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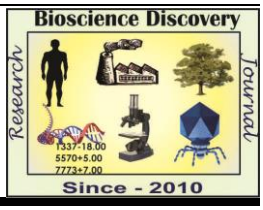

Adaptation to Protease Inhibitors shown by *Spodoptera*

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Abstract

Spodoptera spp. is a serious pest of several legumes, cotton and other cash crops. A variety of crop damage at various levels is attributed to this pest worldwide. This pest had become resistant to almost every pesticide, resulting in heavy loss even on use of them. In the current study attempt has been made to identify protease inhibitors of this pest from several sources, among the tested plant seeds *Citrullus, Ipomoea, Dryptes, Cassia* showed inhibition in all three concentrations used whereas *Lepidium iberis, Piper nigrum, Pimpinella anisum*. When these inhibitors were fed along with artificial diet x-ray film contact print method reveal two newer protease fail to inhibit the digestive proteolysis suggesting adaption or shifting of gene expression.

INTRODUCTION

Spodoptera has shown resistance against pyrethroids, carbamate, organophosphate and some newer chemistry pesticides like Indoxacarb and Fipronil (Armes *et al*., 1997; Kranthi *et al*., 2002; Sumaira Maqsood**,** 2017), emamectin, indoxacarb, and chlorfenapyr low level of resistance was recorded (Tong *et al*., 2013). In the current study attempt has been made to identify and characterize the protease inhibitors so as to arrest the growth of the insect pest, as it feeds lot of plants, it is logical to look the proteins which are capable of inhibiting the digestive proteases has been extensively searched from non-host plants.

MATERIALS AND METHODS

Insect rearing: Fourth instar onwards the insects were fed on artificial diet incorporated with inhibitors and maintained for 10 days taking weight gain-weight loss as criteria for hampered growth due to digestion.

Extraction of *Spodoptra* gut proteases (SGP): Insects were dissected, washed with buffer, and tissue was homogenized to fineness and extracted in 0.1 mM HCl, it was then stored frozen until needed at 4^0C.

Protein estimation: Folin-Lowry's method used for protein estimation (Lowry et al., 1951) with BSA (250µg/mL) as a standard protein.

Assay of trypsin and trypsin inhibitor activity: Trypsin was assayed according to the modified photometric method of Kakade *et al*., (1969) using the substrate BApNA (data not shown). The assay reaction consisted of 0.5 mL of trypsin solution (40-50 µg of trypsin in 1 mM HCl), 0.5 mL of water and 1.25 mL of the substrate. The reaction was carried out at 37^0C for 10 min and the reaction arrested by adding 0.25 mL of 30% acetic acid.

Trypsin and trypsin inhibitory unit: One trypsin (TU) unit is arbitrarily defined as an increase in absorbance of 0.01 at 410nm under conditions of assay. The trypsin inhibitory unit (TIU) is defined as the number of trypsin units inhibited under the same conditions (Kakade *et al.*, 1969).

Electrophoresis and visualization of inhibitors: Inhibitors were visualized by following method described by Veerapa H. M, et al., 2002.

Statistical analysis: Statistical analysis of data obtained was done by SPSS.

RESULTS AND DISCUSSION

Total 100 samples were screened for inhibitory activity and those which are capable of inhibiting even at lower concentrations were chosen for further studies. Dot blot assay reveals *Citrullus, Ipomoea, Dryptes, Cassia* showed inhibition in all three concentrations used whereas *Lepidium iberis, Piper nigrum, Pimpinella anisum* showed inhibition at two concentrations rest of the samples inhibited minimum two concentrations used.

***In vitro* vs *in vivo* inhibition of proteolytic activity**: Total proteolytic activity was assayed using caseinolytic assay showed a mixed response of the herbivore. SGP was pre-incubated with different inhibitors to test enzyme inhibitory activity, amongst the samples tested the threshold of inhibition was from 15 to 56%, *Ipomoea hederacea* and *Mesua Ferrea* could show one band emergence like hydrolysis pointing a resistant protease working under the influence of inhibitors. This is due to insensitivity towards the fed inhibitor or emergence of new protease in other words its adaptation.

Table 1: *In vitro* Percent inhibition of proteolytic activity µg/g of seed powder

Sample Name	Sample Name	Percent inhibition µg/g
Kadu indravan	*Citrullus colocynthis*	42.34
Abhal	*Juniperus communis*	37.69
Kabab chini	*Piper cubeba*	37.95
Kala dana	*Ipomoea hederacea Jacq.*	15.42
Ajmoda	*Apium leptophyllum*	38.71
Onion	*Allium cepa*	39.43
Nagkesar	*Mesua Ferrea*	30.75
Safad mirch	*Piper nigrum*	31.66
Sheetal chini	*Piper cubeba*	56.84
Kavach bij	*Mucuna pruriens*	56.59
Lal todri	*Lepidium iberis*	44.13

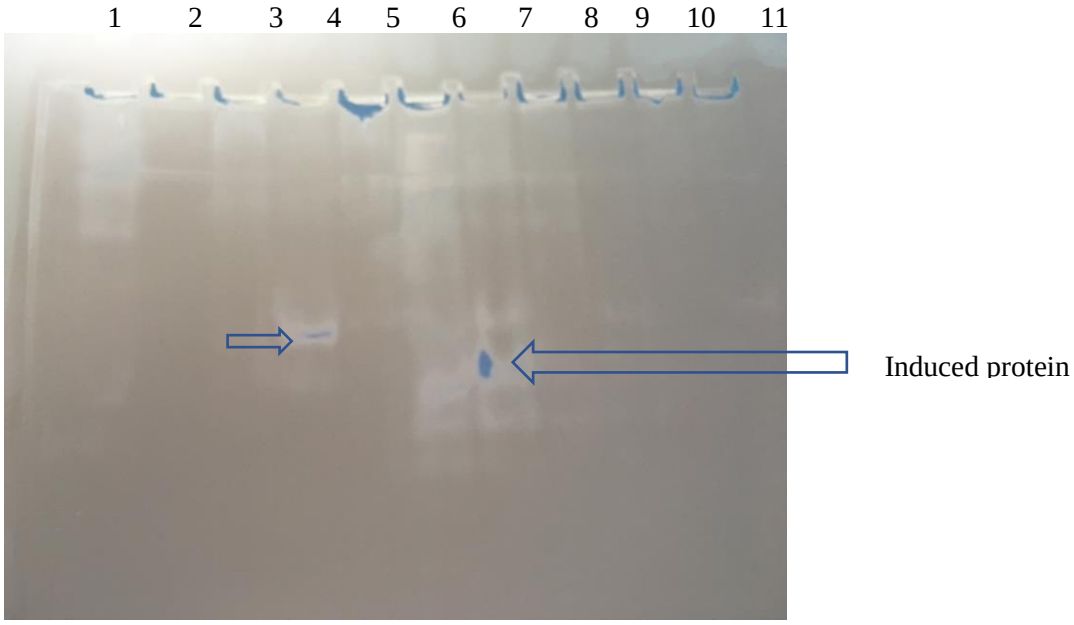

Figure 1 Response of the *Spodoptera* gut proteases to inhibitors fed in diet, hydrolysis shows no inhibition whereas no hydrolysis indicates inhibition. Legends to images are similar numbers in table 1. Lane 9, 10,11 shows all protease forms inhibited while lane 2 only one is not, and rest of the lanes 6 shows partial or mixed response.

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