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Reduction in insect attachment ability by biogenic and non-biogenic ZnO nanoparticles

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Nanomaterials can represent an environmentally safe method to control different insect pests. The present study investigated the effect of biogenic and non-biogenic zinc oxide nanoparticles (ZnO-NPs) on the attachment ability of the Southern green stink bug, *Nezara viridula*, a major agricultural pest. The experiments were conducted on glass surfaces treated with different concentrations of ZnO-NPs, and the attachment ability of adult males of *N. viridula* was measured through traction force experiments. The results showed that both biogenic and non-biogenic ZnO-NPs reduced the attachment ability of *N. viridula*, with a significant decrease in traction force observed at concentrations of 12.5 mg L⁻¹ and above. SEM analyses revealed that biogenic and non-biogenic ZnO-NPs aggregated on the attachment devices of *N. viridula*, including the pulvilli, the hairy pad and claws, disrupting the attachment mechanism. This study suggests that ZnO-NPs, particularly biogenic ZnO-NPs obtained from plant extract, have potential as a natural and eco-friendly pest control agent to reduce insect attachment and damage to crops.

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Environmental significance

Phytophagous insects cause considerable damage to plants, heavily reducing crop production. Insect pests are controlled mainly by insecticides, which present health and environmental issues. The ability to firmly adhere to plant surfaces along the whole life cycle is of pivotal importance for insect survival and reproductive success. In this context, the development of antiadhesive coatings can represent an alternative method to reduce insect pest impact in agriculture. Here we demonstrate that both biogenic and non-biogenic ZnO-nanoparticles can reduce the attachment ability of the green stinkbug *Nezara viridula*, a major pest worldwide. ZnO-NPs aggregate on the insect attachment devices (pulvilli, hairy pad and claws), disrupting the insect attachment mechanism. This study may help to develop sustainable, nontoxic methods that can be alternatives to insecticides.

Introduction

Nearly half of the approximately 1 million described insect species are phytophagous insects,¹ and many represent insect pests that cause considerable damage to plants, with crop losses of about 18 and 16%.² The need to control pest insects in agriculture to prevent crop diseases or damage led to the development of synthetic organic insecticides, mainly represented by organophosphates, carbamates, synthetic pyrethroids, and neonicotinoids.³ Despite their beneficial impact on crop production, these chemicals can have acute and chronic effects on humans if they reach the trophic chain when reaching and accumulating in non-target organisms,

and they can also pose more than one risk to environmental health.^{4,5} Consequently, the indiscriminate use of pesticides can result in environmental pollution, the emergence of agricultural pests and pathogens, and the loss of biodiversity.

Alternative and eco-friendly strategies have been developed over the last few decades, and many studies and research are still on course to offer sustainable and less environmentally impacting solutions to face the above problems that plague cropping systems. Among the approaches to protect plants from insects and environmental biotic and abiotic stresses, inert powders (particle film technology) are regarded for the coating of plants with chemically inert mineral particles. In particular, they create a porous surface film that does not interfere with plant gas exchanges and can be easily removed after harvest (see the review in ref. 6). In addition, the possibility of reducing insect attachment capacity is exploited as a suitable way to decrease their potential damage to crops, thus reducing crop losses. For these reasons, innovative, nanostructured and also bio-inspired materials have been developed. They are still the

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Fig. 1 SEM images of biogenic ZnO-NPs (a) and non-biogenic ZnO-NPs (b).

for significant factors using Dunnett's method.³⁷ The Shapiro–Wilk test was used to verify the normality distribution of the data. The traction forces recorded in the first 60 s on untreated and treated surfaces with a concentration of 50 mg L⁻¹ were compared using a *t*-test for dependent samples. The relationship between the percentage of glass surface covered by NPs and the different tested NP concentrations was tested using linear regression analysis.³⁷ The 50% inhibitory concentration (IC₅₀ values for the ZnO-NP concentration exhibiting antiadhesive properties were calculated using publicly available software on the AAT Bioquest website (<https://www.aatbio.com/tools/ic50-calculator>).

Results

The biogenic ZnO-NPs on glass (concentration of 200 mg L⁻¹), visualized with SEM (Fig. 1a), show nanometric dimensions and a spherical shape, with aggregates of diameters ranging from 40 to 200 nm. In addition, the non-biogenic ZnO-NPs on glass (Fig. 1b) show nanometric dimensions ranging from 20 to 200 nm and a lamellar morphology with an irregular shape. Both NPs showed a clear tendency to aggregate following the application and drying on the surfaces investigated (Fig. 1).

The experiments to test the attachment ability of *N. viridula* adult males to different glass surfaces, untreated or treated with biogenic ZnO-NPs, were carried out according to the following sequence: untreated glass, treated glass, untreated glass after 0 h, and untreated glass after 24 h. The insect traction force on treated glass was significantly lower than that recorded on untreated glass, starting from a concentration of biogenic ZnO-NPs of 12.5 mg L⁻¹. At a lower concentration (6.25 mg L⁻¹), the insect attachment ability on treated glass was not significantly different from that recorded on glass (Fig. 2). In traction force experiments performed using *N. viridula* adults pulling on untreated glass at different time intervals after having walked on treated glass, *i.e.*, after 0 h and after 24 h, there was no difference in the insect traction force recorded on untreated glass both after 0 and after 24 h and the initial insect traction force exerted by insects pulling on untreated glass (Fig. 2) (6.5 mg L⁻¹: $F = 1.79$; d.f. = 3, 39; $p = 0.173$. 12.5 mg L⁻¹: $F = 17.50$; d.f. = 3, 27; $p < 0.001$. 25 mg L⁻¹: $F = 64.33$; d.f. = 3, 53; $p < 0.001$. 50 mg L⁻¹: $F = 45.80$; d.f. = 3, 55; $p < 0.001$. 100 mg L⁻¹: $F = 39.69$; d.f. = 3, 58; $p < 0.001$. 200 mg L⁻¹: $F = 49.01$; d.f. = 3, 48; $p < 0.001$).

The experiments to test the attachment ability of *N. viridula* adult males to different glass surfaces, untreated or treated with non-biogenic ZnO-NPs, were conducted according to the following sequence: untreated glass, treated glass, untreated glass after 0 h, and untreated glass after 24 h. The insect traction force on nanoparticle treated glass was significantly lower than that recorded on untreated glass, starting from a concentration of biogenic ZnO-NPs of 12.5 mg L⁻¹. In contrast, at a lower concentration (6.25 mg L⁻¹) the insect attachment ability on treated glass was not significantly different from that recorded on untreated glass (Fig. 3). In traction force experiments performed using *N. viridula* adults pulling on glass at different time intervals after having walked on treated surfaces, *i.e.*, after 0 h and after 24 h, in insects tested immediately after walking on treated glass (untreated

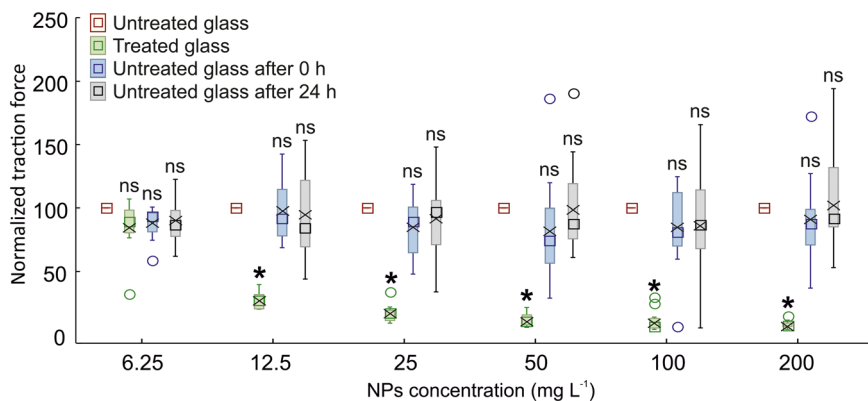


Fig. 2 Normalized traction force obtained in traction force experiments with *Nezara viridula* pulling on untreated glass, treated glass at different biogenic ZnO-NP concentrations (mg L⁻¹), untreated glass after 0 h, and untreated glass after 24 h. Boxplots show the interquartile range and the median (square), whiskers indicate the 1.5 × interquartile range, and X shows the arithmetic mean. Boxplots with an asterisk are significantly different from the untreated glass at $p < 0.05$, 1-way repeated measures ANOVA, Dunnett's method *post hoc* test.





Fig. 3 Normalized traction force obtained in traction force experiments with *Nezara viridula* pulling on untreated glass, treated glass at different non-biogenic ZnO-NP concentrations (mg L^{-1}), untreated glass after 0 h, and untreated glass after 24 h. Boxplots show the interquartile range and the median (square), whiskers indicate the $1.5 \times$ interquartile range, and X shows the arithmetic mean. Boxplots with an asterisk are significantly different from the untreated glass at $p < 0.05$, 1-way repeated measures ANOVA, Dunnett's method *post hoc* test.

glass after 0 h), the traction force on glass was significantly lower than that recorded on untreated glass only at concentrations similar to or higher than 100 mg L^{-1} . At lower concentrations, there was no significant difference in the traction force on glass before and after walking on a treated surface (Fig. 3). In insects tested 24 h after walking on treated glass (untreated glass after 24 h), there was no significant difference in the traction force on untreated glass before and after walking on treated glass at any concentration investigated (Fig. 3) (6.5 mg L^{-1} : $F = 1.18$; d.f. = 3, 29; $p = 0.343$. 12.5 mg L^{-1} : $F = 19.45$; d.f. = 3, 25; $p < 0.001$. 25 mg L^{-1} : $F = 43.55$; d.f. = 3, 54; $p < 0.001$. 50 mg L^{-1} : $F = 45.48$; d.f. = 3, 53; $p < 0.001$. 100 mg L^{-1} : $F = 34.93$; d.f. = 3, 54; $p < 0.001$. 200 mg L^{-1} : $F = 45.77$; d.f. = 3, 51; $p < 0.001$).

The reduction in the traction force of *N. viridula* walking on glass treated with biogenic and non-biogenic Zn-NPs at different concentrations expressed as percentages in comparison with the traction force exerted by insects walking on untreated glass reached

70% at a concentration of 12.5 mg L^{-1} and remained high (around 90%) starting from a concentration of 50 mg L^{-1} (Fig. 4). The 50% inhibitory concentration (IC_{50}) values for the concentration exhibiting antiadhesive properties related to insect attachment ability were very similar for biogenic and non-biogenic ZnO-NPs, and, in particular, it was 8.53 for biogenic ZnO-NPs and 9.03 for non-biogenic ZnO-NPs.

The reduction of the traction force on treated surfaces (with a concentration of 50 mg L^{-1}) was not significantly different during the duration of the traction force measurements both for biogenic ($F = 2.45$; d.f. = 3, 53; $p = 0.079$) and non-biogenic ($F = 2.36$; d.f. = 3, 45; $p = 0.092$) ZnO-NPs (Fig. 5). Moreover, in the first 60 s the traction forces recorded on biogenic ($t = 8.79$; d.f. = 13; $p < 0.001$) and non-biogenic ($t = 10.62$; d.f. = 12; $p < 0.001$) ZnO-NP treated surfaces were significantly lower than those recorded on untreated surfaces.

The real distribution on the surface of NPs follows the increasing concentrations for both biogenic ($F = 12.15$; d.f. = 1, 5; $p < 0.001$), ($Y = 0.1094 \times X + 0.5736$; $P = 0.0001$) and non-biogenic ($F = 29.7$; d.f. = 1, 5; $p = 0.003$), ($Y = 0.1096 \times X + 1.965$; $P = 0.0028$) ZnO-NPs with a linear relationship (Fig. 6).

The tarsal attachment organs of the adult of *N. viridula* are represented by smooth pulvilli, a pair of pretarsal claws and a basitarsal hairy pad. The latter bears on its ventral side a hairy pad with numerous adhesive setae (see ref. 38). The SEM observations of the tarsi of insects after they walked on glass treated with biogenic and non-biogenic ZnO-NPs reveal that at a concentration of 6.25 mg L^{-1} the attachment devices are not contaminated (Fig. 7a and f), while at a concentration of 100 mg L^{-1} both biogenic (Fig. 7b and d) and non-biogenic (Fig. 7g and i) ZnO-NPs aggregate on the distal portion of the claws, on the ventral surface of pulvilli and among the adhesive setae of the hairy pad. Furthermore, the EDX analysis confirms a high amount of Zn in these NPs (Fig. 7c, e, h and j).



Fig. 4 Reduction in the traction force of *Nezara viridula* walking on glass treated with different concentrations of biogenic and non-biogenic ZnO-NPs expressed as percentage in comparison with the traction force exerted on untreated glass.





Fig. 5 Traction force obtained every 60 s during the traction force experiments with *Nezara viridula* pulling on treated glass with ZnO-NPs at a concentration of 50 mg L⁻¹. Boxplots show the interquartile range and the median (square), whiskers indicate the 1.5 × interquartile range, and X shows the arithmetic mean. Boxplots with the same letter are not significantly different at $p < 0.05$, 1-way repeated measures ANOVA.

Discussion

Concerning the need to overcome problems associated with conventional agriculture (pests, fertilizers, environmental pollution, phytopathogens, etc.), nanostructured materials are showing potential to become a new-age material. Some NPs can prompt substantial benefits in crops already at low concentrations, improving germination, photosynthesis, biomass production, chloroplast development and functionality and antioxidant defensive mechanisms.^{39–45} In this frame, zinc-based nanomaterials are attracting some interest, as this element is an essential micronutrient for plants.²⁶ Recent studies have shown that ZnO-NPs can be used for treating crops, thus improving seed germination, plant vigor index, physiological and biochemical traits, and biomass production.^{42,46,47} In addition, the biogenic ZnO-NPs obtained from duckweed (the same used in the present study) revealed a beneficial effect in maize and olive tree explants.^{21,29} As for maize, the biogenic ZnO-NPs positively

influenced the chlorophyll and carotenoid content and the cellular redox state, and stimulated biomass production. Concerning olive explants, ZnO-NPs stimulated the production of shoots, fresh and dry weight, and pigment and soluble protein contents, positively affecting total phenols and ROS scavenging activity.

The results presented in this study demonstrate that the attachment ability of the adult of *N. viridula* to glass surfaces can be significantly reduced by applying both biogenic and non-biogenic ZnO-NPs. The SEM images of the treated surfaces show that after the application, the biogenic ZnO-NPs maintained a spherical shape, according to ref. 21, but after drying, the nanostructured material tends to aggregate. The same happens for the non-biogenic ZnO-NPs, which showed a lamellar nanostructure but aggregation after the application and drying on the glass surface.

Furthermore, our SEM observations reveal that the reduction in attachment ability is due to the contamination of insect attachment devices with the ZnO-NPs. Indeed, the pulvilli and the hairy pad with adhesive setae of *N. viridula* are not contaminated at a concentration of 6.25 mg L⁻¹ (not impairing the bug attachment ability), but at higher concentrations, both biogenic and non-biogenic ZnO-NPs aggregate on the distal portion of the claws, the ventral surface of pulvilli and among the adhesive setae of the hairy pad, interfering with adhesion of insects showing on their legs both smooth or hairy attachment devices. A similar effect in reducing insect attachment ability is induced by plant waxes, according to the contamination hypothesis.^{48,49} However, we cannot exclude some roles of ZnO-NPs in reducing insect adhesion owing to the absorption of the pad fluid due to the structured ZnO particles, as it happens for plant wax coverage.⁵⁰

The concentration of both biogenic and non-biogenic ZnO-NPs required to significantly reduce the attachment ability of *N. viridula* to glass surfaces is around 12.5 mg L⁻¹, and the reduction in attachment ability is very strong (about 70% reduction of the insect attachment ability), reaching 90% reduction of the initial force from a concentration of 50 mg L⁻¹. Hypothesizing that *N. viridula* walks on an inclined plane, considering the maximum value of traction force recorded on treated surfaces at a concentration of 12.5 mg L⁻¹, the insect is not able to attach to treated surfaces with an inclination $\geq 75^\circ$ ($\alpha = \arctan(F/m \cdot g)$). This means that ZnO-NP treatment can prevent the insect presence on different inclined natural surfaces such as leaves or fruits. Moreover, the reduction of the traction force on surfaces treated with ZnO-NPs was not different during 240 s of the traction test, thus suggesting that 1) attachment pad contamination is effective since the beginning of pad contact with the treated surface and does not increase during traction on treated glass surfaces and 2) insects cannot self-clean their attachment devices when walking on treated surfaces. The IC₅₀ values for the concentration exhibiting antiadhesive properties related to insect attachment ability were very similar for biogenic and non-biogenic ZnO-NPs, indicating



Fig. 6 Linear relationship between the percentage of glass surface covered by NPs and the different tested NP concentrations.





Fig. 7 Ventral side of the tarsal attachment devices of *Nezara viridula* males represented by smooth pulvilli (P), a pair of pretarsal claws (C), and a basitarsal hairy pad (HP) just after they walked on glass treated with biogenic (a–e) and non-biogenic (f–j) ZnO-NPs at concentrations of 6.25 mg L⁻¹ (a and f) and 100 mg L⁻¹ (b–e and g–j), visualized with SEM (backscattered electrons) (a, b, d and f–i) and EDX analysis (c, e, h and j) highlighting in red the presence of Zn on the ventral surface of claws, pulvilli (c and h) and hairy pad (e and j).

that the biogenic synthesis of the NPs did not significantly affect their anti-adhesive properties.

The results also show no difference in the insect traction force recorded on untreated glass after 0 and after 24 h, and the initial insect traction force exerted by insects pulling on untreated glass. This indicates that the ZnO-NPs do not have a residual effect on the insect attachment ability. This is probably due to the self-cleaning properties of the insect adhesive fluid, which plays an important role not only in increasing capillary forces allowing insect attachment ability⁵¹ but also in “washing-out” contaminating particles.⁵² Indeed measurements of the attachment ability of stick insects (smooth pads) and dock beetles (hairy pads) on glass after contamination with microspheres showed that insect pads recovered high levels of adhesion after only eight simulated steps.⁵² The only

exception is represented by non-biogenic ZnO-NPs, which, starting from a concentration of 100 mg L⁻¹, tend to significantly reduce the attachment ability of insects pulling on untreated glass after walking on treated glass. In any case, this reduction is no longer visible after 24 h. Such a slightly more persistent antiadhesive effect of the non-biogenic ZnO-NPs, compared to the biogenic NPs, is probably due to the different shapes of the NPs. Indeed, the lamellar shape of the non-biogenic NPs could be more difficult to remove from the insect attachment devices than the spherical ones typical of biogenic Zn-NPs.

In conclusion, this study suggests that biogenic and non-biogenic ZnO-NPs can be promising materials for developing anti-adhesive surfaces for insect control. Further studies are necessary to evaluate the antiadhesive properties of ZnO-NPs on natural surfaces such as plant



leaves, where typical surface plant features such as waxes or trichomes can interfere with insect attachment ability^{34,53} and with the effectiveness of antiadhesive coatings as shown for kaolin particle films.⁹ We cannot also exclude the involvement of ZnO-NPs in insect oviposition and feeding deterrence or in reducing the insect survival rate and mating success, as highlighted for other nanoparticle films such as kaolin.^{54–63} Moreover, this study demonstrates that biogenic ZnO-NPs, synthesized using an extract from an invasive aquatic species, the duckweed, have potential as a non-toxic and environmentally friendly insect repellent. However, further studies are needed to evaluate their effectiveness against other insect species and to investigate their potential impacts on non-target organisms.

Ethical approval

This article does not contain any studies with human participants or animals (other than insects) performed by any of the authors.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author contributions

All authors conceived and designed the research. DD synthesized the NPs. MR and GS conducted the traction force experiments. MR and SP performed the SEM observations. GS analyzed the data. MR and DD wrote the manuscript. All authors read and approved the manuscript.

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

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