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Histochemical analysis of the digestive tract of larvae of Potato tuber moth (*Phthorimaea operculella zell*) on the basis of their food habits

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Abstract

In this study the potato tuber moth (Phthorimaea operculella zell), one of the most common pest of potato crops (solanaceous sp.) was investigated to determine the impact of food habit on the histochemical analysis of the digestive tract of its larval form. Histochemical observations were made on amino acids (tyrosine and tryptophane), lipids (neutral lipids and phospholipids), enzymes (alkaline phosphatase and acid phosphatase) and basic proteins (fibrin and reticulin). These histochemical observations reveal a very significant correlation to the food habit of the moth.

Keywords: *Phthorimaea operculella zell*, tyrosine, tryptophane, neutral lipids, phospholipids, alkaline phosphatase, acid phosphatase, fibrin and reticulin.

Introduction

Class insect constitute a major threat to sustainable agriculture. This threat is associated with cultivation of new crops, intensification of agriculture production systems and spread through human trade. The potato tuber moth (*Phthorimaea operculella zell*) is an oligophagous pest (an insect feeding on restricted range of food plants) feeds on crops belonging to the family solanaceae (mainly potato and tobacco). Potato tuber moth affects production, reduces quality of the produce and increases the risk of infection by plant pathogens. The common name potato tuber worm is given to its damaging larval stage.

It is a cosmopolitan pest and was introduced into India in 1906 along with potato seed brought in from Italy. This well-known pest has a worldwide distribution and is found both in field and in godowns. In India it occurs in almost all potato producing states and is considered to be a major potato pest in several states. In south India, it is more a store pest attacking store potatoes in the vegetable godowns. In some cases upto 100% loss has been reported in storehouses.

Damage to potato tubers first become evident in the "Eyes". In the field, larvae bores the leaf blades of potato plants and gradually forms a mine. Larvae usually find its way to the petiole and then bore downwards. The tunnelling causes the wilting of the affected parts. Young plants are very susceptible and die if heavily damage. The pest is active in the planes throughout the year and passes its life cycle on potato plants in the field from November to March.

Up to the present, little information is available about the histochemical changes in the tissues of digestive tracts of potato tuber moth induced by its feeding habits.

Therefore, the objective of the present study was to determine the impact of food on the histochemical analysis of the digestive tract of *Phthorimaea operculella zell* under laboratory conditions.

Materials and methods

Freshly laid eggs and larvae of *Phthoramaea operculella zell* were collected from the potato plants. They are placed for their further growth or rearing in wooden cages measuring 30X25X18 cm. with mashes on five sides, a wooden base and a cardboard door. Fresh, moist leaves of host plant were provided to them every day. Growth changes in larvae were carefully noted. The mature larvae were taken out and killed in killing bottles. The alimentary canals of larvae were dissected out and fixed in chilled acetone for 24 hrs. (for histochemical analysis of enzymes) and in 10% neutral formaline for 16 hrs. at room temperature (for histochemical analysis of basic proteins, amino acids and lipids). After dehydration by two changes of fixative, alimentary canals were cleared in benzene and embedded in paraffin wax. Paraffin section were prepared and subjected to the following histochemical tests–

Amino acids: Tyrosine – The Millon's reaction[1]. Tryptophane – DMAB-nitrite method[2].

Lipid: Neutral lipids- Sudan black- B staining[3]. Phospholipids- Acid haematin test[4].

Enzymes: Alkaline phosphatase- Calcium Cobalt method[5]. Acid phosphatase- Lead Nirate method[5].

Basic proteins: Fibrin- Mallory's PTAH method[6]. Reticulin- Silver impregnation method[7].

Results and discussion

Histochemical analysis of various regions of alimentary canal of *Phthorimaea operculella zell* for localization of amino acids, lipids, enzymes and basic proteins show following results-

Amino acids: Localization of tyrosine: *P. operculella* showed dull activity in pharynx, oesophagus and proventriculus whereas the crop region show no localization of tyrosine. Mesenteron exhibited moderate activity. Ileum and colon regions show dull activity whereas rectum did not show any response.

Localization of tryptophan: Pharynx, oesophagus and proventriculus showed dull activity whereas there is no localization in crop region. Mesenteron reacted strongly. Ileum and colon regions showed dull activity whereas there is no localization in rectum region of *P. operculella*.

Gautam and Masih reported that larvae preferred to feed on plants having amino acid contents which definitely increase the amount of amino acid in the alimentary canal[8].

Table-1: Localization of Amino Acids (Tyrosine and Tryptophane) in the Alimentary canal of the larvae of *Phthorimaea operculella zell.*

Parts of Alimentary canal		Localization of Tyrosine	Localization of Tryptophane
Stomodaeum (Foregut)	Pharynx	Dull Activity	Dull Activity
	Oesophagus	Dull Activity	Dull Activity
	Crop	Nil activity	Nil activity
	Proventriculus	Dull Activity	Dull Activity
Mesenteron (Midgut)		Moderate activity	Strong activity
Proctodaeum (Hindgut)	Ileum	Dull Activity	Dull Activity
	Colon	Dull Activity	Dull Activity
	Rectum	Nil activity	Nil activity

Lipids: Localization of neutral lipids: In *P. operculella*, moderate reaction was observed in crop, midgut and ileum regions while proventriculus exhibited a poor reaction. No response was observed in pharynx, oesophagus and rectum regions of the alimentary canal.

Localization of phospholipids: The presence of phospholipids was noticed in pharynx and crop regions while midgut and proventriculus exhibited poor and dull reactions respectively.

Gautam and Sharma has worked on the distribution of lipids in the alimentary canal of grasshopper and observed that lipids varied in various parts of alimentary canal due to different food plants[9].

Table-2: Localization of Lipids (Neutral Lipids and Phospholipids) in the Alimentary canal of the larvae of *Phthorimaea operculella zell*.

Parts of Alimentary canal		Localization of Neutral Lipids	Localization of Phospholipids
Stomodaeum (Foregut)	Pharynx	Nil activity	Moderate activity
	Oesophagus	Nil activity	Nil activity
	Crop	Moderate activity	Moderate activity
	Proventriculus	Dull Activity	Dull Activity
Mesenteron (Midgut)		Moderate activity	Dull Activity
Proctodaeum (Hindgut)	Ileum	Dull Activity	Nil activity
	Colon	Nil activity	Nil activity
	Rectum	Nil activity	Nil activity

Enzymes: Localization of Alkaline Phosphatase: Strong to moderate activity was seen in the proventriculus and ileum of moth larvae and comparatively higher in the midgut whereas the other regions of alimentary canal exhibited poor activity.

Localization of Acid Phosphatase: Proventriculus and ileum showed strong to moderate activity. Mesenteron appeared reacting strongly. But the other regions of alimentary canal showed dull activity.

Hiromu reported that the acid phosphatase helps in metabolism and transphosphorylation[10]. The lysosomal enzymes undergo metabolic transformation in vivo, resultingin change of substrate of specificity was carried out by Furquhar et al.[11].

Basic proteins: Localization of Fibrin: Pharynx, crop and oesophagus exhibited moderate to dull activity whereas the proventriculus and mesenteron reacted strongly. Ileum, colon and rectum showed moderate, dull and nil activities respectively.

Localization of Reticulin: Pharynx, crop and proventriculus exhibited moderate activities whereas oesophagus showed dull activity. Mesenteron reacted strongly. Ileum, colon and rectum showed moderate, dull and nil activities respectively.

Thorteinson reported host selection of phytophagus insects[12]. The connective tissues usually consist of cellular proteinsin an enveloping framework of non-cellular substance which is made up of basic proteins.

The connective tissue fibres like fibrin and reticulin were observed in the present investigation. Plants proteins are

converted in to animal proteins after they fed upon and the alimentary canal is expected to receive maximum impact from the type of food intake.

Table-3: Localization of Enzymes (Alkaline Phosphatase and Acid Phosphatase) in the Alimentary canal of the larvae of *Phthorimaea operculella zell*.

Parts of Alimentary canal		Localization of Alkaline Phosphatase	Localization of Acid Phosphatase
Stomodaeum (Foregut)	Pharynx	Dull Activity	Dull Activity
	Oesophagus	Dull Activity	Dull Activity
	Crop	Moderate activity	Moderate activity
	Proventriculus	Strong activity	Moderate activity
Mesenteron (Midgut)		Strong activity	Strong activity
Proctodaeum (Hindgut)	Ileum	Moderate activity	Moderate activity
	Colon	Dull Activity	Dull Activity
	Rectum	Nil activity	Nil activity

Table-4: Localization of Basic Proteins (Fibrin and Reticulin) in the Alimentary canal of the larvae of *Phthorimaea operculella zell.*

Parts of Alimentary canal		Localization of Fibrin	Localization of Retiulin
Stomodaeum (Foregut)	Pharynx	Moderate activity	Moderate activity
	Oesophagus	Dull Activity	Dull Activity
	Crop	Moderate activity	Moderate activity
	Proventriculus	Strong activity	Moderate activity
Mesenteron (Midgut)		Strong activity	Strong activity
Proctodaeum (Hindgut)	Ileum	Dull Activity	Moderate activity
	Colon	Dull Activity	Dull Activity
	Rectum	Nil activity	Nil activity

Conclusion

For amino acids, mesenteron of the larvae of potato tuber moth reacted strongly because in it complex food molecules are catabolized into free amino acids to be circulated throughout the body.

The observations made on lipids reflect the changes in surface tension and mechanical properties of the tissue. The topographical changes in the distribution of lipids observed during analysis justified the morphological alteration.

The presence of alkaline phosphatase in plasma membrane plays important role in transport of ions through cellular membrane. Mesenteron showed maximum activity in comparison to other parts of alimentary canal that suggests the amount of alkaline phosphate is directly proportional to the amount of transfer across cell boundaries.

Acid phosphatase pre-eminently regarded as the marker enzyme and has been found in golgi-cisternae and lysosomes. The lysosomal enzymes undergo metabolic transformation resulting in change of substrate specificity.

Moth larvae preferred to feed on plants having high basic protein contents, which will definitely increase the amount of basic protein in their alimentary canals. The connective tissues usually consist of cellular proteins in an enveloping framework of non-cellular substance which is made up of basic proteins.

Fibrous proteins like fibrin and reticulin were observed in the present investigation. Plants proteins are converted in to animal proteins after they fed upon and the alimentary canal is expected to receive maximum impact from the type of food intake. That's why we find stronger activity in regions performing the major functions of converting plant proteins into animal proteins.

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