Carbon nanotubes as control agents against the Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae)

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1 Work presented at the 1st International Conference on Agricultural Sciences - Virtual (1st ICAS-V, 2020)

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ABSTRACT: This may be attributed to the facts that nanotechnology has shown an excellent ability to control the release pattern of active ingredients of pesticides, so that it can achieve long-term functions more effectively, thus overcoming the problems of agricultural runoff and residual pesticide accumulation. In addition, Nanopesticides have shown greater solubility and stability of active ingredients, which can effectively control pests (Akhtar et al., 2020). In this study, the insecticidal relative toxicity of carbon nanotubes (CNTs) synthesized from seeds of Iraqi date palm *Phoenix dactylifera* L. using a chemical vapor deposition (CVD) method was investigated. These Nanoparticles were evaluated against adult and larvae of the Khapra beetle, *Trogoderma granarium*. The results indicated that CNTs (25, 50 and 100 ppm) caused mortality of the Khapra beetle under laboratory conditions. Additionally, the germination percentage of wheat, *Triticum aestivum* L. grains, has not been affected by the carbon nanotube treatments at 25-100 ppm. This study demonstrates the potential of CNTs as a technology for population control of *T. granarium* because of their toxicity to larvae and adults.

Key words: insecticides; multiwalled carbon nanotubes; seed germination; survival

Nanotubos de carbono como agentes de controle contra o besouro Khapra, *Trogoderma granarium* Everts (Coleoptera: Dermestidae)

RESUMO: Isso pode ser atribuído ao fato de que a nanotecnologia tem demonstrado excelente capacidade de controlar o padrão de liberação de princípios ativos de agrotóxicos, de forma que possa atingir funções de longo prazo de forma mais eficaz, superando, assim, os problemas de escoamento agrícola e acúmulo de pesticidas residuais. Além disso, os nanopesticidas têm demonstrado maior solubilidade e estabilidade dos ingredientes ativos, que podem controlar efetivamente as pragas (Akhtar et al. 2020). Neste estudo, a toxicidade relativa inseticida de nanotubos de carbono (CNTs) sintetizados a partir de sementes de tamareira iraquiana *Phoenix dactylifera* L. usando um método de deposição química de vapor (CVD) foi investigada. Estas nanopartículas foram avaliadas contra adultos e larvas do besouro Khapra, *Trogoderma granarium*. Os resultados indicaram que os CNTs (25, 50 e 100 ppm) causaram a mortalidade do besouro Khapra em condições de laboratório. Além disso, a porcentagem de germinação dos grãos de trigo, *Triticum aestivum* L., não foi afetada pelo tratamento com nanotubos de carbono a 25-100 ppm. Este estudo demonstra o potencial dos CNTs como tecnologia de controle populacional de *T. granarium* devido à sua toxicidade para larvas e adultos.

Palavras-chave: inseticidas; nanotubos de carbono de paredes múltiplas; germinação de sementes; sobrevivência
Introduction

Nanotechnology is a term that involving a wide range of technologies dealing with structures and processes at the nanometer scale (Stadler et al., 2018). This principal is employed to enhance the efficacy or reduce the environmental contamination (Sabry & Ragaei, 2018; Al-Rudainy & Khalel, 2019). The application of synthetic nanomaterials has increased recently in different fields such as medicine, agriculture, and food conservation (Beyene et al., 2017; Vangijzegem et al., 2019). Currently, more than 800 commercially available products contain some types of nanoparticles (NPs). Scientific data have been shown the toxic potential against a wide number of arthropod pests (Sundararajan & Kumari, 2017; Athanassiou et al., 2018; Mao et al., 2018; Manimegalai et al., 2020). Although insecticides have contributed to reduce pest infestation, they can also have negative consequences for human health and the environment (Thompson et al., 2020). Furthermore, there has been a growing development of insecticide resistance in targeted insects to conventional synthetic insecticides (Nauen, 2007; Dang et al., 2017; Sudo et al., 2018; Shaffer, 2020; Struelens & Silvie, 2020). Therefore, there is a need to find plant protection methods that are safe for humans and environment. The use of nanopesticides can reduce the loss of organic solvents and the movement of unwanted pesticides by increasing the dispersion of these formulations (Bergeson, 2010). Recently, technologies such as nanocapsules, nanoemulsions, nanocontainers and nanocages have been reported to provide nanopesticides to protect plants from pests (Bouwmeester et al., 2009; Lyons & Scrinis, 2009).

Carbon nanotubes (CNTs) are pure carbon macromolecules consisted of sheets of carbon atoms covalently bonded as cylindrical shapes (Zhou et al., 2019). Carbon nanotubes are classified into two classes: single-walled (SWNTs) and multiwalled nanotubes (MWNTs) (Garcia-Gallastegui et al., 2012; De et al., 2020). The SWNTs are generally narrower than the MWNTs with diameters typically in the range of 1-2 nm, and tend to be curved rather than straight (Saifuddin et al., 2013; Cheng et al., 2020; Rudyak et al., 2021). MWNTs are composed of multiple SWNTs that are placed concentrically within each other (Shen et al., 2011; Pontoreau et al., 2020). There are three techniques that have been developed to produce CNTs: discharge, laser ablation, and chemical vapor deposition (CVD) (See & Harris, 2007; Purohit et al., 2014; Taylor et al., 2021). The basic elements for the formation of carbon nanotubes are: catalysts, a source of carbon, and enough energy (Moothi et al., 2012). Catalysts play a key role in the CVD synthesis of CNTs and therefore improving the desired characteristics of catalyst will improve the obtained CNTs quality as well as the process yield (Saifuddin et al., 2013; Chen et al., 2021; Saleh, 2021). Carbon nanostructures are commonly synthesized using transition metal nanoparticles as catalysts, such as Fe, Ni, Co, and Mo (Thess et al., 1996). Fossil-based hydrocarbon and plant-based hydrocarbon are the main carbon sources for the synthesis of CNTs (Moothi et al., 2012; Shah & Tali, 2016). The energy source may be electricity from an arc discharge, heat from a furnace (~900°C) for CVD, or the high-intensity light from a laser ablation (Guray, 2016). Different ecotoxicological researches showed that exposure of parasites and insects to (CNTs) result to oxidative stress, induced reactive oxygen species (ROS) production and reduction in levels of intracellular ATP (Guo et al., 2013; Fu et al., 2014; Benelli, 2018). Interestingly, Raduw & Mohammed (2020) found that there is an insecticidal efficacy of silicon oxide, aluminum oxide, and zinc oxide against T. granarium at 50, 100 and 200 mg kg⁻¹, and all these nanoparticles showed various effects against second instars. This effect was significantly higher on barley and wheat than on rice and maize. Khapra beetle insect is a pest of stored grains that damages the economy by infesting stored agricultural products (Mishra et al., 2012; Knorr et al., 2013). The growth of insect larvae does not occur at temperatures below 21 °C, but it can happen at very low humidity, such as at 25 °C and 2% RH (CABI, 2021). The life cycle of the Khapra beetle larvae under laboratory conditions of 25±5°C and 70±5% RH was more than 27 days (Musa & Dike, 2009). However, it grows faster in hot and humid conditions. For example, it takes about 18 days at 35°C and 73% RH (Ahmad et al., 2014). Some studies have stated that diapause process in T. granarium mature larvae provides resistance to several insecticides and allows a rapid adaptation to extreme temperatures, periods of drought and inadequate food (Tabunoki et al., 2016; Kavallieratos et al., 2019).

Their macroscopic shape and the nano-scale dimensions of CNTs might reduce their environmental toxicity (Sharghi et al., 2012). However, there are few studies on the use of carbon nanotubes (CNTs) for seed germination. Haghighi & Da Silva (2014) reported that CNTs at 10 - 40 mg L⁻¹ improved tomato germination, while at 40 mg L⁻¹ they had a deleterious and toxic effect on onion and radish seed germination.

In this study, we tested carbon nanotubes prepared from palm date seeds to control the Khapra beetle, T. granarium, and we checked the possibility of adverse effect of CNTs on seed germination of wheat, Triticum aestivum L. grains as safe indicative on their safe use in horticulture crops.

Materials and Methods

Ethical approval

All procedures performed in this study involving animals were in accordance with the ethical standards of the University of Kufa.

Insect culture

Trogoderma granarium insect culture was obtained from Entomology laboratory in the Faculty of Agriculture / University of Kufa. The culture was maintained on whole sterilized wheat (Triticum aestivum L.) grains. Insects were reared on wheat grains which was placed in 300-mL plastic jars (200 g per jar) secured with a muslin cloth and rubber bands, and maintained in an incubator (Binder Ltd., Suffolk,
UK) at 30 ± 2°C and 65 ± 3% RH in continuous darkness. The bioassay experiments on larvae and adults were carried out separately in different Petri dishes.

**Synthesis of carbon nanotubes**

In this research, CNTs was prepared by modified CVD on homemade ceramic boats which are used as a support without a catalyst. The purity of this method was around 85% and the range of CNTs diameter was 50-60 nm. The method of synthesizing CNTs relies on the study of Ordoñez-Casanova et al. (2013), which was based on the releasing of carbonaceous substances as a gas phase from date palm seeds that are non-volatile, which were considered as energy sources to generate carbonaceous materials. By using ceramic boats as a supporter, the first phase in this segment was processed and it was placed at the center of the tube furnace (SX2-2-17TP, XIN YOO), which is the optimum location for precipitation. In the combustion chamber, the prepared seed samples were positioned with a complete connection to the rest of the reactor. Nitrogen gas was purged to complete the elimination of the air from all the reaction chamber systems before switching the furnace on. The conditions of CNTs synthesis were: 750°C, reaction time of 30 min., and nitrogen flow rate of 100 cm³ min⁻¹. The N₂ gas flow was steadily reduced to a rate of 50 cm³ min⁻¹ when the furnace reached the desired temperature. By switching on the combustion heater and running in the form of batches, a waste date palm sample was then applied to the reaction. The furnace was turned off after deposition and allowed to cool down under a continuous N₂ flow to room temperature, then the product was collected for purification prior to characterization processes. Two steps were used in the purification processes of the produced CNTs: the first step was to heat the product in an oven at 150°C for 4 h, and the second step was to oxidize the remaining product with 30 % of H₂O₂ at 50°C for 4 days. Characterization of the synthesized CNTs has been done by using a Scanning electron microscopy (SEM) (Kahdum et al., 2016).

**Scanning electron microscope (SEM)**

100 ml of the stock solution (100 ppm) were dried at 50°C for 3 days in an oven (UF260, Memmert, Büchenbach, Germany). A sample, then, was sent to the University of Kashan, Kashan, Iran, for examination the composition and diameter of the tested CNTs under a scanning electron microscope (Tescan Mirax SEM, Tescan, Fuveau, France).

**Effect of tested carbon nanotubes on adults and larvae of T. granarium beetles**

**Drenching method**

Wheat grains were treated with the tested carbon nanotubes for protection against larvae (~1–2 weeks old), and adults (~1–2 days old) of *T. granarium* at concentration levels of 25, 50 and 100 ppm of CNTs. Nanoparticle powder (0.1 g) was firstly dissolved in 7.5 ml of 35% hydrochloric acid and then this finished to 1 L water. The mixture was placed on a hotplate with magnetic stirrer (Thermostat Hotplate HPL-500-050M, Gallenkamp, England) for 15 min at 60°C. Deionized water was added to a volume of 1 L and placed on the hotplate magnetic stirrer for an additional 30 min to prepare a stock solution of 100 ppm. Solutions at 25 and 50 ppm were prepared by diluting the stock solution with deionized water. Each concentration was applied in five replicates each consisting of 10 insects. The treatment of wheat grains was carried out by drenching wheat grains in carbon nanotubes solution at the tested concentration for 15 min and spread the grains on top of plastic sheets to dry for 2h under laboratory physical conditions. The negative control was carried using water with 0.7% HCL (of 35%), while the positive control was 100 ppm of Deltamethrin (2.5%). Then, 10 adults and larvae per replicate of *T. granarium* were transferred to treated wheat grains, which were put in a one small Petri dish and kept at 28 ± 2°C and 70 ± 5% RH according to the method described by Kestenholz et al. (2007). The number of dead adults and larvae in each treatment was counted each day for 10 days, and the percentage of insect mortality was recorded.

**Spray method**

In this method, 1 mL from each CNT concentrations (25, 50 and 100 ppm) was sprayed onto each replicate using a small hand-held sprayer, which was calibrated by spraying the solution into a volumetric scale. Each replicate comprised 10 insects (adults and larvae) in one small Petri dish. Insects were treated by direct spraying from a 30 cm distance with 1 mL-volume of the tested solution. The insects were left to dry for 2 h to prevent any contamination, then the Petri dishes were incubated at 28 ± 2°C and 70 ± 5% RH (Lu et al., 2017).

**Seed germination**

In order to check the possible side effect of CNTs, the wheat grains were treated with same concentrations used in the insect treatments (25, 50 and 100 ppm). The treated seeds were then dried to prevent infestation by reducing the moist content (Mobolade et al., 2019) Ten randomly selected grains from each treatment were placed on filter papers inside a Petri dish, 9 cm in diameter to assess their germination. Each treatment was replicated five times. The germinated seeds in each Petri dish were counted after 10 days after treatment, and expressed in percentages described by Mamun & Shahjahan (2011).

**Statistical analysis**

Insect mortality at 8-day evaluation, and percentage of seed germination at 10 days were submitted to one-way ANOVA followed by the Tukey test to compare differences in the effect of various concentrations of CNTs (Minitab v.19, State College, PA, USA).

**Results and Discussion**

To the best of our knowledge, this is the first study to report an advantage in using CNTs synthesized from date palm
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seeds to control *T. granarium* insects. The results indicated that the carbon nanotubes synthesized from Iraqi date palm seed is multi-walled (Figure 1).

**Scanning electron microscope (SEM)**

Scanning electron microscope image of CNTs synthesized from the thermal decomposition of waste seeds from Iraqi date palm *Phoenix dactylifera* L. is shown in Figure 1. From this image, the average length of the synthesized CNTS was 1.25μm in length. In addition, the average diameter of CNTs was 50-60 nm. According to this value, the studied CNTs can be assigned to multiwall carbon nanotubes MWNTs.

**Effect of tested carbon nanotubes on adults and larvae of *T. granarium* beetles – drenching method**

The results revealed that there is insecticidal activity against larvae and adults that feed on wheat grains by drenching CNTs (Figure 2). Current experiments that carried out to test the efficacy of CNTs against *T. granarium* adults and larvae revealed that feeding on drenching wheat grains at different concentrations (25, 50 and 100 ppm) of CNTs resulted in a significant impact on the survival of adults (*F* 4,20 =75.04, p=0.00) and larvae (*F* 4,20 =36.57, p=0.00). Cumulative mortality rates were 70%, 82%, and 100% for adults, and 46%, 58%, 88% for larvae after 8 days at 25, 50, and 100 ppm, respectively (Figure 2), compared to 0.0% mortality in the negative control and 96-100% mortality in the positive control.

The rate of resistance increases in greenhouses and storages, where insects reproduce quickly, and there is little or no immigration of susceptible individuals (Rafter et al., 2017). Therefore, the CNTs can be used efficiently for some generations of an insect as a novel approach to control pests (Alshukri, 2018). A possible explanation for CNTs actions may be attributed to: First, it leads to oxidative stress and induced reactive oxygen (ROS) in the target organism which can lead to the cytotoxicity and DNA damage (Fu et al., 2014). Second, CNTs may lead to reduction in the total lipid of the pest body. For example, Memarizadeh & Sharifi (2020) found a negative relationship between CNTs concentration and the lipid level in the body of *Glyphodes pyloalis* insects. However, in another study, no effect was detected of the CNTs on the mortality of Jamaican field cricket, *Gryllus assimilis*, but they had negative effects in the neurosecretory region of the brain (Zacouteguy et al., 2021).

**Effect of tested carbon nanotubes on adults and larvae of *T. granarium* beetles – spreading method**

The 4th-instar larvae and adults of *T. granarium* treated with different concentrations of CNTs exhibited significant dose-dependent decreases in survival compared to the control treatment (adults: *F* 4, 20 =117.41, p=0.00; larvae, *F* 4, 20 = 62.75, p=0.00). Adult mortalities were 58%, 70% and 72%, while larvae mortality were 32%, 50%, and 70% at 8 days post-spraying with 25, 50 and 100 ppm, respectively (Figure 3), compared to 0.0 and 100% mortality in the negative and positive control respectively. The current results revealed an adverse effect of CNTs against adults and larvae of *T. granarium* by spraying method. This might happen because nanoparticles can penetrate the exoskeleton of insects and

![Figure 1. SEM image for the synthesized CNTs.](image1)

![Figure 2. Survival curves of *T. granarium* fed with carbon nanotube using drenching method.-Note: lines that do not share a letter are significantly different (p < 0.05).](image2)
interact with thiol (−SH) groups of amino acid cysteine and produce the reactive oxygen species (ROS) which may cause damage to DNA, RNA, and proteins, and may cause cell death (Banerjee et al., 2010; Rai et al., 2014). One study has revealed that adults of *Drosophila* spp. exposure to dry CNTs has led to loss locomotion and caused mortality (Liu et al., 2009b).

Comparing the drenching application of the CNTs with the spraying one, the results showed that there is a significant difference between these two methods on the survival of adults, but not for larvae after 8 days ($F_{1,28} = 7.76$, $p=0.009$, for adults; $F_{1,28} = 0.81$, $p=0.377$ for larvae, Figure 4). The survival means of adults were 48.33% and 59.5% using drenching and spraying methods, respectively, while it was 61.8% and 66.6% for larvae respectively at different concentrations tested. It can be seen from the figures below that the spraying method caused more mortality than drenching one, and also we can see, in general, that the adults were more susceptible than larvae to various CNTs, and this suppose that the CNTs used in this study act better as contact toxicants than stomach poison. The findings of the current study are consistent with those of Bartlett (1964) who studied the toxicity of Some pesticides to different life stages of the green lacewing, *Chrysopa carnea*.

**Seed germination**

The results show that wheat grains treated with different concentrations (25, 50 and 100 ppm) of CNTs were not negatively affected compared to control treatment, where the percentage of seed germination in these concentrations reached 98, 95, and 96% respectively, compared to 98% in control after 10 days (Figure 5). One of the possible explanation of the CNTs mechanism on the seed germination is they could penetrate intact plant cell walls and transport different loads into plant cell organelles (Liu et al., 2009a). One of the Studies on toxicity of multi-walled carbon nanotubes on *Arabidopsis* (Lin et al., 2009) showed that large nanotubes (up to several tens of μm) could not easily penetrate the cell clusters, whereas smaller ones (up to a few hundred μm) had a greater interaction with wall proteins and polysaccharides which enabled easier penetration.

The findings of this study suggest that, targeting this insect by carbon nanotubes can be an effective approach to pest control, and may provide a novel pesticide for future control strategies.
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Compliance with Ethical Standards

Author contributions: Conceptualization: BMA; Data curation: MTA, BMA; Formal analysis: MTA; Investigation: BMA; Methodology: BJK; Resources: BJK; Software: MTA; Supervision: MTA, BMA; Validation: MTA, BMA; Writing – original draft: MTA, BMA; Writing – review & editing: MTA.

Conflict of interest: The authors declare there is not any possible conflict of interests (professional or financial) that might affect the manuscript.

Financing source: The authors declare there is no funding sources for the described research.

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Figure 5. Effect of CNTs on seed germination. Means with different letters are significantly different in the seed germination wheat (p < 0.05, one way ANOVA, post-hoc Tukey test.)

Conclusions

This study can be considered the first effort of using carbon nanotube as an insecticide against T. granarium. The results suggest that the carbon nanotube can be a new approach in pest management with a positive relationship with the concentrations tested. Furthermore, wheat seed germination was not affected by CNTs treatment.
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