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ORIGINAL ARTICLE

Toxicity of Nano and non-nano Boron Particles on *Apis mellifera* (honey bee)

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ABSTRACT
Toxicity of boron particles was investigated on Apis mellifera invertebrate organisms exposed during 96 hours to nano and non-nano boron particles at concentrations 0.001, 0.01, 0.1 and 1 mg/L. Under these treatment, 48 and 96 hours to LC50 values of nano and non-nano boron particles, respectively 229.099 mg/L, 0.339 mg/L and 62.330 mg/L, 4.694. According to these results, Nano boron is highly toxic for 96 hours. Generally, toxic effects of boron particles increased longer exposure time.
Key words: *boron particles, Apis mellifera, nanoparticles, nanotoxicology*

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INTRODUCTION

The nanoparticles in the atmosphere emanate from either constant or dynamic sources. Some of these nanoparticles, when some are refrigerated from the direct combustion sources and some others are cooled up to the ambient temperature of hot oversaturated vapours, become formed through the process of nucleation and condensation. Separately, chemical reactions in the atmosphere may lead to chemical forms with rather a low saturation pressure by generating nucleation particles (1, 2, 3, 4). Therefore, any environmental or meteorological factors playing a role in these processes, such as temperature, relative humidity, atmospheric turbulence, etc., may affect the concentration of nanoparticles in the atmosphere (4). At the same time, the physicochemical characteristics of these nanoparticles from different sources may also be different. These nanoparticles interacting with organisms may affect those organisms more and more, since their toxicity was found to be dependent mainly on the size and surface area (5, 4). For this reason, the ecological effects of the nanoparticles generated through the rapid development of nanotechnology along with the risks they pose on potential health have caused great concern (5, 6).

In recent years, nanotoxicology has been introduced as a new term in the scientific world. Nanotoxicology is expressed as a new branch of toxicology in which health-threatening factors caused by nanoparticles are described (7) The energy released as the result of the metal oxidation of some forms of nanoparticles, for instance boron nanoparticles, has been used as a potential fuel source and also in medical researches (8), which is regarded as a solid propellant for rockets and as a gun propellant due to its rapid energy release and the desired combustion heat (9, 10; 11). There are rather limited number of studies on boron nanoparticles in the literature, and of those conducted so far is the study by (12) in which the acute toxicity of titanium dioxide, aluminum and boron nanoparticles in *Daphnia manga* was evaluated. In this study, EC_{50} values of boron nanoparticles were found to be in the range of 56-66 mgL^{-1} and were classified as harmful for aquatic organisms. Another study by Petersen et al. 2008 was a boron-neutron capture therapy during which the boron nanoparticles were injected into the tumorous region referred to as B16-OVA. With this injection, it was reported that the tumour progression was delayed when an irradiation by means of thermal neutrons was performed a day later, and that the mice treated with boron had survived longer than 10 days when compared with those untreated with boron. Apart from an increasingly great number of studies conducted on the toxicity of nanoparticles in recent years, there have been some other toxicological studies, while once focused on the aquatic environments, are now being initiated and conducted on terrestrial environment and organisms.

(13) studied the acute toxic effects of Ag-TiO_2, ZnO-TiO_2 and TiO_2 nanoparticles in *Apis mellifera.* Also in this study were honey bees used. The reason for this is that honey bees are one of the most important groups among pollinator species around the world in terms of being both economic and superior to other ones. In vegetative production, 35% of the world is dependent on these pollinators (14).
This article presents results as to the ecotoxicity of nano boron and non-nano boron particles. The primary objective of this study is to present comparatively the acute toxicity of nano boron and non-nano boron particles on *Apis mellifera* (honey bee). The risk evaluation of the pesticide toxicity for honey bees is predicted commonly by the laboratory studies and at times when the risk coefficient indicates the value as being above 50 (application rating/LD50), or through both semi-field and field trials, or when the behaviours have a specific mode or even when there are signs of indirect effects like delayed behaviours (19). The behavioural effects of the pesticides on honey bees may cause major effects on the development of the colonies (15, 16, 17,18). The effects in question demonstrated by honey bees against the pesticides are also assumed to be likely to be seen in nanoparticles in the same way.

MATERIALS AND METHODS

Bee Material

A month before the start of the experiment, the queen bee was caged on an embossed honey comb and was made to spawn, and then the spawning ground of the queen bee was recorded. No chemical pest control against diseases was performed within the colony used in the acute toxicity study. During the closed brood period, the honeycombs were taken to the grates and the young worker bees of the same age and the same race that emerged from the comb cells were randomly distributed into the cages. The worker bees taken into the cages were left hungry for 2 hours prior to their treatment with the test groups. Before the start of the test, the worker bees that were about to die/perish were replaced by the healthy ones.

Test chemicals

Boron 95, elemental amorphous boron powder (with 95-97% boron content), nano boron powder, elemental amorphous nano boron powder (with> 98.5% boron content) were used in toxicology experiments. Nano boron and non-nano was obtained in pavezyum chemical industry.

The Preparation of the Test Test Solution

In order to prepare the stock solutions at desired concentrations, the test substances, nano boron and non nano-boron were prepared in a deionized water through the medium of dispersion. Afterwards, this solution was vortexed for 20 seconds, and while the stock solutions of the nanoparticles were being prepared, the ultrasonic water bath (Bandelin, sonorex) was used and was made sonicated for 30 minutes in order to increase the dispersion in water and ensure the maximum distribution of nanoparticles in the water. The test concentrations determined in the wake of all of these stages were prepared from the stock solution via dilution. From the test solutions prepared, a sucrose solution at one-to-one proportion was also prepared for the purpose of feeding the bees throughout the testing period. This solution was prepared by adding 1 gram of sugar into 1 ml of solution. The sucrose solution was prepared as fresh to feed the bees each day.

The Studies and the experimental setup of Acute Toxicity

In order to shelter the bees for 96 hours in vitro, 20x5 cm long and 8 cm wide self-covered plastic containers were utilized. Randomly selected 50 bees were put into each container. On one side of these containers, tiny holes were made to let the air in for the bees during the experiment, after which the sides of the cover were rubbed with emery in order not to let the cover open during the experiment. 2x1 cm hole was opened from the lower side of the plastic container to collect the bees that perished during the experiment, thanks to which the bees were easily collected. To feed the bees, on the other hand, 1 ml volume of droppers (pasteur pipettes) were used. The droppers were fixedly and vertically placed on the upper part of the containers, by means of which the bees were made to be fed easily from the droppers. The sucrose solution was put into the droppers with the help of a syringe. In this way, the groups exposed to the test were made to be fed with a test solution every four hours. On the other hand, the control group members were fed with the sucrose solution prepared only with deionized water. The test room was conducted at 25±2 ˚C temperature and in the dark. The relative humidity which was normally 50-70% was recorded throughout the test. No behavioural disorder or mortality was observed in the control groups all through the test. The perished bees in the test groups were counted at the 24^{th}, 48^{th}, 72^{nd} and 96^{th} hours. This study was carried out in 3 repetitions, independent of each other.

Statistical Analyses

All the experiments were repeated three times independently, and the data were recorded on average by means of standard deviation. The LC_{50} value was calculated through the probit statistical analysis of EPA. The other analyses were performed through ANOVA and TUKEY multiple comparison analysis.

DISCUSSION AND CONCLUSION

(12) destroyed all the *Daphnia* during the 24-hour-exposure at the concentration, the boron nanoparticles of which were over 80 mgL^{-1}. LD50 value is 19.5 mgL^{-1} for 24 hours and 6.7 mgL^{-1} for 48 hours. The daphnia exposed to 2.5 mgL^{-1} boron nanoparticles after 24 hours were observed to have shown a swimming process similar to that in the daphnia within the control group, whereas the animals exposed to 8 mgL^{-1} concentration were seen to have been less active and to have had a little amount of these in their digestive systems. On the other hand, their swimming activities at 25 mgL^{-1} concentration was rather slow and the particles in their digestive system are relatively in greater amounts.

There are very few studies on boron nanoparticles in the literature, whereas some of the studies conducted on this matter so far are those performed by (12), in which the acute toxicity of titanium dioxide, aluminum and boron nanoparticles in *Daphnia manga* was evaluated. In this study, LD50 values for 24 and 48 hours were calculated. The titanium dioxide nanoparticle had shown a low level of toxicity, and LD50 value could not be calculated. On the contrary, EC50 value of the boron nanoparticles was found to be in the range of 56-66 mgL^{-1}, which can be classified as harmful for aquatic organisms. (8) evaluated the boron nanoparticles in B16-OVA tumour cells for the boron-neutron capture therapy. When an irradiation towards the tumorous region was performed by means of thermal neutrons a day after an injection was made into that region, the tumour development was delayed, and the mice treated with boron were seen to have survived 10 days longer than those untreated with boron. it was reported that the boron nanoparticles caused a delay in *in vivo* development of the significantly aggressive B16-OVA tumour.

Acute toxicity/ LC_{50} study

In this study where the lethal concentration against the nano boron and non-nano boron, on *Apis mellifera* is determined, the 48 and 96-hour- LC_{50} value was calculated through the probit analysis by taking the test results as the basis of the study. These values were shown in the following table along with the regression graphic in it. Throughout the test, no mortality or behavioral abnormalities were observed within the control group. Whether there was any difference depending on the concentration and the time in the mortality rate for these three substances performed at the same concentrations to be able to give the comparison was determined according to the statistical analyses.

Tablo 1:96 and 48 hour LC/EC values calculated for particles non-nano boron

Points	Exposure time	
	48 hour	96 hour
LC/EC 1.00	0.000	0.000
LC/EC 5.00	0.003	0.000
LC/EC 10.00	0.027	0.000
LC/EC 15.00	0.117	0.001
LC/EC 50.00	**62.330**	**4.694**
LC/EC 85.00	33079.063	17893.178
LC/EC 90.00	145956.094	125876.203
LC/EC 95.00	1316416.625	2266089.500
LC/EC 99.00	81451600.000	%512583.000

After exposure 48 h and 96 h to non-nano boron LC_1 values couldn't be calculated. LC_{50} values for 48 h and 96 h were calculated 62.330 and 4.694 respectively. These values demonstrate that toxic effect of Pd/PVF^{+} nanoparticle depends on exposure duration. Because, an excessive reduction (approximately 13.2-fold) was found in the concentration (required concentration to kill at least 50% of the testing organism) required to show the same toxic effect when exposure duration prolonged from 48 h to 96 h (Table 1).

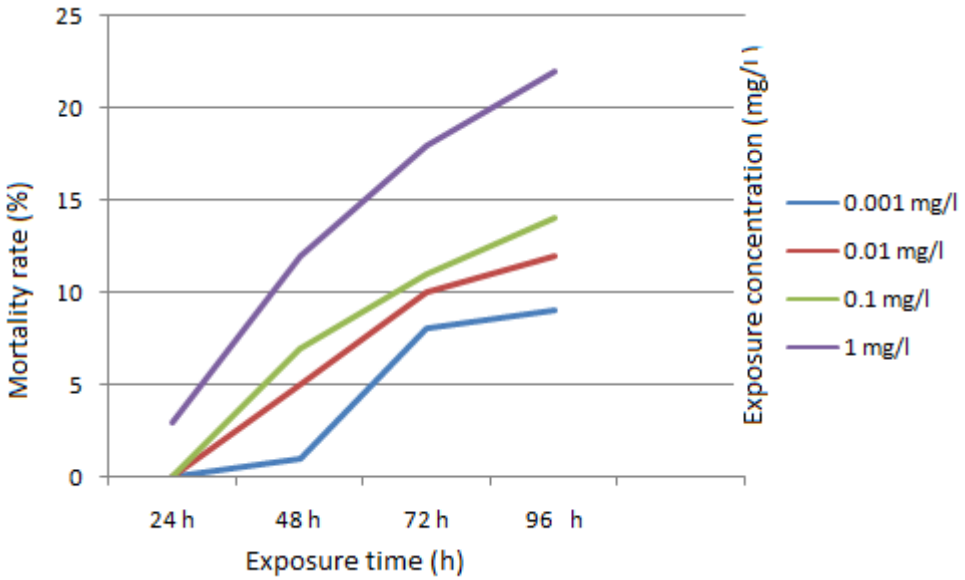

Figure 1: Mortality rates of non-nano boron on *Apis mellifera* depending on time and duration

In the variation graph based on lethal exposure duration and concentration for *Apis mellifera* of non-nano boron in the Figure 1; mortality rates parallel increased for each concentration of 0.001, 0.0, 0.1 and 1 mg/l as exposure duration for these concentrations increased,

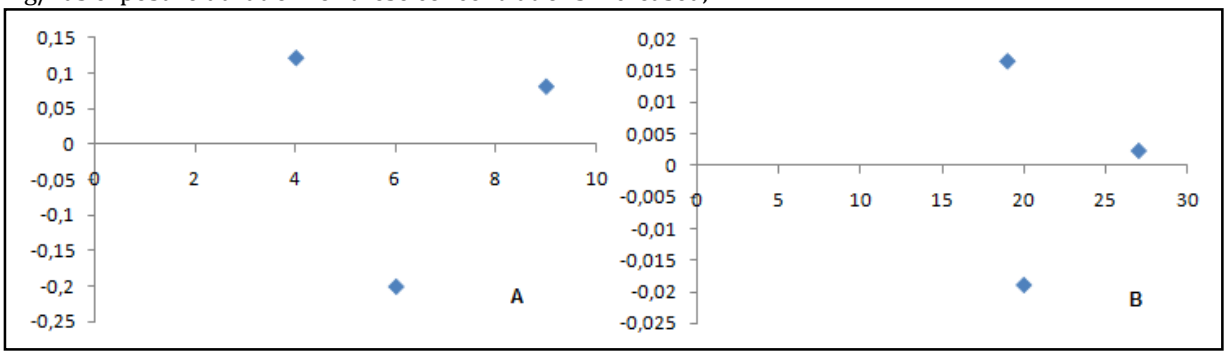

Figure 2: Regression distributions for non-nano boron nanoparticles a) for 48 hours b) for 96 hours.

Table 2: 96 and 48 hour LC/EC values calculated for nano boron particles

Points	Exposure time	
	48 hour	96 hour
LC/EC 1.00	0.000	0.000
LC/EC 5.00	0.000	0.000
LC/EC 10.00	0.002	0.000
LC/EC 15.00	0.018	0.000
LC/EC 50.00	**229.099**	**0.339**
LC/EC 85.00	2997501.750	243.544
LC/EC 90.00	28231326.000	1154.772
LC/EC 95.00	%783092608.000	11586.925
LC/EC 99.00	%398508097536.000	875619.813

In the exposure study conducted at 48 h and 96 h for the same concentrations of boron nanoparticles $LC_{1,5}$ values at 48 h and 96 h and couldn't be calculated, respectively. LC_{50} values at 48 h and 96 h were calculated 1987.567 and 7849373.500, respectively. Toxic effect of K_2PdCl_4 increased by exposure duration (Table 2)

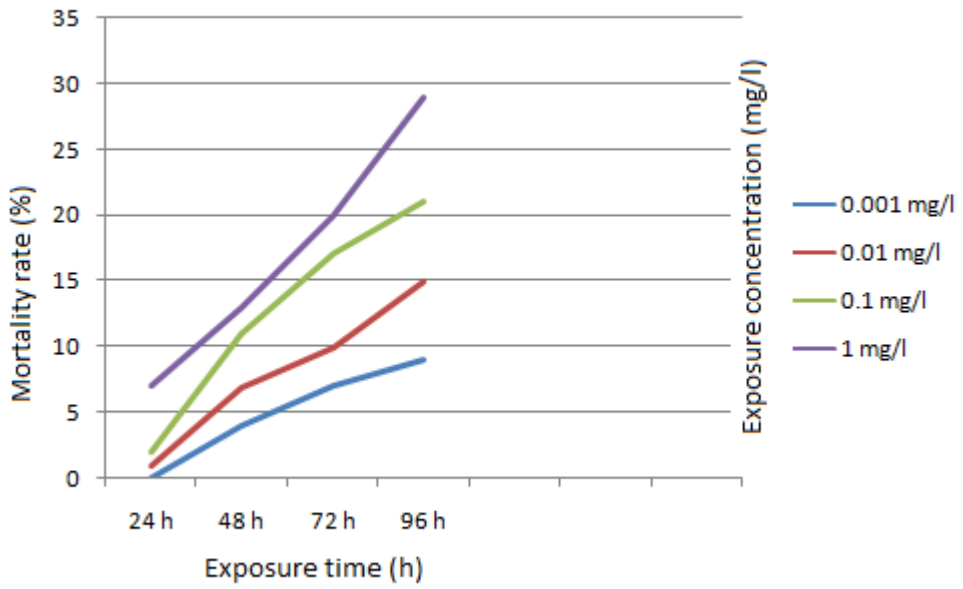

Figure 1: Mortality rates of nano boron on *Apis mellifera* depending on time and duration

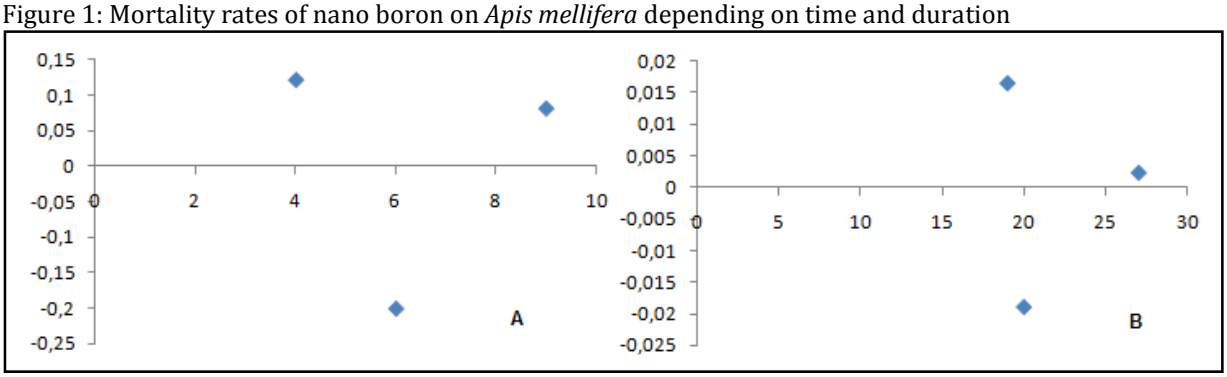

Figure 2: Regression distributions for nano bor a) for 48 hours b) for 96 hours

Analysis of exposure durations and concentration groups

Table 3: The TUKEY test results of the differences in the mortality rates between the different concentration groups and exposure time of non-nano boron.

Time (Hours)	N	Subset for alpha = 0.05			Con. (mg/L)	N	Subset for alpha = 0.05		
		1	2	3			1	2	3
24	15	0.0107[a]			0*	12	0.0000[a]		
48	15	0.1027[ab]	0.1027[ab]		0.001	12	0.0867[ab]	0.0867[ab]	
72	15		0.1880[bc]	0.1880[bc]	0.01	12		0.1383[b]	
96	15			0.2293[c]	0.1	12		0.1633[bc]	0.1633[bc]
Sig.		0.100	0.143	0.719	1	12			0.2750

Con.: concentration

In table 3, the group which made the most difference in all the concentration and time groups of non-nano boron was the 0.1 mg/l concentration and 96 hours.

Table 4: The Difference between the non-nano boron concentrations groups according to ANOVA multiple comparison tests

(I) Con. (mg/l)	(J) Con. (mg/l)	Mean Difference (I-J)	Standard Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.001	1	0.18833*	0.04183	0.000	0.3063	0.0704
0.1	0*	0.16333*	0.04183	0.002	0.0454	0.2813
0*	0.001	0.18833*	0.04183	0.000	0.0704	0.3063
	0.01	0.13667*	0.04183	0.015	0.0187	0.2546
	1	0.27500*	0.04183	0.000	0.1570	0.3930

(J) Time (hours)	(J) Time (hours)	Mean Difference (I-J)	Standard Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
24	72	-0.17733*	0.03922	0.000	-0.2812	-0.0735
	96	-0.21867*	0.03922	0.000	-0.3225	-0.1148
48	96	-0.12667*	0.03922	0.011	-0.2305	-0.0228

0*: control group, Con.: concentration, Sig.: significance

According to the results of the anova test; among the mortality rates according to the concentration groups at the end of the exposure period of nano boron, there were significant differences found between 0.1 mg/l and 0* mg/l, 0* mg/l and 0.01 mg/l, at $P<0.05$ level, whereas such differences were at $P< 0.01$ level between 1 mg/l and 0.001 mg/l, 0* mg/l and 0.001 mg/l, 0* mg/l and 1 mg/l concentrations (Table 4). Among the mortality rates according to the time groups at the end of the exposure period of non-nano bor, there were significant differences found between 48-h and 96-h groups at the level of $P<0.05$, whereas this difference was at $P<0.01$ level between 24-h and 72-h, 24-h and 96-h groups (Table 4).

Table 5: The TUKEY test results of the differences in the mortality rates between the different concentration groups and exposure time of nano boron

Con. (mg/l)	N	Subset for alpha = 0.05				Time (Hours)	N	Subset for alpha = 0.05		
		1	2	3	4			1	2	3
0	12	0.0000[a]				24	15	0.0387[a]		
0.001	12	0.1017[ab]	0.1017[ab]			48	15	0.1400[ab]	0.1400[ab]	
0.01	12		0.1667[bc]	0.1667[bc]		72	15		0.2200[bc]	0.2200[bc]
0.1	12			0.2500[cd]	0.2500[cd]	96	15			0.2960[bc]
1	12				0.3500[d]	Sig.		0.204	0.404	0.449
Sig.		0.232	0.664	0.427	0.247					

In table 5, the group which made the most difference in all the concentration and time groups of nano boron was the 1 mg/l concentration and 96 hours.

Table 6: The Difference between the nano boron concentrations groups according to ANOVA multiple comparison tests

(I) Con. (mg/l)	(J) Con. (mg/l)	Mean Difference (I-J)	Standard Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.1	0.001	0.14833*	0.04829	0.026	0.0121	0.2845
1	0.01	0.18333*	0.04829	0.003	0.0471	0.3195

0*	0.01	0.16667*	0.04829	0.009	0.3029	0.0305
	0.1	0.25000*	0.04829	0.000	0.3862	0.1138
	1	0.35000*	0.04829	0.000	0.4862	0.2138

(I) Con. (mg/l)	(J) Con. (mg/l)	Mean Difference (I-J)	Standard Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
24	72	0.18133*	0.05095	0.004	0.3162	0.0464
	96	0.25733*	0.05095	0.000	0.3922	0.1224
48	96	0.15600*	0.05095	0.017	0.2909	0.0211

0*: control group, con.: concentration, Sig.: significance

According to the results of the anova test; among the mortality rates according to the concentration groups at the end of the exposure period of nano boron, there were significant differences found between 0.1 mg/l and 0.001 mg/l, 1 mg/l and 0.01 mg/l, 0* mg/l and mg/l at P<0.05 level, whereas such differences were at P< 0.01 level between 0* mg/l and 0.1 mg/l, 0* mg/l and 1 mg/l concentrations (Table 6). Among the mortality rates according to the time groups at the end of the exposure period of TiO_2, there were significant differences found between 24-h and 72-h groups and 48-h and 96-h groups at the level of P<0.05, whereas this difference was at P<0.01 level between 24-h and 96-h groups.

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