

# Fungus-synthesized nanoparticles and their target and nontarget effects on stored bean pest beetles and their parasitoid

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## Research Article

**Keywords:** Bruchinae, Bruchidae, Pteromalidae, sex difference, biosynthesized nanopesticides, nontarget effect

**Posted Date:** September 27th, 2022

**DOI:** <https://doi.org/10.21203/rs.3.rs-2097277/v1>

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# Abstract

Whether green nanotechnology can be effectively used with biological control agents in integrated pest management is currently unknown. We biosynthesized 8–33 nm nanoparticles (NPs) using extracts from the fungus *Fusarium solani*: silver (AgNPs), selenium (SeNPs), silicon dioxide (SiO<sub>2</sub>NPs), copper oxide, titanium dioxide (TiO<sub>2</sub>NPs) and zinc oxide (ZnONPs). They were applied to the adult, egg, and larval stages of *Callosobruchus chinensis* (*Cc*) and *Callosobruchus maculatus* (*Cm*) directly or via azuki beans *Vigna angularis*, and the adult stage of their parasitoid wasp *Anisopteromalus calandrae*. Susceptibility to the nanoparticles was species-dependent and differed among developmental stages; adults and eggs were more susceptible than larvae in the beetles except for *Cc* adults. Female adult longevity was shortened by SeNPs in *A. calandrae* and by SeNPs and AgNPs in *Cm*. In contrast, ZnONPs elongated *Cc* adult longevity and SeNPs elongated male *Cm* longevity. When eggs were treated, SeNPs and TiO<sub>2</sub>NPs reduced *Cc* egg hatchability and TiO<sub>2</sub>NPs reduced *Cm* larva-to-adult survivorship, leading to lower numbers of emerged adult offspring. When the bean surface was treated, there was a pronounced toxicity of SeNPs on the fecundity and egg hatchability of *Cm* and of SiO<sub>2</sub>NPs on egg hatchability of both beetle species. When beans containing beetle larvae were treated, no toxicity was detected. *Cc* adults were not negatively affected by any of the nanoparticles, which might be the consequence of endosymbiotic infection by *Wolbachia* that are abundant in the adults. The biosynthesized SeNPs demonstrated a strong potential for control of major stored bean pests but can also affect their biocontrol agent.

## Key Message

- Whether green nanotechnology can be effectively used with parasitoids is unknown
- Six nanoparticles (NPs) were biosynthesized using the extract of the fungus *Fusarium solani*
- The beetle *Callosobruchus maculatus* was more vulnerable than *Wolbachia*-infected *C. chinensis*
- Selenium NPs were the most effective NPs on the beetles but also toxic to their parasitoid
- Selenium NPs enhanced male *C. maculatus* longevity and zinc oxide NPs adult *C. chinensis* longevity

## Introduction

The world's population will reach 8 billion in 2022 and is projected to reach a peak of 10.4 billion by the 2080s (United Nations Department of Economic and Social Affairs, Population Division, 2022). One of the most significant crops for the world's population is *Vigna* beans (cowpeas *Vigna unguiculata*, mung beans *Vigna radiata*, azuki beans *Vigna angularis*, etc.), but this food supply is impacted by storage losses caused by insect pests for a number of people (Mesterházy 2020). *Callosobruchus* beetles, represented by cowpea beetle *C. maculatus* in tropical areas and azuki bean beetle *C. chinensis* in temperate areas, are important stored product pests with wide host ranges that can cause severe losses to dried beans (Tuda et al. 2005, Tuda 2007). Although chemical insecticides such as fumigants and dusts are effective for controlling *C. maculatus* and *C. chinensis*, they are not suitable at the farm level

since farmer's storage facilities are not airtight, and it is not acceptable to mix pesticides with stored grain for insect pest prevention (Bekele et al. 1995). To safeguard the environment, animals, and humans, scientists and governments throughout the world are shifting from chemical-based agriculture to green agriculture.

Nanotechnology has become one of the most promising new approaches for the control of pests to protect crops in recent years (Athanasios et al. 2018). Nanoparticles (NPs) have an atomic or molecular size of less than 100 nm and possess exceptionally novel properties such as a high surface-area-to-volume ratio, high reactivity, and enhanced catalytic and biological properties (Ranjani et al. 2020). All these unique properties make the NPs appropriate for a variety of applications including their usage in agriculture (Deka et al. 2021; Upadhyay et al. 2020; Yaqoob et al. 2020). Metal and metallic oxide NPs such as silver (Ag), zinc oxide (ZnO), copper oxide (CuO), silica (silicon dioxide, SiO<sub>2</sub>), titanium dioxide (TiO<sub>2</sub>), gold (Au), and aluminum oxide (Al<sub>2</sub>O<sub>3</sub>) are being produced for use in pest and disease control (Piesik and Wenda-Piesik 2015). Ag, Cu and ZnO NPs have antimicrobial activity. SiO<sub>2</sub>NPs have been demonstrated to have physisorption in cuticle lipids of insects which promote insect mortality (Barik et al. 2008) and are promising tools for biological applications owing to their excellent biocompatibility, low toxicity to humans, and thermal stability. Additional benefits of SiO<sub>2</sub>NP application were found in controlling *Spodoptera* spp.; SiO<sub>2</sub>NPs promoted volatile emission from infested soybean plants, which attracted the predator *Paederus* spp. (Thabet et al. 2021). Selenium nanoparticles (SeNPs) have antioxidant (Hamza 2012), antibacterial (Vera et al. 2016), anticancer (Zhang et al. 2013), neuroprotective (Turovsky et al. 2022), antimicrobial (Menon et al. 2019), and plant-growth-promoting (Nazari et al. 2022) effects and used in the treatment of diabetes, bioremediation, and as a biosensor (Kumar and Prasad 2021). The mode of action of NPs against insects are summarized in Benelli (2018) for Ag, SiO<sub>2</sub>, and metal NPs. The insecticidal effect of SeNPs has recently been shown for *Spodoptera litura* (Arunthirumeni et al. 2021). SeNPs can be biosynthesized by bacteria (Visha et al. 2015; Menon et al. 2019) and fungi (e.g. *Mariannaea* sp., Zhang et al. 2019). The fungus *Fusarium* sp. was used for the extracellular biosynthesis of AgNPs (Singh et al. 2015).

The application of biosynthesized NPs is expected to transform the field of integrated pest management (IPM) in future (Bhattacharya et al. 2020; Duhan et al. 2017; Rai et al. 2018) (Supplementary Table S1). Compared to chemical synthesis, the biological synthesis of nanopesticides using plant extracts and microbes is greener—environmentally friendly, non-toxic, and inexpensive without using high temperature, high pressure, high energy, and toxic chemicals and without producing by-products toxic to humans (Gunalan et al. 2012; Devi et al. 2014; Kapinder and Verma 2021; Selvan et al. 2018; Narayanan et al. 2021).

There have been few studies on the use of NPs in managing stored product pests (Abo-Arab et al. 2014; El-Naggar et al. 2020; Jasrotia et al. 2022; Supplementary Tables S1, S2). For example, AgNPs synthesized using the aqueous leaf extracts of *Euphorbia prostrata* proved to have potential to control adult *Sitophilus oryzae* (Zahir et al. 2012). The insecticidal effects of SiO<sub>2</sub> and Ag NPs (Rouhani et al. 2013)

and phyto- (*Pongamia pinnata* leaf extract) or entomopathogen- (*Bacillus thuringiensis*) coated ZnONPs on *C. maculatus* has been reported (Malaikozhundan et al. 2017; Malaikozhundan and Vinodhini 2018). Al<sub>2</sub>O<sub>3</sub> and SiO<sub>2</sub> NPs are more effective than TiO<sub>2</sub> and ZnO NPs in the control of the rice weevil *S. oryzae* (Goswami et al. 2010). Green-synthesized Ag and lead (Pb) NPs using mangrove (*Avicennia marina*) plant extract also have insecticidal effects against *S. oryzae* (Sankar and Abideen 2015). Green-synthesized AgNPs also have more larvicidal effects against *Tribolium castaneum* and *C. maculatus* than pure AgNPs or *Nerium oleander* leaf extract (Jafer and Annon 2018). AgNPs synthesized using the leaf extracts of *Myriostachya wightiana* exhibited insecticidal activity against *S. oryzae*, *T. castaneum*, and *Rhyzopertha dominica* (Vadlapudi and Amanchy 2017). Rock powder and ZnONPs showed high efficacy against *S. oryzae* with respect to mortality rate and progeny of adults (Hamza 2012).

Non-selective insecticides can affect not only pests but also their natural enemies. For example, AgNPs have been demonstrated to impact a predator *Podisus maculiventris* (spined soldier bug) of lepidopteran pests (tobacco hornworm *Heliothis virescens* and cabbage looper *Trichoplusia ni*), whereas impact on the pests were negligible (Afrasiabi et al. 2016). Little side-effect on honeybees was confirmed for a botanical pyrethrum-based nanoinsecticide (Oliveira et al. 2019). However, still, little is known about the non-target effects of nanopesticides on beneficial insects compared to their target effects on pests. This study aimed to test the efficacy of biosynthesized NPs of Ag, CuO, Se, SiO<sub>2</sub>, TiO<sub>2</sub>, and ZnO by *Fusarium solani* extract as insecticides against two *Callosobruchus* beetle species at different developmental stages and their non-target effects on their parasitoid. We hypothesized that the biosynthesized nanoparticles decrease the fecundity and survival of the different developmental stages (and consequently the number of emerging adults of both sexes) of the two pest beetles, irrespective of direct/indirect application, and the adult longevity of both sexes of their parasitoid.

## Materials And Methods

### Fungal culture

Fungal culture used in this study for the biosynthesis of the different NPs were isolated from the soil. An agricultural soil samples were collected from the potentially contaminated pots used for the experimental studies at the Laboratory of Insect Natural Enemies, Faculty of Agriculture, Kyushu University. Fungal strains were isolated by the direct plating method (Dhingra and Sinclair 1995) and morphologically differentiated according to Smith and Onions (1983). Further molecular classification was performed to complete the differentiation according to Henry et al (2000) and detailed in the following section.

### Biosynthesis of nanoparticles by fungi

To prepare biomass for biosynthesis of metal and non-metal NPs, fungal culture was grown aerobically in liquid media containing 3.0 g malt extract, 10.0 g glucose, 2.0 g yeast extract, 5.0 g peptone, 20.0 g agar-agar and 1.0 L distilled water, with pH adjusted to 6.2 as per Helmy and Mekawey (2014). Fungal culture was filtered aseptically and incubated in sterilized deionized water aerobically for 72 h.

Silver (Ag) NPs were prepared by adding 500 mg L<sup>-1</sup> of AgNO<sub>3</sub> solution to cell-free water extract of the fungal isolate. The reduction of Ag ions to AgNPs was confirmed by color transformation of the mixture to brown (El-Naggar et al. 2014) (Fig. 1a). Copper oxide (CuO) NPs were prepared by adding 500 mg L<sup>-1</sup> of Cu/ISO<sub>4</sub> solution to cell-free water extract of the fungal isolate. The reduction of Cu ions to CuONPs was confirmed by color transformation of the mixture to blue green (Fig. 1b). Selenium (Se) NPs were prepared by adding 500 mg L<sup>-1</sup> of Na<sub>2</sub>SeO<sub>3</sub> solution to cell-free water extract of the fungal isolate. The reduction of Se ions to SeNPs was confirmed by color transformation of the mixture to red (Fig. 1c). Silicon dioxide or silica (SiO<sub>2</sub>) NPs were prepared by adding 500 mg L<sup>-1</sup> of SiO<sub>2</sub> solution to cell-free water extract of the fungal isolate. No color transformation of the mixture was observed to confirm the reduction of Si ions to SiO<sub>2</sub>NPs (Fig. 1d). Titanium dioxide (TiO<sub>2</sub>) NPs were prepared by adding 500 mg L<sup>-1</sup> of TiO<sub>2</sub> solution to cell-free water extract of the fungal isolate. The reduction of Ti ions to TiO<sub>2</sub>NPs was confirmed by color transformation of the mixture to deep white colloidal solution (Fig. 1e). Zinc oxide (ZnO) NPs were prepared by adding 500 mg L<sup>-1</sup> of ZnSO<sub>4</sub>·7H<sub>2</sub>O solution to cell-free water extract of the fungal isolate. No color transformation of the mixture was observed to confirm the reduction of Zn ions to ZnONPs (Fig. 1f). The characterization of the resulted NPs produced by the fungal isolate was carried out using visual observation, transmission electron microscopy (TEM) photographing, electron diffraction (ED), and energy-dispersive X-ray (EDX) as follows.

#### Characterization of nanoparticles

*Particle size and shape.* Size and shape of different NPs produced by the fungal isolate were determined using TEM (Philips Tecnai-G2 20, Japan). Samples for TEM were prepared by placing a drop of well dispersed NP solution onto conventional carbon coated copper TEM grids (150 µm meshes, Plano GmbH, Germany), allowing the drop to dry overnight in a desiccator before imaging. The TEM images of the samples were obtained using an accelerating voltage of 200 kV. Three images with multiple NPs were taken to have a clear representation of its morphology and particle size.

*Identification of elemental compositions.* To examine the elemental chemical composition of the NPs, surface binding elements of the sample NPs were analyzed with X-ray photoelectron spectroscopy, using EDX system coupled with the TEM (Tecnai-G2 20). The NPs excited by an electron beam showed the peak values percentage of elements in comparison to other surface binding elements.

ED coupled with the conventional TEM (ED/TEM) (JEM-2100HCKM, JEOL, Tokyo, Japan) was used to characterize the diffraction pattern of different NPs produced by the fungal isolate. Samples for ED were prepared by placing a drop of well dispersed NPs solution separately onto conventional carbon coated copper TEM grids (150 µm meshes) as described above. Images of the ED analyses of the samples were obtained using an accelerating voltage of 200 kV.

#### Molecular identification of fungi

The fungal isolate was identified based on the ITS rDNA sequence with primers ITS1 and ITS4 (Henry et al. 2000). First, DNA was extracted by freezing and thawing a small sample of the colony of the fungal isolate dissolved in TE buffer. PCR was conducted with an annealing at 53°C using KOD One (Toyobo, Tokyo, Japan), following the manufacturer's protocol. The PCR products were purified and subjected to Sanger sequencing. Sequence data were then searched for matches in the database nr using BLASTn (NCBI, MD, USA).

#### Toxicity test on parasitoid wasp adults

A culture of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) originating from Japan was used as a parasitoid of *Callosobruchus* beetle larvae and pupae (Tuda 2007; Zhang et al. 2021, 2022). It has been maintained with *C. chinensis* for more than 70 years in the laboratory at 30°C (Nakamichi et al. 2020; Zhang et al. 2021). Five females and five males emerged within 24 h were introduced to a petri dish (6 cm diameter). Twenty µl biosynthesized NP solution or distilled deionized water as control was then applied. We monitored the number and sex of dead wasps every 24 h at 30°C, 60% r.h. and a 16L:8D photocycle. Each treatment was replicated for nine times. A total of 628 adults were used for this experiment.

#### Toxicity test on pest bean beetles

We used two stored bean pest beetle species, *Callosobruchus chinensis* (Coleoptera: Chrysomelidae: Bruchinae) strain jC that originated from Japan and maintained on azuki beans in the laboratory condition at 30°C for more than 70 years (Tuda 1993; Tuda and Shimada 2005). The other is *Callosobruchus maculatus* strain tQ that originated from the Middle East and maintained on azuki beans *Vigna angularis* var. *angularis* (Fabaceae) in the laboratory condition at 30°C for more than 30 years (Toquenaga and Fujii 1991). We applied each of these NPs directly to the egg, larva, or adult stage, or indirectly to the crop (azuki beans) before oviposition of each beetle species. Twenty µl of 500 mg L<sup>-1</sup> of each NP solution synthesized by the fungal isolate or distilled deionized water as control was applied to eggs, larvae, or adults of each beetle species or 30 µl to the beans before oviposition by the beetles. Each treatment was replicated for 9–12 times at 30°C, 60% r.h. and 16L:8D.

*Direct application of nanoparticles to adults.* Five females and five males emerged within 24 h were introduced to a petri dish (6 cm diameter). Twenty µl (10 µg) biosynthesized NP solution or distilled deionized water was then applied. We monitored the number and sex of dead beetles every 24 h. A total of 630 adults for *C. chinensis* and 640 adults for *C. maculatus* were used for this experiment.

*Direct application of nanoparticles to eggs.* Eggs were deposited for 2 h on azuki beans by females that emerged within 24 h. Beans with 1–2 eggs were chosen. Seven to eight beans with a total of 10 eggs of 24 h old were introduced to a petri dish (6 cm diameter) and 20 µl (10 µg) biosynthesized NP solution or distilled deionized water was applied and eggs were reared. After eight days from application, hatched eggs were counted. Emerged adults were counted after 36 days from egg deposition. A total of 650 eggs for *C. chinensis* and 650 eggs for *C. maculatus* were used for this experiment.

*Application of nanoparticles to larvae.* Twenty  $\mu\text{l}$  (10  $\mu\text{g}$ ) biosynthesized NP solution or distilled deionized water was applied to seven to eight azuki beans infested by a total of 10 fourth instar larvae (14 days old) at the density of 1–2 larvae/bean in a petri dish (6 cm diameter). After 23 days under the same condition, emerged adults were counted. A total of 640 larvae for *C. chinensis* and 620 larvae for *C. maculatus* were used for this experiment.

*Application of nanoparticles to beans.* A pair of adults (a female and a male) emerged within 24 h was introduced to a petri dish (6 cm diameter) with 10 azuki beans (1.8 g) that were applied with 30  $\mu\text{l}$  (15  $\mu\text{g}$ ) of biosynthesized NPs (corresponding to  $8.3 \text{ mg kg}^{-1}$ ) or distilled deionized water. After 14 days from the NP application (and the introduction of pairs), hatched and unhatched eggs were counted. Emerged adults were sexed and counted after 37 days from the NP application. A total of 71 pairs for *C. chinensis* and 68 pairs for *C. maculatus* were used for this experiment.

## Statistics

The effect of NPs on the longevity of adults was analyzed with a survival analysis with a best-fit Weibull distribution with NP element, sex, their interaction, and replicates as explanatory variables, followed by posthoc comparisons with the control. The effect of NPs on the numbers of laid eggs, emerged adults and emerged females was analyzed with generalized linear models with a Poisson distribution and a log link function and an overdispersion parameter included with NP element as an explanatory variable. Posthoc tests were performed to compare with the control. Logistic regression analyses were performed on the survival rates of eggs (hatchability), larva to adult, and egg to adult, and on sex ratio of emerged adults, followed by posthoc comparisons with the control. All statistical tests were performed using JMP 14.2.0.

## Results

According to morphological differentiation and genotypical identification based on the ITS sequence obtained, the fungal isolate was identified with 100% certainty as *Fusarium solani* (Hypocreales: Nectriaceae). The size and shape of the six types of metal NPs produced by the *F. solani* isolate were characterized using TEM (Fig. S1), ED, and EDX (Fig. S2). The diameter of the spherical shaped AgNPs produced by this fungal extract was  $15.3 \pm 0.2 \text{ nm}$  (mean  $\pm$  SE). The diameter of the spherical shaped CuONPs was  $11.7 \pm 0.3 \text{ nm}$  and that of the spherical shaped SeNPs was  $20.0 \pm 0.1 \text{ nm}$ . The size of the amorphous  $\text{SiO}_2$ NPs was  $32.9 \pm 2.6 \times 75.1 \pm 8.9 \text{ nm}$ . The diameter of the spherical shaped  $\text{TiO}_2$ NPs was  $15.4 \pm 0.2 \text{ nm}$  and that of the ZnONPs was  $8.1 \pm 0.5 \text{ nm}$ .

## Toxicity on parasitoid

When adults were treated with NPs, adult longevity of *A. calandreae* was significantly affected by the interaction effect between NP element and sex (Table 1); SeNPs shortened the adult longevity of both sexes (females by 12.4%, males by 22.6%) (female,  $P < 0.001$ ; male,  $P < 0.001$ ) and  $\text{SiO}_2$ NPs reduced the longevity of only males by 4.2% ( $P = 0.010$ , Fig. 2), compared to the control.

Table 1

Results of survival analysis on adult longevity of the parasitoid *Anisopteromalus calandrae* and the beetles *Callosobruchus chinensis* and *Callosobruchus maculatus*. Nanoparticle element indicates six biosynthesized nanoparticles or control. One replicate stands for 10 adults, five individuals of each sex.

|                                    | <i>Anisopteromalus calandrae</i> |    |         | <i>Callosobruchus chinensis</i> |    |         | <i>Callosobruchus maculatus</i> |    |         |
|------------------------------------|----------------------------------|----|---------|---------------------------------|----|---------|---------------------------------|----|---------|
|                                    | $\chi^2$                         | df | P       | $\chi^2$                        | df | P       | $\chi^2$                        | df | P       |
| Nanoparticle element               | 54.51                            | 6  | < 0.001 | 34.41                           | 6  | < 0.001 | 2.96                            | 6  | 0.814   |
| Sex                                | 116.69                           | 1  | < 0.001 | 301.79                          | 1  | < 0.001 | 126.15                          | 1  | < 0.001 |
| Nanoparticle element $\times$ sex  | 16.20                            | 6  | 0.013   | 3.46                            | 6  | 0.749   | 29.46                           | 6  | < 0.001 |
| Replication [nanoparticle element] | 95.52                            | 56 | < 0.001 | 83.11                           | 56 | 0.011   | 75.40                           | 57 | 0.052   |

#### Toxicity on pest bean beetle *Callosobruchus chinensis*

*Treatment on adults of C. chinensis.* When adults were treated with NPs, adult longevity was significantly affected by NP element and sex (Table 1); ZnONPs elongated longevity by 13.5% compared to the control ( $P = 0.008$ , Fig. 3). Significant main effect of sex showed that females lived longer than males (when they were not allowed to lay eggs) (Table 1, Fig. 2).

*Treatment on eggs of C. chinensis.* When eggs with density per bean controlled were treated, there was a significant effect of NP element on hatchability of eggs (likelihood ratio  $\chi^2_6 = 19.09$ ,  $P = 0.004$ ); SeNPs and TiO<sub>2</sub>NPs reduced the egg hatchability by 22.8% and 17.7%, respectively (posthoc comparison with the control, SeNPs,  $P < 0.001$ ; TiO<sub>2</sub>NPs,  $P = 0.008$ , Fig. 3). Larva-to-adult survival rate was not affected by NP element (likelihood ratio  $\chi^2_6 = 5.09$ ,  $P = 0.533$ , Fig. 3), while egg-to-adult survival rate was affected (likelihood ratio  $\chi^2_6 = 13.06$ ,  $P = 0.042$ ); SeNPs reduced egg-to-adult survival rate compared to the control ( $P = 0.021$ ). The number of emerging adults was not affected by NP element (likelihood ratio  $\chi^2_6 = 0.205$ ) but the posthoc comparison test indicated that it was reduced by SeNPs by 18.0% ( $P = 0.047$ , Fig. 3).

*Treatment on larvae of C. chinensis.* When larvae were treated with NPs, there was no difference among the NP elements and the control in larva-to-adult survival rate (likelihood ratio  $\chi^2_6 = 5.53$ ,  $P = 0.477$ , Fig. S3).

*Treatment on beans before oviposition by C. chinensis.* When azuki beans were treated *a priori* with each of the NPs, the number of laid eggs was not affected by the NP element (likelihood ratio  $\chi^2_6 = 2.22$ ,  $P = 0.898$ ). Egg hatchability was reduced by SiO<sub>2</sub>NPs by 10.6% (likelihood ratio  $\chi^2_6 = 19.97$ ,  $P = 0.003$ ; posthoc comparison with the control,  $P < 0.001$ , Fig. 4). Larva-to-adult survival rate was reduced by

AgNPs by 6.6%, CuONPs by 7.3%, and ZnONPs by 8.0% (likelihood ratio  $\chi^2_6 = 9.73$ ,  $P = 0.136$ ; posthoc comparison with the control, AgNPs,  $P = 0.026$ ; CuONPs,  $P = 0.027$ ; ZnONPs,  $P = 0.007$ , Fig. 4). Consequently, egg-to-adult survival rate was reduced by SiO<sub>2</sub>NPs and ZnONPs by 15.7% and 9.9% (likelihood ratio  $\chi^2_6 = 15.00$ ,  $P = 0.020$ ; posthoc comparison with the control, SeNPs,  $P < 0.001$ ; ZnONPs,  $P = 0.005$ ). The number of emerged adults was not affected by the NP element (likelihood ratio  $\chi^2_6 = 1.89$ ,  $P = 0.929$ , Fig. 4). Offspring female ratio was not affected by any of the NPs (likelihood ratio  $\chi^2_6 = 8.91$ ,  $P = 0.179$ ).

#### Toxicity on pest bean beetle *Callosobruchus maculatus*

*Treatment on adults of C. maculatus.* When adults were treated with NPs, adult longevity was significantly affected by the interaction effect between NP element and sex (Table 1); SeNPs shortened the longevity of female adults by 28.3% of the control, while lengthened the longevity of male adults by 9.1% (posthoc pairwise tests with the control; female,  $P < 0.001$ ; male,  $P = 0.016$ , Fig. 2). TiO<sub>2</sub>NPs shortened the longevity of male adults by 6.1% ( $P = 0.013$ , Fig. 2). AgNPs shortened the longevity of female adults by 11.6% ( $P = 0.036$ , Fig. 2) but not males'.

*Treatment on eggs of C. maculatus.* When eggs were treated with NPs, there was no significant effect of NP element on hatchability of eggs (likelihood ratio  $\chi^2_6 = 6.21$ ,  $P = 0.400$ ), larva-to-adult survival rate ( $\chi^2_6 = 9.56$ ,  $P = 0.144$ ), egg-to-adult survival rate ( $\chi^2_6 = 10.56$ ,  $P = 0.103$ ) and the number of emerged adults ( $\chi^2_6 = 9.15$ ,  $P = 0.165$ ) (Fig. 3). However, posthoc tests indicated that larva-to-adult survival rate and egg-to-adult survival rate were reduced by TiO<sub>2</sub>NPs by 10.8% and 15.0%, respectively, compared to the control (larva-to-adult survival,  $P = 0.011$ ; egg-to-adult survival,  $P = 0.034$ ; emerged adults,  $P = 0.021$ , Fig. 3).

*Treatment on larvae of C. maculatus.* When larvae were treated with NPs, there was no difference among the NP elements and the control in larva-adult survival rate (likelihood ratio  $\chi^2_6 = 2.64$ ,  $P = 0.852$ , Fig. S3).

*Treatment on beans before oviposition by C. maculatus.* When azuki beans were treated *a priori* with each of the NPs, the number of laid eggs was reduced by SeNPs by 32.9% (likelihood ratio  $\chi^2_6 = 16.79$ ,  $P = 0.010$ ; posthoc comparison with the control, SeNPs,  $P < 0.001$ , Fig. 4) and egg hatchability was reduced by CuO, Se, and SiO<sub>2</sub> NPs by 13.9, 11.7, and 11.2% of the control, respectively (likelihood ratio  $\chi^2_6 = 22.05$ ,  $P = 0.001$ ; posthoc comparison with the control, CuONPs,  $P < 0.001$ ; SeNP,  $P = 0.002$ ; SiO<sub>2</sub>NP,  $P = 0.005$ , Fig. 4). Egg-to-adult survival rate did not differ among NP elements (likelihood ratio  $\chi^2_6 = 9.7$ ,  $P = 0.140$ ) but was reduced by CuONPs by 14.6% of the control (posthoc comparison with the control,  $P = 0.024$ ). The number of emerged adults differed among NP elements (likelihood ratio  $\chi^2_6 = 12.76$ ,  $P = 0.047$ ) and was reduced by CuONPs by 24.6% and by SeNPs by 37.1% of the control (posthoc comparison with the control, CuONPs,  $P = 0.042$ ; SeNPs,  $P = 0.003$ , Fig. 4). Larva-to-adult survival rate (likelihood ratio  $\chi^2_6 = 5.26$ ,  $P = 0.511$ , Fig. 4) and offspring female ratio (likelihood ratio  $\chi^2_6 = 1.32$ ,  $P = 0.971$ ) were not affected by any of the NPs.

## Discussion

We hypothesized that nanoparticles biosynthesized by the fungal extract of *F. solani* decrease the fecundity and survival of the different developmental stages (and consequently the number of emerging adults) of *C. chinensis* and *C. maculatus*, irrespective of direct/indirect application of the nanoparticles, as well as the adult longevity of their parasitoid *A. calandreae*, and that these effects do not differ based on sex. Overall, susceptibility to the biosynthesized NPs were species-dependent and also differed among developmental stages and between sexes. The adult beetles and eggs were more susceptible than the last-instar larvae, which were protected by the seed coat, suggesting that direct contact with nanopesticides is critical for control. However, adults of *C. chinensis* were not affected by any of the NPs, which might be the consequence of endosymbiotic infection by *Wolbachia* that are most abundant at the adult stage (Kondo et al. 2011; Ijichi et al. 2002; Miksanek and Tuda, under review). The bacteria may be able to provide the host resistance against NPs as some symbiotic bacteria and fungi are known to detoxify/degrade insecticides (Shen and Dowd 1991; Kikuchi et al. 2012; Lauzon et al. 2003; Cheng et al. 2017; Liu and Guo 2019). When female adult longevity was compared, negative effects were found of SeNPs on *A. calandreae* and of SeNPs and AgNPs on *C. maculatus*. A sex-dependent effect of SeNPs was also detected in the adult longevity of *C. maculatus*. When beetle eggs were treated, SeNPs and TiO<sub>2</sub>NPs reduced either egg hatchability or larva-to-adult survival rate, leading to a reduction in the number of emerged (survived to adult) offspring by SeNPs in *C. chinensis* and by TiO<sub>2</sub>NPs in *C. maculatus*. When the bean surface was pretreated, the effects of the different NPs on the two beetle species varied, with pronounced toxicity of SeNPs and CuONPs on the fecundity and/or egg hatchability of *C. maculatus* and toxicity of SiO<sub>2</sub>NPs on egg hatchability of both beetle species. When beans containing beetle larvae were treated, no toxicity was detected. The biosynthesized NPs, particularly SeNPs, showed the potential to control major stored bean pests but also affected their biocontrol agent. The influence of dose dependency remains to be tested (e.g. Thabet et al. 2019).

This is one of the first demonstrations of the insecticidal effects of SeNPs (Arunthirumeni et al. 2021). The reduced egg-to-adult survival may be due to cumulative stress during long exposure, which appeared at a later developmental stage. Additionally, the biosynthesized SeNPs may have attractive properties because the solution had a characteristic odor and adult beetles were observed drinking the solution (M.T., personal observation). Since Se is known for its toxicity against insects (Mechora 2019), our results demonstrating sex-specific differences in resistance and elongation of male longevity in *C. maculatus* is a novel finding (c.f. Miksanek and Tuda, under review). The mode of action of Se is a chemical form and positive and negative effects are observed dose dependently (Wrobel et al. 2016). An optimal concentration range of Se for its positive effects is narrow (Wrobel et al. 2016). Se-based organic molecules can produce reactive oxygen species (ROS) and trigger apoptosis or autophagy of cancer cells (Zhang et al. 2021). The mechanism of the antimicrobial effect of SeNPs still remains largely unexplored (Huang et al. 2021). Spherical SeNPs coated with chitosan killed the nematode *Bursaphelenchus xylophilus* with ROS by effectively penetrating the pine tree to the nematode at an LC<sub>50</sub> of 15.6 mg L<sup>-1</sup> (Shang et al. 2022). Sodium selenite induced dose-dependent mortality and dose-dependent

accumulation of selenium in the Malpighian tubules of the mealworm beetle *Tenebrio molitor* but was not observed in the digestive and reproductive organs (Hogan and Razniak 1991).

The biosynthesized NPs of Se, TiO<sub>2</sub>, SiO<sub>2</sub>, CuO and Ag with a diameter less than 50 nm can be used as insecticides against *C. maculatus* (Se and Ag NPs on adults, TiO<sub>2</sub>NPs on eggs, and Se and CuO NPs for pretreating beans) and *C. chinensis* (Se and TiO<sub>2</sub> NPs on eggs), as the increased mortality of one or more developmental stages was observed at the tested concentration. However, the following effects must be taken into consideration in biological control practice: the positive effects of ZnONPs on the adult longevity *C. chinensis* and of SeNPs on the longevity of male *C. maculatus*, and the negative effects of SeNPs on the adult longevity of the parasitoid *A. calandrae*. These positive effects might be attributed to hormesis, i.e., the beneficial or stimulatory effects of low doses of stressors such as chemicals or radiation (Cutler et al. 2022). Current results and earlier investigations of SiO<sub>2</sub>NPs (Rouhani et al. 2013; Arumugam et al. 2016) and AgNPs (Rouhani et al. 2013) demonstrate that the application of NPs to beans, adults, or eggs (or 1st instar larvae) could increase mortality and reduce fecundity for *C. maculatus*. The egg mortality caused by SiO<sub>2</sub>NPs might be attributed to the dehydration of eggs. Possible mechanisms of the toxic action of Ag and CuO NPs are documented by Athanassiou et al. (2018), Benelli (2018) and Duhan et al. (2017). The activity of these NPs also depends on the morphology, structure, size, and other properties of the particles. NPs that are smaller in size and more spherical in shape tend to be more effective and have superior properties than larger ones, in terms of antimicrobial, cytotoxic, larvicidal, and antioxidant activity (Salem and Fouda 2021) and thus there is a greater number of potential applications (Shaheen and Fouda 2018).

The biosynthesized NPs demonstrated and compared in the current study can be used as an alternative to the more lethal insecticides in controlling both species of beetles as had been shown for other biosynthesis/application methods and target stored product insect pests (Supplementary Table S1). Although the environmental effects and phytotoxicity to plants of our NPs should be studied further, our findings suggest that SeNPs can reduce the fecundity and survival of female *C. maculatus* and egg hatchability of *C. chinensis*. Used at the correct concentrations and targeting developmental stages of the pest, we believe that the biosynthesized NPs can help maintain the densities of bean beetles below damaging levels and without significantly disrupting biological control.

## Declarations

### Acknowledgments

We thank O. Nishi (Institute of Biological Control, Kyushu University) for advice on fungal DNA analysis, Y. Fukunaga (Ultra-microscopy Research Center, Kyushu University) for technical support, J. R. Miksanek for insightful comments, and the Ultra-microscopy Research Center.

### Ethical Approval

Not applicable.

## Competing interests

No conflicts of interest or competing interests.

## Authors' contributions

EAMH and MT conceived the idea and design of the study; YZZ, PPS, EAMH and MT performed the experiment; EAMH and MT performed data analyses and wrote the original draft; MT and CA reviewed and edited the manuscript. All authors read and approved the final manuscript.

## Funding

This study was supported by KAKENHI (19K06840) from JSPS (Japan Society for the Promotion of Science) and Initiative for Realizing Diversity in the Research Environment from MEXT (Ministry of Education, Science and Technology) to MT, JSPS Invitational Fellowship (L22516) to CA, and JDS (Project for Human Resource Development Scholarship by Japanese Grant Aid) to PPS.

## Availability of data and materials

The datasets associated with the current study are available from the primary corresponding author (M.T.) on reasonable request.

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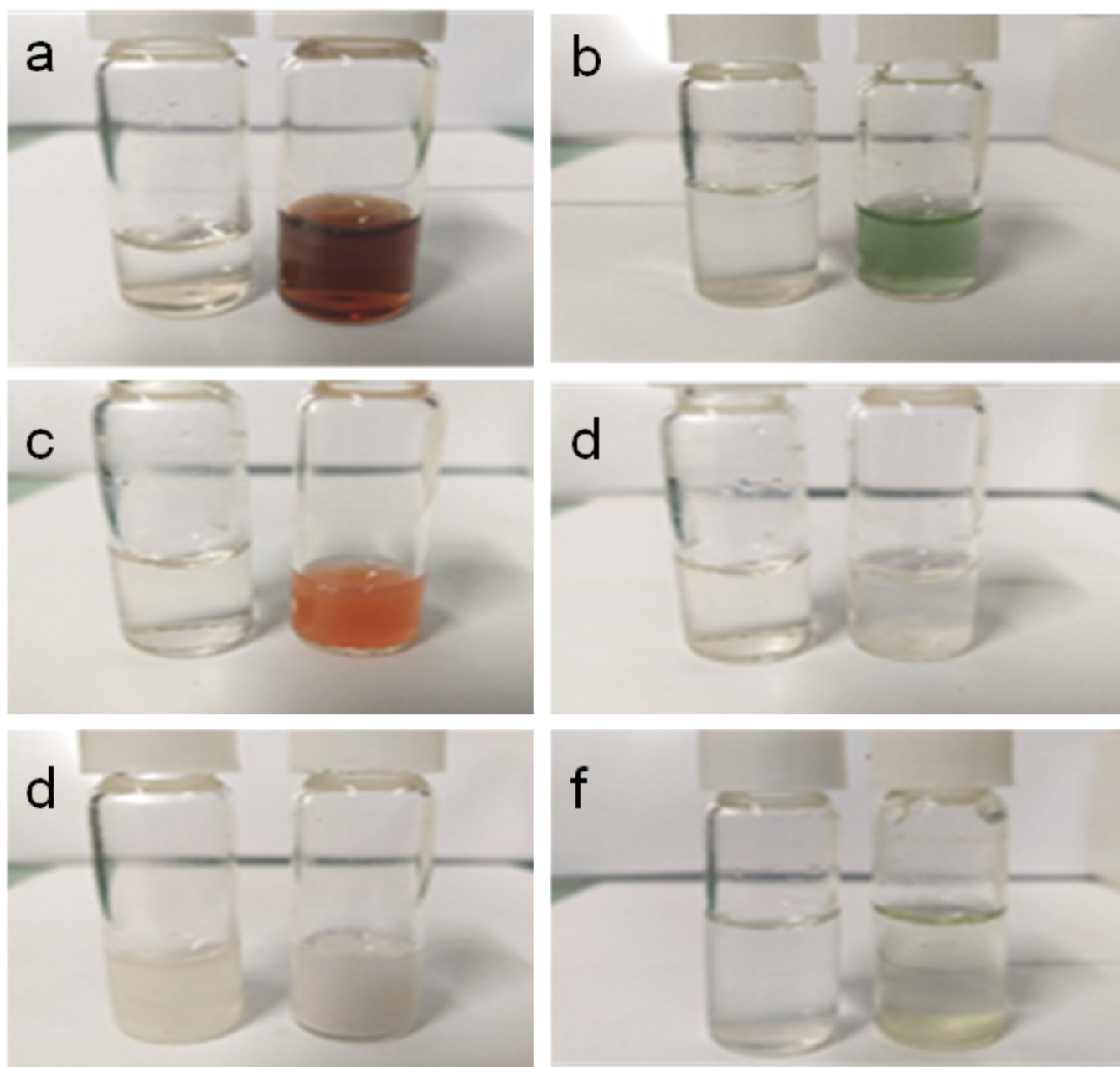
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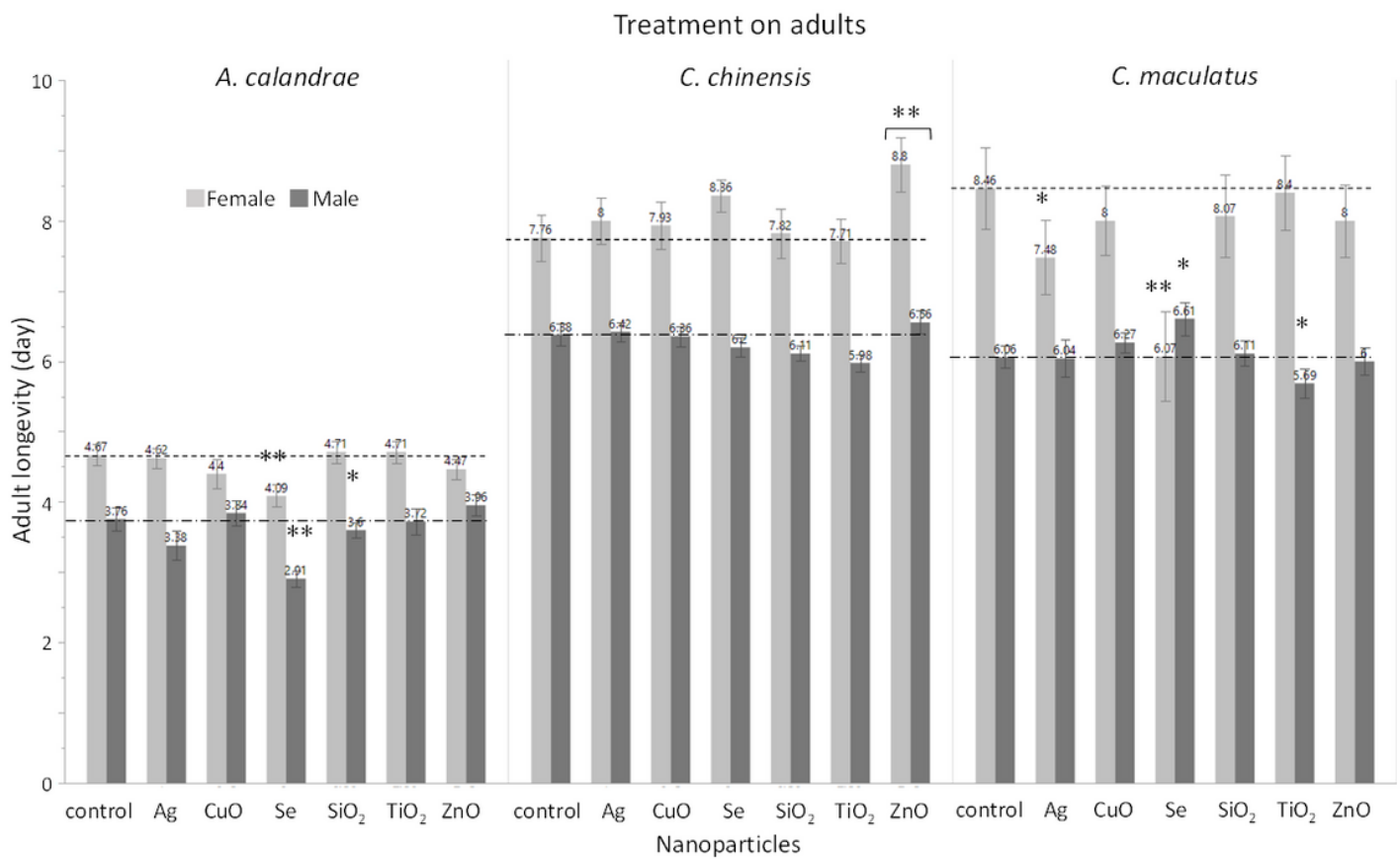
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## Figures



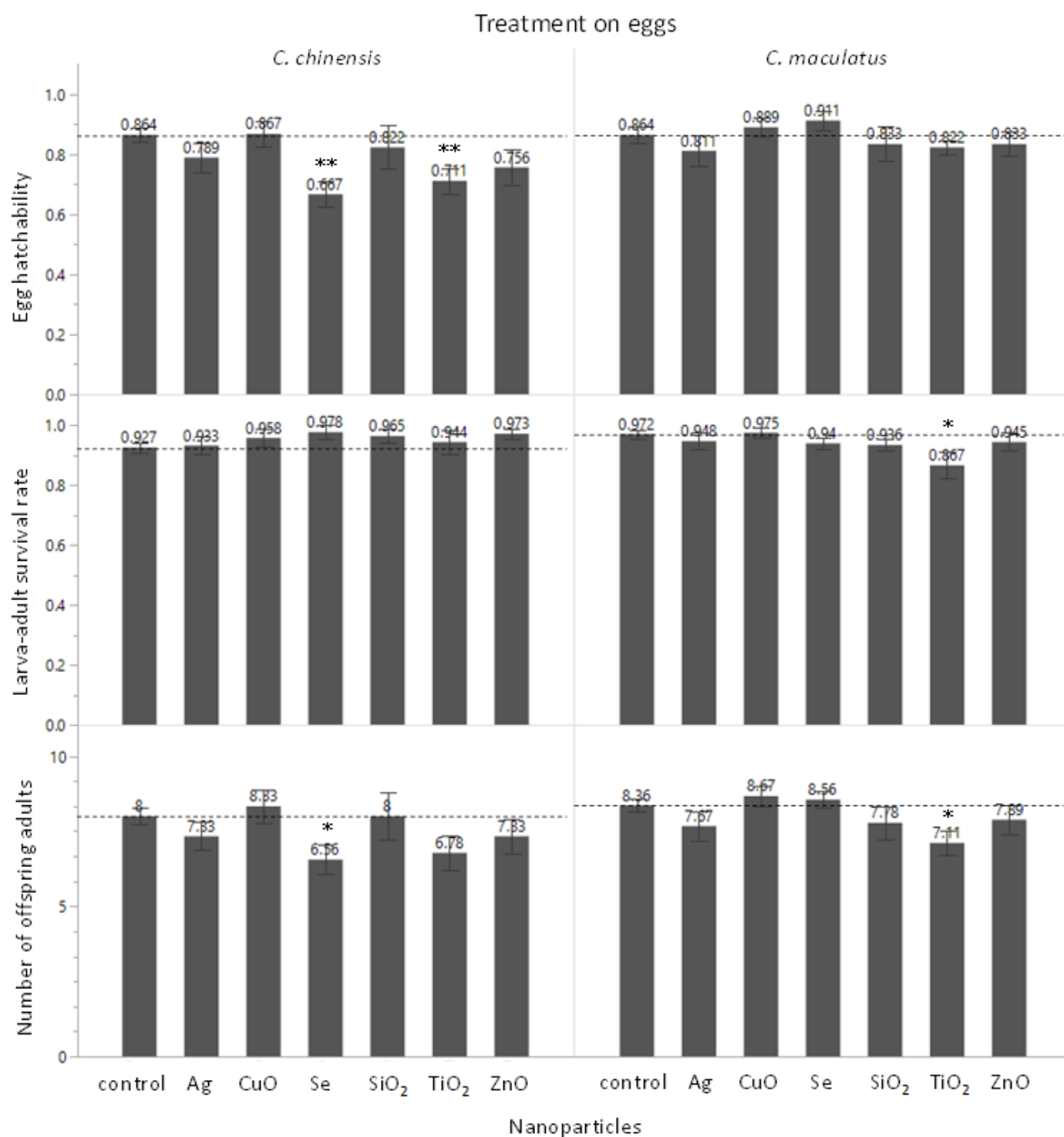
**Figure 1**

Synthesis using the extract from the fungus *Fusarium solani* of (a) Ag nanoparticles, (b) CuO nanoparticles, (c) Se nanoparticles, (d) SiO<sub>2</sub> nanoparticles, (e) TiO<sub>2</sub> nanoparticles and (f) ZnO nanoparticles. Left bottles: before the synthesis of nanoparticles. Right bottles: after the synthesis.



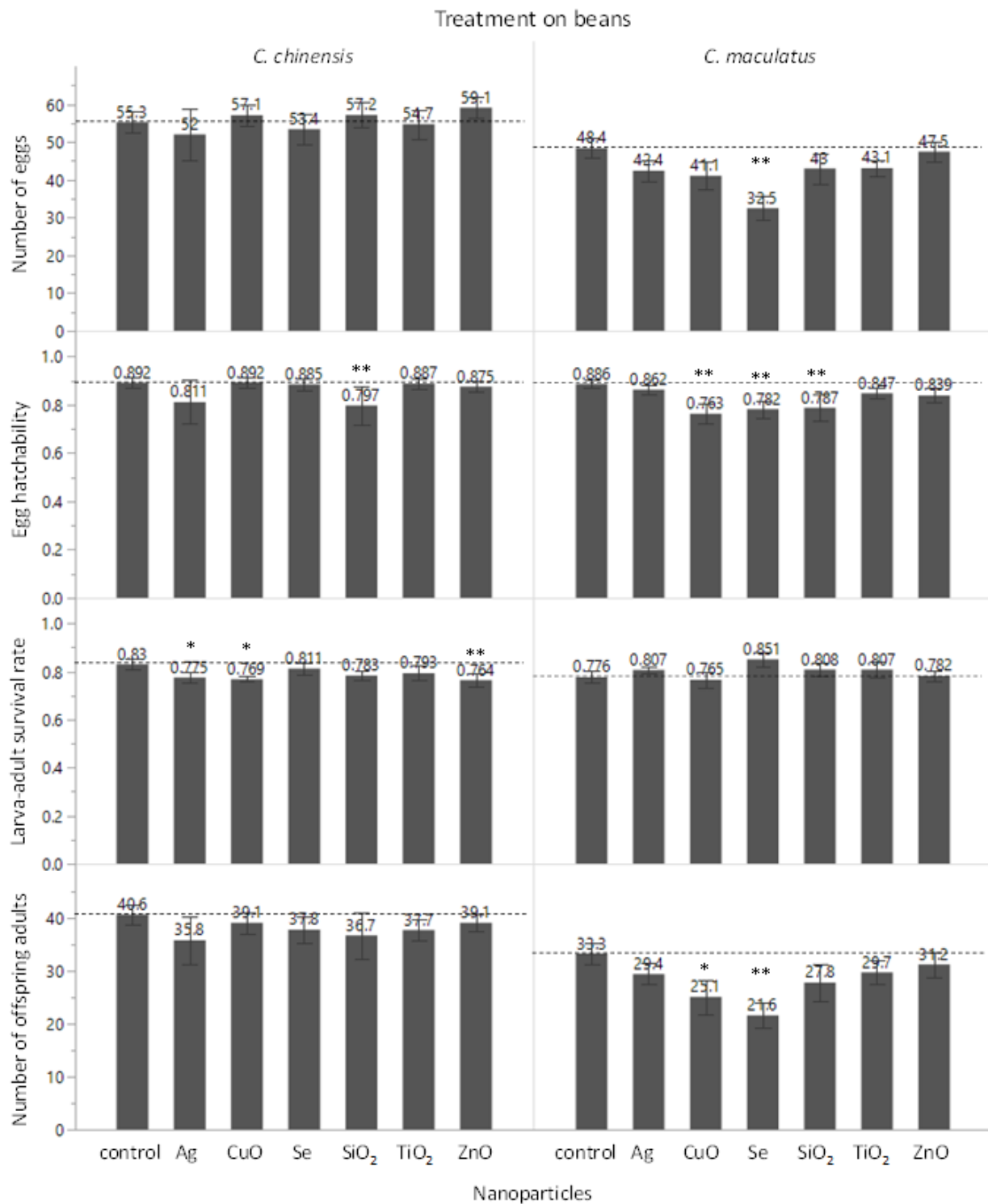
**Figure 2**

Adult longevity (mean  $\pm$  SE) (days) of the parasitoid *Anisopteromalus calandrar* and the *Callosobruchus* beetles when adults were treated with different biosynthesized nanoparticles. The numbers on the bar indicate the mean values. Horizontal broken lines: the control means in females and males. \*:  $P < 0.05$ , \*\*:  $P < 0.01$ .



**Figure 3**

Survival rates and emerged adults (mean  $\pm$  SE) of the *Callosobruchus* beetles when eggs were treated with different biosynthesized nanoparticles. The numbers on the bar indicate the mean values. Horizontal broken lines: the control means. \*:  $P < 0.05$ , \*\*:  $P < 0.01$ .



**Figure 4**

Deposited eggs, survival rates and emerged adults (mean  $\pm$  SE) of the *Callosobruchus* beetles when provided with beans treated with different biosynthesized nanoparticles. The numbers on the bar indicate the mean values. Horizontal broken lines: the control means. \*:  $P < 0.05$ , \*\*:  $P < 0.01$ .

## Supplementary Files

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- [HelmySupplementaryTablesandFigs.docx](#)