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RELATIVE SENSITIVITY OF ANGIOSPERMIC POLLEN TO ENDOSULPHAN

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PESTICIDES play a vital role in crop protection, but at times they cause phytotoxic effects such as stunting of growth, scorching of foliage, abnormal development of flower, inhibition of pollen germination/tube growth and reduced seed setting[1,2]. To ensure safe use of pesticides during flowering and seed formation, a simple system is required to evaluate toxicity and predict impairment in the reproductive process which seriously affect the ultimate productivity.

The use of pollen grains provides a convenient system for monitoring atmospheric pollutants[3], bioassay for toxic substances[4] and studies on mutation[5]. Some studies are available on the effect of pesticides applied as vapour or liquid spray during flower development yielding less viable and damaged pollen grains[6–9]. The effect of endosulphan on the pollen germination of *Catharanthus roseus* (Linn.) G. Don. was reported earlier[10] and this communication deals with the response of another three angiospermic plants viz. *Vigna radiata* (Linn.) *Wilczek*; *Trigonella foenum-graecum* Linn. and *Brassica campestris* Linn.

The pollen grains were collected from mature anthers early morning just before dehiscence. The effects of endosulphan were studied by sowing the pollen grains on sterilized cavity slide in drops (≅ 50 μl) of 10% sucrose culture media containing endosulphan in concentrations ranging from 10 to 1000 ppm. The slides so prepared were incubated for 4 h in a petri plate lined with moist filter papers at room temperature of 22–30°C and relative humidity of 49–70% under diffuse light. Parallel control experiments were conducted without endosulphan. After incubation, the pollen cultures were scanned under light microscope in ten fields (15 × 10 magnification) to determine germination and measure the pollen tube length by calibrated ocular micrometer.

Table 1 gives comparative data of percentage pollen germination in *C. roseus*, *V. radiata*, *T. foenum-graecum* and *B. campestris* on treatment to varying endosulphan concentrations. There was a drop in germination with increase in concentration of the insecticide. At 500 ppm the pollen of *B. campestris* failed to germinate while in the other three species germination was uniformly low. *Catharanthus* and *Vigna* pollen were more resistant than *Trigonella* and *Brassica* as the percentage inhibition in germination was more severe in the latter group.

Table 1 *Behaviour of pollen germination after premixing endosulphan in 10% sucrose culture media*

Endosulphan concentration (ppm)	Pollen germination (%)			
	C. roseus	*V. radiata*	*T. foenum-graecum*	*B. campestris*
0	85	88	95	85
10	65 (24)	75 (12)	70 (33)	55 (35)
100	45 (47)	50 (41)	25 (76)	20 (76)
500	10 (88)	20 (67)	5 (95)	NG (100)
1000	NG (100)	5 (94)	NG (100)	NG (100)

The values are mean of 10 replicates; Figures in parentheses indicate percentage of inhibition; NG, No germination.

Table 2 shows pollen tube growth in different species after endosulphan treatment. The pattern of inhibition in tube growth was similar to that found in germination in response to different concentrations of endosulphan. However, the degree of inhibition was more severe in tube growth than in germination. The present observations support the view that pollen germination and tube growth are independent processes governed by separate conditions[11]. Of the two processes the activation phase of pollen germination was less sensitive to endosulphan. Some effects were also observed on the initiation phase as the time for sprouting was delayed (data not presented in tables) with increasing endosulphan concentration in the treatment medium. However, severe effects were observed on the exponential phase.

The mechanism of inhibition of pollen germination and tube elongation is not clear but by equating with some of the known phytotoxic effects of endosulphan on seed germination and seedling growth[12–14] a plausible explanation may be offered.

Table 2 *Effect of endosulphan on pollen tube growth*

Endosulphan concentration (ppm)	Pollen tube growth (μ) after 4 h incubation			
	C. roseus	*V. radiata*	*T. foenum-graecum*	*B. campestris*
0	1023 ± 32.4	740 ± 15.7	1235 ± 18.5	625 ± 12.5
10	587 ± 45.2 (43)	450 ± 19.4 (39)	750 ± 14.2 (39)	305 ± 7.5 (51)
100	330 ± 12.2 (68)	285 ± 11.8 (61)	125 ± 6.7 (90)	75 ± 8.6 (88)
500	72 ± 12.4 (83)	145 ± 4.5 (80)	62 ± 9.5 (95)	NG (100)
1000	NG (100)	60 ± 7.3 (92)	NG (100)	NG (100)

The values are mean of 10 replicates; ± Standard deviation; Figures in parentheses indicate percentage of inhibition; NG, No germination.

Subsequent reaction after imbibition of pollen includes metabolic changes resulting in membrane permeability and regulation of promotor inhibitor ratio to provide conditions favourable for germination. Endosulphan alters membrane permeability in plant cell[15] and any such effect on pollen may result in the loss of germination potential. Following germination, there is tube initiation for which transcription and/or translational processes are required which are regulated by auxin independently or in combination with other hormones. Earlier studies showed that auxin regulated elongation of barley coleptile sections was suppressed by endosulphan[16]. Pollen germination also includes mostly cell elongation and rarely cell division, therefore, inhibition in tube growth may be the effect of endosulphan observed earlier on cell elongation. However, detailed biochemical studies are necessary to confirm this. Formation and growth of the pollen tube is one of the most fascinating problems in plant physiology. The growth of the pollen tube is generally rapid and requires activation of enzymes, fresh synthesis of protein and polysaccharide for wall formation[17,18]. The present results reveal that the effect of endosulphan on the structural integrity, hormonal control or biosynthetic processes reported earlier[12,13,16,19] independently or in combination may be responsible for the inhibition in pollen germination and tube growth. Among the species tested *B. campestris* was most sensitive towards endosulphan followed by *T. foenum-graecum*, *C. roseus* and *V. radiata*.

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DISTINCT DEHYDROGENASE ISOENZYMES IN STRAINS AND SPECIES OF SIMIAN MALARIAL PARASITES AS EVIDENT BY GRADIENT POLYACRYLAMIDE GEL ELECTROPHORESIS

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ENZYME electrophoresis is a valuable tool for identifying genetic variations among different species and strains of parasites[1–3]. Among malarial parasites, the technique was first applied to rodent species of *Plasmodium*[4,5] and subsequently to human and primate malarial parasites[1,6,7]. Earlier studies on isoenzymes were mainly confined to employing starch gel or agarose gel electrophoresis. Polyacrylamide gradient gel electrophoresis, which yields much greater resolution of proteins in comparison to other supports like starch or agarose, has recently been used successfully for isoenzymic studies of malaria[7], leishmaniasis[8] and amoebiasis[9]. The present report describes the isoenzymic patterns of glutamate dehydrogenase (E.C.1.4.1.4, GDH) and malate dehydrogenase (E.C.1.1.1.37, MDH) of simian malarial parasites, separated on polyacrylamide gradient gel electrophoresis.

Two species of simian malarial parasites viz. *Plasmodium knowlesi* and *P. cynomolgi bastianelli* were used. Three strains of *P. knowlesi*, one from Philippines (P-strain) and the other two from Malaysia (H-strain and W_1 strain), which are known to induce fatal infections in rhesus monkeys were used. *P. knowlesi* P and H strains were kindly provided by Dr L. H. Miller, Malaria Section, Laboratory of Parasitic Diseases, NIH, Bethesda, MD, USA. *P. knowlesi* W_1 strain was obtained from Prof. P. C. C. Garnham, Imperial College, London, UK. The other species of simian malaria, *P. cynomolgi* B producing less severe non-fatal infection in rhesus monkeys was obtained from Dr William Collins, Centre for Disease Control, Atlanta, Georgia, USA. Both these species also infect man.

Rhesus monkeys (*Macaca mulatta*) of either sex weighing about 3–6 kg were infected by intravenous infusion of 1×10^6 schizonts of *P. knowlesi*/*P. cynomolgi* and the percentage of parasitaemia was determined by examining Giemsa stained thin blood films. Blood from infected monkeys was collected in acid citrate dextrose when most of the parasites were at schizont stage. The schizonts were purified on Percoll gradient and extracted as described earlier[7]. The enzyme activities of GDH[10] and MDH[11] were determined. The protein contents of the parasite and normal erythrocyte ghost extracts were estimated by the method of Lowry *et al*[12] as modified by Deans *et al*[13].

Polyacrylamide gel electrophoresis of parasite and normal erythrocyte extracts was done under non-dissociating conditions following Davis[14] with certain modifications. The separating and the stacking gels were used as described earlier[15]. 5.0–7.5% separating and 3% stacking gels (without sodium dodecyl sulphate) were made in 0.375 M Tris-HCl buffer pH 8.8. Electrophoresis was carried out at 20 mA for 12–14 h in the case of GDH and 18–20 h in the case of MDH. The temperature was maintained at 4–8°C with a cold water bath circulator. Specific staining of MDH and GDH was done as described by Carter[4,5].

In the present study, the technique of polyacrylamide gradient gel electrophoresis has been used to study the isoenzymic patterns of GDH and MDH of different strains and species of simian malarial parasites. Electrophoresis was done for varying lengths of time to get better separation of the isoenzymic bands. A running time of 12–14 h was sufficient to separate the GDH isoenzymes as further increase in the running time did not affect the separation of the isoenzymic bands though it did result in some loss of GDH activity. On the other hand, for getting better separation of MDH isoenzymes, the gel had to be run for 18–20 h. Zymograms of GDH and MDH are shown in figures 1 and 2 while figure 3 shows the diagrammatic representation of the same. Only one major isoenzymic band of GDH in *P. cynomolgi* and different strains of *P. knowlesi* was observed, but they differ in their