Experiment No 12. Estimation of Amylase Activity in Haemolymph of Bivoltine and Multivoltine races.

Aim: To estimate the amylase activity in haemolymph of bivoltine and multivoltine silkworm strains.

Principle: When amylase acts on starch, it is converted in to glucose units. The resultant glucose units react with 3, 5-dinitrosalicylic acid (DNS) in alkaline solution to give rise to orange coloured complex, which can be measured at 540 nm.

Reagents Required:

1. **Phosphate buffer 0.1 M pH 7.8:**
   a. 0.1 M KH_2PO_4: Dissolve 1.360 g of KH_2PO_4 in 100 ml of distilled water.
   b. 0.1 M K_2HPO_4: Dissolve 1.7418 g of K_2HPO_4 in 100 ml of distilled water.

Mix the solutions a and b at 1:1 ratio and adjust the pH 7.8.

2. **Haemolymph Sample:** Dilute 100 µl of silkworm haemolymph with 1900 µl phosphate buffer containing 1mM thiourea.
3. **Substrate (1% starch):** Dissolve 1 g soluble starch in 90 ml of distilled water and boil to get clear solution. Make up to 100 ml with water.
4. **DNS reagent:** Please refer experiment number 11.

Procedure for Standard Curve: For preparation of standard curve please refer experiment number 11 or same standard curve may used here for the estimation of amylase activity.

Procedure for Experiment:

1. **Blank:** Take 2 ml of phosphate buffer, 0.5 ml of substrate and 0.5 ml of inactivated haemolymph sample or distilled water in a clean dry test tube.
2. **Test 1:** Take 2 ml of phosphate buffer, 0.5 ml of substrate and 0.5 ml of multivoltine haemolymph samples in a clean dry test tube.
3. **Test 2:** Take 2 ml of phosphate buffer, 0.5 substrate and 0.5 ml of bivoltine haemolymph samples in a clean dry test tube.
4. Mix the contents of the tubes by vortexing / shaking the tubes and incubate for 30 min at 37°C.

5. Now add 0.5 ml of DNS to all the test tubes, mix the contents of the tubes by vortexing / shaking the tubes and incubate for 10 min in a boiling water bath and cool to room temperature.
6. Then to the cooled test tubes add 0.5 ml of distilled water, mix the contents and record the absorbance at 540 nm against blank.

Observations and Calculations

	Buffer (ml)	Substrate (ml)	Enzyme sample (ml)	Mix, incubate at 37°C for 30'	DNS (ml)	Incubate for 10 min in a boiling water bath and cool	D W (ml)	A_{540}
Blank	2	0.5	0.5 IE*		0.5		0.5	0.00
Test 1 (MV)	2	0.5	0.5		0.5		0.5	
Test 2 (BV)	2	0.5	0.5		0.5		0.5	

*IE-Inactivated Enzyme

Amylase activity = $\frac{\text{Standard Curve Value X 60 X Dilution Factor (20)}}{\text{Sample taken (ml) X Time of incubation (min)}}$

= -----µg of glucose released/ml of haemolymph sample/hour at 37°C

Result: The given multivoltine and bivoltine haemolymph samples shown ----- and ----- µg /ml / hour at 37°C respectively.
