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Monitoring Movement Behaviour of *Caenorhabditis elegans* in Response to Formaldehyde at Low Concentrations

M. Golam Mortuza,[1, 3*] C. W. Ji,[2] Fahad A. Al-Misned[1] and T.-S. Chon[2]
[1]*Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia*
[2]*Department of Biological Sciences, Pusan National University, Busan 609-735, Republic of Korea*
[3]*Department of Zoology, Faculty of Life and Earth Science, Rajshahi University, Rajshahi 6205, Bangladesh*

Abstract.- This study describes a new approach for assessing toxic response behaviour of *Caenorhabditis elegans* by automatic recognition of line movement through an image-processing system. The movement behaviour of this nematode is different even at low concentrations of formaldehyde. A comparison of the response behaviour of this animal at different concentrations of formaldehyde has shown concentration-dependent toxicity. This study has identified some sequential line-movements on nematodes that confirmed the toxicological effect on nematode behaviour.

Key words: Monitoring, movement behaviour, formaldehyde, *Caenorhabditis elegans.*

INTRODUCTION

The soil nematode *Caenorhabditis elegans* is a widely used model organism for studying animal development, genetics and neurobiology (Rankin, 2002; Roussel *et al*., 2007). Its advantages include a rapid life cycle (3.5 days from fertilized egg to fertile adult at 20°C), hermaphrodite nature, simple body architecture, availability of forward and reverse genetic approaches (Jorgensen and Mango, 2002), knowledge of the complete cell lineage (Sulston and Horvitz, 1977; Sulston *et al*., 1983), simplicity of the nervous system (302 neurons, whose precise position, cell lineage and synaptic connectivity are known), its wiring (White *et al*., 1986) and fully sequenced genome (The *C. elegans* Sequencing Consortium, 1998). The species garnered a special attention as an indicator species in the field of ecotoxicology (Gerhardt *et al*., 2002).

In various studies, the movement of *C. elegans* was shown to be a promising toxicity parameter for different toxicants, metals and organic compounds, using a computer tracking system (Williams and Dusenbery, 1990; Anderson *et al*., 2001). The movement pattern was found to be at least as sensitive as other sublethal endpoints and the method showed to be less time consuming than measuring other toxicity endpoints, such as growth and reproduction (Dhawan *et al*., 1999; Anderson *et al*., 2001). However, the body of *C. elegans* is in a line-shape and line movements have not been used for monitoring in response to toxic chemicals, although behavioural monitoring has been used as an alternative tool for risk assessment with various techniques (Kramer *et al*., 1989; Staaks, 1996; Staaks *et al*., 1999; Gerhardt *et al*., 1998, 2005; Kwak *et al*., 2002; Borcherding, 2006; Untersteiner *et al*., 2003; Chon *et al*., 2004; Park *et al*.,. 2005). The monitoring studies stated above, however, are mostly based on detection of point estimation according to the centroid of the body since the specimens used for tests were round or elliptic.

Continuous monitoring of movement behaviour requires automatic recognition of the line body shape for *C. elegans*. Tracking of the line-movement was reported by using the technique of machine vision (Butcher *et al*., 1999; Hardaker *et al*., 2001; Baek *et al*., 2002; Geng *et al*., 2003, 2004; Feng *et al*., 2004; Ajie *et al*., 2005; Stephens *et al*., 2008).

In the present study, line-tracking is used to detect the response behaviour of nematodes treated with low concentrations of formaldehyde. We also have demonstrated the sequential line-movement of nematodes after toxic chemical treatment and efficiently characterize the stressed behaviour of the specimens.

* Corresponding author: mortuzaru@yahoo.com
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MATERIALS AND METHODS

Nematode

Caenorhabditis elegans wild type, strain N_2 was collected from the Molecular Ecotoxicology Laboratory for Environmental Biomarker Research, University of Seoul, South Korea. The specimens were cultured in agar medium with *E. coli* (strain Op_{50}) as food on crystal-grade polystyrene Petri dish (60 mm x 15 mm) under dark condition at 20±3°C. To obtain synchronized cultures for the experiments, larval stage-4 was transferred to a new agar plate, allowed to develop up to young adult stage for 12 h and finally young adult individuals were selected for the experiments.

Toxic chemicals and concentrations

K-media (2.38g KCl and 3g NaCl salt dissolved in 1 liter DDW) was used as the control to accommodate the individuals in the arena. For treatment, 37% formalin was diluted in K-media to make 0.1 mg/l and 1mg/l formaldehyde.

Observation system

The movement of *C. elegans* was observed and recorded under an observation system consisting of an observation arena (1.5mm in depth, 4 mm in diameters), a camera, and a desktop computer with software for image recognition. Experimental animal was transferred to the observation arena filled with K-media (control) or low concentrations of formaldehyde (treatment). Each experimental animal was allowed to acclimate for 5 minutes before tracking. After the individual was transferred to the arena, cover glass was placed on top of the arena carefully to prevent the air bubbles formed within the arena. By this method noise (*i.e.* reflection from liquid surface) was eliminated for recognition of line movement through the image recognition system. The movement behaviour of the specimens were similar with and without the cover glass according to preliminary observation. The amount of oxygen was sufficient in the arena during the observation period and hypoxia of specimens was not observed. The observation arena was placed on a Petri-dish (9 cm) filled with water used as a cooling plate for the arena. The observation arena was surrounded by a channel which was also filled with same liquid as the observation arena to prevent evaporation of the formaldehyde solution (Fig. 1).

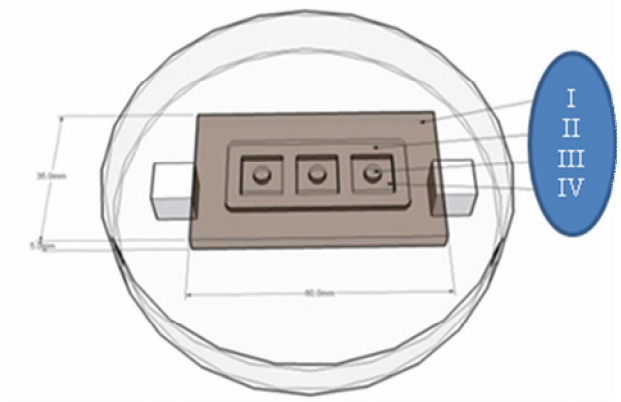

Fig. 1. Petri dish with observation plate; I, cover glass; II, channel; III, observation arena; IV, observation stage.

Detection of line movement and analysis

The movement of the specimens was tracked for 3 h from top view at every 0.25 second interval using a CCTV (Kukjae Electronics Co. Ltd.; IVC-841) camera both for the control and treated animals. The back light condition (back light; 0.2 Watt green diodes) was provided underneath the observation cage to enhance visual recognition of the specimens.

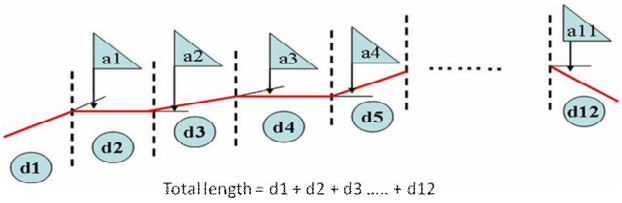

Fig. 2. Schematic diagram of *Caenorhabditis elegans* body is divided into twelve sub-segments (d1through d12) and the angles (a1through, a2).

Considering the complexity in calculating the line movement of long animals (Baek *et al.*, 2002; Geng *et al.*, 2003; Stephens *et al.*, 2008), we intended to demonstrate the structural changes originated from segments (Fig. 2). The parameters such as speed, acceleration, stop duration, stop number, turning rate and meander were measured

for each point by using MATLAB (version 7.0.1.). The lines presenting the body of the specimens were extracted based on subtraction from the background image and skeletonizing, The head and tail of the line body were recognized according to the activity and movement sequences identified (Baek *et al*., 2002; Geng *et al*., 2003; Stephens *et al*., 2008). After extraction of the line body shape, the line was evenly divided into twelve segments and, points were assigned from 1 to 13 from head to tail (Fig. 2). The length of each segment and the curvatures between the neighboring segments were recorded at each time interval.

RESULTS

To analyzing of the sequential line-movements of *C. elegans*, six parameters on each 13 points were check. The average values of speed (Fig. 3 A-B), acceleration (Fig. 3 C-D), stop time (Fig. 3E-F), stop duration (Fig. 3G-H), turning rate (Fig. 3I-J), and meander (Fig. 3K-L), were compared with control and treatment at 0.1 and 1 mg/l of formaldehyde.

At the higher concentration of formaldehyde (1 mg/l), the average values of six parameters were overall higher than that of lower concentration (0.1 mg/l), except speed and acceleration. At 1 mg/l formaldehyde, the effect of chemical treatments appeared to be more toxic response behaviour from *C. elegans*. All variables were clearly different at all points of the body among all treatments. However the average values of speed, acceleration, stop duration stop number; and meander differed more clearly at all points along the body before and after treatment with 1 mg/l formaldehyde.

Speed and acceleration decreased in a higher degree at end of the body after the treatments with 1 mg/l formaldehyde, while stop number and meander increased at the points located in the center of the body. This indicated that body movement was changed after the chemical treatments; more curvature occurred in the center along with higher speed at the end. In exposure to formaldehyde at 0.1 mg/l, this type of partial difference was also observed, although the degree of difference was not as great as in 1 mg/l formaldehyde.

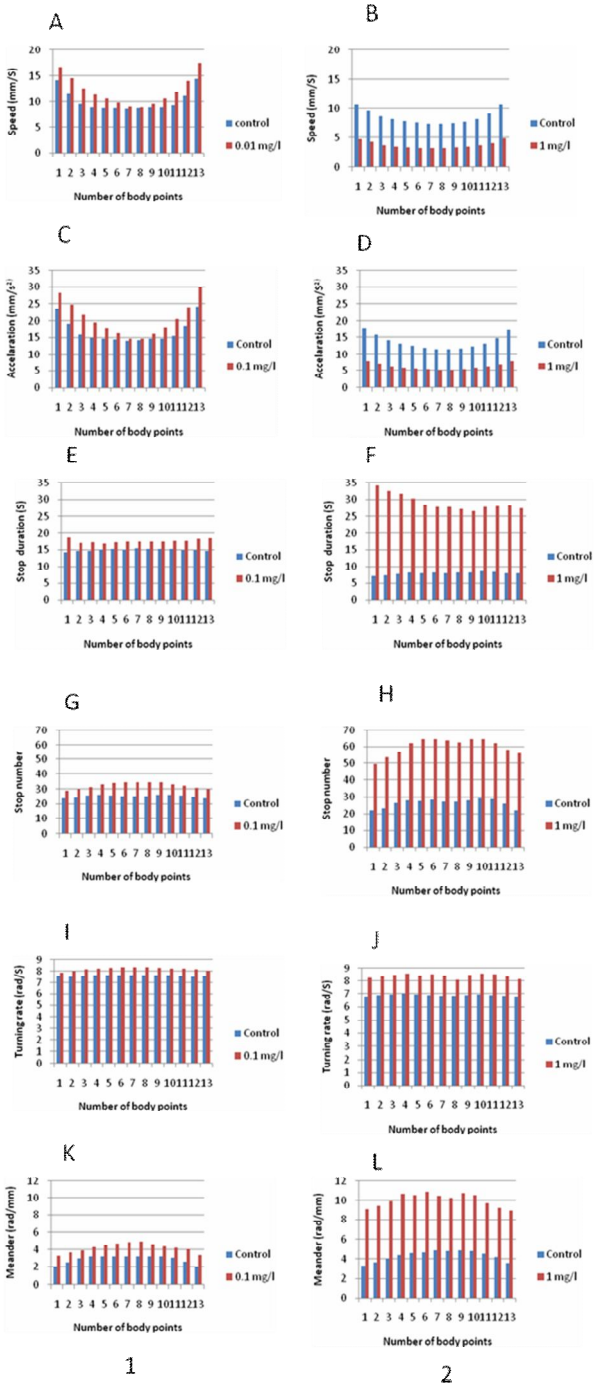

Fig. 3. Comparison of line tracking parameters of *C. elegans* treated with formaldehyde at 0.1 mg/l formaldehyde (1) and 1 mg/l formaldehyde (2); A-B, speed; C-D, acceleration; E-F, stop duration; G-H, stop number; I-J, turning rate and K-L, meander.

Angle values for control and treatments were almost smaller at the centre of the body (Fig. 4). All angles, a1~a11, were matched with Figure 2. But after treatment of formaldehyde at 1 mg/l, the values of angles were relatively increased in the center position. The angle values at 1 mg/l formaldehyde were different with other groups at all points. Although the angle values in 0.1 mg/l formaldehyde 0.1 were also different at different points, the discrepancies between the experiments were relatively higher in the center positions. This indicated that the body of the treated nematode tended to contract and fold strongly after the treatments with 1 mg/l formaldehyde.

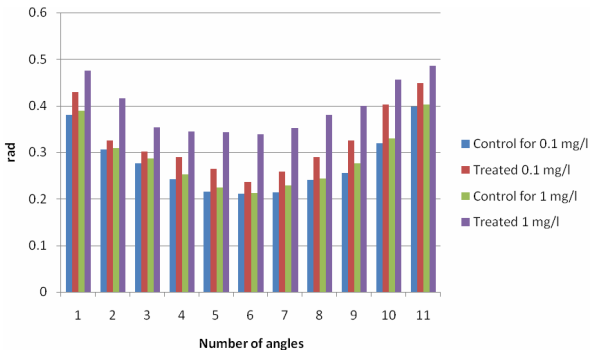

Fig. 4. Comparison of angles of the sub-segments of *C. elegans* treated with formaldehyde before and after the treatments.

DISCUSSION

Sequential line-movements of *C. elegans* were used to detect toxic response behaviour. Movement parameter and body shapes were useful for quantifying the response behaviour. In the case of line-movement parameters, we noted the partial differentiations between the control group and those formaldehyde treated, while all parameters increased little after treatment of formaldehyde except speed and acceleration in 1 mg/l formaldehyde. Speed and acceleration decreasing directly after treatment of 1 mg/l formaldehyde indicates a toxic effect on locomotive behaviour. The values of speed and acceleration decreased due to the toxic effect of formaldehyde and thus the movement pattern of *C. elegans* show greater toxic response to 1 mg/l formaldehyde.

The angles of the body segments showed more significant differences than movement parameters. Angle values were comparatively higher in the middle part of body with 1 mg/l formaldehyde treatment, especially at a2, a5, a6, a7, a8, a9 and a10. This indicated that the body of the treated nematode tended to contract and fold strongly after the treatments with 1mg/l.

C. elegans has been shown to be a suitable test organism for ecotoxicological assessments of sediments and soils (Hoss *et al*., 2001). Researchers reported that this animal has several locomotives behaviour such as rapid withdrawal, crawling, body reversal and helical swimming. These are stereotyped behaviour that can be used for sub-lethal toxicology (Rogge and Drewes, 1993; Ding *et al*., 2001). This study has identified some sequences of line-movements of the nematode that confirmed the toxicological effect on the nematode's behaviour.

CONCLUSIONS

Behaviour has been extensively used as an alternative tool for environmental risk assessment. A number of studies have used behavioural monitoring of *C. elegans* to assess toxicity. The present work focused on evaluating the toxicity of formaldehyde on the locomotive behaviour of this nematode. The speed and acceleration decreased due to the toxicity of 1 mg/l formaldehyde, and higher values of angles in the center points of the body indicated strong curvature or sharp body movement. The method of analysis used in this study would be an efficient tool for identifying response behaviour patterns of indicator specimens and could be used as an *in-situ* real-time monitoring device for toxicological study in future.

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