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Effect of Endosulfan, Malathion, and Permethrin on Sexual Life Cycle of *Chlamydomonas reinhardtii*

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Biological activities of insecticides do not remain restricted to target organisms but also extend to non-target organisms which play an important role in the ecosystem. Algal cells constitute a major portion of nontarget organisms and thus, the understanding of likely effects of insecticides on these cells is of importance. For such studies, algal cells of Chlamydomonas reinhardtii can be used as a model system as the laboratory conditions for the sexual and asexual growth of this organism are well established (Hudock 1976; Levin and Ebersold 1960). Results from this paper provide information on output of gametes and formation, maturation and meiotic division of zygotes of Chlamydomonas reinhardtii following single treatment of synthetic insecticide (endosulfan, malathion or permethrin) to both or one of the two opposite mating types at one of the four developmental stages in sexual life cycle. Reports concerning possible effects of insecticides on sexual propogation of algal cells are few in literature (Cain and Cain 1984).

MATERIALS AND METHODS

Cell cultures of Chlamydomonas reinhardtii mating type 90 (mt^+, female) and 89 (mt^-, male) were obtained from Dr. Elizabeth Harris, Chlamydomonas Genetic Centre, Department of Botany, Duke University, Durham, NC 27706, U.S.A. mt^+ and mt^- cells were grown in yeast extract-acetate medium in continuous light (3750 lux) for 96 hr at 25°C (Sueoka 1960). mt^+ and mt^- gametes were obtained by suspending the respective vegetative cells in nitrogen deficient medium for 18 hr in light (Chiang et al. 1970). Conditions for fusion of mt^+ and mt^- gametes to form young zygotes, maturation of young zygotes, meiotic division of mature zygotes and formation of visible colonies were followed as described by Sager and Granick (1954) and Levin and Ebersold (1960). The efficiencies of gamete and zygote formations were found to be in the range of 99 to 100 per cent (Martin and Goodenough 1975). Vegetative cells, gametes and zygotes in suspension were counted by haemocytometer. Maturation of young zygotes on plates was monitored microscopically by using two parameters; (i) enlargement in size (Levin and Ebersold 1960) and (ii) change in colour from

green to pale orange (Smith 1976). Disappearance of the nucleolus in mature zygote (Haskell 1960), one of the specific criteria of the prophage - the first stage of meiosis, was selected to ascertain the commencement of meiosis. Presence or disappearance of the nucleolus in mature zygotes was determined microscopically after staining of the zygotes with crystal violet, a known nucleolus specific stain (Haskell 1960).

Endosulfan and malathion were obtained from Excel Industries, Bombay, India, and permethrin was acquired from The Alkali Chemical Corporation (India) Limited, Calcutta, India. The concentration of each insecticide expressed in the experiment is based upon its purity (around 94 per cent). Solutions of endosulfan and malathion were prepared in dimethyl sulfoxide (DMSO, AR grade, Sigma Chemical Company, U.S.A.). Solution of permethrin was prepared in absolute ethyl alcohol. In all experiments, the concentration of these solvents in the growth medium was maintained at or less than 2 per cent. In a separate set of experiments, it was confirmed that DMSO and ethyl alcohol at the concentration of 2 per cent in the medium do not affect the sexual life cycle of Chlamydomonas cells.

Endosulfan, malathion or permethrin treatment to cells was carried out at 0.25×10^{-4} M, 1×10^{-3} M and 1×10^{-3} M concentration, respectively. The presence of each insecticide at the respective concentration in the growth medium inhibits the vegetative growth (mitotic cell division, growth measured at 72 hr) of Chlamydomonas cells completely (results not included). Cells treated for 2 hr with endosulfan (0.25×10^{-4} M) or malathion (1×10^{-3} M) show 50 per cent inhibition in vegetative growth (measured at 72 hr after inoculation of the treated cells), whereas, those treated with permethrin, for a period as long as 72 hr, exhibit the growth similar to that of untreated cells (results not included).

Treatment of endosulfan (0.25×10^{-4} M), malathion (1×10^{-3} M) or permethrin (1×10^{-3} M) for 2 hr period was accorded to opposite mating types (both, or either one of the two) at one of the four developmental stages [vegetative stage, after gametogenesis (gametes), during the fusion of mt^+ and mt^- gametes and immediately after the fusion of gametes (young zygotes)] in sexual life cycle. As the treatment of endosulfan (0.25×10^{-4} M, 2 hr) resulted in loss of population of vegetative cells, gametes of opposite mating types, and young zygotes (45, 45 and 20 per cent, respectively), the respective segment of population sustaining the treatment was used in the experiments. However, the treatment of these insecticides accorded during the fusion of mt^+ and mt^- gametes did not affect the level of zygote formation. Treatment (2 hr) of malathion or permethrin to vegetative cells, gametes and young zygotes did not show loss in respective population.

RESULTS AND DISCUSSION

It is discernible from data given in table 1, 2 and 3 that treatment of endosulfan, malathion or permethrin to both or either one

of the opposite mating types, before (vegetative cells) and after gametogenesis (gametes), and during the fusion of gametes, does not affect the levels of gamete and zygote formations. This would imply that prior treatment of these insecticides to male (mt^-) and female (mt^+) cells may not influence the following biochemical developmental processes occurring during gametogenesis and fusion of mt^+ and mt^- gametes: degradation of ribosomal RNA (Wiese 1976), extension in flagellar length (Ensing et al. 1983), division of gametes (Martin and Goodenough 1975), synthesis of glycoproteins required for recognition of opposite mating types for pair formation (Martin and Goodenough 1975), induction of lytic enzymes needed for shedding of gamete cell wall (Snell 1982), synthesis of proteins required for formation of tubular structure and chanoid body on mt^+ and mt^- gametes, respectively (Triemer and Brown 1975). That the young zygotes formed after the treatment of endosulfan, malathion or permethrin to cells, as mentioned above, can complete the process of zygote maturation was observable by microscopic examination of the zygotes [increase in size (Levin and Ebersold 1960) and change in colour from green to pale orange (Smith 1976) (results not included)]. This indicates that prior exposure of these insecticides to cells may not interfere with the processes of zygote maturation, like - synthesis of ribosomal RNA, zygote wall formation and dedifferentiation of chloroplast structure (Sano et al. 1984; Wiese et al. 1977). Thus, from the results described so far, it appears that expression of sex alleles and genome regions involved in the formations of gametes, young zygotes, and maturation of young zygotes occur in endosulfan, malathion or permethrin treated cells as in untreated controls.

Although, the treatment of one of the three insecticides to both the mating types does not interfere with the formation and maturation of the young zygotes, all of the mature zygotes thus formed do not divide on the first day as in untreated controls. It is discernible (Table 1,2,3) that in the populations of mature zygotes formed after the treatment of (i) endosulfan, (ii) malathion or (iii) permethrin to both the mating types at the developmental stages - before gametogenesis, after gametogenesis and during the fusion of gametes (Aiii, Biii, C of the tables) - (i) 0,0,0, (ii) 65, 70, 50 and (iii) 65, 80, 65 per cent of the zygotes undergo meiotic division on the first day, respectively (in untreated controls, 100 per cent of the mature zygotes divide on the first day). Thus, potential of endosulfan in swaying away more number of mature zygotes from meiosis in normal period is significantly higher than that of malathion or permethrin. Between malathion and permethrin, such potential of the latter is slightly less than that of the former. Likewise, differences in such potentials of the three insecticides are also discernible following their exposures to young zygotes. Data (D of Table 1, 2, 3) show that 0, 50 and 100 per cent of the mature zygotes formed after the treatment of endosulfan, malathion or permethrin to young zygotes, divide on the first day, respectively.

Additionally, the results described so far point out that opposite mating type cells at four stages of development (before and after gametogenesis, during and immediately after the fusion) in sexual life cycle exhibit differential sensitivity (concerning meiotic division of mature zygotes on the first day) to three insecticides (Aiii, Biii, C, D, of Table 1, 2, 3). The cell stages - during and immediately after the fusion of gametes - show maximum sensitivity to malathion. With permethrin, the cell stage - before gametogenesis (vegetative cells) - exhibits such sensitivity. On the other hand, stages of the cells - after gametogenesis (gametes) and immediately after the fusion of gametes (young zygotes) - show minimum sensitivity to malathion and permethrin, respectively. Data (Aiii, Biii, C, D of Table 1) on endosulfan do not help to understand whether opposite mating types at different stages of development respond differentially to this insecticide. In each of the four populations of mature zygotes formed after the exposure of endosulfan (0.25×10^{-4} M) at different stages of cellular development, the percentage of the first day dividing zygotes stands out to be the lowest (zero per cent). The nondiffering high magnitude of the effect on each of the four populations of mature zygotes may possibly arise through the high concentration (0.25×10^{-4} M) of endosulfan used in the treatment. In view of this, similar experiments were carried out with low concentration of endosulfan (1×10^{-5} M). The results show that unlike malathion or permethrin, opposite mating types at three developmental stages (before and after gametogenesis, and during the fusion of gametes), do not exhibit differential sensitivity to endosulfan (the percentage level of the first-day dividing zygotes in these endosulfan treated groups remains the same, 50 per cent). However, the cell stage - immediately after the fusion of gametes (young zygotes) - shows the least sensitivity to endosulfan (results not included).

Data from Table 1, 2 and 3 (Ai, Aii of tables) show that 65, 85 and 75 per cent of the mature zygotes formed after the treatment of endosulfan, malathion or permethrin to either mt^+ or mt^- vegetative cells, divide meiotically on the first day, respectively. Thus, the treatment of respective insecticide to either male (mt^-) or female (mt^+) vegetative cells, do not exhibit difference in the percentage of first day dividing zygotes. However, the levels of these percentages decrease significantly when the treatments are accorded to both, male and female, vegetative cells (Aiii of Table 1, 2, 3).

As a consequence of the treatment of endosulfan, malathion or permethrin to either mt^+ or mt^- gametes, 100, 100 and 80 per cent of the mature zygotes undergo meiosis on the first day, respectively (Bi, Bii of Table 1, 2, 3). As seen with the vegetative cells, again, the percentages of the first day dividing zygotes in the populations formed after the treatment of the respective insecticide to either male or female gametes do not change. These results also show that all of the mature zygotes from the populations formed after the treatment of endosulfan or malathion to

only mt^+ or mt^- gametes, undergo meiotic division on the first day (Bi, Bii of Table 1, 2). However, after their (endosulfan or malathion) treatment to the gametes of both the mating types, this percentage level (100 per cent) of the first-day dividing zygotes lowers to 0 and 70 per cent, respectively (Biii of Table 1, 2). On the other hand, permethrin does not display such difference in the percentages of the first day dividing zygotes in the populations formed following its exposure to both or either of the male and female gametes (Bi, Bii, Biii of Table 3). These treated groups exhibit the same percentage (80 per cent) of the first-day dividing zygotes.

Data from table 1, 2 and 3 show that the mature zygotes which do not undergo meiotic division on the first day as a result of single treatment of endosulfan, malathion or permethrin to both or either one of the opposite mating types at any one of the four developmental stages in sexual life cycle of Chlamydomonas cells, remain meiotically undivided for the period of five days and endure meiosis on the sixth day. Microscopic examination of these zygotes for the period of five days show absence of the prophase - the first stage of the process of meiosis. The delay in the commencement of the prophase in these zygotes may arise through temporary inhibitory action of these insecticides and/or their breakdown products on the synthesis of prophase functional proteins. Requirement of specific proteins for the functions of unwinding and reannealing of DNA strands in the prophase of meiosis has recently been reported (Hotta and Stern 1978; Stern 1980). The mature zygotes which do not exhibit commencement of the prophase for the period of five days display the same on the sixth day. From the onset of the prophase in these zygotes, the subsequent stages of meiosis and formation of visible colonies occur in normal period of five days.

In summary, the studies presented in this paper show that single exposure of endosulfan, malathion or permethrin to opposite mating types at any one of the four developmental stages (before and after gametogenesis, and during and after the fusion of gametes) in sexual life cycle of Chlamydomonas reinhardtii, delays meiosis in significant number of zygotes for five days. However, these zygotes undergo meiosis after the delay period. This delay in meiosis appears to be due to delay in the onset of prophase. The proportion of such zygotes in the populations formed after the exposure of endosulfan is much higher as compared to that of malathion or permethrin. However, these insecticides do not influence output of gametes, and formation and maturation of zygotes. Cells of opposite mating types at different stages of development exhibit differential sensitivity (concerning meiosis of zygotes) to endosulfan, malathion and permethrin.

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TABLE I. Effect of endosulfan treatment on sexual life cycle of Chlamydomonas reinhardtii

Stage of cell development		Mating combination		Formation of gametes	Formation of young zygotes	Meiosis of mature zygotes in the population after exposure to light on the	
		Treated	Untreated	(%)	(%)	first day (%)	sixth day (%)
A. Vegetative cells (before gametogenesis)	(i)	mt^+	mt^-	100	100	65	35
	(ii)	mt^-	mt^+	100	100	65	35
	(iii)	mt^+ & mt^-		100	100	0	100
B. Gametes (after gametogenesis)	(i)	mt^+	mt^-	-	100	100	-
	(ii)	mt^-	mt^+	-	100	100	-
	(iii)	mt^+ & mt^-		-	100	0	100
C. During the fusion of gametes		mt^+ & mt^-		-	100	0	100
D. Young zygotes*		Young zygotes		-	-	0	100

Cells were treated with endosulfan for 2 hr at 0.25×10^{-4} M concentration. In untreated groups, formation of gametes, zygotes and meiotic division of mature zygotes on the first day of light exposure was 100 per cent. The mature zygotes in treated groups which remain meiotically undivided on the first day of light exposure, endure meiosis on the sixth day. Each value is an average of six replicates of seven independent experiments.

* Immediately after the fusion of gametes (before maturation of zygotes).

TABLE 2. Effect of malathion treatment on sexual life cycle of Chlamydomonas reinhardtii

Stage of cell development		Mating combination		Formation of gametes	Formation of young zygotes	Meiosis of mature zygotes in the population after exposure to light on the	
		Treated	Untreated	(%)	(%)	first day (%)	sixth day (%)
A. Vegetative cells (before gametogenesis)	(i)	mt^+	mt^-	100	100	85	15
	(ii)	mt^-	mt^+	100	100	85	15
	(iii)	mt^+ & mt^-		100	100	65	35
B. Gametes (after gametogenesis)	(i)	mt^+	mt^-	-	100	100	-
	(ii)	mt^-	mt^+	-	100	100	-
	(iii)	mt^+ & mt^-		-	100	70	30
C. During the fusion of gametes		mt^+ & mt^-		-	100	50	50
D. Young zygotes*		Young zygotes		-	-	50	50

Cells were treated with malathion for 2 hr at 1×10^{-3} M concentration.

* Immediately after the fusion of gametes (before maturation of zygotes).

Other details are as described in foot-note to table 1.

TABLE 3. Effect of permethrin treatment on sexual life cycle of Chlamydomonas reinhardtii

Stage of cell development		Mating combination		Formation of gametes	Formation of young zygotes	Meiosis of mature zygotes in the population after exposure to light on the	
		Treated	Untreated	(%)	(%)	first day (%)	sixth day (%)
A. Vegetative cells (before gametogenesis)	(i)	mt^+	mt^-	100	100	75	25
	(ii)	mt^-	mt^+	100	100	75	25
	(iii)	mt^+ & mt^-		100	100	60	40
B. Gametes (after gametogenesis)	(i)	mt^+	mt^-	-	100	80	20
	(ii)	mt^-	mt^+	-	100	80	20
	(iii)	mt^+ & mt^-		-	100	80	20
C. During the fusion of gametes		mt^+ & mt^-		-	100	65	35
D. Young zygotes*		Young zygotes		-	-	100	-

Cells were treated with permethrin for 2 hr at 1×10^{-3} M concentration.

* Immediately after the fusion of gametes (before maturation of zygotes).

Other details are as described in foot-note to table 1.

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