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EFFECT OF ULTRA VIOLET IRRADIATION AND CHROMOSOMAL ABERRATIONS IN *EUDRILUS EUGENIAE*

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ABSTRACT
Earthworms are generally coined as farmer's friend as they have a great role to play in enhancing soil quality. *Eudrilus eugeniae* was mass cultured in the prepared vermibed for cocoon production. The cocoons were collected, maintained in plastic containers and exposed to UV light at different time intervals viz., 5, 10 and 15 minutes. A batch of 10 cocoons was maintained as control. The cocoons were preweighed before UV exposure. The average number of hatchlings from UV exposed samples was 1.6, 2.8 and 3.1 respectively but it was 5.4 in the control. The biomass of juveniles in the control and experimental samples on 14th day did not exhibit much variation but on the 28th day there was a loss of weight of 1.433, 1.253, 1.264 respectively on 5, 10, 15 minutes of UV exposure. Adult clitellated worm developed from UV exposed cocoon and that of the control were collected and their impact was studied by chromosome spread. Interpretation of the morphometric data of the chromosome is carried out. The total and relative length of the chromosome in *Eudrilus eugeniae* indicated that there exists an aberration in the chromosome due to UV irradiation. The cytogenetic studies on the earthworm paves way for genetic upgradation which results in new morphs, with different genetic makeup which ultimately fetches more profit to vermibiotechnologist and also to the economy of the country.

KEYWORDS: *Eudrilus eugeniae*, Cocoons, UV irradiation, Chromosomal aberrations, Cytogenetics.

INTRODUCTION

Earthworms play a vital role in the soil environment where they contribute to the complex process of decomposition while affecting aeration, water transport and soil structure. (Van Hook 1974) concluded that earthworms could serve as useful biological indicators of contamination. Earthworm biomass containing higher content of protein can serve as feed for live stocks (Sabine, 1983). *Eudrilus eugeniae* has become widely distributed around the warmer parts of the world and is called as African night crawler. The worm appears dark pink in color. The length of the body ranges from 8-12cm with 145-196 segments with the incubation period for the cocoon ranging from 16-17days. Cytogenetics relies on the preparation of karyotype where individual chromosome can accurately be identified. A karyotype is the characterization and analysis of a chromosome complement at metaphase within the nucleus of given species (Blaxhall, 1983). The result of this study will provide information for the establishment of gene banks by which genetic potential of economically valuable and endangered species can be conserved for future generation.

MATERIALS AND METHODS

Culture of *Eudrilus eugeniae*

Eudrilus eugeniae was mass cultured in two rectangular plastic troughs of (12"x17"x5" size), where 3Kg of cow dung served as feed for the earthworms. Fifteen adult clitellated worms were introduced manually into the trough, which was watered every day to maintain the moisture content.

Preparation of vermi beds for cocoons

The cow dung was used as a bedding material which was procured form nearby locality, dried, sieved and watered for acclimatization of the earthworms with regard to the animal palatability.

Collection and maintenance of cocoon

The collected cocoons were exposed to UV light in a batch of 10 numbers at different time intervals (5, 10 and 15 minutes) in plastic boxes with cow dung as the medium.
Ten cocoons were maintained as control. In a couple of weeks, the cocoons got hatched and the emerged juveniles were carefully counted. The juveniles were introduced into separate four rectangular troughs with cow dung and weighed on 14th and 28th days respectively and the readings were tabulated.

Chromosome preparation

The adult clitellated earthworms were injected intramuscularly with 0.03-0.05% of colchicine solution at a dosage of 0.1 unit which was left for 3-5hrs in the medium for the arrest of mitotically dividing cell at the subsequent stage. Active tissue and parts such as spermatheca, ovary and tail were removed, mixed and placed in 1% hypotonic solution (KCl). The material was fixed in Cornoy's fixative (3:1 methanol: acetic acid) for the duration of 30 min and centrifuged. This process was repeated until clean transparent cell suspension was obtained.
The cell suspension was incubated in a refrigerator at 0° C over night. The fixed cells were softened in 50% acetic acid for 2 min. The cell suspension was dropped on a clean glass slide and air dried. This technique was recommended by (Crozier, 1968). Later air dried slide

was kept in 5% Giemsa stain and washed in running water and examined under microscope.

RESULTS AND DISCUSSION

Biomass of cocoons before UV exposure

Freshly laid cocoons of forty in numbers were collected and weighed (Table 1). The availability of sufficient food is of prime importance for maintaining a high production rate (Reinecke *et al*., 1999). Earthworm fed on any type of Nitrogen rich diets grow faster and produce more cocoons (Evans and Guild, 1948).

TABLE 1. Average weight of the cocoon of *Eudrilus eugeniae* before UV exposure

S. No	Treatments	Average weight of cocoons (gm)
1	Control	0.202
2	5 min	0.196
3	10 min	0.249
4	15 min	0.218

Number of hatchlings per cocoon

The mean of hatchlings per cocoon exposed to UV at different period of exposures (5, 10 and 15min) and for the control is tabulated in Table 2. The control and UV exposed cocoons started to hatch from 14th day onwards. The control cocoons showed a maximum number of hatchlings of 5.4. Generally in most earthworm species, only one juvenile successfully hatched from each cocoon (Edwards and Lofty, 1977). The number of hatchlings per cocoon varied from 1-7 with an average of 3.9 in diary waste sludge cake (Hatanaka *et al.,* 1983). The number of hatchlings were decreased with the increasing concentration of paper mill sludge(Umamaheswari,2003).

TABLE 2. Average number of hatchling per cocoon of *Eudrilus eugeniae*

S. No	Treatments	No of hatchling per cocoon
1	Control	5.4 ± 2.90
2	5 min	1.6 ± 1.0
3	10 min	2.8 ± 1.32
4	15 min	3.1 ±1.7

Results represents mean±SD of replicates

Biomass of juveniles of the experimental cocoons

The weight of the juveniles in the control and UV exposed troughs are recorded in Table 3. On the 14th day, not much variation with regard to the weight of the hatchlings in the two samples was observed but on the 28th day, there was a loss of weight in the hatchlings obtained from the 10 and 15 minutes UV exposed cocoons on comparison to the control. Maborta *et al*., (1999) found the presence of lead to have an inhibitory effect on the growth of earthworm *Eudrilus eugeniae.*

TABLE 3. Weight gain of juveniles of *Eudrilus eugeniae* after UV exposure

Exposure of	Weight of juveniles (gm)	
UV (min)	After 14th day	After 28th day
0	0.471±0.017	1.382±0.026
5	0.489± 0.015	1.433± 0.046
10	0.464± 0.012	1.253± 0.038
15	0.529± 0.011	1.264± 0.033
F	1.84^{NS}	0.54^{NS}

Results represent mean ± SD of replicates, NS – Not significant

Chromosomal study

Colchicine treatment and hypotonic treatment

In the present study, colchicine concentration ranging from 0.05% to 0.07% were injected intramuscularly and left for 3-5 hrs. Table 4 indicates best result obtained from 0.05% colchicine solution and the two hypotonising agents like potassium chloride and trisodium acetate with a concentration ranging from 0.9% to 1.5% used for a period of 2 hrs. Better results were obtained at the dosage of 1% potassium chloride solution (Table 4).

About 15 metaphase spreads of the control *Eudrilus eugeniae* were observed for the karyotype study. In Giemsa stained metaphases, the karyotype consisting of 4 subtelocentric and 7 telocentric pairs were observed 4ST+7T, NF=30.

About 10 metaphase spreads of *Eudrilus eugeniae* exposed to UV for 5 min were observed in which the karyotypes consisting of 3 subtelocentric and 8 telocentric pairs were observed in the Giemsa stained metaphases and the following chromosome formula was established 3ST+8T,NF=30. Bakhtadze; *et al.,* (2008) stated that *Eisenia fetida* have the diploid chromosome (2n=11).

TABLE 4. Effect of concentration and method of Colchicine and Hypotonic treatment on quality of chromosome spread in *Eudrilus eugeniae*

Solution used	Concentration (%)	Quality of spread
Colchicine	0.05	Good
	0.06	Better
	0.07	More overlapped
Hypotonic solution	0.9	Poor
(KCl)	1.0	Good
	1.5	Overlapped

TABLE: 5 Morphometric chromosome of *Eudrilus eugeniae* Control

	Length of chromosome			AL	AL x	LA / SA	100 +1/ R
Pair	LA	SA	TL		1.521RL %	A / R	CL %
1	1.5	0.2	1.7	0.85	1.29	7.50	11.76
2	1.0	0.3	1.3	0.65	0.98	3.30	23.07
3	0.9	0.5	1.4	0.70	1.06	1.80	35.71
4	1.0	0.3	1.3	0.65	0.98	3.30	23.07
5	0.8	0.3	1.1	0.55	0.83	2.60	27.27
6	0.8	0.4	1.2	0.60	0.91	2.00	33.33
7	1.1	0.5	1.6	0.80	1.21	2.20	31.25
8	0.7	0.4	1.1	0.55	0.83	1.75	36.36
9	0.2	_	0.2	0.10	0.15	_	_
10	0.4	_	0.4	0.20	0.30	_	_
11	0.5	_	0.5	0.25	0.38	_	_
12	0.4	_	0.4	0.20	0.30	_	_
13	0.5	_	0.5	0.25	0.38	_	_
14	0.4	_	0.4	0.20	0.30	_	_
15	0.5	_	0.5	0.25	0.38	_	_
16	0.7	_	0.7	0.35	0.53	_	_
17	0.4	_	0.4	0.20	0.30	_	_
18	0.3	_	0.3	0.15	0.22	_	_
19	0.3	_	0.3	0.15	0.22	_	_
20	0.5	_	0.5	0.25	0.38	_	_
21	0.7	_	0.7	0.35	0.53	_	_
22	0.6	_	0.6	0.30	0.45	_	_

LA- long arm, SA-short arm, AL- arm length, RL- relative length, TL- total length.

TABLE 6: Morphometric chromosome of *Eudrilus eugeniae* (5min UV exposure)

	Length of chromosome			AL	AL x	LA / SA	100 +1/ R
Pair	LA	SA	TL		1.521RL %	A / R	CL %
1	0.4	0.2	0.6	0.30	0.45	2.0	33.33
2	0.5	0.3	0.8	0.40	0.60	1.6	37.50
3	0.3	0.2	0.5	0.25	0.38	1.5	40.00
4	0.4	0.3	0.7	0.35	0.53	1.3	42.85
5	0.3	0.2	0.5	0.25	0.38	1.5	40.00
6	0.2	0.2	0.4	0.20	0.30	1.0	50.00
7	0.4	0.2	0.6	0.30	0.45	2.0	33.33
8	0.4	0.2	0.6	0.30	0.45	2.0	33.33
9	0.4	_	0.4	0.20	0.30	_	_
10	0.3	_	0.3	0.15	0.22	_	_
11	0.3	_	0.3	0.15	0.22	_	_
12	0.3	_	0.3	0.15	0.22	_	_
13	0.3	_	0.3	0.15	0.22	_	_
14	0.3	_	0.3	0.15	0.22	_	_
15	0.3	_	0.3	0.15	0.22	_	_
16	0.3	_	0.3	0.15	0.22	_	_
17	0.3	_	0.3	0.15	0.22	_	_
18	0.4	_	0.4	0.20	0.30	_	_
19	0.4	_	0.4	0.20	0.30	_	_
20	0.3	_	0.3	0.20	0.30	_	_
21	0.4	_	0.4	0.20	0.30	_	_
22	0.5	_	0.5	0.25	0.38	_	_

LA- long arm, SA-short arm, AL- arm length, RL- relative length, TL- total length.

Likewise 20 metaphase spreads of *Eudrilus eugeniae* exposed to UV for 10 min were observed wherein Giemsa stained metaphase, karyotypes consisting of 3 subtelocentric and 8 telocentric pairs were observed 3ST+8T,NF=29.

Observance of 12 metaphase spreads of karyotypes of *Eudrilus eugeniae* on exposure to UV for 15 min indicated the karyotypes consisting of 2 subtelocentric and 9 telocentric pairs 2ST+9T,NF=27 in the Giemsa stained metaphase. The morphometric data of the chromosome is interpreted in the Table 5, 6, 7 and 8. The rapid development took place, as in cytogenetics of all animals in general, after including novel cytogenetic procedure that is colchicines inhibition of dividing cells of metaphase stage and hypotonic free treatment of cells for spreading the chromosome in early 60's (Roberts, 1967).

CONCLUSION

The karyotype study revealed that the chromosome spread of the control *Eudrilus eugeniae* differed from the other UV treated *Eudrilus eugeniae*. The mutated earthworm chromosomes rendered some changes in its chromosome due to UV mutation which clearly revealed that there prevails chromosome aberrations predicted in the karyotypic studies on the earthworm, *Eudrilus eugeniae*.

REFERENCES

Blaxhall, P.C. (1975) Fish chromosome technique - A review of selected literature. *J. fish. Bio.* **7**, 315-321.

Bakhtadze, N.G., R. Vitturi, N.G. Bakhtadze, G.I. Bakhtadze and E. Kvavadze (2008) The chromosome numbers of Georgian envs. (Oligochaeta: Lumbricidae). *Comp. cytogenetics.* **2**, 79- 83.

Crozier, R.H. (1986) An acetic acid dissociation air drying technique or insert chromosomes with acetolacto orcein staining. *Stain Tech*. **43**, 171.

Edwards, C.A. and J.R. Lofty (1977) Examines the earthworm through morphology, taxonomy, biology and look at its environmental role; in *Biology of earthworm.* Chapmen and Hall, London, pp. 333.

Evans, A.C. and W.J. Grild (1948) Studies on the relationship between environment and soil fertility IV on the life cycles of some Lumbricidae. *Ann. Appl. Biol*. **35,** 471 – 484.

Hatanaka, K., Y. Ishioka and E. Furuichi (1983) Cultivation of *E. fetida* using dairy waste sludge cake in J.E. Satchell (Ed): *Earthworm Ecology. Chapmen and Hall*, London, pp 323- 329.

Maborta, M.S. and S.A. Reinecke (1999) The effect of low lead levels on the growth and reproductions of the African earthworm, *E. eugeniae*. *Biol. Fert. Soils.* **30,** 113 – 116.

Reinecke, A.J. and M.S. Maboeta (1999) Cocoon production and viability as end points in toxicity testing of heavy metals with three earthworm species. *Pedobiologia.* **45,** 61-68.

Roberts, F.L. (1967) Chromosomal polymorphism in North American land clocked salon. *Solar. Can. J. Genet. Cytol.,* **10,** 865-875.

Sabine, J.R, (1983) Earthworms as a source of food and drys in earthworm ecology in J.E. Satchell (Ed) *Earthworm Ecology. Chapmen and Hall*, London, pp 285-296.

Umamaheswari. S. (2003) Ppaper mill sludge treatment through app of vermi and mycotechnology. Ph. D. Thesis, Manonmaniam Sundaranar University. Tirunelveli, India.

Van Hook. R.I. (1974) Cadmium, lead and zinc distribution between environment and soils: potentials for biological accumulation. *Bull. Environ. Contain toxicol*. **2,** 509-512.