TUBIFEX TUBIFEX MÜLL. – PHOTOSENSITIVE ORGANISM

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SUMMARY

The worm *Tubifex tubifex* Müll. (Tubificideae, Oligochaeta) is a suitable organism for the research of the biological effect of various pollutants. This pilot study deals with the responds of the organism to the treatments of two photosensitizers (bengal rose B, quinidine) and UVA radiation. The activity of the photosensitizers was evaluated by the comparison of the surfaces of tested worms and dark controls. The results showed that *T. tubifex* Müll. could be a suitable organism for the studies of phototoxicity. This species demonstrated relatively strong sensitivity to the effect of the selected photodynamically active substances.

Key words: Tubifex tubifex, photosensitizers, phototoxicity, scanning electron microscopy

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INTRODUCTION

Tubifex tubifex Müll.(Tubificideae) is a widely distributed freshwater Oligochaete. Its nutrients are various bacteria and other microorganisms of benthos (1). Oligochaete worms are bilaterally symmetrical segmented coelomates with bundles of chaetae on every segment except the first (prostomium) (2). The length of the worm is 20 – 85 mm (about 130 segments). The body wall consists usually of five layers. An outer, non-cellular colourless cuticule overlies the epidermis which is composed of one layer of cuboidal to columnar cells of various types. Beneath the epidermis there are two layers of muscles which are bounded internally by a thin peritoneal epithelium. Alimentary canal consists of an anterior, ventrally opening mouth cavity composed of very thin epithelium, a pharynx, and a simple tubular hindgut that extends trough the length of the body and terminates in a posterior ventral anus. Vascular system is formed with dorsally large pulsating blood vessel, which runs through the entire length of the body, and other vessels. Blood flows in the dorsal vessel anteriorly, in the ventral vessel posteriorly, dorsally through the alimentary plexus and ventrally through the connectives. Blood is red because it contains dissolved haemoglobin (2). The exchange of gases between the organism and water is performed by diffusion through the epidermis. In hypoxic environment *T. tubifex* Müll. can survive about 48 hours.

This species has been commonly used for various water pollution testing especially for effects of some metal compounds (3-5). QSAR analysis was carried out for some predictions of acute toxicity of alcohols (6) and the correlation of the acute toxicity with a partition coefficient of these chemicals between n-octanol and water was also investigated (7). *T. tubifex* Müll. was found to be useful as a sensitive alternate model for studying UVB induced

phototoxicity (8, 9), possible mechanisms of its action and also the effect of some photosensitizers (10).

The aim of this study is to assess the effect of two well-known phototoxins and UVA radiation on *T. tubifex* Müll. The structures of the photosensitizers are very different (Fig. 1). Their molecules contain conjugated multiple bonds or aromatic system. Bengal rose B is a derivate of xanthene which is used for pigmentation of histological preparations. Isoquinolic alkaloid quinidine is current in cardiology. Its source is the bark of the tree *Cinchona succirubra* Pav. (Rubiaceae).

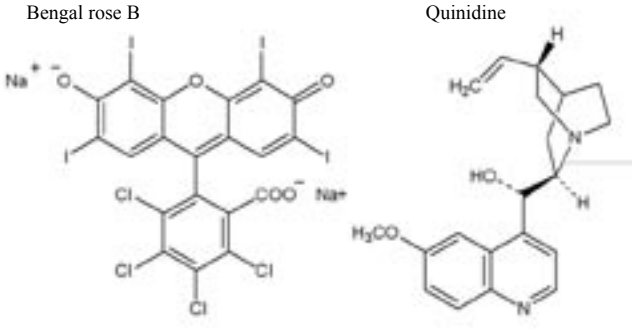

Fig. 1. *The structures of the photosensitizers.*

MATERIAL AND METHODS

Chemicals

Bengal rose B, quinidine and $MnCl_2.4H_2O$ were purchased from FLUKA & Riedel-de Haën Comp. The chemicals were puriss. p.a. quality at least.

Experimental Animals

Worms of species *Tubifex tubifex*. Müll were reared in the aquarium with a 4 cm layer of sand, and 8 cm overlaying fresh water (the concentration of dissolved $O_2 = 8$ mg.l^{-1} at least, pH = 7.5 ± 0.1). The aquarium was gently aerated, maintained on a 10:14 h light dark cycle and at ambient room temperature (20 ± 2 °C). The animals tested were not fed and they were kept in dark and constant temperature 20 ± 0.1 °C 24 hours before beginning the experiment.

Procedures of the Experiment

The aqueous solutions or microcrystalline suspensions of bengal rose B or quinidine were prepared by sonication for 15 min in an ultrasonic bath. The Tubifex experiment was arranged according to Tichý M. et al. (11). 5 concentrations (dilution coefficient = 1/2) were tested with 4 repetitions at least. Every experiment was repeated three times at least. The organism was irradiated 30 min by UV 365 nm, 0.3 mW.cm^{-2} radiant flow density. The control set of worms was kept in dark. The results were obtained immediately after the end of the irradiation. After stereomicroscopic evaluation of the number of damaged organisms (e.g. destruction of epidermis) the worms were fixed for further examination by scanning electron microscopy. The organisms were fixed by a standard process. The negative control was fresh water. The sensitivity of the organism was specified by the solution of $MnCl_2$. The mortality was determined after 1 h.

RESULTS AND DISCUSSION

Interpretation of the results was based not just on the values of EC_{50} (Table 1) but also on the evaluation of morphological changes of the worm *Tubifex tubifex* Müll. body surface by scan microscopy (Fig. 2-5).

However differences between the non-irradiated and irradiated worm are minimal, without the presence of the phototoxins the epidermal structures of the irradiated animal corrugate more distinctly. It is generally assumed that this species is very sensitive to the effect of UVA radiation.

Bengal rose B caused no direct destruction of the non-irradiated epidermal structures but deformations of the body shape are visible. On the other hand, the deep ruptures of the epidermis and total deformation of the cephalic part become patent after exposing to this photosensitizer and UVA irradiation.

Table 1. *EC_{50} values (95% Confidence Intervals) of phototoxic activity (bengal rose B, quinidine) and standard toxin ($MnCl_2$)*

Toxins	Time of irradiation	EC_{50} [mmol/l] (95% Confidence Interval)
Bengal rose B	0 min	0.72 (0.50-1.03)
	30 min	0.31 (0.22-0.46)
Quinidine	0 min	1.88 (0.79-2.23)
	30 min	1.25 (0.71-2.19)
$MnCl_2$ (mortality)	0 min	68.04 (56.20-82.37)

EC_{50} is such concentration of the phototoxins that causes the damage at 50% of the worms.

Quinidine brings about more distinct surface body corrugation of the non-irradiated animal, but the entirety of the epidermis is saved. The irradiated worm shows marked differences of its surface compared to the control animal. Natural corrugation of its body disappears and its cephalic part exhibites total destruction of the epidermal structures.

The EC_{50} values of the toxicity of bengal rose B are higher compared to EC_{50} values of quinidine effect in both cases – the activities independent and also dependent on UVA radiation, but 95% confidence intervals are relatively broad. This problem is necessary to be dissolved by more experiments and their repetitions and more tested concentrations of the substances. The EC_{50} values of the phototoxicity of bengal rose B are twice more potent at least in comparison to the dark control. The phototoxic activity of quinidine is not too evident in comparison to EC_{50} values of the irradiated animals and dark control.

CONCLUSION

It is a safe assumption that this organism could be suitable for the scanning microscopy studies of the activity of photosensitizers. Since this species demonstrated relatively strong sensitivity to the effect of the selected photodynamically active substances it might

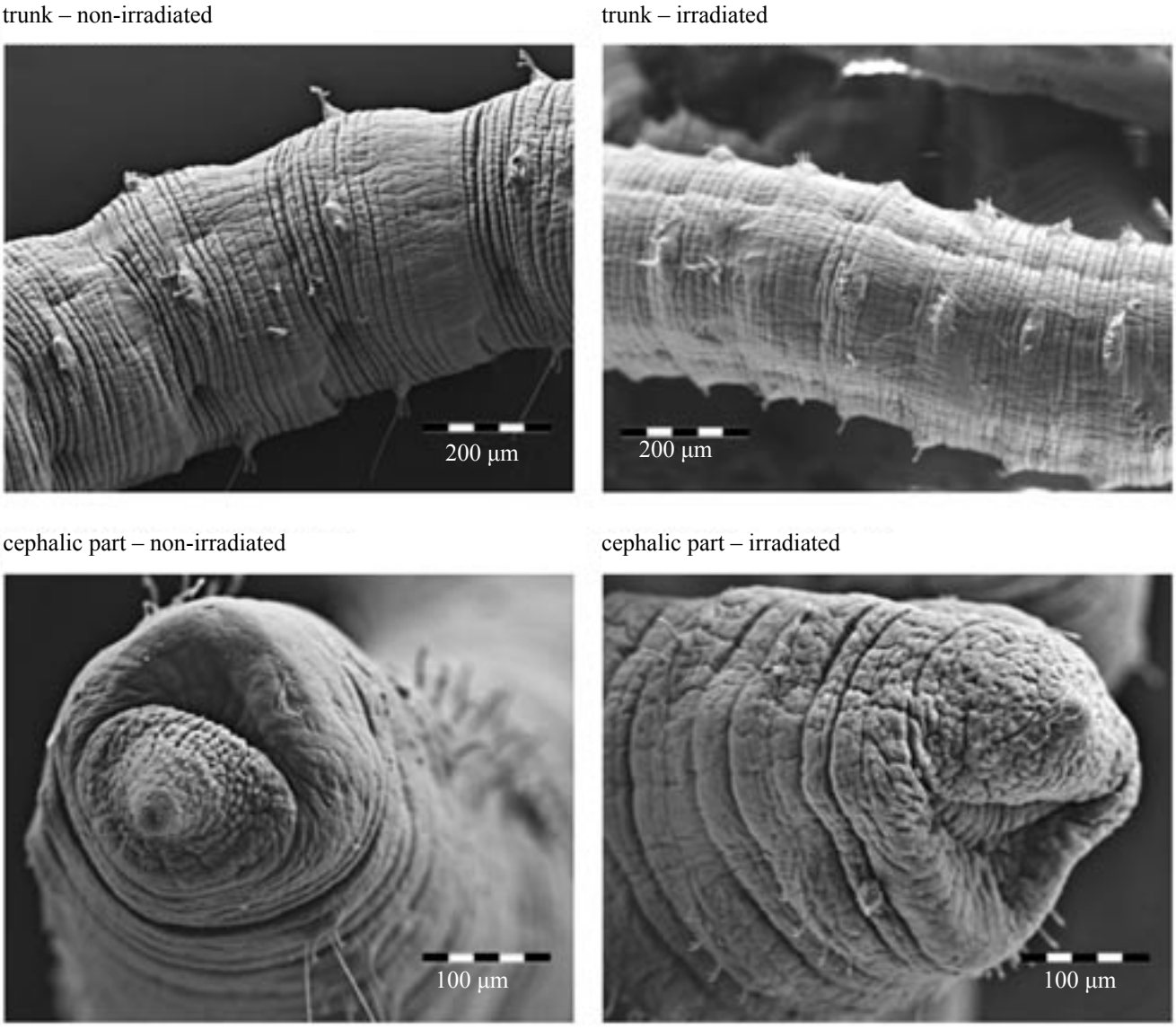

Fig. 2. *Changes of the T. tubifex body surface after 30 min of the UVA irradiation.*

bridge a gap of the phototoxicity research after standardisation of the experimental conditions.

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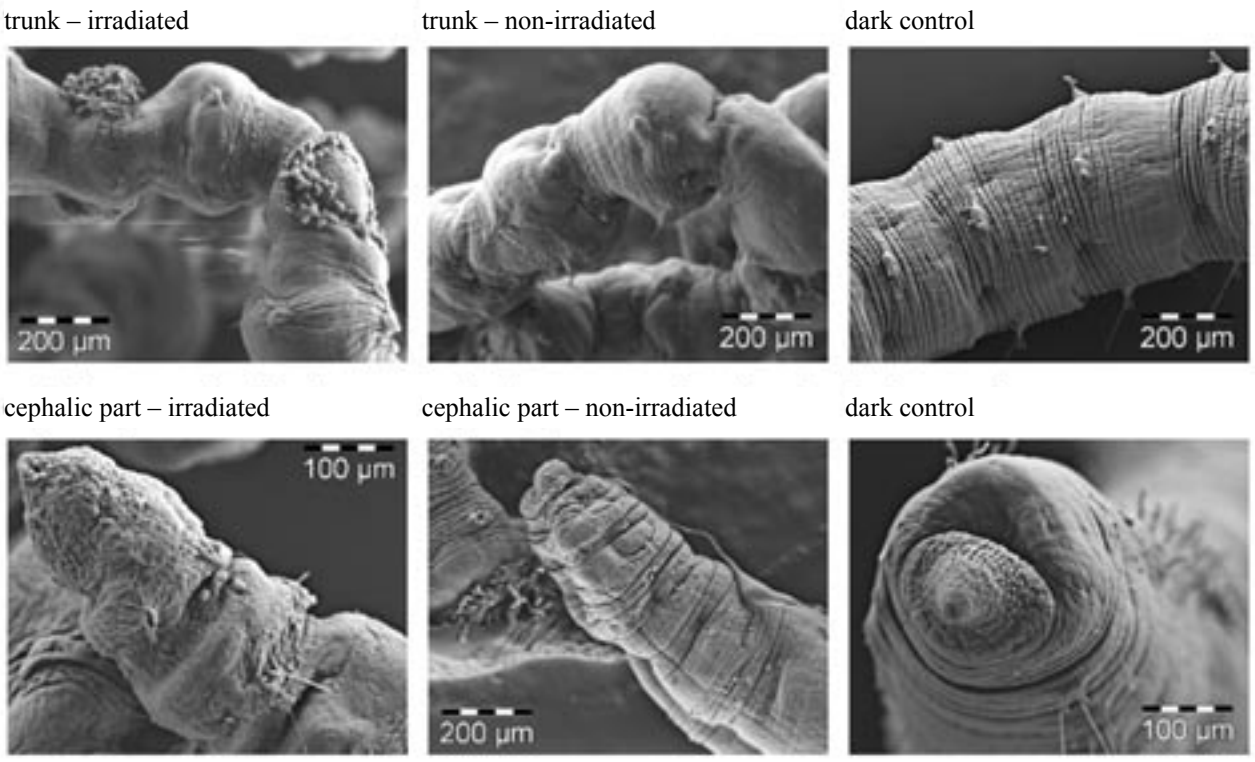

Fig. 3. *The effect of bengal rose B and UVA radiation on T. tubifex.*

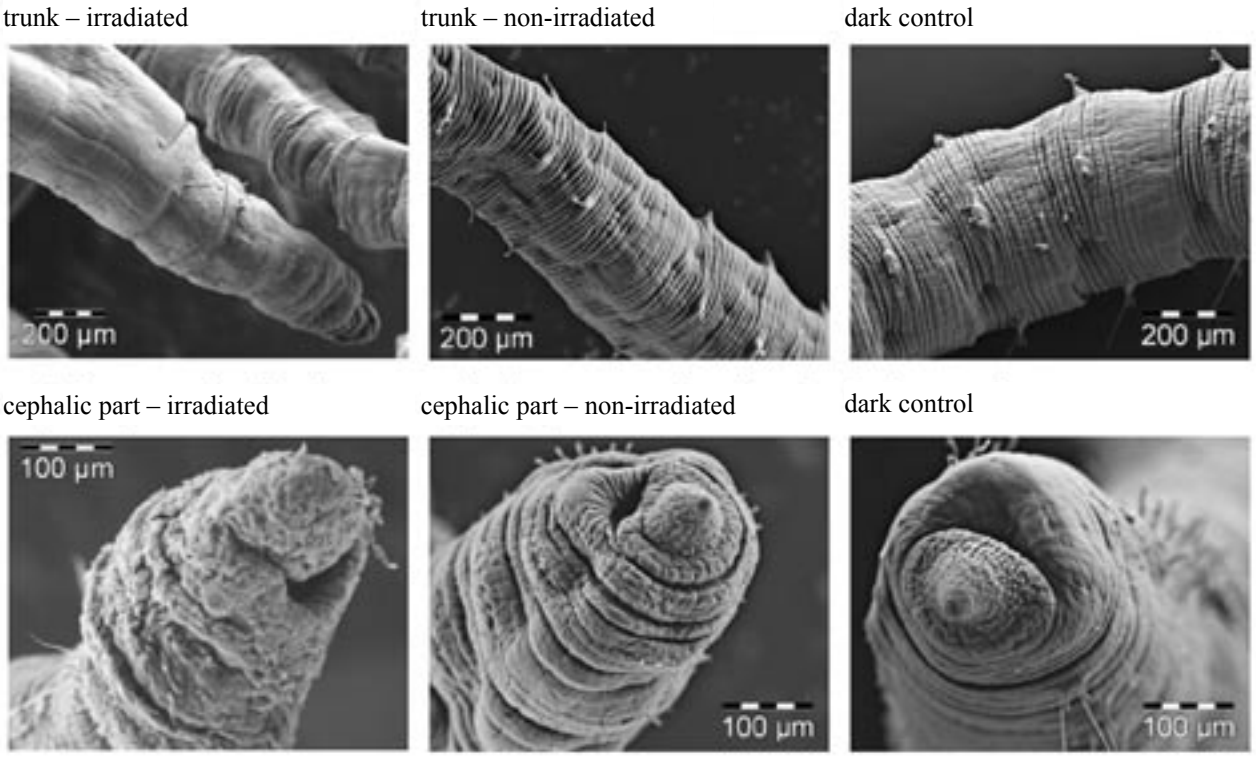

Fig. 4. *The effect of quinidine and UVA radiation on T. tubifex.*

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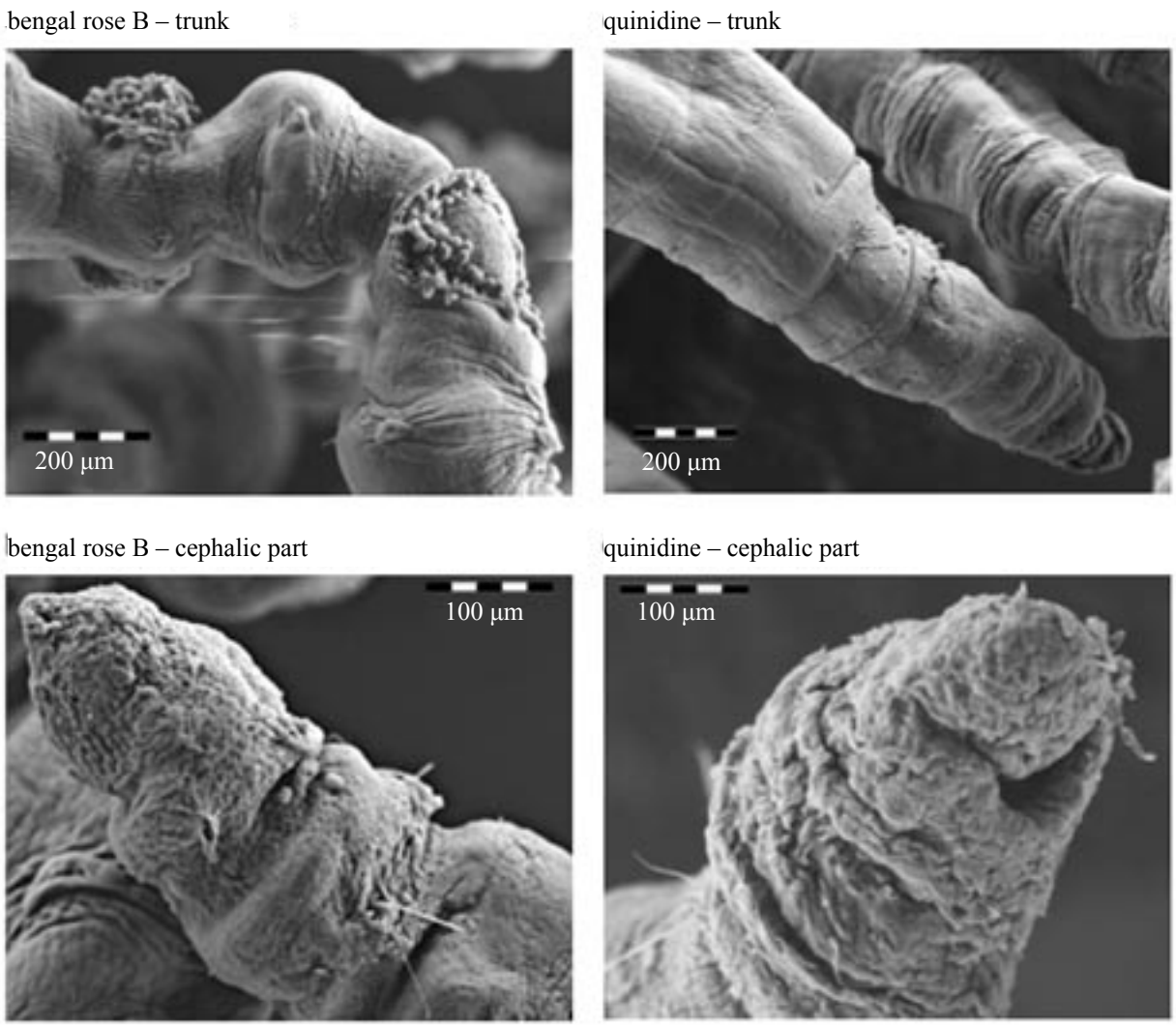

Fig. 5. *Toxic effect of bengal rose B and quinidine independent of UVA radiation.*