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IMPACT OF MULTI-WALLED CARBON NANOTUBES ON VIABILITY AND PATHOGENICITY OF ENTHOMOPATHOGENIC NEMATODES

WPŁYW WIELOŚCIENNYCH NANORUREK WĘGLOWYCH NA ŻYWOTNOŚĆ I PATOGENNOŚĆ OWADOBÓJCZYCH NICIENI

Abstract: The investigations aimed at recognizing the impact of multi-walled carbon nanotubes on the activity of enthomopathogenic nematodes under laboratory conditions. Two kinds of nanotubes were applied to treat infective juveniles of two enthomopathogenic nematode species. The obtained results allowed for a conclusion that multi-walled carbon nanotubes do not cause mortality of the analysed nematode infective juveniles (IJs). However, they partially reduce their activity towards test insects.

Keywords: carbon nanotubes, enthomopathogenic nematodes

Nanotechnology is a field of science allowing for manufacturing and use of the structure sized between 1 and 100 nm. Nanotechnology makes possible manufacturing products and objects atom after atom, molecule after molecule without any wastes [1, 2]. One of the nanotechnology products are carbon nanotubes. Research has been conducted for many years to determine the properties of carbon nanotubes [3]. They are known to possess high mechanical resistance, conductivity of metallic or semiconductor type dependent on the kind of nanotube, low electrical resistance and excellent thermal conductivity, many times higher than copper [2, 4].

There are *single-walled carbon nanotubes* (SWCNT) and *multi-walled carbon nanotubes* (MWCNT). They differ in their shapes, length and properties [5, 6].

Carbon nanotubes are used in various fields of science. Research conducted by scientists from Mexico [7] revealed that single-walled carbon nanotubes may be used

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for detecting and removal of disease agents in contaminated potable waters. They also find numerous applications in medicine. Nanotubes are empty inside and once opened these empty channels provide a possibility to use them as storage for various substances (eg some medicines or aromas) protecting them against the unfavourable effect of external factors [1, 2].

Recently nanotechnology has been applied also in food technology, eg for crushing solid substances into nanoparticles or encapsulation of some nutrients, reserves or dyes which contributes to their release according to needs or after a determined period of time [8].

There are presumptions that nanotechnology may be also used in agriculture for crop protection. It has been known that some properties of nanoproducts allow for their use to fight dangerous pathogenic microorganisms [3]. Pike-Biegunski [9] stated that crystalline structure of nonionic nanoparticle preparations and their large active surface make them most efficient in controlling pathogens such as: fungi, bacteria and viruses, because they do not produce typical defensive mechanisms. Research conducted so far has revealed that copper products manufactured by nanotechnology have been already successfully used in agriculture for controlling fungi infecting numerous crops, such as potatoes, tomatoes or fruit [2]. However, it is still unknown whether nanoproduct application in agriculture may not contribute to reducing the numbers of organisms which are not the object of control (beneficial organisms, such as enthomopathogenic nematodes). Enthomopathogenic nematodes occur numerously in the unpolluted environment and are most sensitive to pollutants [10]. For this reason they may be used as indicator organisms. Nematodes also play an important role in biological pest control, therefore a growing number of preparations containing these organisms are created [11].

Rapidly developing technologies and potential which nanotechnology provides make necessary conducting further research enabling more precise identification of nanotube activity in the agricultural environment.

Material and methods

The analyses were conducted under laboratory conditions at the Department of Agricultural Environment Protection of the University of Agriculture in Krakow.

The initial stage of the experiment focused on the effect of two kinds of multi-walled carbon nanotubes (MWCNT) and carboxylated multi-walled carbon nanotubes (MWCNT(COOH)) on viability and behaviour of nematode infective juveniles. 1 cm^3 of multi-walled carbon nanotubes was added to 1.5 cm^3 glass vessels. Subsequently about 30 infective juveniles of *Steinernema feltiae* enthomopathogenic nematodes originating from Owinema®, Nemasys and Nemaplus, and nematodes of *Heterorhabditis bacteriophora* species originating from Nematop preparation were added to each vessel. Nematodes placed in glass vessels with distilled water provided the control. The nematodes were observed under a magnifying glass for five subsequent days since the experiment outset in order to determine their viability and their motion activity in the analysed solutions. Motion activity of the nematode infective juveniles was determined on a 6-degree scale. After 5 days of observations nematode activity was checked against *Tenebrio molitor* L. test insects. Therefore, the nematodes were moved to filter paper and after rinsing with distilled water and draining they were moved to moist three-layer filter papers placed in plastic Petri dishes. 10 *Tenebrio molitor* test insect larvae were put in each dish prepared in this way. For the following 7 days mortality of the test insect larvae was observed. The results were analysed statistically using the Statistica Programme, ANOVA analysis was conducted and Neuman-Keuls critical intervals were computed and the value of the final step was used for differentiating means at the significance level $p < 0.05$.

Results

The first stage of the experiment aimed to check vitality of nematode larvae under the influence of applied two kinds of multi-walled carbon nanotubes. It was observed that nematodes kept in the vessels with nanotubes retained a 100 % vitality after 5 days, the same as in the control (Table 1). Moreover, no significant differences in nematode larvae behaviour was spotted under the influence of nanotubes, and their motion activity was approximate to the larvae in the control.

Table 1

Vitality of *S. feltiae* and *H. bacteriophora* infective juveniles (IJs) originating from Owninema, Nemasys, Nemaplus and Nematop preparations kept in solutions containing multi-walled carbon nanotubes

Combination	IJs viability after 96-hour contact with nanotubes [%]			
	Kind of IJs			
	Owinema	Nemasys	Nemaplus	Nematop
Control	100	100	100	100
MWCNT	100	100	100	100
MWCNT(COOH)	100	100	100	100

The subsequent stage of the research comprised determining the activity of investigated nematodes treated with various kinds of multi-walled carbon nanotubes towards the test insect larvae. Already after 24 hours the nematodes kept in the control caused significantly higher mortality of the test insect larvae in comparison with the nematodes kept in the solution containing nanotubes. The observations were conducted for the 7 subsequent days. Mortality of the test insects for individual nematodes during the period was presented in Fig. 1.

The average life span was calculated for IJ-treated test insects previously kept in the analyzed solutions containing multi-walled carbon nanotubes (Fig. 2). Despite that fact that multi-walled carbon nanotubes contributed to a delayed IJs activity towards the test insect larvae, statistical analysis did not reveal any significant differences in the life span of *T. molitor* larvae between the applied combinations.

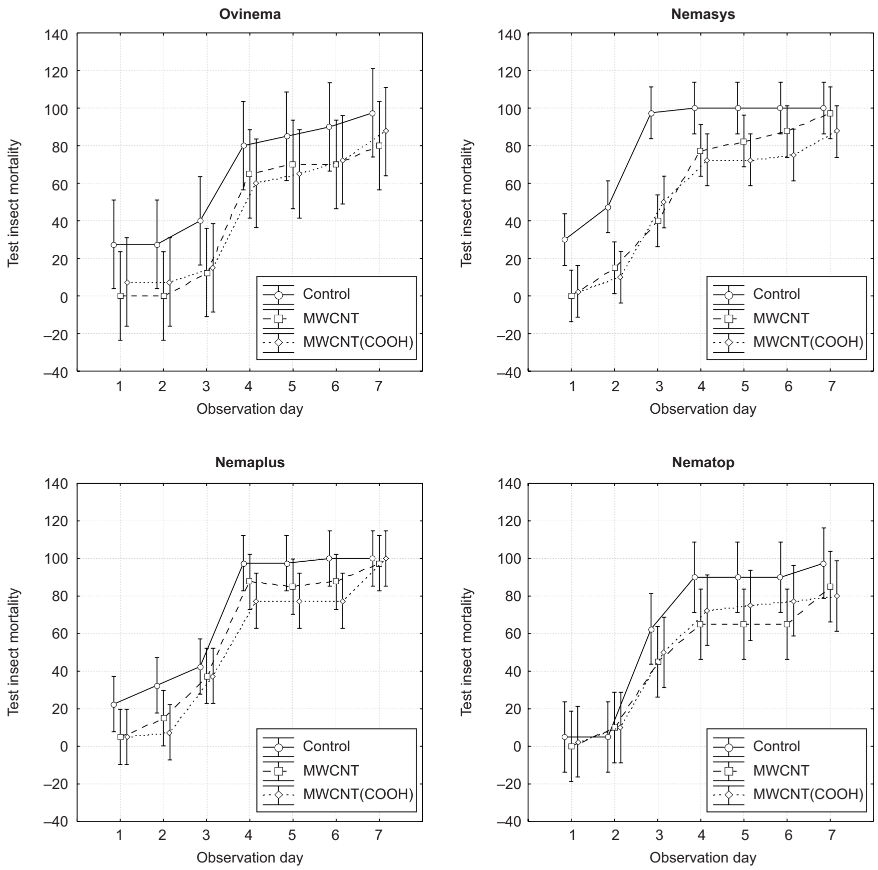

Fig. 1. Mortality of *T. molitor* L. larvae for 7 days under the influence of *S. feltiae* and *H. bacteriophora* infective juveniles (IJs) activity, originating from Owinema, Namasys, Nemaplus and Nematop biopreparations, kept in solutions containing multi-walled carbon nanotubes

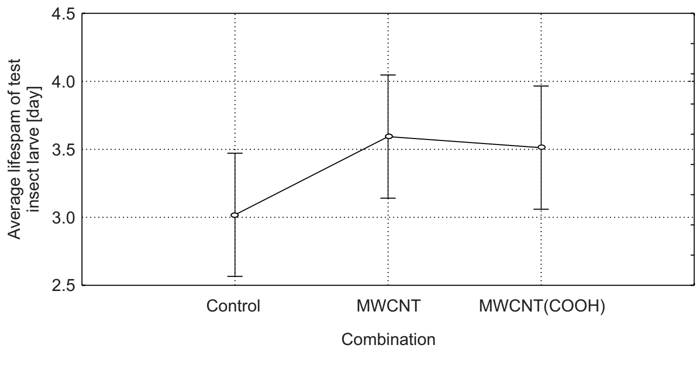

Fig. 2. Lifespan of *T.molitor* L. larvae caused by activity of *S. feltiae* and *H. bacteriophora* nematode infective juveniles (IJs), originating from Owinema, Namasys, Nemaplus and Nematop biopreparations, kept in solutions containing multi-walled carbon nanotubes

Discussion

Enthomopathogenic nematodes are organisms commonly present in the unpolluted natural environment [10]. Heavy metal accumulation in the environment where the nematodes live contributes to limiting their activity and sometimes also reduces their numbers. Wang et al [10] revealed in their studies that some nanocompounds, such as Nano-ZnO, Nano-Al_2O_3 or Nano-TiO_2 are instrumental in diminishing nematode body length, limiting the number of eggs laid in test insects and considerably decrease nematode larvae reproduction. The present Authors obtained similar results in their own experiment, where the analyzed multi-walled carbon nanotubes played a part in inhibiting the activity of investigated IJs towards *T. molitor* test insects. The research conducted by Gorczyca et al [3] demonstrated that carbon nanotubes significanty stimulate surface growth of *Paecilomyces fumosoroseus* mycelium but at the same time cause a reduction in its sporulation in comparison with the control samples. Nanotubes also pose a hazard for fish in the aquatic environment. Investigations carried out by American scientists [12] revealed that carbon nanotubes supplied to the aquatic environment in which rainbow trout existed irritated their branchiae and caused changes in their brains. Research conducted by Muller et al [13] on rats demonstrated that nanotubes caused considerable damage to these rodents' lungs. Moreover, currently American scientists have warned against some nanoparticle products. The risk is associated with the size of the particles which, inhaled by humans, may penetrate to various cells in human organism and accumulate there causing various illnesses. It has been demonstrated that nanotubes may damage lungs whereas their structure and size make impossible their complete removal from the organism. There are reports in literature also pointing to a toxic effect of such nanoparticles as: titanium, cobalt, iron, wolfram or silver [1].

Despite the fact that nanotubes seem to pose a considerable hazard to the surrounding environment, they find applications in more modern fields of science. They are most useful in medicine eg for combating neoplastic diseases.

The observable technological progress making use of nanotechnology makes some people optimistic, while others remain anxions. The opinions on nanoproducts are divided, which may be settled by conducting more extensive research towards a better understanding [14]. Therefore further investigations in this field seem both necessary and justified.

Conclusions

1. Multi-walled carbon nanotubes (MWNCT and MWCNT(COOH)) are non-toxic for the infective juveniles of enthomopathogenic nematodes of *Steinernema feltiae* (Owinema, Namasys, Nemaplus) and *Heterorhabditis bacteriphora* (Namatop) species.

2. Multi-walled carbon nanotubes (MWNCT and MWCNT(COOH)) are a factor in limiting the activity of infective juvenile larvae of enthomopathogenic nematodes of *Steinernema feltiae* (Owinema, Namasys, Nemaplus) and *Heterorhabditis bacteriphora* (Namatop) species.

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Abstrakt: Celem przeprowadzonych badań było poznanie wpływu wielościennych nanorurek węglowych na aktywność nicieni owadobójczych w warunkach laboratoryjnych. W badaniach zastosowano dwa rodzaje wielościennych nanorurek węglowych, którymi traktowano larwy inwazyjne dwóch gatunków nicieni owadobójczych. Uzyskane wyniki pozwoliły stwierdzić, że wielościenne nanorurki węglowe nie powodują śmiertelności badanych larw inwazyjnych (IJs) nicieni. Powodują jednak częściowe ograniczenie ich aktywności względem owadów testowych.

Słowa kluczowe: nanorurki węglowe, nicienie owadobójcze