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Effect of Toxicity of Silver Nanoparticles on Rice Weevil, (*Sitophilus Oryzae*)

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ABSTRACT

Due to the increasing demand for organic produce and the widespread adoption of rules by businesses to reduce the use of residual chemical pesticides, non-chemical control techniques have become more significant in integrated pest management. Nanomaterial synthesis can be achieved by a variety of methods, such as chemical, biological, and physical processes. To create silver nanoparticles from silver nitrate, the biologically green method was selected. Euphorbia prostrata leaf extracts were used to create silver nanoparticles (Ag NPs), which are a safe, easy-to-manage, and environmentally beneficial green material. In the Laboratory of the Biology Department, Faculty of Education-Derna, temperatures of 32°C, 1°C, and 80 % relative humidity (RH) were used to assess the pesticide activity and determine the efficacies of aqueous leaf extracts of E. prostrata, silver nitrate ($AgNO_3$) solution (1 mM), and synthesized AgNPs against the adult of Sitophilus oryzae L. Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), X-ray diffraction (XRD), and UV-visible spectroscopy were used to characterise the synthesised nanoparticles. The size range of the rod-shaped nanoparticles was 25–80 nm, with an average of 52.4 nm. For fifteen days, pesticide bioassay testing was carried out at various concentrations. The lethal concentration (LD50 values) and mortality percentages of Sitophilus oryzae were found to rise with increasing exposure days (up to 15 days) and concentrations of AgNPs (up to 250 µg/ml). According to these findings, synthetic Ag NPs may out to be a perfect environmentally benign method of controlling S. oryzae. This is the first report on the use of synthetic nanoparticles with plant extracts as pesticides.

1. INTRODUCTION

One of the most significant food crops in the world is rice, *oryza sativa* L. (Poaceae). The most significant species of insects that harm rice grains is the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) **(Costa *et al.,* 2023).** About half of the world's population gets their sustenance from the rice harvest. A staple crop in many parts of the world, rice supplies more than 21% of the calories needed by humans and can make up as much as 76% of the caloric diet of people living in Southeast Asia (Zhao *et al.,* 2020).

More than half of the world's population depends on this crop as their main source of calories; in poor nations, it accounts for 27% of dietary energy and 20% of dietary protein (Xu *et al.,* 2015). However, insect pests attack stored grains and their products, resulting in significant annual crop losses (Ashamo 2009). Over half of the world's population depends on rice for regular consumption and food security. Global rice production is expected to increase by 58 to 567 million tonnes (Mt) by 2030. In addition to being high in calories, rice is a good source of many important vitamins, minerals, and other nutrients. It has more nutrients than potatoes, wheat, and corn combined. Along with carbs, thiamine, calcium, folate, iron, and vitamins E and B5, it is also an excellent source of these nutrients. Various phenolic chemicals, including phenols, sterols, flavonoids, terpenoids, anthocyanins, tocopherols, tocotrienols, and oryzanol, are present in rice, including phytic acid. These substances have been demonstrated to help prevent diabetes and cardiovascular disease and are positively associated with antioxidant qualities (Mohidem et al., 2022). For more than half of the world's population (Liu et al., 2020), domesticated rice is the most often consumed staple food among cereal grains, especially in Asia and Africa. After sugarcane and maize, it is the agricultural product with the third-highest production in the globe (FAOSTAT, 2020). In many places of the world, rice is considered a staple food. Nonetheless, the rice industry finds losses from pest infestations to be extremely concerning (Biancolillo et al., 2019). Auxiliary techniques are necessary to effectively manage pests in rice grains due to the pests' growing resistance to insecticides (Benhalima et al., 2004). Because it feeds internally on seeds, the rice weevil, Sitophilus oryzae, is a difficult pest to control on grains that have been stored (Jalaeian et al., 2021). The most significant species of insects that harm rice grains when they are being stored is the rice weevil, Sitophilus oryzae (L.) (Nwaubani et al., 2014). According to Souza et al. (2012), damage can result from mite and fungal infection, germination loss, reduction in grain weight, and loss of the product's commercial value. By internally feeding on seeds, the rice weevil Sitophilus oryzae (L.) (Coleoptera: Curculionidae) has the potential to significantly reduce grain yield (Tian et al., 2023). After harvest, storage and maintenance of agricultural products are crucial tasks. Inadequate storage and processing facilities are causing a significant percentage of food grains to spoil after harvest (Singh and Satapathy, 2003). Pests cause 5–30% of postharvest grain to be damaged, making grain loss due to insects a global issue (Zhang et al., 2021). Losing weight, designating rice as animal feed, or requiring extra insect control can all lower the value of rice. One of the most destructive pests to stored grains is the rice weevil, which feeds on rice, corn, wheat and a variety of other cereals. It may be found in all parts of the world, but it is more common in warmer climates. As a main pest of grains, Sitophilus oryzae targets healthy, undamaged grains. Grain damage makes it more vulnerable to subsequent pests, which exacerbates issues even more (Vijay and Bhuvaneswari, 2018; Charles Kasozi et al., 2018). For the sake of future generations and food security, grain storage in agriculture is crucial. Because they result in significant losses and have an impact on the storage fate of grains, insect pests are significant from an economic standpoint. Reducing or eliminating insect pest populations while storing food can help meet the world's increasing needs. Synthetic pesticides have traditionally been used to control a wide range of insect pests, which pose a threat to the ecosystem and environment in several ways, such as by eradicating natural enemies, causing insect resistance and resurgence, contaminating soil, water, and air, and leaving behind residual effects that can lead to a variety of disorders or diseases in both humans and animals (Akbar et al., 2021). One of the most common insects attacking stored goods worldwide is Sitophilus oryzae (Kły´s et al., 2020). Conventional pesticide formulations and delivery methods have several unfavourable effects on the environment as well as human health. Therefore, it is always necessary to look for techniques that are safe, effective, biodegradable, and ecologically friendly. One such cutting-edge tactic that can be applied in this situation to disperse pesticides with accuracy, control, and targeted dispersion is nanotechnology (Urvi et al., 2023). These days, one of the finest methods for controlling pests is nanotechnology, which deals with materials and particles as small as 10-9 nm (Bhattacharyyal et al., 2010). A new generation of environmental remediation technologies known as nanoparticles may be able to solve some of the most difficult environmental clean-up issues at a reasonable cost (Chinnamuthu and Murugesa, 2009). The objectives of green nanotechnology are to create nanomaterials and products that do not damage the environment or human health, as well as to create nanoproducts that address environmental issues. It makes use of current green chemistry and green engineering principles to produce nanomaterials and nanoproducts at low temperatures with no hazardous components while consuming less energy and renewable resources (Gnanasangeetha and Thambavani, 2014). Because it is less hazardous, more economical, and less harmful to the environment than chemical and physical approaches, "green synthesis," or "biogenic synthesis," of nanoparticles, demonstrates superior advancement (Vidya et al., 2013). Therefore, the main objective of this research was to investigate effect of toxicity of silver nanoparticles on rice weevil, (Sitophilus oryzae).

2. METHOD

Insects rearing

S. oryzae was isolated from infected rice purchased from a local market and raised in glass jars in a Laboratory of the Biology Department, Faculty of Education-Derna with constant darkness and temperatures of 32°C, 1°C and 80 5% relative humidity (RH). Sodium chloride saturated solution was used to maintain the relative humidity (Winston and Bates, 1960). The tests employed adults who were less than 24 hours old after emerging from the pupal stage.

The preparation of silver nanoparticles

A known amount of sodium hydroxide (0.3 g) was dissolved in water, and then the alkali solution was gradually added also to a known amount of raw rice (2 g) starch while stirring. The mixture is left under continuous stirring till complete solubilization of starch. Keeping into your consideration, the pH of the previous solution was kept at 11 and the of this solution was raised to 60oC. Meanwhile, different concentrations of silver nitrate (0.25 and 0.5 g) were dissolved in 100 ml distilled water. The silver nitrate solution with different concentrations was added drop-wise to the starch paste solution while stirring at 60oC and pH 11. After 15 min, and with the addition of silver nitrate solution, the colour was gradually turned from an obscure white colour to a transparent yellow colour signifying the formation of silver nanoparticles (AgNPs). The colour strength of AgNPs was attributed to the volume of the silver nitrate added. The deeper the color formed, the higher the concentration of former AgNPs formed (El-Rafiea et al., 2013 Abdelsalam et al., 2019). Based on the experimental route, two concentrations of AgNPs were nominated as AgNPs-2000 and AgNPs-4000 ppm.

Pesticide Bioassay

Rice in each jar was mixed individually with aqueous leaf extracts of E. prostrata, 1mM AgNO3 solution and synthesized AgNPs. Different concentrations of 1000, 800, 600, 400, and 200 mg/kg-1 of aqueous extract, AgNO3 and synthesized Ag NPs in the concentrations ranging from 250, 200, 150, 100, 50 mg/kg-1 rice were prepared. Then, the jars were shaken manually for approximately 1 minute to achieve an equal distribution on rice (Subramanyam and Roesli, 2000). For each dose, there were five replicates. The control papers were impregnated with aqueous plant extract, AgNO3 and synthesized Ag NPs with rice. The jars were kept for 24 hrs before 20 unsexed adults of S. oryzae were introduced into each jar. All bioassays were performed at 30°C ± 1°C, 75 ± 5% R.H. Insect mortality was checked after 1, 3, 6, 9, and 15 days as per the method of Debnath et al.(2010). After the bioassay was complete, all the live insects were removed and the treated boxes were retained for two months beyond the experiment to check if there were any fresh S. oryzae progeny in the rice.

Characterization of the synthesized nanoparticles

UV-vis spectroscopy makes it simple to examine the synthesis of Ag NPs from leaf extract. By routinely sampling aliquots (1 mL) of the aqueous component after 20 times dilution and analysing the solution's UV-vis spectra, the bioreduction of the Ag+ ions in solutions was observed. On a Schimadzu 1601 spectrophotometer operating at a resolution of 1 nm, the UV-vis spectra of these aliquots were monitored as a function of reaction time. The reaction mixture was also centrifuged for 40 minutes at 60,000 g; the pellet that was produced was then dissolved in deionized water and filtered through a Millipore filter (0.45 m). To do X-ray diffraction (XRD) and Fourier transform infrared (FTIR) analyses, an aliquot of this filtrate containing Ag NPs was employed. The pictures of the nanoparticles were analyzed using scanning electron microscopy (SEM; JEOL, Model JFC-1600), and the measurements were operated at an accelerating voltage of 120 kV and later with an XDL 3000 powder. For the electron microscopic examinations, 25 L of the sample was sputter-coated on the copper stub. The Perkin-Elmer Spectrum One instrument was used to measure the materials' FTIR spectra in the diffuse reflectance mode with a resolution of 4 cm"1 in KBr pellets. Powder samples for the FTIR were created like that of powder diffraction tests. Analysis of the FTIR spectra of leaf extracts collected both before and after the production of Ag NPs led to a discussion of potential functional groups that could have contributed to the development of Ag NPs. Dried nanoparticles were coated on an XRD grid for XRD research, and spectra were captured using a Phillips PW 1830 instrument running on CuKa1 radiation at a voltage of 40 kV and a current of 30 mA.

Statistical analysis

The data were analyzed statically by the SAS program (SAS, 1999). Duncan's test was used at a significant level of 5% for the comparison of means.

3. RESULT

A) Laboratory experiments

Sitophilus oryzae, the rice weevil, has a larval mortality percentage that rises with rising AgNPs concentrations and steadily rises with longer exposure times.

Results from the pesticide activity showed that the synthesized Ag NPs using *E. prostrata* were more effective than the aqueous extract and $AgNO_3$ solution (Table 1). Complete mortality (100%) was observed on 15 days for the synthesized AgNPs but in aqueous extract and the $AgNO_3$ solution the same mortality was observed after 15 days (Table 1). However,

increased mortality percentages of adults *Sitophilus oryzae* with increasing period (after 15 days) increasing concentration of silver nanoparticles (250 μg /ml), however, mortality (%) after 24 h at different concentrations were (50±2.24, 43±2.88, 37±1.58, 22±0.45 and 16±0.90 %), after three days were (63±1.22, 52±2.19, 41±1.58, 28±1.14 and 24±0.54 %), after six days (88±1.67, 72±1.92, 60±2.00, 51±1.48 and 33±1.52 %), after nine days (100±0.00, 92±1.14, 85±2.00, 71±1.64 and 60±1.12%), after 15 days (100±0.00, 97±0.84, 91±1.23, 80±1.58 and 71±1.48 %),
respectively, as compared with control (untreated) which did not record any deaths of rice weevil, *Sitophilus oryzae* at different exposure days (one day, 3days, 6days, 9days and 15ddays) and concentrations (250, 200, 150, 100 and 50 μg/ml). As indicated in the Tables (2), AgNPs were shown to be superior to killing *Sitophilus oryzae*. The LC_{50} value for adults *Sitophilus oryzae* at concentrations (250, 200, 150, 100 and 50 μg/ml) of AgNPs were (213.32, 229.75, 234.33, 247.90 and 44.69 μg/ml), respectively.

4. DISCUSSION

The findings data make it abundantly clear that nano silver has no effect on adults; this could be because of their cuticles, but it has strong entomotoxic potential against larval stages. This reaction may be related to the several documented toxicity mechanisms of Ag. Reactive oxygen species production, oxidative stress, membrane disruption, unfolding of proteins, and/or inflammation are a few examples of these pathways (Meng *et al.,* 2009; Donaldson *et al.,* 2009). According to Abo-Arab *et al.* (2014), dehydration, tracheal and spiracle blockage, or digestive system malfunction may be the cause of insect mortality. Furthermore, compared to the effects of free silver ions, it has been observed that the oxidative stress and gene detoxification induced by silver nanoparticles is much more effective (Nair *et al.,* 2013). The collapse of the epithelial lining in the midgut vesicles and disruption to the membrane at the apical surface of the epithelial cells may be the cause of the harmful effect of nanoparticles (Foldbjerg *et al.,* 2015; Sultana *et al.,* 2018). Furthermore, compared to the effects of free silver ions, it has been observed that the oxidative stress and gene detoxification induced by silver nanoparticles is much more effective (Nair *et al.,* 2013). The collapse of the epithelial lining in the midgut vesicles and disruption to the membrane at the apical surface of the epithelial cells may be the cause of the harmful effect of nanoparticles (Foldbjerg *et al.,* 2015; Sultana *et al.,* 2018). According to Sultana *et al.* (2018), disruption of the apical membrane of the epithelial cells and partial synapsis of the midgut epithelial cells may also contribute to the toxicity of NPs. By physically harming the bug and absorbing into the cuticular lipids, they destroyed it (Barik *et al.,* 2008). NPs typically harm insects by piercing the exoskeleton and entering the intracellular area (Rai *et al.,* 2014). NPs' sizes, coatings, concentrations, and exposure times all affect how effective they are (Jiang *et al.,* 2015). Our results clearly show that nano silver has great entomotoxic potential on larval stages but is ineffective on adults, possibly due to the cuticle. This reaction may be associated with one of the recognized Ag toxicity pathways. Meng *et al.* (2009) and Donaldson *et al.* (2009) list the synthesis of reactive oxygen species, oxidative stress, membrane disruption, unfolding of proteins, and/or inflammation as instances of these pathways. AgNPs can be hazardous in a number of ways, including surface adherence and changed membrane properties that eventually reduce cell permeability (Morones *et al.,* 2005). Physical and chemical properties of AgNPs can be altered by their interactions with biochemical and physiological structures and processes (Nel *et al.,* 2006; Kim *et al.,* 2009).
This was consistent with previous research by Kaveri (2014), who found that S. oryzae completely died at 1500 mg kg^{-1} on day 15 in stored maize. Additionally, at 2500 mg kg^{-1} of cowpea seeds on day 14, 75% of C. maculatus F. deaths were recorded by Rouhani *et al.* (2012). However, at lower doses of 250 and 100 mg kg^{-1} of rice on 14 DAT, respectively, full mortality of S. oryzae was recorded (Zahir *et al.,* 2012, Sankar and Abideen, 2015). On 15 DAT, the powdered sweet flag rhizome showed total mortality. Similarly, it has been observed that treating sorghum with sweet flag rhizome powder against *S. oryzae* results in 97% mortality (Gadewar *et al.,* 2017). In the current observation, pungam oil was discovered to provide 98.66 percent of rice weevil mortality. In a similar vein, pungam oil's effectiveness has been linked to a decrease in adult emergence following six months of storage (Deb, 2016).
Although it was noted that the weevils could survive for more than a day without feeding, damage to their mouthparts was not the primary cause of insect mortality, since mortality was seen at all SNP treatment doses within a 24-hour period (Debnath *et al.,* 2012). AgNPs adhesion to the rice weevil's body resulted in cuticle splits and scratches. Due to the disruption of the water barrier in the cuticle and subsequent desiccation, this resulted in the loss of water through dehydration. Additionally, silica nanoparticles induced damage to the insect cuticle by adsorbing cuticular lipids (Ebeling, 1971). Similar harm is caused by sorption and abrasion to the insect's waxy protective covering on the cuticle (Debnath *et al.,* 2011). Reduced fertility may result from adult insects suffering from desiccation and spiracle blockage brought on by exposure to silica nanoparticles (Arumugam *et al.,* 2016). The adult weevils' bodies are covered in AgNPs, which would have stopped them from mating again. According to (Arumugam *et al.,* 2016), insects are known to exude a greasy substance on their body surface that may be used in their physical contacts with other species, particularly during mating. According to Voigt (2009), lubricants are crucial for the base's attachment when males attach to females during mating. Debnath *et al.* (2011) reported that dehydration or tracheal and spiracle blockage can cause surface enlargement of the integument.

Consequently, it became evident that the mortality seen in this study might be related to cuticle abrasion and waxy depletion. These findings are consistent with those of Abo Arab *et al.* (2014), who demonstrated that when compared to titanium oxide (TiO_2) nanoparticles, Al_2O_3 had the greatest deterrent impact on *S. oryzae* and *S. zeamais*. Furthermore, compared to Al_2O_3 and ZnO nanoparticles, Malathion had the greatest impact on mortality progeny and weight loss in *T. castaneum*, according to Salem *et al.* (2015). Furthermore, they showed that Al_2O_3 affected the tested insect more than ZnO did. According to the results of the current investigation, the investigated materials had varying effects on the *S. oryzae* insect. Nanoalumina was effectively sprayed by Stdlere *et al.* (2010) against two stored grain pests, *S. oryzae* and *R. dominica* (F.).

The cause of insect death may be ascribed to digestive system impairment or integument surface expansion brought on by dehydration or tracheal and spiracle blockage. On 15 DAT, AgNPs showed total rice weevil mortality at 2000 mg kg^{-1} of sorghum seeds. This was consistent with previous research by Kaveri (2014), who found that *S. oryzae* completely died at 1500 mg kg^{-1} on day 15 in stored maize. Additionally, at 2500 mg kg^{-1} of cowpea seeds on day 14, 75% of *C. maculatus* F. deaths were recorded by Rouhani *et al.* (2012). However, at lower doses of 250 and 100 mg kg^{-1} of rice on 14 DAT, respectively, full mortality of *S. oryzae* was recorded (Zahir *et al.*, 2012, Sankar and Abideen, 2015). On 15 DAT, the powdered sweet flag rhizome showed total mortality. Similarly, when sweet flag rhizome powder is used to treat S, reports of 97% fatality have been made.

Table 1: Effect of synthesized AgNPs on percentage of mortality of Sitophilus oryzae

AgNPs Conc. (µg/ml)	**Mortality (%)**				
	Exposure days				
	1day	**3day**	**6day**	**9day**	**15day**
250	50±2.24	63±1.22	88±1.67	100±0.00	100±0.00
200	43±2.88	52±2.19	72±1.92	92±1.14	97±0.84
150	37±1.58	41±1.58	60±2.00	85±2.00	91±1.23
100	22±0.45	28±1.14	51±1.48	71±1.64	80±1.58
50	16±0.90	24±0.54	33±1.52	60±1.12	71±1.48
Control	0	0	0	0	0

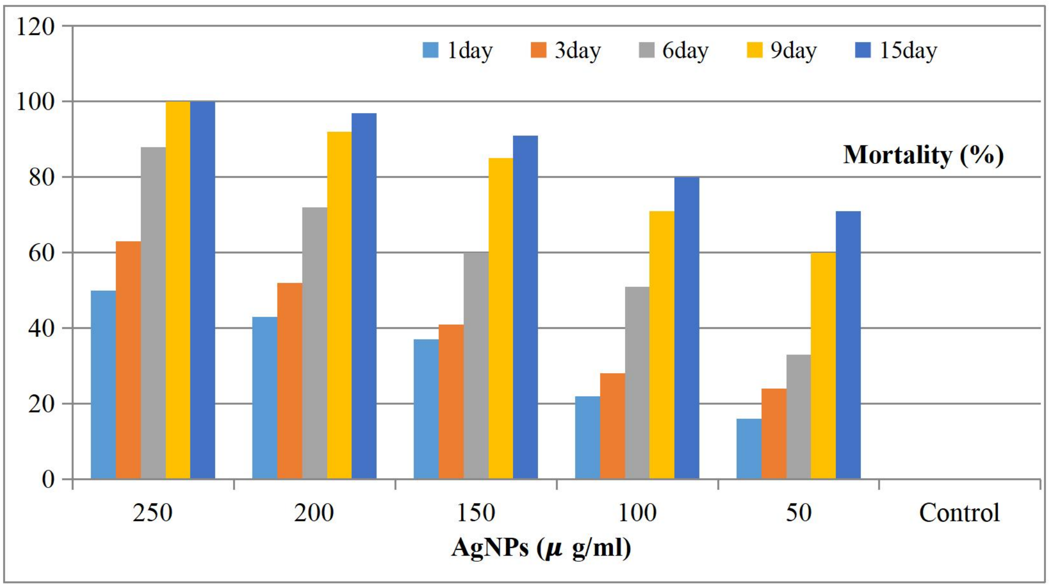

Fig. (1): Effect of synthesized AgNPs on percentage of mortality of *Sitophilus oryzae*

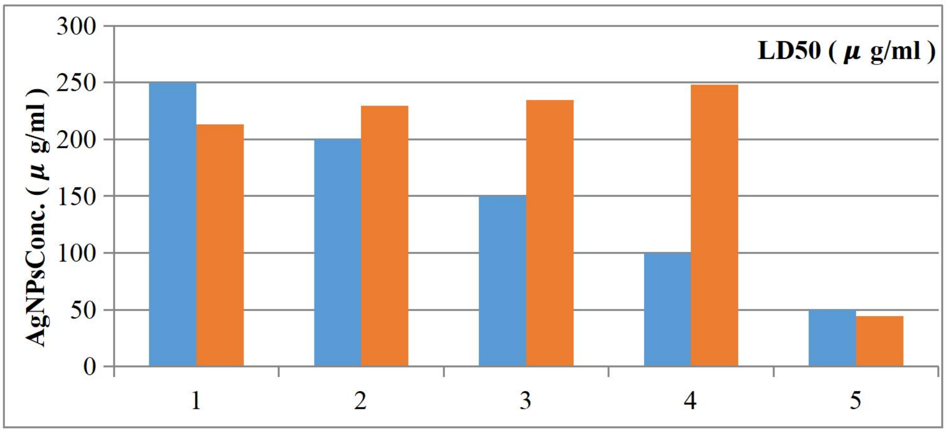

Fig. (2): Effect of synthesized AgNPs on LD_{50} (μg/ml) of *Sitophilus oryzae*

Table (2): Effect of synthesized AgNPs on LD_{50} (μg/ml) of *Sitophilus oryzae*

AgNPsConc. (μg/ml)	LD_{50} (μg/ml)
250	213.32
200	229.75
150	234.33
100	247.90
50	44.69

$$Mortality\ \ percentage = \frac{X - Y}{100 - Y} \text{X } 100$$

X = Percentage mortality in the treatments
Y = Percentage mortality in the control

5. CONCLUSION

Unlike sprayable formulations of traditional pesticides that leave residues on stored grain, the nanocides can be eliminated by a standard milling procedure. Therefore, if used with appropriate safety precautions, silver nanoparticles have a good potential as a seed protector, stored grain, and chemical insecticide substitute. Additionally, using a minimal amount that is still effective on insects and selecting a product that is effective at lower rates are two ways to reduce the negative impacts of inert dusts. Ultimately, more research is required to determine the precise mechanisms of action of nanocides and assess the effects of the examined nanoparticles on other species in other settings.

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