Bull. Environ. Contam. Toxicol. (1992) 49:285–289

Environmental Contamination and Toxicology

Effects of Lindane on Growth of Cellular Slime Mold *Dictyostelium discoideum*

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The persistence of the pesticides in the soil renders them amenable to uptake by soil microorganisms. Dictyostelium discoideum, the cellular slime molds form an important constituent of soil ecosystem. Its growth and differentiation cycles are mutually exclusive and that offers an important advantage for studying the effects of pesticide on cell growth and differentiation (Bonner 1982). Lindane (γ - isomer of Hexachlorocyclohexane) is an extensively used insecticide in India and is used to control a wide range of agricultural pests (Atwal 1976). The purpose of the present work was to study the effects of lindane on the growth stage of D. discoideum.

MATERIALS AND METHODS

The axenic strain (Ax2) of D. discoideum obtained from Dr. Robert Kay (University of Cambridge, U.K.) was grown axenically at 23°C (Ashworth and Watts 1970).A 0.01gm stock solution of lindane (purchased from John Baker Inc., U.S.A.) was prepared in 10 ml of 50% acetone and the exponentially growing Dictyostelium cells were treated with different concentrations (60ppm, 80ppm and 100ppm). The controls recieved equivalent volume of solvent. Following the treatment of the cells with lindane for 20 min the cells ($1x10^6$ cells/ml) were put back into 10ml of axenic medium and the growth was monitored by haemocytometer counting.In another set of experiment,the growth of Dictyostelium cells were monitored in continuous presence of lindane (5ppm, 10ppm and 20ppm) in axenic medium. Controls received equivalent volume of the solvent.

The growth and behaviour of lindane treated cells were checked by putting the cells in multiwell test plates containing axenic medium and observed microscopically at regular intervals. The growth was also monitored by

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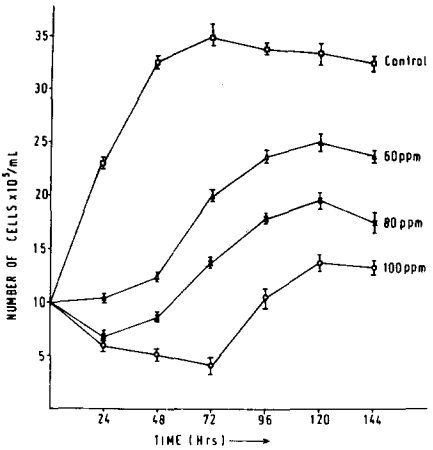

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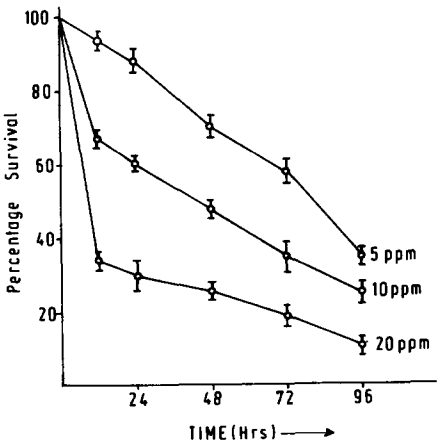

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Figure 1. Survival of Dictyostelium cells treated with lindane for 20 min in axenic medium

Figure 2. Survival of Dictyostelium cells in axenic medium containing lindane

plating the lindane treated cells on Escherichia coli seeded nutrient agar. When the plaques started to appear the colony blots were made and stained with Ponceau S Red.

Spores were collected and treated with different doses (20ppm, 60ppm, 80ppm and 100ppm) of lindane for 3 hours and then put back into 20ml of axenic medium, following which the germination and growth were recorded. All the experiments were repeated at least three times.

RESULTS AND DISCUSSION

Dictyostelium cells treated for 20 min with lower doses of lindane (60ppm) showed a slower growth rate as compared to control. Cells treated with higher doses of lindane (80ppm and 100ppm) showed an initial decline followed by a gradual increase (Fig.1). Cells treated with 100ppm showed approximately 50% survival.

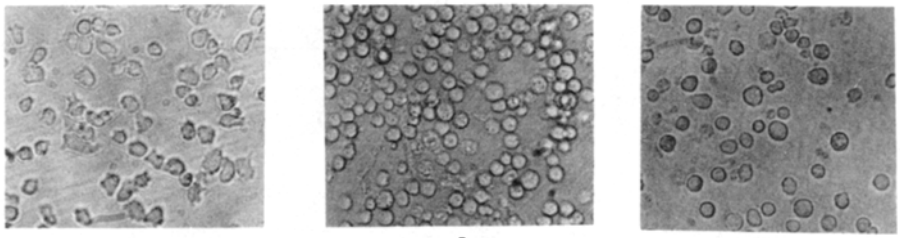

Figure 3.Cytomorphology of Dictyostelium cells in axenic subcultures at 48hrs (a) control, (b) cells treated for 20min with 100ppm lindane and (c) cells in continuous presence of 20ppm lindane

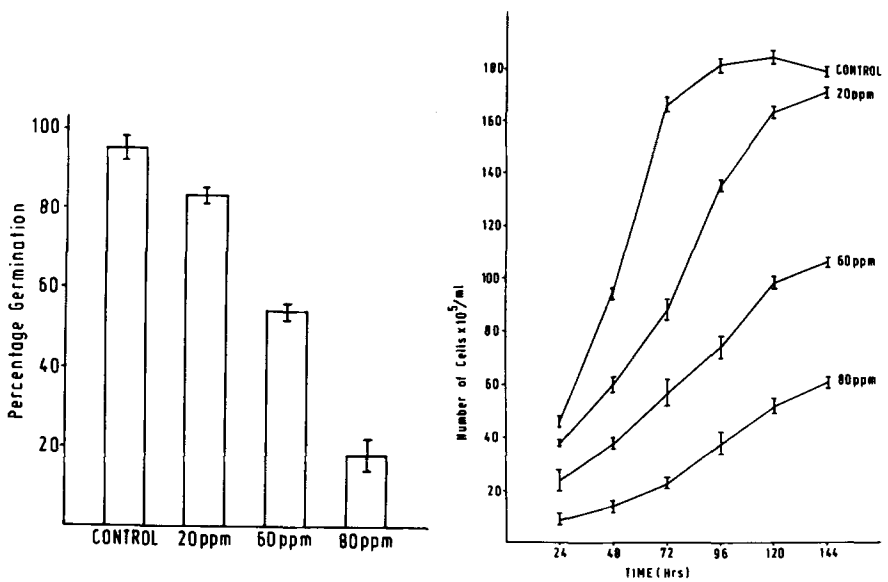

Figure 4. Germination of lindane treated spores (a) and the growth of germinated cells from the treated spores (b)

When the cells were cultured in continuous presence of lindane even the lower doses of lindane (5ppm, 10 ppm) were found to be lethal to the cells (Fig.2). The cells treated for 20 minutes with lindane (80ppm ,100ppm)

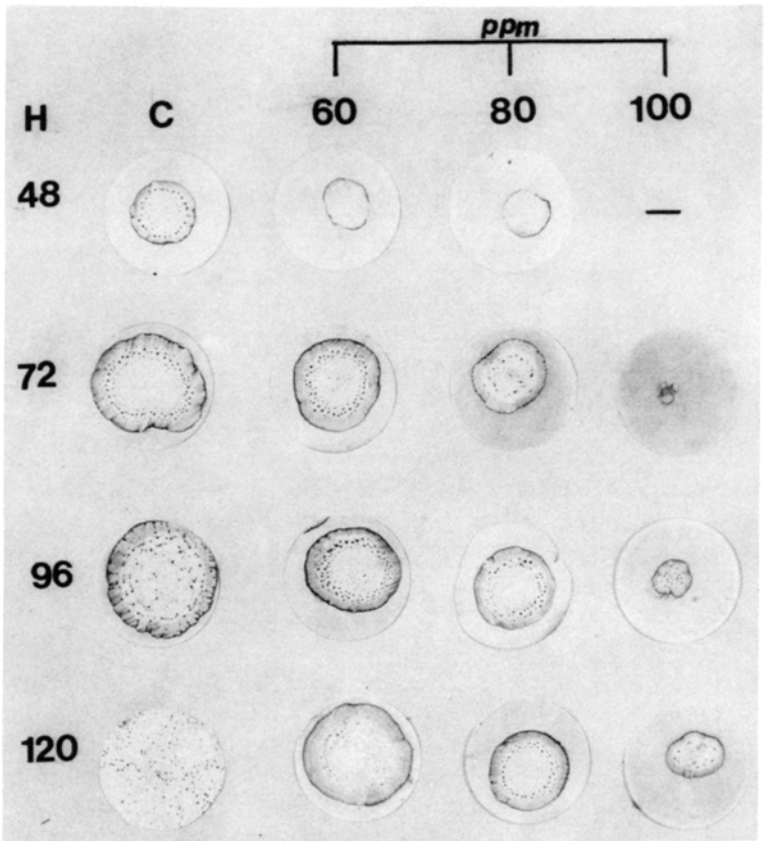

Figure 5.Colony blots of control (C) and lindane treated (ppm) Dictyostelium cells at different hours (H) from nutrient agar

were initially spherical (Fig.3b) and later recovered their amoeboid shape. The cells present in continuous presence of lindane (20ppm) remained spherical and loosely attached to substratum (Fig.3c)

Spore germination was totally inhibited when treated with 100ppm of lindane for 3 hrs, while there was approximately 50% germination in 60ppm treated spores (Fig.4a). Further the cells germinated from lindane treated spores have a slower growth rate (Fig.4b).

The severe effect of this pesticide on growth was also reflected when treated cells were placed on E. coli seeded nutrient agar. The treated cells took much longer time for plaque formation (control-24hrs, 100ppm -72hrs) and the plaque size was also smaller as compared to control (Fig.4).

Alteration of membrane structure due to lipophilic nature of this pesticide (Tripathy 1988) would affect the growth and cellular morphology. It has been reported that lindane affects the growth, cell shape and size of ciliated protozoan (Mathur and Saxena 1984; 1988), blue green algae and bacteria (Ray 1989).Amphidinium carteri treated with lindane showed decrease in number of microtubules (cited in Lal and Dhanraj 1987) which in turn might affect the cell duplication and cell morphology. Further, our unpublished observations have also shown the altered profiles of endocytic activities in lindane treated cells. In conclusion, it can be stated that exposure to lindane poses significant hazards on cellular growth of Dictyostelium discoideum.

Acknowledgment. One of us (RG) was supported by Council of Scientific and Industrial Research during the tenure of this work.

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Received September 5, 1991; accepted February 10, 1992.