leaf was maintained by placing cotton stubs below the leaf in petri plate and petiole of the leaf was covered with cotton dipped in agar solution.

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

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

Results and Discussion

Biosynthesis of silica nanoparticles from rice husk

The characterization of biosynthesized silica nanoparticles for identification of its size, morphology and functional group are explained below.

The results of zetasizer revealed that average particle diameter of silica nanoparticles was 26.19 nm (Fig. 1). Results are in line with the findings of Das *et al.*, (2014).

The UV-Visible spectrum of SNPs recorded maximum absorption band edge of 310 nm in 1.95 absorbance (Fig. 2). These optical features are similar to those obtained in previous reports and attributed to Si-O-Si bond confirming the presence of silica nanoparticles (Vaccaro *et al.*, 2013; Djangang *et al.*, 2015).

The X-ray diffraction pattern of silica nanoparticles which is characterized by a broad halo band of absorbance at about $2\theta=15-25^\circ$ region which confirms the amorphous structure of the biosynthesized silica nanoparticles (Fig. 3).

Similar results were recorded by Djangang *et al.*, (2015) who reported amorphous nature of silica nanoparticles from rice husk.

SEM analysis data showed that uniformly distributed silica nanoparticles were in the agglomerated form with spherical shape (Plate 1). Results are in accordance with findings of Vaccaro *et al.*, (2013) Djangang *et al.*, (2015).

Mortality of aphids caused by silica nanoparticles

At one day after treatment, the mortality of aphids ranged between 30.00 to 56.67 per cent among different treatments (Table 1). The metal Si nanoparticle @ 2000 ppm (T₁₀) recorded highest mortality (56.67%) and was significantly superior to all other treatments.

The next highest mortality of 53.33 per cent was observed in metal Si nanoparticle @ 1500 ppm (T₈) and green Si nanoparticle @ 2000 ppm (T₉) which were on par with each other (Fig. 4).

At three days after treatment, cent per cent mortality was observed in chemical check dinotefuran @ 0.3 g/l (T₁₁) which proved to be significantly superior to all other treatments (Table 1). This was followed by 83.33 per cent mortality in metal Si nanoparticle @ 2000 ppm (T₁₀) treatment. The metal Si nanoparticle @ 1500 ppm (T₈) and green Si nanoparticle @ 2000 ppm (T₉) recorded 73.33 per cent mortality. Untreated control recorded the lowest mortality of 3.33 per cent (Fig. 4).

At five days after treatment mortality of aphids ranged from 6.67 to 100.00 per cent among various treatments (Table 1).

The green Si nanoparticle @ 2000 ppm (T₉), metal Si nanoparticle @ 2000 ppm (T₁₀) and dinotefuran @ 0.3 g/l (T₁₁) recorded cent per cent mortality.

Lowest mortality was recorded in untreated control with 6.67 per cent (Fig. 4).

Table.1 Effect of silica nanoparticles on cotton aphid, *Aphis gossypii*

Treatment details	Dosage	Per cent mortality of aphids at different intervals		
		1 DAT	3 DAT	5 DAT
T ₁ : Green Si nanoparticle	250 ppm	30.00 (33.21) ^{g*}	46.67 (43.09) ^h	63.33 (52.73) ^h
T ₂ : Metal Si nanoparticle	250 ppm	36.67 (37.27) ^f	53.33 (46.91) ^g	70.00 (56.79) ^g
T ₃ : Green Si nanoparticle	500 ppm	36.67 (37.27) ^f	53.33 (46.91) ^g	73.33 (58.91) ^f
T ₄ : Metal Si nanoparticle	500 ppm	43.33 (41.17) ^e	56.67 (48.83) ^f	76.67 (61.12) ^e
T ₅ : Green Si nanoparticle	1000 ppm	43.33 (41.17) ^e	60.00 (50.77) ^e	76.67 (61.12) ^e
T ₆ : Metal Si nanoparticle	1000 ppm	50.00 (45.00) ^c	66.67 (54.74) ^d	83.33 (65.91) ^d
T ₇ : Green Si nanoparticle	1500 ppm	46.67 (43.09) ^d	66.67 (54.74) ^d	90.00 (71.57) ^c
T ₈ : Metal Si nanoparticle	1500 ppm	53.33 (46.91) ^b	73.33 (58.91) ^c	96.67 (79.48) ^b
T ₉ : Green Si nanoparticle	2000 ppm	53.33 (46.91) ^b	73.33 (58.91) ^c	100.00 (90.00) ^a
T ₁₀ : Metal Si nanoparticle	2000 ppm	56.67 (48.83) ^a	83.33 (65.91) ^b	100.00 (90.00) ^a
T ₁₁ : Dinotefuran 20 % SG	0.30 g/l	46.67 (43.09) ^d	100.00 (90.00) ^a	100.00 (90.00) ^a
T ₁₂ : Untreated control	--	0.00 (0.00) ^h	3.33 (10.52) ⁱ	6.67 (14.96) ⁱ
S.Em±		0.16	0.21	0.15
CD @1%		0.62	0.82	0.59

n=30 second instar nymphs

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

Table.2 Effect of silica nanoparticles on cotton mealybug, *Phenacoccus solenopsis*

Treatment details	Dosage	Per cent mortality of mealybugs at different intervals		
		1 DAT	3 DAT	5 DAT
T ₁ : Green Si nanoparticle	250 ppm	23.33 (28.88) ^{h*}	40.00 (39.23) ^j	50.00 (45.00) ^g
T ₂ : Metal Si nanoparticle	250 ppm	30.00 (33.21) ^g	43.33 (41.17) ⁱ	56.67 (48.83) ^f
T ₃ : Green Si nanoparticle	500 ppm	33.33 (35.26) ^f	50.00 (45.00) ^h	56.67 (48.83) ^f
T ₄ : Metal Si nanoparticle	500 ppm	36.67 (37.27) ^e	50.00 (45.00) ^h	63.33 (52.73) ^e
T ₅ : Green Si nanoparticle	1000 ppm	36.67 (37.27) ^e	53.33 (46.91) ^g	70.00 (56.79) ^d
T ₆ : Metal Si nanoparticle	1000 ppm	43.33 (41.17) ^d	56.67 (48.83) ^f	83.33 (65.91) ^c
T ₇ : Green Si nanoparticle	1500 ppm	46.67 (43.09) ^c	60.00 (50.77) ^e	83.33 (65.91) ^c
T ₈ : Metal Si nanoparticle	1500 ppm	46.67 (43.09) ^c	63.33 (52.73) ^d	90.00 (71.57) ^b
T ₉ : Green Si nanoparticle	2000 ppm	50.00 (45.00) ^b	66.67 (54.74) ^c	100.00 (90.00) ^a
T ₁₀ : Metal Si nanoparticle	2000 ppm	53.33 (46.91) ^a	73.33 (58.91) ^b	100.00 (90.00) ^a
T ₁₁ : Buprofezin 25 SC	1.00 ml/l	46.67 (43.09) ^c	83.33 (65.91) ^a	100.00 (90.00) ^a
T ₁₂ : Untreated control	--	0.00 (0.00) ⁱ	0.00 (0.00) ^k	3.33 (10.52) ^h
S.Em±		0.19	0.15	0.19
CD @ 1%		0.77	0.61	0.77

n=30 second instar nymphs

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

Fig.1 Average particle diameter of green silica nanoparticles

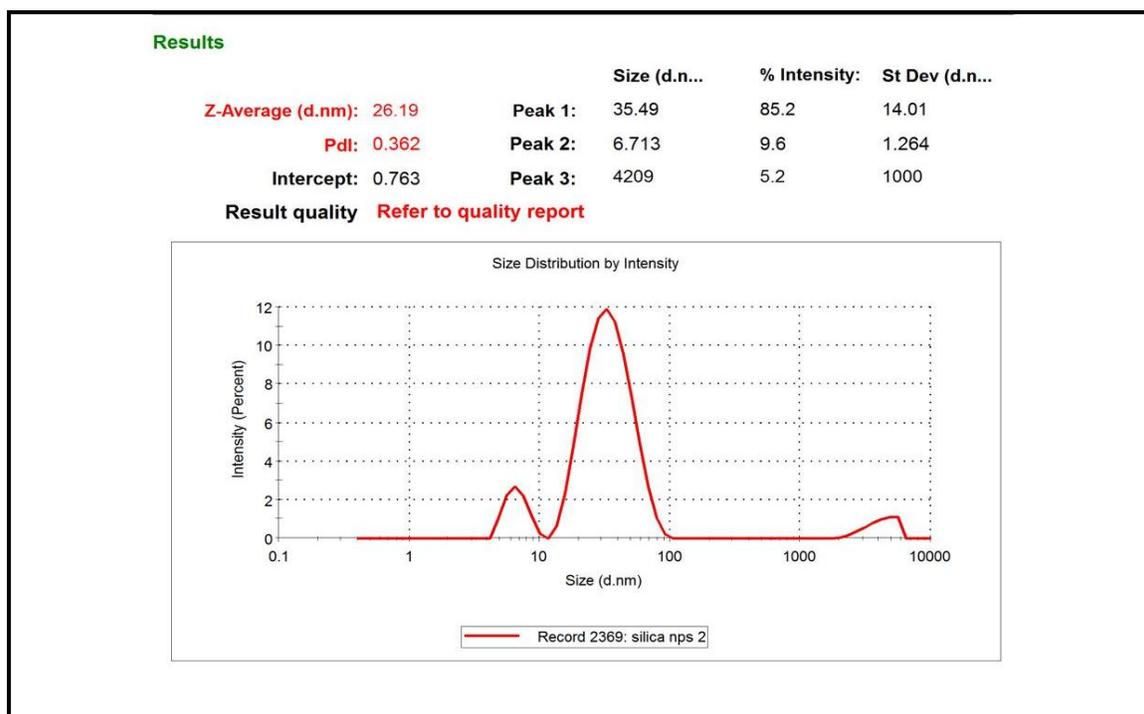


Fig.2 UV - Visible spectrum analysis of green silica nanoparticles

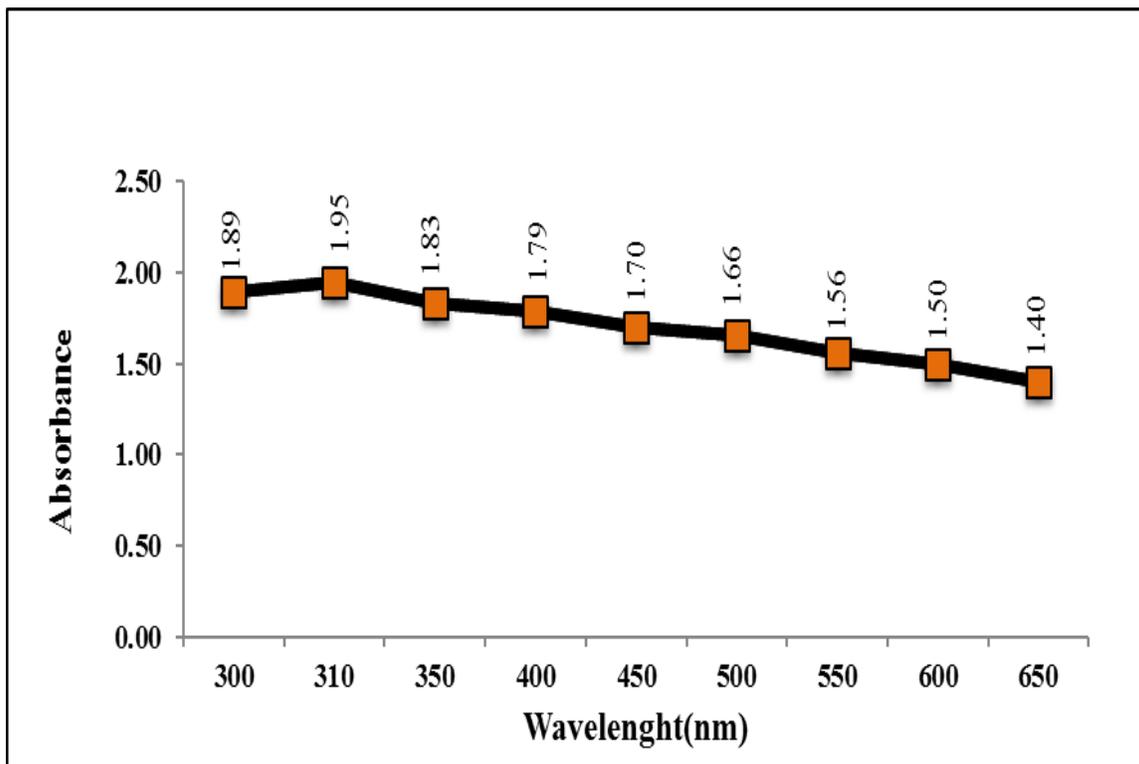


Fig.3 X- ray diffraction of green silica nanoparticles

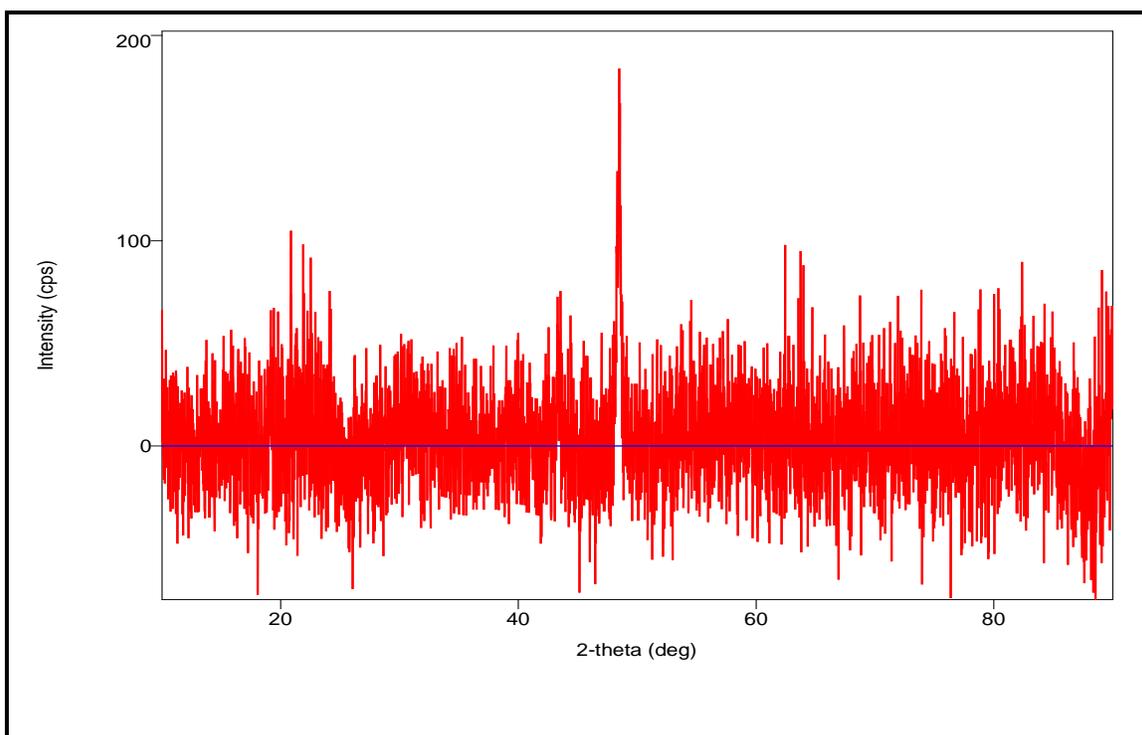
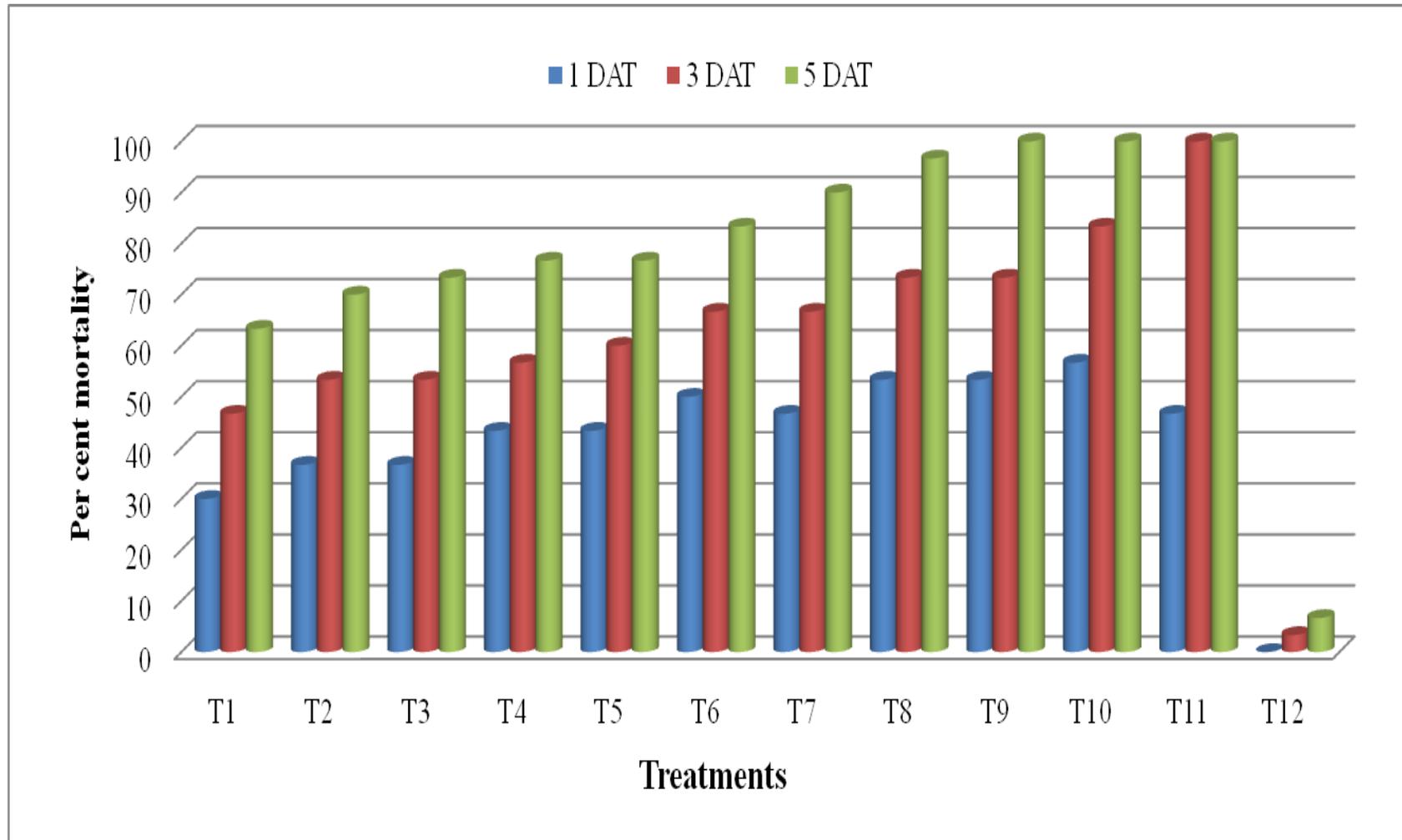


Fig.4 Mortality of cotton aphid, *Aphis gossypii* caused by silica nanoparticles

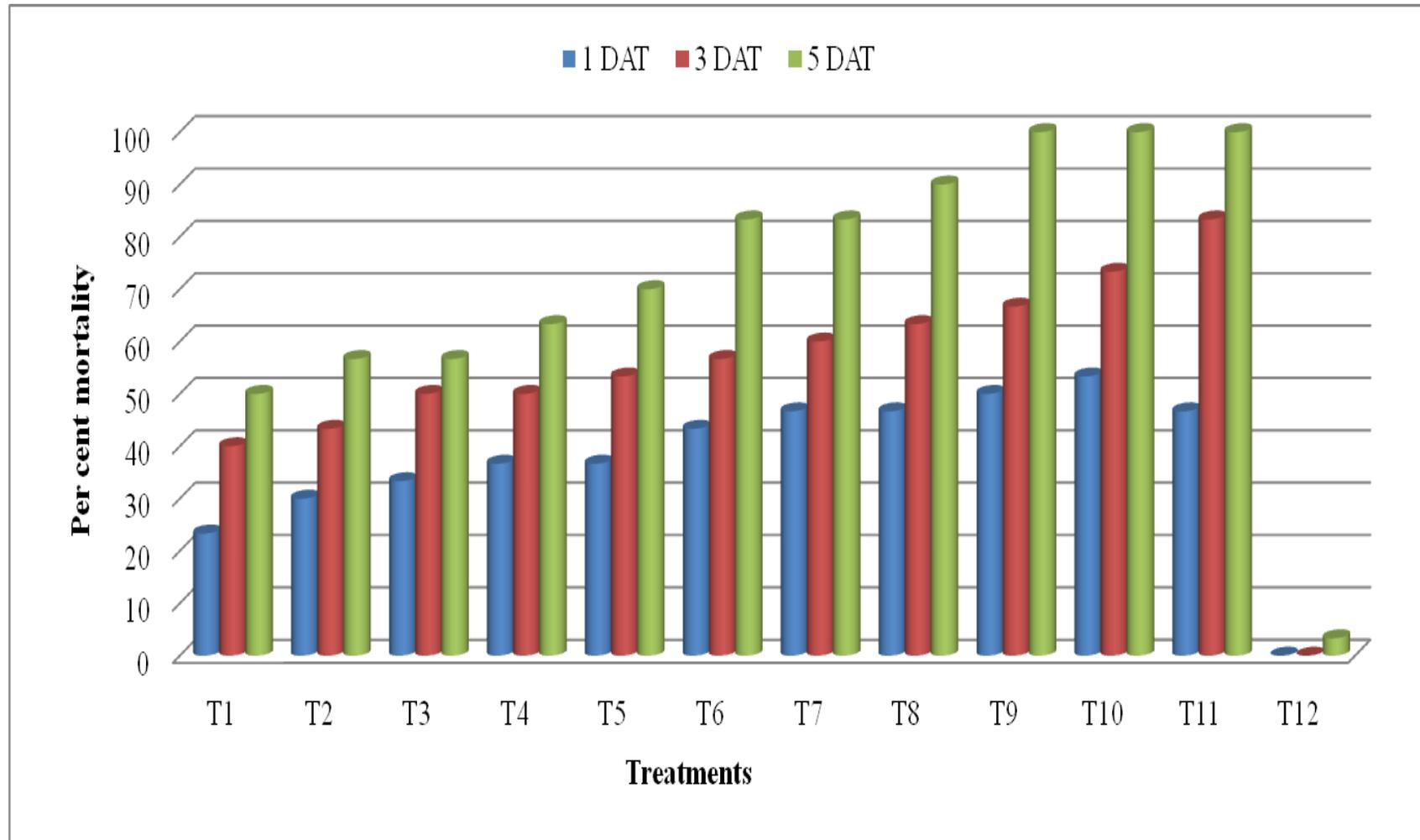


T₁: Green Si nanoparticle @ 250 ppm
 T₄: Metal Si nanoparticle @ 500 ppm
 T₇: Green Si nanoparticle @ 1500 ppm
 T₁₀: Metal Si nanoparticle @ 2000 ppm

T₂: Metal Si nanoparticle @ 250 ppm
 T₅: Green Si nanoparticle @ 1000 ppm
 T₈: Metal Si nanoparticle @ 1500 ppm
 T₁₁: Dinotefuran 20 % SG @ 0.30 g/l

T₃: Green Si nanoparticle @ 500 ppm
 T₆: Metal Si nanoparticle @ 1000 ppm
 T₉: Green Si nanoparticle @ 2000 ppm
 T₁₂: Untreated control

Fig.5 Mortality of cotton mealybug, *Phenacoccus solenopsis* caused by silica nanoparticles

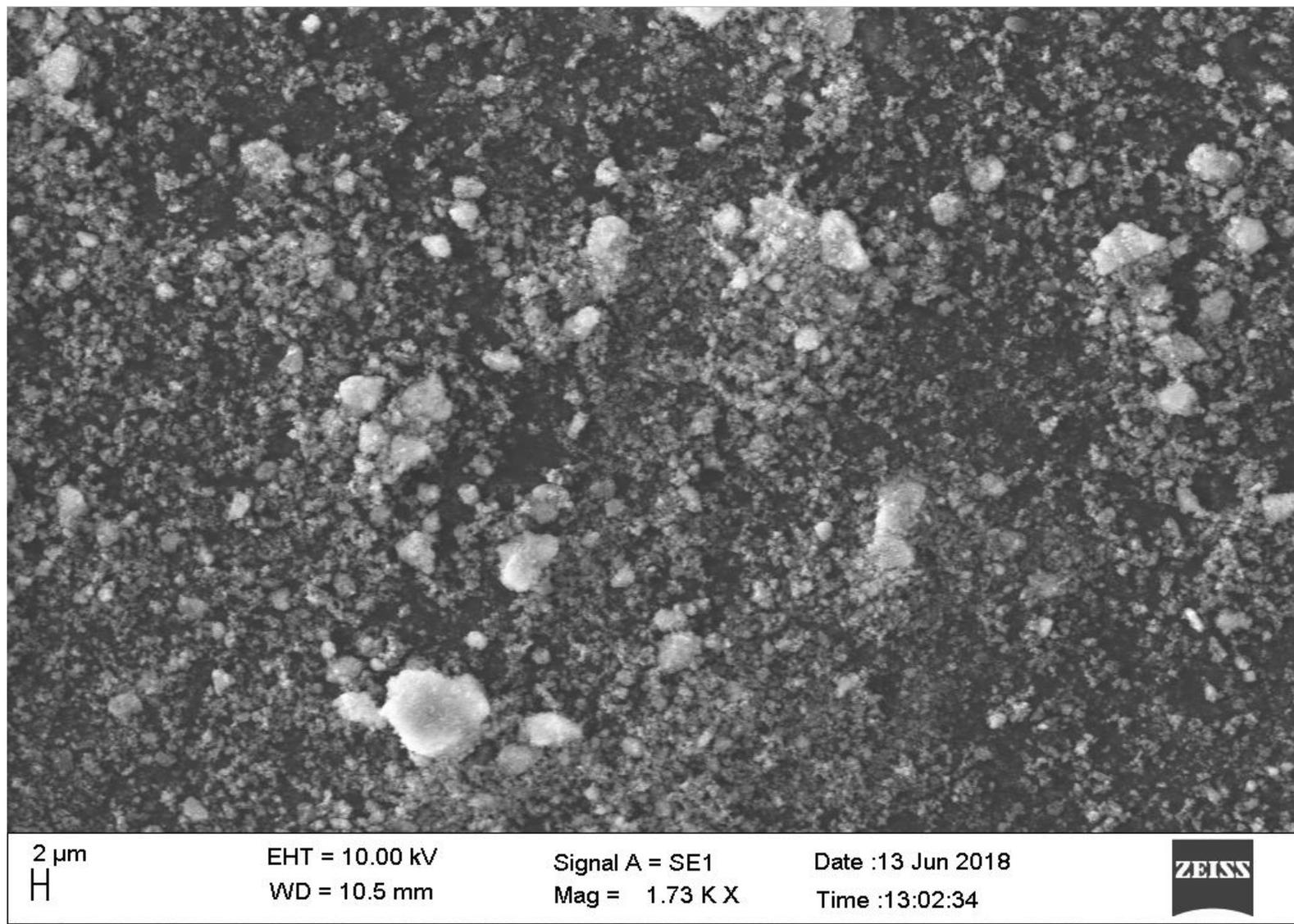


T₁: Green Si nanoparticle @ 250 ppm
 T₄: Metal Si nanoparticle @ 500 ppm
 T₇: Green Si nanoparticle @ 1500 ppm
 T₁₀: Metal Si nanoparticle @ 2000 ppm

T₂: Metal Si nanoparticle @ 250 ppm
 T₅: Green Si nanoparticle @ 1000 ppm
 T₈: Metal Si nanoparticle @ 1500 ppm
 T₁₁: Buprofezin 25 SC @ 1ml/l

T₃: Green Si nanoparticle @ 500 ppm
 T₆: Metal Si nanoparticle @ 1000 ppm
 T₉: Green Si nanoparticle @ 2000 ppm
 T₁₂: Untreated control

Plate.1 Scanning electron microscope image of green silica nanoparticles



Nearer to present findings Abd El-Wahab *et al.*, (2016) reported a lower dose of 500 ppm of hydrophilic nano silica could be an effective pest control approach for species *Myzus persicae*, *Acyrtosiphon pisum* and *Aphis craccivora*. Finally, it could be concluded that silica had moderate control affect against tested aphid species. In line with present findings Rouhani *et al.*, 2012, Nitai *et al.*, 2010 and Lee *et al.*, 2005 found that silica accumulates intracellular in plants and prevent sucking.

Mortality of mealybugs caused by silica nanoparticles

At one day after treatment mortality of mealybugs ranged from 23.33 to 53.33 per cent (Table 2). Highest mortality of 53.33 per cent was recorded in metal Si nanoparticle @ 2000 ppm (T₁₀) and it was significantly superior to all other treatments. The next best treatment was green Si nanoparticle @ 2000 ppm (T₉) with 50.00 per cent mortality. There was no mortality of mealybugs in untreated control. The lowest mortality of 23.33 per cent was recorded in green Si nanoparticle @ 250 ppm (T₁) treatment (Fig. 5).

At three days after treatment, the mortality rates increased proportionately with increased concentration in all treatments (Table 2). Chemical check buprofezin 25 SC @ 1 ml/l (T₁₁) recorded the highest mortality of 83.33 per cent and was significantly superior to all other treatments. This was followed by metal Si nanoparticle and green Si nanoparticle @ 2000 ppm with 73.33 and 66.67 per cent mortality respectively (Fig. 5).

At five days after treatment, the mortality ranged from 50.00 to 100.00 per cent (Table 2) of which green Si nanoparticle @ 2000 ppm (T₉), metal Si nanoparticle @ 2000 ppm (T₁₀) and buprofezin 25 SC @ 1 ml/l (T₁₁) recorded cent per cent mortality. Next highest mortality was exhibited in metal Si nanoparticle @ 1500 ppm (T₈) with 90.00 per cent. Lowest mortality was witnessed in untreated control with 3.33 per cent (Fig. 5).

Since there are no reviews available on silica nanoparticles against mortality of cotton mealybug, hence reviews of effect of silica green nanoparticles on other insects are discussed here. Rouhani *et al.*, (2013) reported that nanosilica and nanosilver on *Callosobruchus maculatus* F. resulted in 100 and 75 per cent mortality at the highest concentration of 2.5 g kg⁻¹ on day 14. Ziaee and Ganji (2016) reported the highest insecticidal potential of silica nanoparticles could be attributed due to increase in high surface to volume ratio which increased insect contact with particles which led to more cuticle desiccation and death.

The green silica nanoparticle @ 2000 ppm has effectively managed both the sucking pests like cotton aphid and mealybug. Results suggest the possibility of using green silica nanoparticles to eradicate pests and can be used as valuable tools in pest management. While the environmental effects of using silica nanoparticles as insecticide is subject of further study, one obvious advantage of using them as insecticides is the low risk of developing resistance by the insects in long term uses.

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