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Article

Effectiveness of three Commercially Nanoparticles against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) under the Lab. conditions

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ABSTRACT

In recent years, Nanotechnology has become the most promising new insect control method. In this study, laboratory experiments were applied to assess the efficacy of three commercial, Aluminium oxide (ANPs), Zinc oxide (ZNPs) and Nanoparticles Silicon oxide (SNPs) on second fifth-instar larvae and adults of *Tribolium castaneum* (Herb.) as well as the effect of Nanoparticles Silicon oxide (SNPs) on the fecundity of *T. castaneum* adult females. Beetles were fed on a diet of flour treated with nanoparticles SNPs, ANPs, and ZNPs at different concentrations (50, 100 and 200 mg/kg) for each Nanocomposite. The results showed that SNPs and ANPs were significantly more effective than ZNPs at all tested concentrations after 15 days post-treatment. The results also confirmed that Nanoparticle SNPs have significant effects on the number of eggs lying, where the mean number of eggs produced by female adults exposed to dose-respond treated with Nanoparticle at the rate of 50 mg/kg was 0.0, compared with 98.1/ eggs in the control treatment. However, susceptibility showed significant differences in the mortality rate at the end of the experiments between second and fifth-instar larvae and adults treated by all three Nanocomposites. Second and fifth-instar larvae were most susceptible, while adults were least susceptible to nanoparticles. All Nanoparticles SNPs, ANPs, and ZNPs were significant and more effective on insect life at a higher concentration of 200 mg/kg, where the mortality proportion was 100%, 92.5% for 2nd, fifth instar larvae, respectively, and 77.5% for an adult after 15 days post-treatment. Moreover, the result showed that all nanoparticle SNPs, ANPs, and ZNPs can be applied as an alternative tool in the pest management programs of *T. castaneum*.

Keywords: *Tribolium castaneum*, Nanozinc, Nanoaluminium, nano-silica, storage pest

INTRODUCTION

Red flour beetle, Tribolium castaneum (Herbst), is the most important pest in wheat storage[1]. The stored grain pest control methods typically stand on broad-action pesticides and fumigants. Unfortunately, Debnath et al [2]·(2011) proved this leads to food contamination with toxic insecticide residues. Furthermore, the major problem in monitoring insects in the stored grain is resistance to insecticides. Regarding the resistance of grain insects and insecticide residues, biochemical control methods are not a suitable attitude for the management of the population of insects. More methods are used, such as Nanotechnology, entomopathogenic fungi, and parasitic wasps[3,4,5,6,7,8]. According to Korunik[9] (1997), Nanotechnology has become the most effective approach against insects in stored products if it has high silica content with uniform size distribution. In addition, Debnath et al[2]. (2011) proved that several physical features of materials change, for example, the size attitude of the Nanoscale. Nanoparticles demonstrate a new generation of environmental curing technologies that could supply the effective cost of the solution to the most defying environmental cleaning difficulties[10]. Nanoparticle methods help to produce new insect repellents, pesticides, and insecticides[11]. Moreover, researchers consider that Nanotechnology will cause the agricultural revolution with pest management in the future[12]. , there have many studies on the effects of Nanoparticle toxicity on animal pathogens, bacteria, and fungi[13,14,15,16]. Some research has been approved to investigate the effect of Nanoparticle toxicity on pests, particularly storage pest insects. Wan et al· [17]. (2005) studied the effect of the action of a mixture of two Nanoparticles with two chemical insecticides on Epitrimerus pyri (Nal.). According to the authors, cypermethrin and alpha Terhienyl mixed with zinc and copper oxide nanoparticles were effective on the tested mite. Yang et al.[18] (2009) stated that garlic essential oil overloaded with Nanoparticles is a successful treatment against the flour beetle T. castaneum. In addition, Stadler et al.[19] (2010) indicated that Nanoalumina could effectively control stored grain insects.

MATERIALS AND METHODS

Source of insects

Red-flour beetle T. castaneum was mass-reared on wheat flour medium, a mixture of 5% brewer’s yeast and wheat flour mixed 100% by weight in a glass jar covered with a muslin cloth without light and humidity control. The insect cultures were kept in a controlled environment at 28± 2°C and relative humidity of 70± 5%, increasing insect activity. Beetles have five instar larvae to determine the presence of insect stages under laboratory conditions to gain the required instar larvae for each test. A study was carried out using a camel hair brush to distinguish between larval stages. Beetles were placed into a jar for 3 days, after which an adult was transferred, and the jar was left for the hatch of eggs. Instar larval stages of 1st, 2nd, 3rd, 4th, and fifth were identified by observing molts when the insect larvae were laying into plastic Petri dishes (9 cm × 1.6 cm), which were covered with their lids and maintained at 28± 2°C without light. Fresh flour was provided to feed larvae inside the Petri dishes whenever required. From the first instar larvae to the adult stage, the total time was around 28 days. Adults tested were less than two weeks old, and second and fifth instar larvae with the adults were used for the experiments.

Bioassay

Feeding treatments

In three separate experiments, the effects of Nanoparticles zinc oxide (ZNPs), silicon oxide (SNPs), aluminum oxide (ANPs) and on adults and larvae of T. castaneum were treated by contact or feeding at three concentrations of 50, 100 and 200 mg Nanoparticle kg flour. The experiment was statistically analyzed using a Completely Randomized Design (CRD), with 5 replications, each consisting of 10 larvae or adults of T. castaneum in small plastic jars containing 20 g of flour. The flour in each jar was treated separately with Nanoparticles. Then, manually the jar was shaken for nearly 10 min to achieve equal distribution of Nanoparticles in flour[6]. No Nanoparticle was mixed with flour in one additional set, and this set served as a control treatment. Flour treated with Nanocomposites was distributed on Petri dishes. Ten adults of T. castaneum were introduced into each Petri dish after 24 h. Bioassay was achieved at 28±2oC and 70±5% RH. Mortality of insects was checked 1, 3, 5, 7,9,11 and 15 days after treatment for adults and larvae stages.

2.3. Statistical Analysis

The data on mortality were analyzed with GenStat (Ver. 16) software. Using [20], Insect mortality was corrected. Data were transferred using arcsine square root change when needed to meet the normality assumption. The effect of the life stage of T. castaneum on the efficacy of either nanoparticle SNPs, ANPs, or ZNPs was analyzed using two-factor repeated measurement analysis ANOVA. The effect of three concentrations of each Nanoparticle on the mortality of the insect was analyzed individually using two-factor recurrent measurement analysis ANOVA. Mean comparison was achieved using the LSD test at a 1% level of significance ($P < .01$).

RESULTS

Experiment 1: Effect of Nanoparticles Silicon Oxide (SNPs) on the mortality of T. castaneum adults and different instar larvae

In the first experiment with nanoparticle SNPs, significant differences ($P < 0.01$) in T. castaneum mortality were noted with different concentrations for each of the three insect stages 15 days post-treatment. The highest mortality level was noted with the highest Nanoparticle concentration of 200 mg/kg for three insect stages (see tab. 1). All larval and adults were susceptible to silicon oxide at higher concentrations. However, susceptibility decreased with the beetle's age, indicating that older larvae and adults were more tolerant to the Nanoparticles. 2nd and fifth instar larval mortality rates at 200 mg/kg concentrations were 100% and 92.5%, respectively, compared with 77.5% for adults. Additionally, the control mortality of T. castaneum larval stages and adults did not exceed 5.0%. The effect of the period after Nanoparticle SNP application on the mortality level was significant ($P < 0.01$). All treatments achieved the highest kill rates 15 days post-treatment (Table. 1).

Insect stages	**Con.**	**Corrected mortality / Days**							
		1	**3**	**5**	**7**	**9**	**11**	**13**	**15**
2nd instar larvae	50 mg/kg	20.0	40.0	70.0	72.0	77.5	82.5	87.5	92.5
	100 mg/kg	27.5	52.5	75.0	80.0	92.5	100	100	100
	200 mg/kg	32.5	50.0	80.0	100	100	100	100	100
5th instar larvae	50 mg/kg	25.0	37.5	50.0	57.5	72.5	72.5	80.0	82.5
	100 mg/kg	22.5	45.0	52.5	60.0	67.5	77.5	82.5	87.5
	200 mg/kg	25.0	47.5	57.0	62.0	70.0	80.0	87.0	92.5
Adults	50 mg/kg	12.5	15.0	25.0	35.0	42.5	55.0	65.0	70.0
	100 mg/kg	15.0	20.0	25.0	37.5	45.0	55.0	70.0	72.5
	200 mg/kg	20.0	32.5	35.0	40.0	55.0	57.5	72.5	77.5
L.S.D of treatment $_{(0.05)}$ = 2.6; L.S.D$_{(0.05)}$ for time = 4.9; L.S.D$_{(0.05)}$ for concentration = 2.8									

Table 1. The effect of nanoparticle SNPs on the corrected mortality of larval stage and adults of T. castaneum after 1, 3, 5, 7, 9, 11, 13 and 15 days of application.

Experiment 2: The effect of Nanoparticles Aluminium Oxide (ANPs) on the mortality of T. castaneum adults and different instar larvae

The result of nanoparticle ANP tests showed that high mortality levels were recorded for second and fifth-instar larvae after 15 days of treatment (see Tab. 2) compared with the adult stages at the highest Nanoparticle concentration. The highest concentration of 200 mg/kg was more effective at the end of the experiment, and significant differences ($P < 0.01$) were noted with concentrations for each stage. The adults were less susceptible than larvae, with percentage mortality rates at all Nanoparticle concentrations tested. Adults mortality rates were 62.5%, 87.0%, and 82.0% for second- fifth instar larvae. First-instar mortality rates were 86%, 84%, 82% and 80% for third-instar larvae, respectively. These mortality rates mean that the old larvae and adults were more tolerant to Nanoparticles than early larvae. For concentrations, there was a significant difference ($P < 0.01$) in the mortality rates of the insect stage. The effect of the period after nanoparticle ANP application on the mortality level was significant ($P < 0.01$). All treatments achieved the highest kill rates 15 days post-treatment (Table. 2).

Insect stages	**Con.**	**Corrected mortality / Days**							
		1	**3**	**5**	**7**	**9**	**11**	**13**	**15**
2nd instar larvae	50 mg/kg	10.0	25.0	25.0	42.5	57.5	67.5	80.0	92.0
	100 mg/kg	15.0	35.0	45.0	65.0	75.0	80.0	82.5	85.0
	200 mg/kg	20.0	37.5	55.0	65.0	75.0	82.5	87.5	87.0
5th instar larvae	50 mg/kg	7.5	22.5	25.0	47.5	52.5	52.5	57.5	67.5
	100 mg/kg	10.0	25.0	32.5	57.5	60.0	70.0	75.0	77.5
	200 mg/kg	15.0	27.5	42.5	52.5	62.5	72.5	80.0	82.0
Adults	50 mg/kg	0	2.5	15.0	25.0	35.0	37.5	45.0	47.5
	100 mg/kg	2.5	12.5	12.5	25.0	32.5	45.0	50.0	57.5
	200 mg/kg	5.0	10.0	25.0	42.5	50.0	57.5	60.0	62.5
L.S.D of treatment $_{(0.05)}$ = 2.8; L.S.D$_{(0.05)}$ for time = 6.3; L.S.D$_{(0.05)}$ for concentration = 4.5									

3.3.

Table 2. The effect of nanoparticle ANPs on the corrected mortality of larval stage and adults of T. castaneum after 1, 3, 5, 7, 9, 11, 13 and 15 days of application.

Experiment 3: Effect of Nanoparticles Zinc Oxide (ZNPs) on the mortality of T. castaneum adults and different instar larvae

The highest Nanoparticle concentration of 200 mg/kg had the greatest effect at the end of the experiment, and significant effects ($P < 0.001$) of concentration were found for each stage. For each stage, there was an important difference ($P < 0.001$) among the efficacies of the different ZNP concentrations. However, the adults were less susceptible than the larvae. Percentage mortality rates at the concentration of 200 mg/kg for an adult were 60.0%, 80%, and 87.5% for fifth and second instar larvae, 15 days after post-treatment (see Tab. 3). The mortality rates above mean that the early larvae stages were less tolerant to Nanoparticles concentration than the adults. The effect of the period after Nanoparticles ZNPs application on the mortality level was significant ($P < 0.01$). All treatments achieved the highest kill rates 15 days post-treatment (Table. 3).

Insect stages	**Con.**	**Corrected mortality / Days**							
		1	**3**	**5**	**7**	**9**	**11**	**13**	**15**
2nd instar larvae	50 mg/kg	10.0	22.5	32.5	45.0	57.5	67.5	77.5	80.0
	100 mg/kg	12.5	20.0	32.5	47.5	60.0	70.0	80.0	82.5
	200 mg/kg	20.0	37.5	50.0	62.5	72.5	80.0	82.5	87.5
5th instar larvae	50 mg/kg	7.5	12.5	20.0	22.5	27.5	37.5	52.5	57.5
	100 mg/kg	7.5	17.5	25.0	35.0	45.0	55.0	67.5	77.5
	200 mg/kg	15.0	20.0	27.5	45.0	55.0	65.0	77.5	80.0
Adults	50 mg/kg	5.0	7.5	12.5	15.0	20.0	25.0	32.5	45.0
	100 mg/kg	7.5	10.0	15.0	20.0	25.0	27.5	35.0	47.5
	200 mg/kg	10.0	15.0	25.0	27.5	32.5	42.5	57.5	60.0
L.S.D of treatment $_{(0.05)}$ = 2.6; L.S.D$_{(0.05)}$ for time = 5.7; L.S.D$_{(0.05)}$ for concentration = 4.1									

Table 3. The effect of Nanoparticles ZNPs on the corrected mortality of larval stage and adults of T. castaneum after 1, 3, 5, 7, 9, 11, 13 and 15 days of application.

Controlling stored product insects stands usually on the use of chemicals pesticides, and long-term application of the synthetic compounds grows insecticide resistance. [21,22,23] reported that Nanocomposite has conventional attention to managing pathogens in agriculture. Recently, the application of Nanotechnology in agriculture is still at an elementary stage. [24] presented that nanoparticle ANPs in groundwater stop the growth of cabbage, carrot, cucumber, soybean and corn.

3.4. Effect of Nanoparticles silicon oxide (SNPs) on the fecundity of T. castaneum

The fecundity of T. castaneum was affected by the 50 mg/kg SNP treatment. Adult females in the control treatment produced 98.1/eggs, higher than those of the nanoparticle SNPs treatment at 0.0/eggs. There was a significant difference between the control and SNP nanoparticle treatments in the hatched eggs percentage (Table 3).

Treatment	No. of eggs per female (Fecundity)	Hatched eggs (%)
SNPs Nanoparticles	0.0	0.0
Control	98.1± 1.5	89.30± 4.9
L.S.D$_{(0.05)}$	2.45	3.5

Table 3. Effect of SNPs Nanoparticles on the mean numbers of eggs produced per T. castaneum adult, the proportion of hatched eggs compared to the treatment of control.

DISCUSSION

[19]. successfully used the Nanoparticles of aluminum oxide (ANPs) against two stored grain insects. Nevertheless, these experiments showed that nanoparticle SNPs, ANPs, and ZNPs could be used to facilitate integrated pest management against stored grain insects such as T. castaneum. A comparison of these results with earlier research [2] shows that the application of Nanoparticles SNP could significantly increase the mortality effect of NPs by increasing the time after application. They expose that SNPs have a high likelihood as an insecticide. The potential reason could be the age-old practice of using silica dust as a protective agent for stored products worldwide.

Based on our results, the mortality effect of nanoparticle SNPs, ANPs, and ZNPs was less active in insect stages. That mortality could be attributed to the impairment of the digestive tract[26]. Furthermore, it states their particularly increased exposure surfaces that could interact with insects' cuticles. [2,27] provides that SNPs and ANPs Nano pesticides act by obligated the insect's cuticle followed by physical sorption of lipids and waxes, leading to dehydration of insect. According to the experimental findings, it is obvious from the results that SNPs could be applied as a good agent for managing this insect. This should be done to determine whether nanoparticle SNPs, ANPs, and ZNPs can act as a potential control method and bring stored grain insect groups under control following EIL. Several researchers have investigated the effect of nanoparticles, particularly against pathogens[2,14,15]. The results are consistent with many researchers' reports[2,19,28]. [2], prove that the mortality of S. oryzae was significantly affected by Sio2 nanoparticles. The finding of [19] was consistent with this study. They confirmed the insecticidal effect of Nanalumina on Rhyzopertha dominica (Fabricius) and S. oryzae. In addition, [28] stated that nanoparticles ZnO-TiO2-Ag have chemical action on the Frankliniella occidentalis (Perg.) and also showed that the most mortality effect related to 2% Ag- and 28% ZnO-70% TiO2 (LC50=195.27 mg/L). For the application of nanoparticles SiO2 and the use of compounds as a novel insecticide to proceed, additional investigation is needed on the safety of use and human health[28].

The decrease in the average egg production of T. castaneum could be recognized as histological and cytological injuries to the ovaries[29]. Insects infected by SNP nanoparticles might affect calling and mating at the beginning of the Nano silicon damage the testes of exposed males. This might explain the lack of eggs dropped by the beetles. No research is shown on the sub-lethal consequence of Nano silicon on T. castaneum insect females. Nevertheless, significant effects of Nano silicon on the fecundity of many insects have been informed in this study. SNPs Nanoparticles reduced the whole number of eggs laid per female fecundity of T. castaneum by about 0.0% compared to untreated females 98.1 %[28].

CONCLUSIONS

The study concludes the efficacy of three commercially available Nanoparticles, aluminum oxide (ANPs), silicon oxide (SNPs) and zinc oxide (ZNPs), against different life stages of T. castaneum.

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