Table 3. Segregating pattern of the induced mung bean leaf mutants in M_3 generation

Mutant	Treatment	Total plants	Normal plants	Mutant plants	χ^2 value
Variety PDM-116					
Tetrafoliate	30 kR γ-rays	19	15	4	0.103
Pentafoliate	0.2% EMS	20	14	6	0.260
Variety PDM-11					
Tetrafoliate	45 kR γ-rays	22	15	7	0.480
Pentafoliate	0.1% EMS	20	16	4	0.260

Table value of χ^2 at 1 degree of freedom and 5% level of significance is 3 841.

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Use of slide agglutination test for the detection of nuclear polyhedrosis virus of silkworm, *Bombyx mori* L.

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Specific antibody raised against nuclear polyhedrosis virus (NPV) has been used for the detection of the virus disease in silkworm. The agglutination reactions were positive and could be visualized directly up to a titre of antigen 1×10⁸ polyhedra/ml and antiserum 1:2000.

THE agglutination reaction involving clumping of cellular or particulate antigen by specific antiserum is one of the earliest immunodiagnostic methods[1]. Many sophisticated immunodiagnostic methods have been developed subsequently, but these are expensive and require operational skill, thus limiting the commercial use.

Arakawa[2] used latex agglutination test for detection of nuclear polyhedrosis virus (NPV) in silkworm and concluded that, particularly for NPV, the test was not as sensitive as that for other viruses because of low titre of the antivirus IgG. Slide agglutination test, though less precise, is more rapid and can be adopted by all, including the unskilled workers.

In the present study, an attempt has been made to standardize a simple slide agglutination test for detection of NPV of silkworm. Early detection of the NPV-induced disease using this method can be of great help to the sericulture industry for adopting preventive measures.

Polyhedral particles from the haemolymph of NPV-infected silkworm larvae were collected by centrifugation and purified using Percoll towards preparation of inoculum as immunogen for raising antiserum. Purified polyhedra (6.0×10^8 polyhedra inclusion bodies per ml (PIB/ml)) suspended in 1 ml of phosphate-buffered saline (PBS) was emulsified with 1 ml of Freund's complete adjuvant and injected intradermally at multiple sites to rabbits. Similar injections were given four times at one-week intervals and the rabbits were bled after seven days of the last injection for the collection of antiserum.

Slide agglutination test was performed using NPV and the antiserum. Different amounts of NPV suspended in PBS, containing 10–10^9 polyhedra/ml, were allowed to react with varying dilutions of antiserum (Table 1). In each case 50 µl of NPV suspension was mixed with 50 µl of antiserum on a microscopic glass slide for agglutination reaction. A control where 50 µl of NPV suspension was mixed with 50 µl of PBS was included in each case. The slides were tilted to and fro and agglutinations were visualized (with naked eye) in the form of curdlings within 2–3 min when the reactions were strongly positive (Figure 1). The curdlings could not be seen in the case of weakly positive reactions but were visualized under a microscope (150×).

The agglutination reactions were detected visually by using antiserum diluted up to 1:2000 for 10^9 and 10^8 polyhedra/ml of antigenic concentrations (Table 1). To employ this technique for disease detection, the total

Table 1. Slide agglutination reactions in various titres of antigen (NPV) and antiserum

NPV (Polyhedra/ml)	Dilution of antiserum					
	1:10	1:100	1:1000	1.2000	1.5000	1.10,000
10^9	V/M	V/M	V/M	V/M	M	M[b]
10^8	V/M	V/M	V/M	V/M	V[a]/M	M[b]
10^7	M	M	M	M	M	M[b]
10^6	M	M	M	M[b]	—	—
$10–10^5$	—	—	—	—	—	—
PBS	—	—	—	—	—	—

Note: The dilutions were carried out in PBS.

[a] Detected with difficulty.
[b] Low agglutination.
V, Agglutination detected visually.
M, Agglutination detected microscopically
— No reaction.

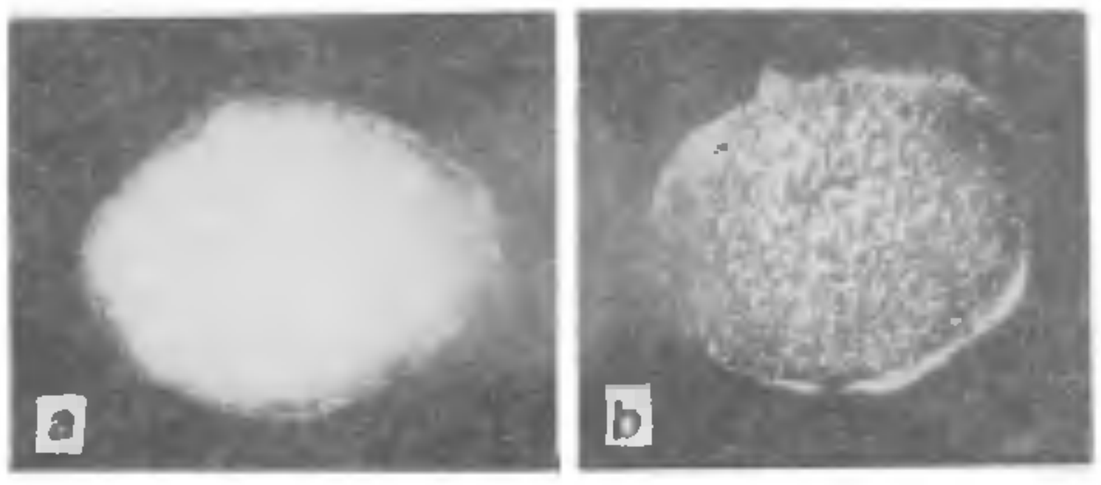

Figure 1. Direct visualization of agglutination reactions. *a*, NPV mixed with PBS solution (negative reaction, control); *b*, NPV mixed with antiserum (positive reaction).

accumulation of PIB in individual larva should be $2.757 \pm 0.092 \times 10^8$/g body weight or $2.946 \pm 0.122 \times 10^8$/ml body volume. The technique which is sensitive up to 10^8 polyhedra/ml enables the detection of infection in larva well ahead of the manifestation of disease symptoms. If the concentration of polyhedra in a single larva is lower, escaping detection, the infection can still be detected in the population from a sample of five to ten larvae.

An early and low level of infection in one lot, prior to manifestation of disease symptoms, could be detected using this simple method. This shows that the method is suitable for even the unskilled farmers.

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Effect of Endosulfan on the integrated density of the erythrocytes of *Channa punctatus* (Bloch)

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Amongst the various organochlorine pesticides used to augment agricultural production in India, Endosulfan is a commonly used pesticide and its toxicity is primarily due to its sulphur content. This paper reports that the integrated density (area in $\mu m^2 \times$density) of nucleus, cytoplasm and cell membrane of the erythrocytes of *Channa punctatus* decreased when exposed to sublethal concentrations of Endosulfan for different durations.

HAEMATOLOGY plays an important role in diagnosing the diseases in fishes and assessing the toxic effects of pollution on them. A normogram to determine different haematological parameters is needed to be developed for different species of fishes on the same pattern as that of human beings[1,2]. Fishes being poikilothermic aquatic vertebrates exhibit changes in their physiology at different stages in their life history, hence complete formation of normogram is very difficult. To understand the deviations from the normal condition, the maximum number of haematological parameters is required to be studied.

The most commonly studied haematological parameters are erythrocyte counts, haemoglobin concentration, haematocrit value, erythrocyte sedimentation rate and blood pH[3,4]. In the present study a freshwater fish *C. punctatus* was exposed to different concentrations of an organochlorine pesticide, Endosulfan, and the effects on the integrated density of nucleus, cytoplasm and cell membrane of erythrocytes were recorded.

Adults of *C. punctatus* were procured from a local pond and transported to the laboratory. The fish were acclimatized to laboratory conditions for 7 days at 25 ± 3°C (SD = 1.81). They were fed with zooplanktons once a day. The LC_{50} value (96 h)[5] of Endosulfan for *C. punctatus* was found to be 0.0070 mg/l (technical grade, i.e. 35% w/w of Thiodan 35 EC).

Endosulfan stock solution of 1 g/l of water was prepared. Aliquots of this stock solution were added to each experimental tank to bring the Endosulfan concentration to 0.0022 mg/l and 0.0035 mg/l (sublethal concentrations), representing 32% and 50% of the 96 h LC_{50}, respectively. A parallel control group was maintained in toxicant-free tap water (pH 6.8; total hardness 86 mg/l and dissolved oxygen 8.91 mg/l). A batch of ten fishes was released in each tank.

After 5 and 15 days of exposure, fish were bathed in clean water and blood smears (2 slides per individual) were prepared and fixed in methanol and stained