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The Acaricidal Impact of Green Synthesized Zinc Oxide Nanoparticles Against *Caloglyphus mycophagous* and *Mycetoglyphus fungivorus* (Acari: Acaridae)

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Abstract

In an era of eco-friendly development, the green synthesis of metal nanoparticles using plant extracts has become a focus of researcher attention. The present work introduces method of a green synthesis of Zinc oxide nanoparticles (G ZnO NPs) using orange peel extract and their size and morphology were examined using high resolution transmission electron microscope (HRTEM). Additionally, its impact in comparison with chemically synthesized Zinc oxide nanoparticles (C ZnO NPs) was examined on females, nymphs and larvae of *Caloglyphus mycophagous* (Megnin) and *Mycetoglyphus fungivorus* Oudemans. Three different concentrations of both types of ZnO NPs (10, 30 and 60 ppm) were utilized. The mortality rate (%) was recorded after 24, 48, 72 and 96 hour (h) post-exposure to each tested concentration. The results of high resolution electron microscope showed that the size of the C and G ZnO NPs were 35 and 3 nm, respectively. Both types of ZnO NPs showed toxicity against tested stages and the mortality rates were increased by concentration and exposure time increase. Chemical ZnO NPs was more effective than G ZnO NPs to all stages of mites post -initial exposures, and then all had the same effect by passing time. Females and nymphs of both tested mites recorded 100% mortality after 72h post-exposure to 60 ppm of both ZnO NPs, while Larvae of M. fungivorus recorded this mortality percentage after 24h post-exposure to all tested concentrations of each ZnO NPs except with GZnO NPs at 10ppm, the mortality was (90%). The same result (100%) was observed in C. mycophagous larvae after 48h post-exposure. The deposited eggs by treated females of each mite were highly significantly decreased and did not hatch at all. Strong adherences of tested ZnO NPS were visualized clearly attached to different parts of dead females by images of Scanning Electron Microscopy (SEM).The result of this work suggests that G ZnO NPs are effective candidate agents against storage mites.

Keywords: Zinc oxide, nanoparticles, synthesized methods, toxicity, storage mite's pests, *Caloglyphus mycophagous*, *Mycetoglyphus fungivorus*.

1. Introduction

Mites of family acaridae are inhabitant a wide range of stored products including cereals and commodities based on it at all stages of manufacturing [1, 2]. Such taxa like *Mycetoglyphus, Caloglyphus, Tyrophagus, Acarus* and *Rhizoglyphus* are responsible for remarkable losses of these products worldwide [3,4]. Cereals mainly wheat, rice, soybean and maize are of the indispensable agricultural products that contribute to the bulk of a global food security basis. Increasing and preserving these cereals is urgent to meet the mankind consumption needs especially in developing countries that the population densities are growing [5]. Manson [6] recorded that *Mycetoglyphus fungivorus* Oudemans on different stored product species like pole beans sp, also Chaopin [7] reported this mite on rice bran, soybeans as well as *Caloglyphus mycophagous* (Megnin) was recorded on rice and others. In Egypt, Mohamed [8] recorded great number of Acaridae mites such as *Caloglyphus sp., Acarus siro* (Linnaeus) and *Tyrophagus sp.* in stored maize. The economic importance of the storage mites being in their small size that are difficult to be seen or detect, fast reproduction in warm and dry conditions as well as in temperate and high moisture, also feces produced by infested live and dead mites [9]. The direct and indirect damage which caused by these mites including changes in nutrition content [10], reduces the cereal germination [11], decreases the seed value and malting as well as induces infection by micro-organisms [12]. Moreover, heavy mite infestation can taint cereals making it non edible to livestock [13]. Also, mites of stored products are allergenic and

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responsible for health risks to food industry workmen [14-16].

Controlling of stored product pests by using synthetic pesticides strategy is facing a great challenge universally due to its complicated side effects which were triggered on living organisms, besides environmental pollution, pest resistance and long persistence, also toxic residues in the treated cereals [17- 18].

Over recent years, nanotechnology has become among the most promising alternative tool in pest control [19], NPs gain unique physical and chemical properties that increase their efficacy as pesticides [20-21]. Many researchers explored the toxic activity of some metal oxide NPs., copper, magnesium, zinc, titanium and iron showed potential toxicity effect against bulb mite, *Rhizoglyphus robini* Claparede (Acaridae family) [22]. Also, Al-Azzazy et al., [23] concluded that silver NPs caused a significant mortality to *Tetranychus urticae* Koch (family: Tetranychidae) and *Aculops lycopersici* (Massee) (family: Eriophyidae) with low side effect on their predators, *Neosiulus cucumeris* Oudemans and *Euseius scutalis* (Athias-Henriot) (family: Phytoseiidae) on plants of tomato under greenhouse conditions. Diatomaceous earth was found to be effective against storage mite, *Tyrophagus putrescentiae* (Schrank) (acaridae family) [24-25].

Among metal oxides that has been efficient for NPs biosynthesis and were preferred in agriculture sector, Zinc oxide (ZnO); it was listed by Food and Drug Administration as safe material, also it used as food additive [26-27]. Moreover, synthesized ZnO NPs own importance due to its antibacterial, antifungal activity and insecticidal activity [20-21, 27-30].

Zinc oxide nanoparticles can be synthesized by using chemical or eco-friendly pollutant-free chemicals (green methods) [20-21, 27, 29-31]. A considerable attention has been paid to ZnO NPs via plant extracts green synthesis due to the benefits to environment via producing valuable components from wastes with lack of toxicity and low cost [27-32]. A study by Ibrahim et al., [29] exhibited a moderate and high toxic effect resulted by ZnO NPs green synthesis against *Sitophilus oryzae* (L) and *Sitotroga cerealella* (Olivier).

In this regard, a comparative toxicity between C and G synthesis of ZnO NPs was explored against females, nymphs and larvae of *C. mycophagous* and *M. fungivorus* mites. The post effect of both types of ZnO NPs was evaluated on hatchability or progeny of deposited eggs by treated females and the adherence of both tested ZnO NPs was examined on tested females using Scan Electron Microscope (SEM).

2. Material and methods

2.1. Chemical synthesis of zinc oxide nanoparticles (CZnO NPs):

According to Menazea et al., [21], using Sol-Gel method mixing stoichiometric moles of zinc chloride and sodium hydroxide in a deionized water also addition of 400 M.Wt polyethylene glycol as a surfactant reagent under vigorous stirrer for 2 h. After that, the white precipitate was obtained, then decantation of supernatant. The precipitate was dried at 60 °C overnight.

2.2. Green synthesis of zinc oxide nanoparticles (GZnO NPs):

According to Samy et al., [20] and Ismail et al., [28], using zinc nitrate as a precursor of zinc and mixed with orange peel extract as reducing and capping reagent (V/V) under vigorous stirring for 2 h. Then centrifuge the solution at 1500 g for 10 minutes to separate the white precipitate. The precipitate was dried at 60 °C overnight.

After that, both samples of each type were calcinated at 400 °C to. High Resolution Transmission Electron Microscope (HRTEM) was performed by JEM-2100F electron microscope with accelerating voltage of 200 kV for sample characterization.

2.3. Source and maintenance of mites

The pest mites were collected from grains stored in the Agriculture faculty farm, Al Azhar- University, Egypt; *C. mycophagous* was found in untreated stored maize and soybeans, while *M. fungivorus* was obtained from wheat, also soybeans. The collected mites were brought to Acarology laboratory at National Research Center (NRC) and identified according to taxonomical rank described by [1]. Each mite was reared for many generations on diet consists of wheat bran mixed with brewer's yeast (1% w/w) placed in rearing unit consists of a Petri dish (3cm. in diameter and 2cm. in length) which base involved with consolidate substrate of charcoal and gypsum mixture (9:1 ratio) [33]. The stock races of both mites were kept under 26±2 oC, 75±5% R.H and continuous darkness. Sufficient diet was introduced continuously to keep culture available. For the experiments, females, nymphs and larvae were chosen based on the body traits.

2.4. Toxicity bioassay

Females of same age were conveyed to experimental units. Each unit consists of cylindrical vial (3cm in diameter and 5 cm in height) with holes at the top for ventilation. Each vial contains 10g of wheat bran dusted by both types of ZnO NPs. Each type of ZnO NPs was utilized at three concentrations (10, 30 and 60 ppm) of rates, 0.01, 0.03 and 0.06 mg/g of wheat bran. Each concentration had 5replicates (20 females/ replicate). Before introducing tested mites, the vials were shaken for few times to ensure that ZnO NPs were distributed well. Female's mortality was recorded after 24, 48, 72 and 96 h. from treatments.

The dead females were removed, while the deposited eggs were remained for additional week then, the vials were checked for each tested mite offspring and counted. A control was included using untreated wheat bran. The testes were repeated two times by preparing new experimental units each time. The same procedure was done for other examined stages, nymphs and larvae. All treated and untreated units of each experiment were kept in incubator under 26±2 ^{o}C, 75±5% R.H.

2.5. Scanning Electron Microscope (SEM) test.

Females of *C. mycophagous* and *M. fungivorus* mites that exposed to 60 ppm of C and G ZnO NPs were subjected to SEM to verify the adherence of each tested material. The scanned females of both tested mites were air dried previously and putted on adhesive stubs. (SEM) (TESCAN, Vega III/Czech Republic) was done in the Electronic Microscope Unite, Central Laboratory, NRC.

2.6. Statistical analysis

The mortality percentages of females, nymphs and larvae of each tested mites at different concentrations were analyzed statistically by using one-way ANOVA (SPSS 14.00 program) and Duncan multiple range test to compare means. Corrected mortality was not calculated as the control mortality was zero.

3. Results

3.1. High resolution transmission electron microscopy (HRTEM)

The morphology and the size of each C and G synthesized ZnO NPs were illustrated in Figure (1); the images cleared that the majority of ZnO NPs are spherical in shape. As shown in Figure (1. A), the size of C ZnO NPs was about 35 nm, while the size of G ZnO NPs was very accurate about 3 nm (Figure, 1. B).

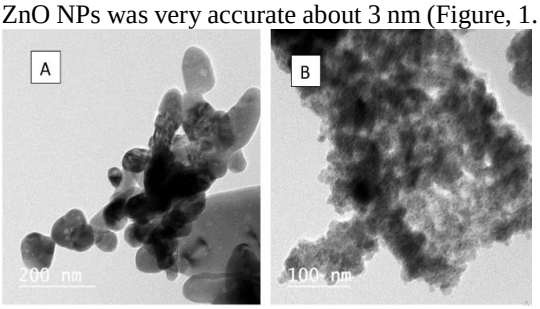

Figure 1: High resolution transmission electron microscope (HRTEM) of ZnO NPs synthesized by A: Chemical method and B: Green method.

3.2. Efficacy of synthesized C and G ZnO NPs on females, nymphs and larvae of *C. mycophagous* mites.

The exposure of females, nymphs and larvae of *C. mycophagous* to different concentrations of both synthesized ZnO NPs verified their acaricidal efficacy. Figure (2) showed that the mortality (%) of tested stages was increased by increasing concentrations and exposure time of C and G ZnO NPs. As shown in Figure (2.A), the mortality rates of females by C ZnO NPs concentrations 10, 30 and 60 ppm after 24h were 48, 65 and 82%, respectively; Differences among mortality rates of females were highly significant (F = 42,3; df = 2, 14; P < 0.000). Also, the mortality rates of nymphs after 24h at the same concentrations were 59, 79 and 93%, respectively and the concentrations were significantly different at (F =37, 3; df = 2, 14; P < 0.000). Figure (2.A) showed also C ZnO NPs concentrations from 10 to 60 ppm caused 96 to 100%, larval mortality, respectively; this difference among concentration effects is statistically not significant (F =1.9; df = 2, 14; P = 0.2). After 72h, all means of mortality rates didn't differ significantly and more than 97% mortality was found in females at 10ppm and 100% at 60 ppm, while 100 % mortality was found in nymphs at all tested concentrations. Figure (2.B) demonstrated that the efficacy of G ZnO NPs was lower than C ZnO NPs, the mortality rate of females, after 24h from exposure to G ZnO NPs concentrations (10, 30and 60 ppm) was 11, 20 and 53%, respectively, while it was in nymphs 22, 31 and 60%, respectively and in larvae 65, 82 and 97%; Differences among mortality rates of females, nymphs and larvae at tested concentrations were highly significant (F =146,7 59, 2 and 49,6 ; df = 2, 14; P < 0.000) , respectively. The highest mortality scored by G ZnO NPs after 72 h in all tested stages was 100% at 60 ppm. After passing tested time, all tested stages at the three concentrations died

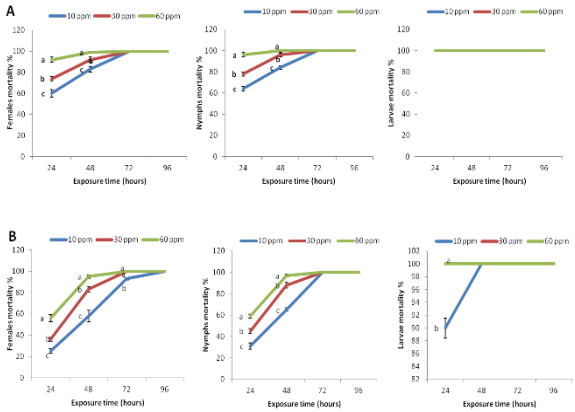

Figure 2: Mean mortality (±S.E.) of *C. mycophagous* females, nymphs and larvae exposed to C and GZnO NPs at three concentrations (ppm) and different exposure times (hours). A= Chemical ZnO NPs, B= Green ZnONPs. Different letters within the same exposure time are significantly different (P<0.05).

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3.3. Efficacy of synthesized C and G ZnO NPs nanoparticles on females, nymphs and larvae of *M. fungivorus* mites.

Figure (3) showed the toxicity effect of C and G ZnO NPs against *M. fungivorus* females, nymphs and larvae. The same trend of what was observed by both ZnO NPs on tested stages of *C. mycophagous* was observed here, C ZnO NPs was more effective than G ZnO NPs after initial exposures and larvae was more sensitive than other tested stages. The mortality rate of

larvae was 100% after 24h from exposure to each tested ZnO NPs at all tested concentrations except with G ZnO NPs at 10 ppm, the mortality rate was 90%. Figure (3.A) revealed that female's mortality significantly increased with the increase of C ZnO NPs concentrations. In addition, the mortality rates of concentrations 10, 30 and 60 ppm after 24h were 60, 74 and 92 %, respectively; differences among females mortality rates were highly significant (F =34,3; df = 2,14; P < 0.000), also the mortality rates of nymphs after 24h at the same arranged concentrations were 64, 78 and 96%, respectively; also, differences were statistically significant among rates of nymphs mortality (F =57,2; df = 2,14; P < 0.000). Figure (3. B) concluded that females and nymphs were influenced by G ZnO NPs concentrations 10, 30 and 60 ppm after initial exposure and the resulted mortality was (25, 36 and 56%) and (31, 45 and 59%), respectively; differences among concentration effects on females and nymphs were statistically significant (F =38 and 34,6; df = 2,14; P < 0.000), respectively. By comparing treatments on females and nymphs at the different concentrations after passing 72h, all caused 100% mortality except with G ZnO NPs treatment on females at 10ppm, the mortality rate was 93%.

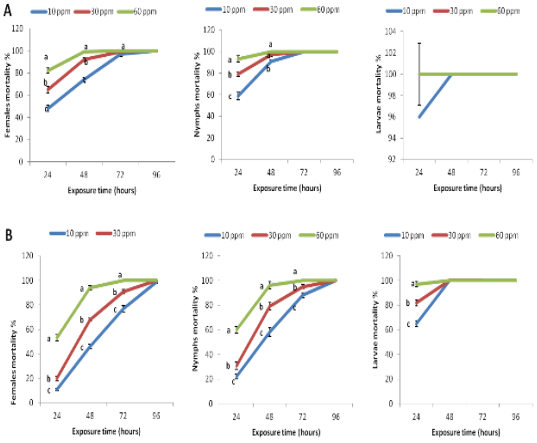

Figure 3: Mean mortality (±S.E.) of M. fungivorus females, nymphs and larvae exposed to C and G ZnO NPs at three concentrations (ppm) and different exposure times (hours). A= chemical ZnO NPs, B= Green ZnO NPS. Different letters within the same exposure time are significantly different (P<0.05).

3.4. Post toxic effect of C and G ZnO NPs on deposited eggs of *C. mycophagous* and *M. fungivorus* females at different concentrations after 96h post –exposure.

Data in Table (1) concluded that there were a highly significant decrease of deposited eggs by the treated females of both storage mites with both tested ZnO NPs compared to that of control ($P \leq 0.05$) and it was observed that by increasing the tested concentrations, the reduction of deposited eggs number was remarkably increased ; as shown in Table (1), the number of deposited eggs by *C. mycophagous* was reduced from 846 egg to 11and 31egg by C and G ZnO NPs, respectively at the lowest concentration (10ppm) and to 0.0and 0.2 egg, respectively at the highest concentration(60ppm). Also, the number of deposited eggs by M. fungivorus was 2 and 11eggs by C and G ZnO NPs, respectively at (10ppm) and zero at (60ppm) compared to that of control (208egg).it was observed that all deposited eggs of treated females of both mites did not hatch.

Table 1: Mean number (±S.E.) of deposited eggs by females of *C. mycophagous* and *M. fungivorus* mites at tested concentrations.

Tested mites	*C. mycophagous*		*M. fungivorus*	
Source	CZnO NPS	GZnO NPs	CZnO NPS	GZnO NPs
10 ppm	11.2±0.9b	31.0±2.0b	2.0±0.7b	11.4±1.1b
30 ppm	0.6±0.6b	9.0±0.7b	0.0±0.0b	9.0±0.7b
60 ppm	0.4±0.2b	1.6±0.5b	0.0±0.0b	0.0±0.0b
Control	845.8±23.8a		207.8±10.3a	
F-Value	1248	1212	405	378

3.5. Scanning Electron Microscope (SEM) test.

SEM images showed that C and G ZnO NPs attached to different parts of dead treated females compared to untreated ones. As shown in (Figure, 4 A and B), C ZnO NPs distributed on ventral side between legs, hairs and head of *C. mycophagous* and *M. fungivorus mites*, respectively compared to untreated females (Figure, 5). Similarly, G ZnO NPs coated storage mite bodies (Figure, 4 C and D). The heavy distribution of particles may penetrate the cuticle of female causing death.

4. Discussion

The current study revealed that G ZnO NPs showed toxic effect equivalent to chemical C ZnO NPs against female, nymphs and larvae of *C. mycophagous* and *M. fungivorus* with high mortality rates based on exposure times and concentrations. It was also observed that females ovipostion of both mites were influenced. Moreover, the deposited eggs did not hatch at all. To our knowledge, this is the first investigation showing the toxic effect of C and G ZnO NPs on the two tested mites. Enhancing the use of ZnO NPs as pesticide is important to develop controlling tools. In addition, using plant extracts in synthesizing metal nanoparticles instead of chemicals as alternative and eco-friendly tools are gaining great attention in recent years [20-21, 27-29, 31].

Some previous studies utilized ZnO NPs as pesticides; same to our results, a study by Korghon et al., [22] on R. robini m,ite showed this acaricidal action of ZnO NPs.

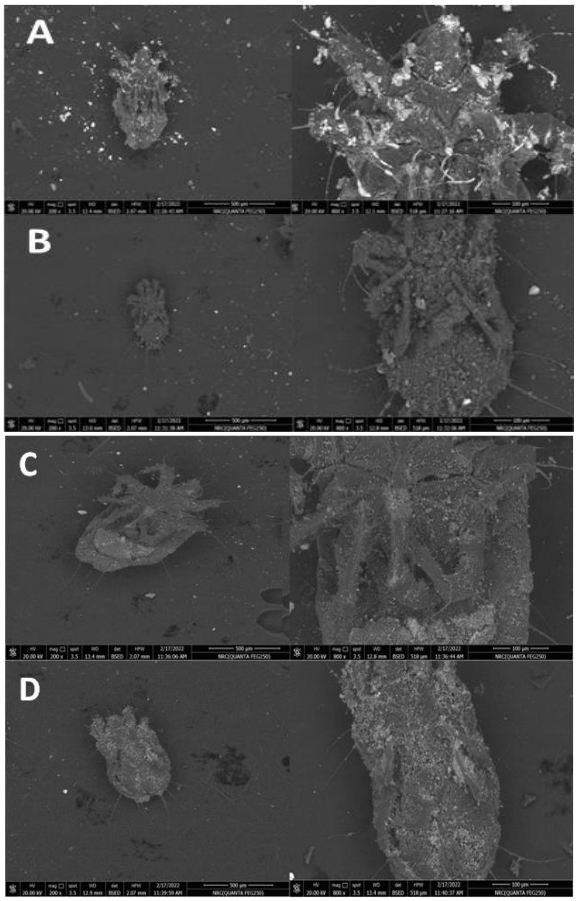

Figure 4: Scanning Electron Microscopic images visualized C ZnO NPs and G ZnO NPs aggregation on *C.mycophagous* and *M. fungivorus*. (A and B), C ZnO NPS on females of *C. mycophagous and M. fungivorus* , respectively. (C and D), G ZnO NPS on females of *C. mycophagous* and *M. fungivorus*, respectively.

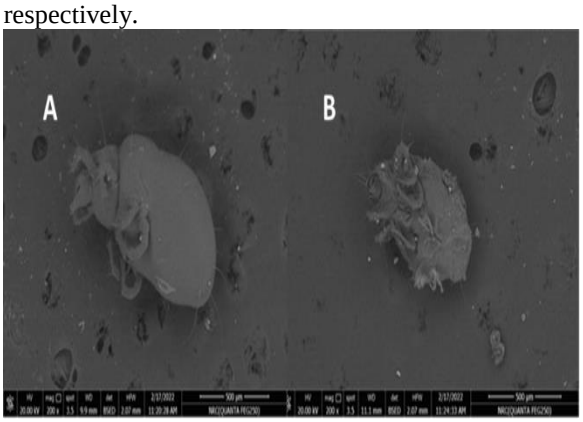

Figure 5: Scanning Electron Microscopic images visualized untreated females of A; *C. mycophagous*, and B; *M. fungivorus*.

Also, G ZnO NPs using Pomegranate peels extract caused high mortality rate and progeny reduction to adults of *S. oryzae* and *S. cerealella* [29]. Moreover, ZnO NPs showed high mortality rates in *S. oryzae*, *Tribolium castaneum* (herbst) and *Callosobruchus maculates* (Fabricius) [34- 36]

The potential activity of both types of ZnONPs as acaricides here could be contributed to their unique chemical and antimicrobial properties [37-38]. Interestingly, G ZnO NPS activity may refers to reducing and capping agents of orange peels extract as plant extract contains reductive compounds like polysaccharides, polyphenols, alkaloids flavonoids, vitamins, amino acids, tannins and terpenoids [32, 39]. Results in excellent acaricidal activity against all stages of both test mites.

Again, the present study concluded that *C. mycophagous* mite was more tolerant than *M. fungivorus* and the larvae were more sensitive than females and nymphs of each mite. The differences in the toxicity rate between the two mites may be referring to differential metabolism and mode of action as well as the pest mite's size and the body surface treats. Based on our observation in the research, *M. fungivorus* mites have small size and hairs more than *C. mycophagous* mites.

In the current study, SEM images exhibited ZnO NPs powder covered different body surface parts of females. This powder distribution may be penetrating the cuticle and drained it via damaging water barrier leading to mite death and this in agreement with Ebeling, [40]; Debnath et al., [41] and Rumbos et al., [42] who demonstrated this mode of action. Similarly, Ibrahim et al., [29] observed ZnO NPs powder on various parts of *S. oryzae* and *S. cerealella* bodies, that confirmed their ability to penetrate and made insect desiccation ended by death. So, The results of this research suggests the possibility of using of ZnO NPS to control both tested storage mites and other storage mites as their mode of action depend on cuticle absorption to these NPS followed by abrasion, water loss , desiccation and pest mite death Moreover, they are decreasing their population density via reducing fecundity and hatchability of eggs.

5. Conclusions

Our findings concluded that green method was ecofriendly and less cost than chemical method. The size of chemical and green zinc oxide NPs were 35 and 3 nm, respectively measured by high resolution transmission electron microscope. Our results showed that G ZnO NPs had the comparable acaricidal effect with C ZnO NPs on females, nymphs and larvae of *C. mycophagous* and *M. fungivorus.* Moreover, the deposited eggs by females were decreased and what was laid did not hatch at all. Although, SEM results suggests the way the ZnO NPs act on storage mites through cuticle absorption and abrasion. The use of ecofriendly ZnO NPs was safer than the use of the traditional pesticides on the stored product. Further studies well needed on the stability of tested material for long time and side effects on non-target organisms.

6. Conflicts of interest

There are no conflicts to declare

7. Formatting of funding sources

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