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Genotoxicity Evaluation of *Steinernema scaptersici*, Nematodes in *Locusta migratoria* (Orthoptera, Acrididae), Using Alkaline Comet Assay

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Abstract

Entomopathogenic nematodes are commonly used in various applications of insect control. Otherwise, the genotoxicity effects of this promising technology should be studied to understand its genetic virulence. This research aimed to investigate the potential genotoxicity of different concentration of *Steinernema scaptersici* nematodes (0-50 IJs/µL) on the brain cells of insects using alkaline comet assay. The level of DNA damaged was significantly higher in insects treated with 10-50 IJs/µL comparing to that from the negative and positive control one with the fold of 0.25 and 0.75 for 50, and 40 IJN/µL at 24 h PI and 1.6, 1.5, 1.6, 1.7, 2.3 -x fold for 10-50 IJN/µL at 48 h PI of S. scaptersici. A strong positive correlation occurred between concentration of *S. scaptersici* nematodes and all comet assay parameters were occurred with linear prediction equations. The possible deleterious impacts of *S. scaptersici* nematodes on the *Locusta migratoria* were discussed. Also, the potential using of comet assay as an accurate and cost-effective monitoring tool of genotoxicity application of entomopathogenic nematodes was proposed. These findings will consider as an essential part for future perspective in pests controlling process using eco-friendly biological technology.

Keywords: Alkaline Comet Assay; DNA Damage; *Steinernema scaptersici*; Entomopathogenic Nematodes; *Locusta migratoria*

Abbreviation: ROS: Reactive Oxygen Species; SCGE: Single Cell Gel Electrophoresis Assay.

Introduction

The environmental pollutants are considered as a big challenge nowadays Abdelfattah [1], one of the most deleterious sources on human and environmental health is the using of traditional pesticides [2-4]. So, the using of eco-friendly pesticides is so essential step to deviate the negative impacts of traditional pesticides on the ecosystem structure and function [5].

Nematodes are considered as a promising alternative biocontrol agent. Where entomopathogenic nematodes live naturally in soil environments and it could recognize their host as a response to carbon dioxide, vibration and other chemical cues [6]. Additionally, the two families (Heterorhabditidae and Steinernematidae) have been effectively used as biocontrol agents in pest management

programs [7]. The only free-living stage of entomopathogenic nematodes is the infective juvenile. Nematodes could penetrate the host body through the natural openings of the insects as mouth, anus and spiracles, then it goes into the insect hemocoel [8]. Both *Heterorhabditis* and *Steinernema* nematodes have symbiotic bacteria of the genera *Photorhabdus* and *Xenorhabdus* [9]. The juvenile stage releases its bacteria into the hemocoel. The bacteria reproduce in the insect, feed on the host tissues the infected host usually dies within 24 to 48 hours. After the death of the host, nematodes continue to feed on the host tissue, mature and reproduce. The progeny nematodes develop through four juvenile stages to the adult, and many infective juveniles are released into environment to infect other hosts. [6].

In this context, nematodes can increase the production of reactive oxygen species (ROS) in organisms [4]. When ROS increased than normal level, and lead to oxidative stress causing macromolecules damage, including DNA damage, protein carbonylation, lipid peroxidation, and enzyme inactivation [4,5,10-15]. DNA damage involves the bases removing process that leads to strand breaks [2]. Single strand breaks of DNA damage can be measured using alkaline comet assay. This method is considered as one of the simplest, most selective method for detecting DNA strands breakages. The top secrets feature of comet assay allows early detection of the stressor deleterious. Recently, the comet assay became as a genotoxicity tool in different living organisms, especially insects [1,2].

Locusta migratory is considered as an agricultural pest; having a daylight phytophagous behavior and a widespread appearance especially in terrestrial ecosystems. However, the role of grasshoppers in biomonitoring environmental pollution, it has a deleterious economic effect on wide range plants especially in intense climate change effects.

Hence, the aim of the study was to evaluate the damage level of DNA, using alkaline comet assay in the brain cells of *Locusta migratoria*, which injected by different concentrations of *S. scaptersici* nematodes (0-50 IJs/µL) after 24 and 48 hours post injection.

Materials and Methods

The grasshoppers (nymphs and adults) were fed on Alfalfa leaves according to 5th stage nymphs were used while, the nematode species *S. scaptersici* were provided from Prof. El-Sadawy laboratory, National Research Centre, Dokki, Giza, Egypt. Entomopathogenic nematodes were propagated on in vitro solid culture according to El-Sadawy, et al. [16].

Experimental Study

Susceptibility of *L. migratoria*i infected with the nematodes *S. scaptersici,* in the form of DNA strand breaks. Different 5th nymphal instars of *L. migratoria*i were injected with (0 (positive control), 10, 20, 30, 40, and 50 IJs / 1 µL distilled water) beside control groups (non-injected, negative control). Briefly, the naïve insect behaves as negative control while the saline-injected insects considered as a positive control. Injection was carried out using a sterile micro-syringe (30-gauge needle); the needle was carefully inserted through the intersegmental membrane of the hind leg. Plastic cups (5×4 cm^2) were used for insect grouping. Each cup contained five individuals of *L. migratoria*, 5th instar with alfalfa leaves for feeding. The accumulative mortality percentage was recorded at 12, and 24 h post-injections. Control group (positive) were injected with 1µL distilled water. Each treatment replicated thrice, 5 larvae for each replicate, incubated at 25 ± 2°C; 50 – 60 % RH (12:12; D:L). After treatment, insects were dissected to isolate brain tissues for further analysis and were stored at -20 °C until use.

The Single Cell Gel Electrophoresis assay (SCGE), known as the Comet assay was used to assess the DNA strand breaks according to Duroudier et al. [17]. The analysis of DNA damage was performed using OPTIKA B-350 fluorescent microscope (OPTIKA, Ponteranica, Italy), with a CCD camera. The image analysis system (Comet IV software) was used to quantify the single strand breaks of DNA by different parameters. Statistical analysis was performed using IBM SPSS Statistics for Windows (Version 17.0. Armonk, NY: IBM Corp). A non-parametric test was carried out using the k independent Kruskal-Wallis test to compare between the effects of different concentration of AgNPs on comet parameters.

Results and Discussion

In the present study, the alkaline comet assay was used to evaluate the genotoxicity of promising entomopathogenic nematode application (Figures 1, 2; Table 1).

The use of comet assay parameters as a biomonitoring of pesticides applications were studied in various studies [18,19]. However, the genotoxicity effect of entomopathogenic nematodes *S. scaptersici on the L. migratoria* is considered as a novel application. As a result of several exogenous and endogenous factors, the levels of ROS production are increased and lead to macromolecules damaged especially for DNA single strands breaks.

Comet parameters	% DNA in tail				% of severed cells			
Time PI (h)	24		48		24		48	
Entomopathogenic nematode concentration (IJs/µL)	Med.	SD	Med.	SD	Med.	SD	Med.	SD
Positive control	0.005	0.001	0.007	0.0001	10a	2	15a	1
Negative control	0.006	0.002	0.005	0.0001	11a	1	14a	2
10	0.007	0.001	0.011	0.0002	25b	3	29	5
20	0.004a	0.001	0.061a	0.0002	26b	5	31	7
30	0.003	0.002	0.062a	0.0001	40	7	41	8
40	0.004a	0.001	0.004	0.0002	50	8	49	9
50	0.004a	0.001	0.003	0.0003	54	10	50	14

Table 1: % of severed cells and % of DNA in tail comet parameters of brain cells of *Locusta migratoria* treated with different concentration of *Steinernema scaptersici* nematodes (0-50 IJs/µL) Median values marked with different small letters significantly different (Kruskal-Wallis test, P < 0.05).

Figure 1 showed that the different concentration of entomopathogenic nematodes *S. scaptersici* lead to increase the level of stand breaks of DNA inform of comet length, tail length, and comet height (Figures 1 A-1C).

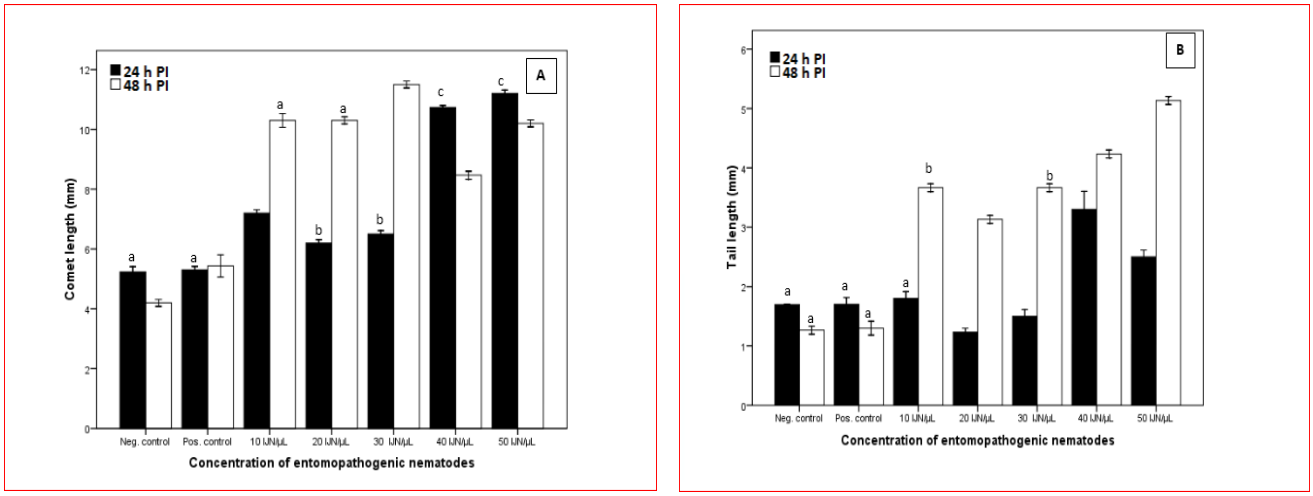
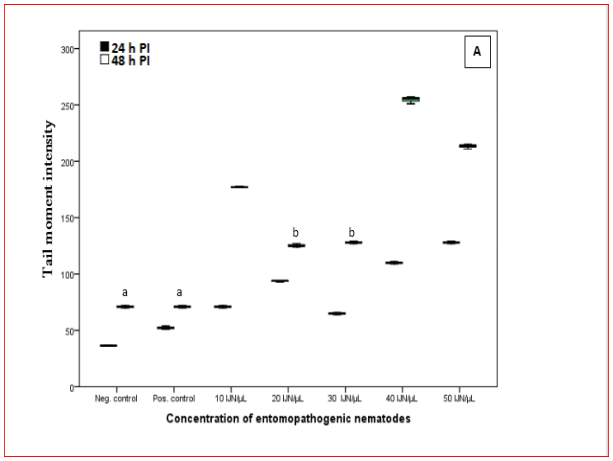
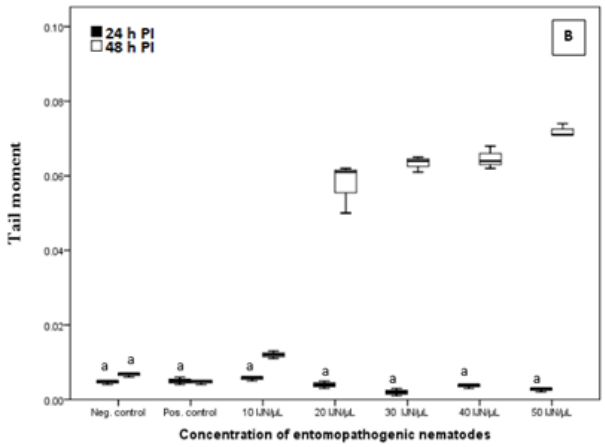


Figure 1: Alkaline comet assay of brain cells of *Locusta migratoria* treated with different concentration of *Steinernema scaptersici* nematodes (0-50 IJs/µL).
Median values marked with different small letters significantly different (Kruskal-Wallis test, P<0.05).

Our results met the explanation of Fridovich [20] study; which approved that when nematodes enter the insect's body, their symbiotic bacteria such as *Heterohabdus* and *Xenorhabdus* are elevated the ROS levels [21]. The accumulation of ROS causes oxidative stress in the host insect cells, and case macromolecules damage. The damage of macromolecules includes lipids peroxidation [4,22,23], proteins carbonylation (Gutierrez-Correa, et al.; Costa et al.; Abdelfattah) [13,24,25], and DNA single strand breaks [2]. This analysis was done according to previous accepted literature [1,2]. The results revealed a significant increase of DNA damage in brain cells of treated insect with different concentration of *S. scaptersici* compared to the positive controls and negative control insects especially in the tail mean intensity, tail moment, and olive moment of brain cells of *L. migratoria*i (Figures 2A-2C).





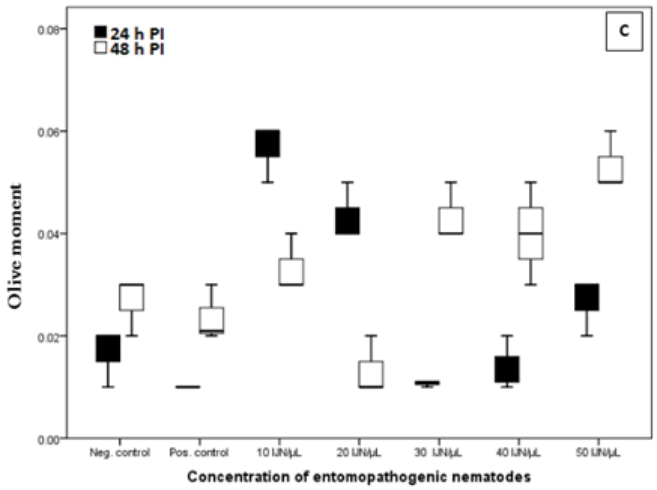


Figure 2: DNA damage of brain cells of *Locusta migratoria* treated with different concentration of *Steinernema scaptersici* nematodes (0-50 IJs/µL).
Median values marked with different small letters significantly different (Kruskal-Wallis test, P<0.05).

The % of DNA in tail and % of severed cells showed a significant increase of DNA damage in treated samples compared with positive and negative control samples (Table 1). The insect brain cells considered as a most sensitive tissue to any effector. Also, it plays a key role in some essential activities as a biomonitor agent [1,2].

These results confirm the hypothesis that *S. scaptersici* increases the level of oxidative stress in *L. migratoria* larva. Also, the study of van Sambeek, et al. [26] showed that the *Heterorhabditis megidis* and *Steinernema feltiae* nematodes can used against the orthopteran insects. The death rate of *Locusta migratoria* and *Schistocerca gregaria* was positively correlated with the nematode-inoculated sand percentage. Also, all these findings suggest that entomopathogenic nematodes *S. scaptersici* exposure may induce oxidative stress, which can be indirectly detected through evaluation of macromolecules damage or the activity of antioxidant enzymes. The deleterious damage of DNA may occur as results of increasing levels of ROS. Besides that, the present results corroborate with those of Lobo, et al. [27], who reported that DNA is considered as key target of free radical attack in the living cells. Abdelfattah, et al. [2] observed genotoxicity effect of different environmental stressors on different tissues of males and female grasshopper *Aiolopus thalassinus.* The relationship between comet parameters and different concentration of AgNPs treatment in the present study showed a unified pattern of a positive correlation.

The endo-symbiotic bacteria of *Steinernema scaptersici* nematodes which include *Xenorhabdus and Photorhabdus* species can be used widely in insect control field. The article of Silva, et al. [28] showed various studies focused on encoding low molecular weight proteins, and secondary

toxin complexes generating genes with insecticide activities, and bacterial infection. Also, several species of symbiotic nematodes bacteria act as a bio-control agent against mosquitoes. In addition to, the toxicological effects of *Heterorhabditis bacteriophora* IJS on 5th nymphs of the desert locust *S. gregaria*, showed a dose- and time-dependent pattern. The susceptibility of locusts to nematode infections was remarkably high reached up to 80% after 120h, at 400 IJs/mL nematodes concentration. The results emphasized that *Steinernema* nematodes is considered as a useful model system to study an insect defensive mechanism against bacterial infection. Also, the ability of using the genotoxicity response parameters of *L.migratoria* as an indicator of entomopathogenic nematodes infection was evaluated. The parameters were selected according to the generally accepted knowledge [1,2,11].

Conclusion

This study demonstrates the effect of entomopathogenic nematode on the oxidative damage parameters especially DNA damage using alkaline comet assay. This experiment was done using different concentration of nematodes (0-50 IJs/µL) on the brain cells of insects. Also, the results showed that A strong positive correlation occurred between concentration of *S. scaptersici* nematodes and all comet assay parameters were occurred with linear prediction equations.

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Conflict of Interest

This study has no conflict of interest

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