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Short Note

Staining Technique for Nuclear Polyhedrosis Virus of Silkworm, *Bombyx mori*

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For obtaining thin sections of any tissue for histopathological studies, the tissues are subjected to various preparatory processes like fixation, dehydration embedding, sectioning and dehydration. Most of the tissues do not retain sufficient colour after processing to make their components clearly visible when observed under the microscope. Moreover, many tissues when impregnated with mounting media become much transparent and it is difficult to observe different structures under the microscope. Hence, colour is added rendering them visible and the process is referred as staining. Generally, haemotoxylin eosin staining is followed (Patki, 1992).

Perfect staining is highly essential to study different structures. When modified Azan technique described by Hamm (1966) was followed, defined contrast between infected cells and uninfected cells could not be obtained. Hence, certain modifications in the procedure were done to get effective staining of polyhedra and other tissues like epicuticle, hypodermis, epithelial cells, fat body, nerve tissues and salivary gland cell.

***Stain Solution* I:** Dissolve 0.1g of azocarmine G in 100 ml of distilled water and boil the solution for 5 minutes. Allow to cool and add 2 ml of glacial acetic acid. Filter before use.

***Stain Solution* II:** Dissolve in 100 ml of distilled water, 1.0 g phosphotungstic acid, 0.1 g aniline blue (water soluble), 0.5 g orange G, 0.2 g fast green FCF.

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Staining procedure

Step	Chemicals/ distilled water	No. of changes	Duration
1.	Xylene 100%	1	5 min.
2.	Xylene alcohol (7:3, 1:1 and 3:7 ratio)	1	3 min. each
3.	Alcohol(30%, 60% and absolute)	1	3 min. each
4.	50 per cent acetic acid	1	5 min.
5.	Distilled water	2	5 sec. each
6.	Azocarmine (Solution I)	2	45 min.22 min.
7.	Distilled water	2	2 sec. each
8.	50 and 70 per cent alcohol	1	5 min. each
9.	0.1 per cent aniline in 95 per cent alcohol	1	2 sec. each
10.	Distilled water	2	2 sec.
11.	Counter staining (Solution II)	1	10 min.
12.	10, 30, 50 per cent alcohol	1	2 sec. each
13.	Absolute alcohol	2	30 sec.
14.	Xylene	3	15 min. each

Differentiation of structures

Structures	Colours
Viral polyhedra	Red
Epicuticle	Red
Endocuticle	Blue
Hypodermis	Red
Mid gut epithelial cells	Green and Blue
Fat body	Yellowish brown with dark green nuclei
Salivary gland	Red
Muscles cells	Green

Though modified Azan technique was highly suitable to inclusion viruses, modifications over this method brought about sharp contrast between infected and non infected cells of *B. mori*.

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Reference

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