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Original Research Article

Effect of Green Zinc and Copper Nanoparticles on Cotton Mealybug, *Phenacoccus solenopsis* Tinsley

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A B S T R A C T

Among the sucking pests ravaging cotton, mealybug is one among them affecting the quality and quantity of cotton. The cotton mealybug reduces its yield to the extent of 40-50 per cent. The green zinc oxide and copper nanoparticles were synthesized from spinach and tulasi leaves respectively and its characterization was done by zetasizer (size: ZnO: 20.67 nm, Cu: 82.41 nm) and scanning electron microscope (ZnO: rod shape, Cu: spherical shape). This study was carried out in the laboratory of Department of Agricultural Entomology during 2017-18. Different concentrations of nanoparticles (250, 500, 1000, 1500 and 2000 ppm) and standard check buprofezin 25 SC @ 1 ml l^{-1} was tested against mealybug *P. solenopsis* and each treatment was repeated thrice. Results revealed that with the increased concentration of nanoparticles the mortality of nymphs increased, wherein, the green zinc oxide and copper nanoparticles @ 2000 ppm recoreded 90.00 and 80.00 per cent mortality respectively at five days after treatment as compared to its metal form which registered 93.33 and 86.67 per cent mortality. Hence, looking at their efficiency the nanoparticles have wide scope in pest management to substitute the presently used synthetic chemicals, of course with a dare concern of safety.

Keywords

Spinach, Tulasi, Green zinc oxide nanoparticles, Green copper nanoparticles, Cotton, *Phenacoccus solenopsis*

Introduction

Cotton, *Gossypium hirsutum* L., is one of the commercially important fibre crops in the world grown as an annual crop in both tropical and warm temperate regions (Ozyigit *et al.*, 2007). Cotton crop as commercial commodity, plays an important role in industrial activity of nation in terms of both employment generation and foreign exchange, hence it is popularly known as "king of fiber", "White Gold" and "Friendly Fiber".

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).

During 2006, the mealy bug, *Phenacoccus solenopsis* caused economic damage, reducing yields by up to 40-50 per cent 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).

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. Nanoparticles are highly stable and quick biodegradable; and can be successfully employed in production of nanocapsules for delivery of pesticides, fertilizers and other agrochemicals. Nanoparticles display slow release of encapsulated functional molecules and reduce its frequent applications.

Materials and Methods

The green zinc oxide and copper nanoparticles were biologically synthesised from spinach and tulasi leaves respectively. These green synthesized nanoparticles were characterized by Zetasizer, UV-Vis spectroscopy, X-ray diffraction (XRD) and Scanning electron microscope (SEM).

Bioassay studies on mealybug

The mealybug nymphs required for the experiment was sourced from the insect culture maintained in the laboratory of Department of Agricultural Entomology, College of Agriculture, Raichur. For bioassay studies the cotton leaves were placed in petri plates as food. Then ten nymphs of mealybugs were released on each petri plate. Later different concentrations (250, 500, 1000, 1500 and 2000 ppm) of 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 buprofezin 25 SC at 1ml/L as chemical check. It was replicated three times under lab conditions.

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 Discussions

The average particle diameter of zinc oxide and copper nanoparticles was found to be 20.67 nm and 82.41 nm, UV-Visible spectrophotometer shown a sharp bands at around 350 nm and 560 nm, X-ray diffraction pattern showed broad halo at about 2θ=20-36^{o} region and 2θ=20-40° region and scanning electron microscope showed that rod shaped and spherical with uniform shape distribution respectively.

Mortality of mealybugs caused by zinc oxide nanoparticles

The mortality of mealybugs increased proportionately with increase in concentration in all the treatments at one day after treatment. Highest per cent mortality of 46.47 was registered by buprofezin 25 SC @ 1 ml/l (T_{11}) and was significantly superior to all other treatments (Table 1). This was followed by 43.33 per cent mortality in metal ZnO nanoparticle @ 2000 ppm (T_{10}).

At three days after treatment, highest mortality was recorded in buprofezin 25 SC

@ 1 ml/l (T_{11}) treatment with 83.33 per cent (Table 1). This was followed by metal ZnO nanoparticle @ 2000 ppm (T_{10}), green ZnO nanoparticle @ 2000 ppm (T_9) and metal ZnO nanoparticle @ 1500 ppm (T_8) and was significantly different with each other by recording 76.67, 66.67 and 60.00 per cent mortality, respectively.

At five days after treatment, mortality of mealybugs ranged from 46.67 to 100.00 per cent. The treatment buprofezin 25 SC @ 1 ml/l (T_{11}) recorded cent per cent mortality and proved to be superior to all other treatments (Fig. 1).

Mealybugs mortality due to nanoparticles appeared as cuticular lysis (Plate 1).

Effectiveness of green zinc oxide nanoparticles may be attributed to the damage to the protective wax coat on the cuticle of insects, both by sorption and abrasion so that the insects begin to lose water and die due to desiccation (Arumugam *et al.,* 2016).

Clausen *et al.,* (2011) investigated the efficiency of $ZnSO_4$ and ZnO nanoparticles on mortality of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Their results confirmed that *R. flavipes* feeding on wood impregnated with ZnO nanoparticles, decreased to less than 4 per cent in comparison to control treatment.

Similarly, Kirthi *et al.,* (2011) reported that the comparison between acaricidal, licicidal and larvicidal activities of zinc oxides and the synthesized ZnO nanoparticles, the mortality effect of ZnO nanoparticles was significant. Malaikozhundun and Vinodhini (2017) reported that the treatment with Pp-ZnO NPs at 25 µg /ml increased the mortality of *Callosobruchus maculatus* to 100 per cent.

Mortality of mealybugs caused by copper nanoparticles

At one day after treatment, highest mortality of mealybugs was registered by treatment buprofezin 25 SC @ 1 ml/l (T_{11}) with 46.67 per cent followed by metal Cu nanoparticle @ 2000 ppm (T_{10}) with 43.33 per cent (Table 2). Treatments metal Cu nanoparticle @ 1500 ppm (T_8) and green Cu nanoparticle @ 2000 ppm (T_9) recorded 36.67 per cent mortality each and were on par with each other.

At three days after treatment, the mortality of mealybugs ranged from 23.33 to 83.33 per cent of which buprofezin 25 SC @ 1 ml/l (T_{11}) recorded highest mortality of 83.33 per cent and was significantly superior to all other treatments (Table 2). The next best treatments were metal Cu nanoparticle @ 2000 ppm (T_{10}), metal Cu nanoparticle @ 1500 ppm (T_8) and green Cu nanoparticle @ 2000 ppm (T_9) which recorded 63.33, 56.67 and 53.33 per cent mortality and were significantly different with each other.

At five days after treatment, cent per cent mortality was recorded in chemical check buprofezin 25 SC @ 1 ml/l (T_{11}) treatment (Table 2). Untreated control recorded lowest mortality of 3.33 per cent followed by green Cu nanoparticle @ 250 ppm (T_1) with 36.67 per cent mortality (Fig. 2).

Since there are no reviews available on copper nanoparticles against mortality of cotton mealybug, hence reviews of effect of copper green nanoparticles on other insects have been used for discussion. Ghidan *et al.,* (2017) evaluated the activity of biologically synthesized copper oxide nanoparticles against the mortality of green peach Aphid, where 86 per cent of nymphal mortality was noticed at 8000 µg/ml. Yang and Watts (2005) reported that nanoparticles could be applied to facilitate pest control management (IPM) of stored grain pests as *C. maculates.*

Table.1 Effect of zinc oxide 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 ZnO nanoparticle	250 ppm	20.00 (26.57)^{i}*	33.33 (35.26)^{j}	46.67 (43.09)^{k}
T_2: Metal ZnO nanoparticle	250 ppm	23.33 (28.88)^{h}	40.00 (39.23)^{i}	53.33 (46.91)^{j}
T_3: Green ZnO nanoparticle	500 ppm	26.67 (31.09)^{g}	43.33 (41.17)^{h}	56.67 (48.83)^{i}
T_4: Metal ZnO nanoparticle	500 ppm	30.00 (33.21)^{f}	46.67 (43.09)^{g}	63.33 (52.73)^{h}
T_5: Green ZnO nanoparticle	1000 ppm	33.33 (35.26)^{e}	50.00 (45.00)^{f}	70.00 (56.79)^{g}
T_6: Metal ZnO nanoparticle	1000 ppm	36.67 (37.27)^{d}	56.67 (48.83)^{e}	73.33 (58.91)^{f}
T_7: Green ZnO nanoparticle	1500 ppm	36.67 (37.27)^{d}	56.67 (48.83)^{e}	83.33 (65.91)^{d}
T_8: Metal ZnO nanoparticle	1500 ppm	40.00 (39.23)^{c}	60.00 (50.77)^{d}	80.00 (63.43)^{e}
T_9: Green ZnO nanoparticle	2000 ppm	40.00 (39.23)^{c}	66.67 (54.74)^{c}	90.00 (71.57)^{c}
T_{10}: Metal ZnO nanoparticle	2000 ppm	43.33 (41.17)^{b}	76.67 (61.12)^{b}	93.33 (75.04)^{b}
T_{11}: Buprofezin 25 SC	1.00 ml/l	46.67 (43.09)^{a}	83.33 (65.91)^{a}	100.00 (90.00)^{a}
T_{12}: Untreated control	--	0.00 (0.00)^{j}	0.00 (0.00)^{k}	3.33 (10.52)^{l}
S.Em±		0.17	0.15	0.19
CD @1%		0.67	0.60	0.76

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 copper nanoparticles on cotton mealybug, *Phenacoccus solenopsis*

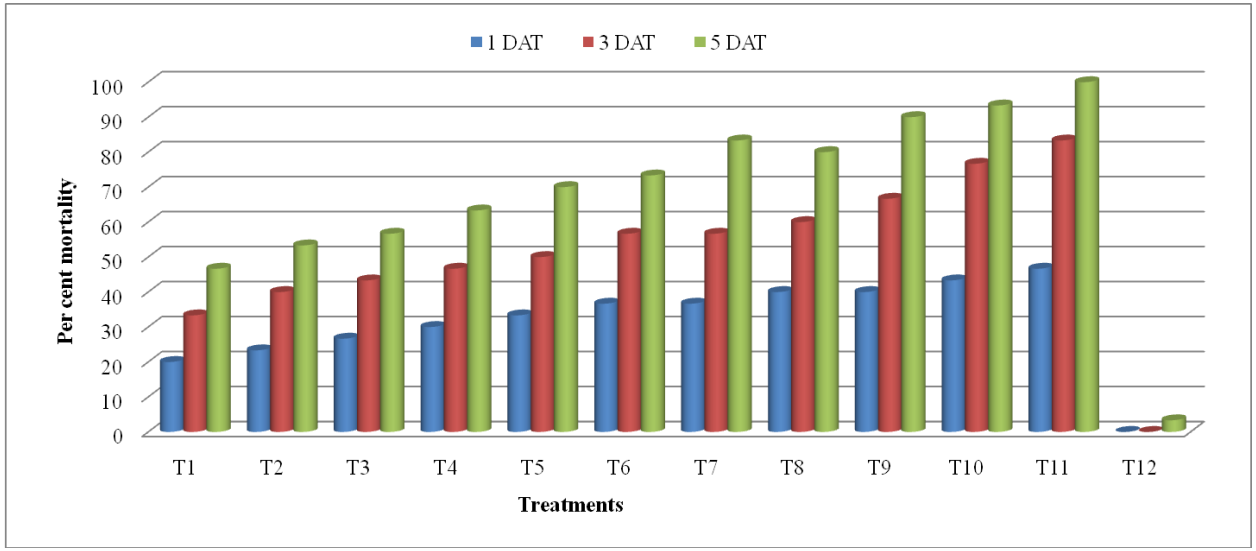
Treatment details	Dosage	Per cent mortality of mealybugs at different intervals		
		1 DAT	3 DAT	5 DAT
T_1: Green Cu nanoparticle	250 ppm	13.33 (21.42)^{i}*	23.33 (28.88)^{j}	36.67 (37.27)^{j}
T_2: Metal Cu nanoparticle	250 ppm	16.67 (24.09)^{h}	30.00 (33.21)^{i}	43.33 (41.17)^{i}
T_3: Green Cu nanoparticle	500 ppm	20.00 (26.57)^{g}	33.33 (35.26)^{h}	43.33 (41.17)^{i}
T_4: Metal Cu nanoparticle	500 ppm	26.67 (31.09)^{f}	36.67 (37.27)^{g}	50.00 (45.00)^{h}
T_5: Green Cu nanoparticle	1000 ppm	26.67 (31.09)^{f}	40.00 (39.23)^{f}	53.33 (46.91)^{g}
T_6: Metal Cu nanoparticle	1000 ppm	33.33 (35.26)^{d}	46.67 (43.09)^{e}	56.67 (48.83)^{f}
T_7: Green Cu nanoparticle	1500 ppm	30.00 (33.21)^{e}	46.67 (43.09)^{e}	70.00 (56.79)^{e}
T_8: Metal Cu nanoparticle	1500 ppm	36.67 (37.27)^{c}	56.67 (48.83)^{c}	76.67 (61.12)^{d}
T_9: Green Cu nanoparticle	2000 ppm	36.67 (37.27)^{c}	53.33 (46.91)^{d}	80.00 (63.43)^{c}
T_{10}: Metal Cu nanoparticle	2000 ppm	43.33 (41.17)^{b}	63.33 (52.73)^{b}	86.67 (68.58)^{b}
T_{11}: Buprofezin 25 SC	1.00 ml/l	46.67 (43.09)^{a}	83.33 (65.91)^{a}	100.00 (90.00)^{a}
T_{12}: Untreated control	--	0.00 (0.00)^{j}	0.00 (0.00)^{k}	3.33 (10.52)^{k}
S.Em±		0.19	0.21	0.15
CD @1%		0.75	0.83	0.59

n=30 second instar nymphs DAT- Days after treatment

*****Figures in the parentheses are "arcsine" transformed values

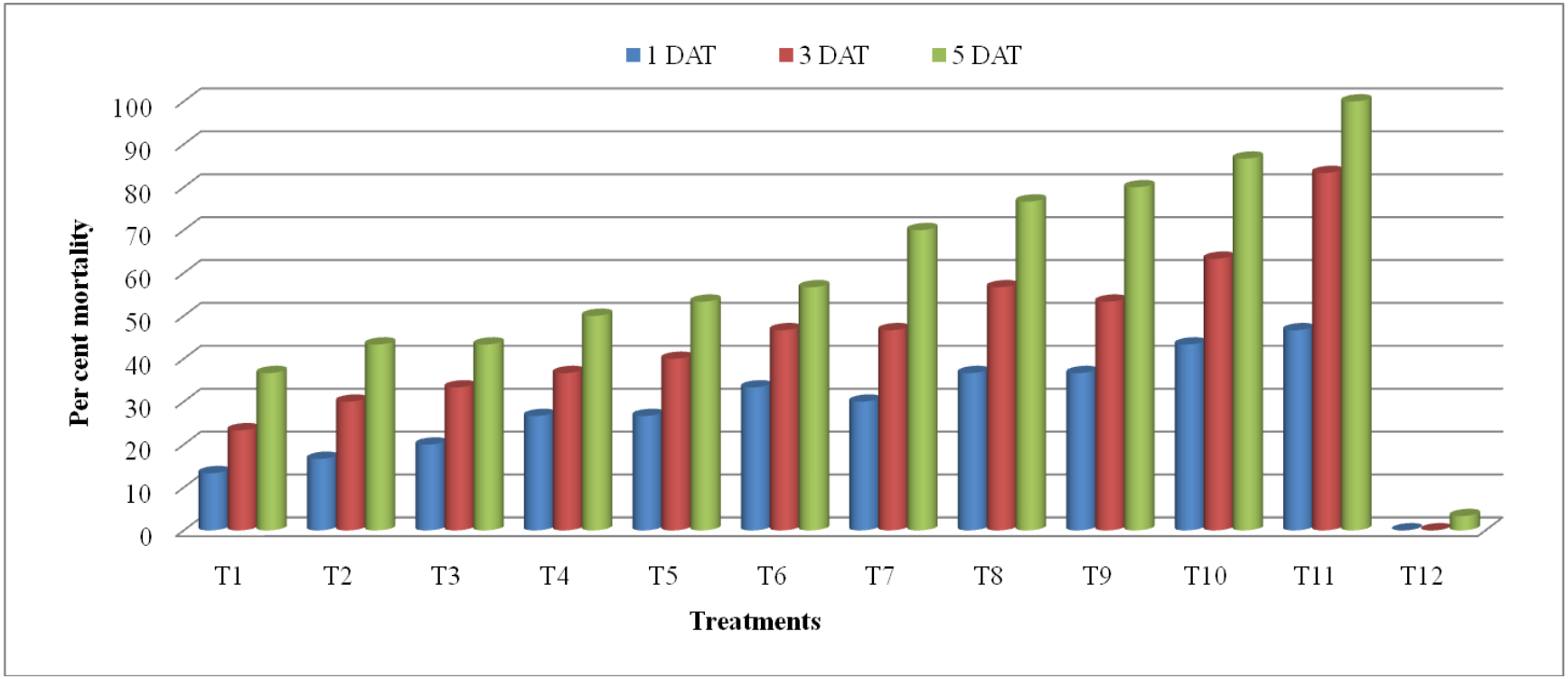
Means followed by same letters in a column are not significantly different (P=0.01) by DMRT

Fig.1 Mortality of cotton mealybug, *Phenacoccus solenopsis* caused by zinc oxide nanoparticles



T_1: Green ZnO nanoparticle @ 250 ppm	T_2: Metal ZnO nanoparticle @ 250 ppm	T_3: Green ZnO nanoparticle @ 500 ppm
T_4: Metal ZnO nanoparticle @ 500 ppm	T_5: Green ZnO nanoparticle @ 1000 ppm	T_6: Metal ZnO nanoparticle @ 1000 ppm
T_7: Green ZnO nanoparticle @ 1500 ppm	T_8: Metal ZnO nanoparticle @ 1500 ppm	T_9: Green ZnO nanoparticle @ 2000 ppm
T_{10}: Metal ZnO nanoparticle @ 2000 ppm	T_{11}: Buprofezin 25 SC @ 1ml/l	T_{12}: Untreated control

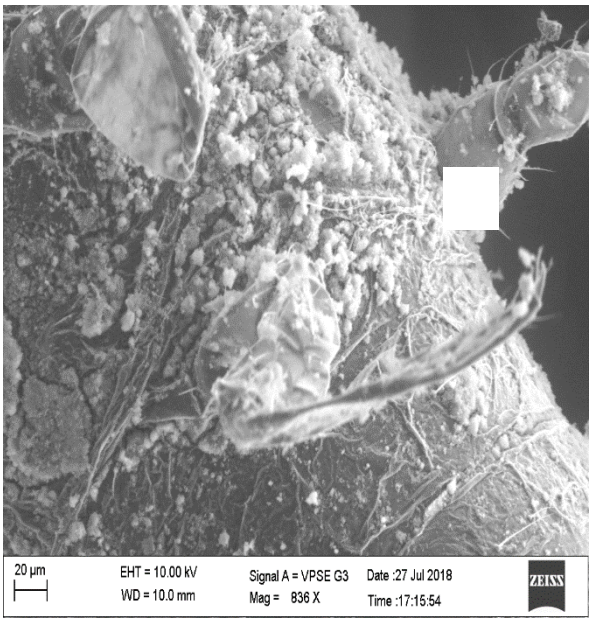
Fig.2 Mortality of cotton mealybug, *Phenacoccus solenopsis* caused by copper nanoparticles

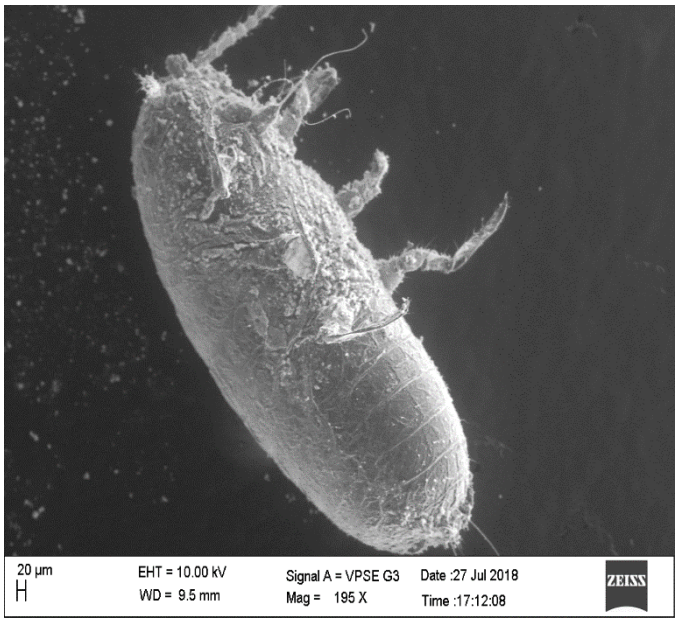


T_1: Green Cu nanoparticle @ 250 ppm	T_2: Metal Cu nanoparticle @ 250 ppm	T_3: Green Cu nanoparticle @ 500 ppm
T_4: Metal Cu nanoparticle @ 500 ppm	T_5: Green Cu nanoparticle @ 1000 ppm	T_6: Metal Cu nanoparticle @ 1000 ppm
T_7: Green Cu nanoparticle @ 1500 ppm	T_8: Metal Cu nanoparticle @ 1500 ppm	T_9: Green Cu nanoparticle @ 2000 ppm
T_{10}: Metal Cu nanoparticle @ 2000 ppm	T_{11}: Buprofezin 25 SC @ 1ml/l	T_{12}: Untreated control

Dead mealybug

Plate.1 Mealybug treated with green nanoparticles





SEM image of cotton mealybug

In conclusion, zinc oxide and copper nanoparticles was tested at various concentrations, the treatment with 2000 ppm concentration resulted in significantly higher mortality of mealybugs 90.00 and 80.00 per cent respectively at five days after treatment. Apart from synthetic insecticides, zinc oxide and copper nanoparticles can be used in the management of sucking pest like mealybug. Use of nanoparticles from plant products minimizes the residual effect of insecticides and manage the insect's pests in an eco-friendly way.

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