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RESEARCH ARTICLE

SPODOPTERA SHOWS ADAPTATION TO PROTEASE INHIBITORS

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ABSTRACT

Spodoptera spp. is a serious pest of several legumes, cotton and other cash crops. Lot of destruction of crop is attributed to this pest worldwide. This pest had become resistant to variety of pesticides, resulting in burden on economy and use of pesticides is well known for health hazards to human beings. In the current study attempt has been made to identify plant protease inhibitors which can inhibit the digestive protease of *Spodoptera* to develop a strategy to control crop plants from pests by introducing a transgene which is responsible for the inhibition. *Spodoptera* gut proteases inhibited by *Piper cubeba, Mucuna pruriens* 56.84% and 56.59%. respectively. *Pimpinella anisum, Citrullus colocynthis, Juniperus communis,Piper cubeba, Apium leptophyllum, Cassia absus, Ipomoea hederacea* Jacq. and *Dryptes roxburghii* could inhibit them partially. Trypsin inhibitory potential of different seeds was also carried out which shows *Mesua Ferrea* 387.2 ± 5.91TIU, *Mucuna pruriens* 4.9±3.44 was lowest. Protein content in seeds revealed *Piper longum* 0.432±0.22 µg/g and *Lepidium iberis* 0.775±2.21µg/g.

Key words: Spodoptera, Trypsin inhibition, adaptation.

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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).

MATERIALS AND METHODS

Insect rearing: Insects were fed on artificial diet incorporated with inhibitors and maintained for 10 days.

Extraction of *Spodoptra* gut proteases (SGP): Insects were dissected from segment 1-6, washed with buffer, tissue was homogenised to fineness and extracted in 0.1 mM HCl, it was then stored frozen until needed.

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, 40 mg of BApNA was dissolved in 2 mL dimethylsulfoxide (DMSO) and then diluted (1:100) in 50 mM Tris-HCl buffer, pH 8.2, prior to enzyme assay. 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 *et al.,* 2002.

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

RESULTS AND DISCUSSION

Total 128 samples were screened for inhibitory activity and those which are capable of inhibiting even at lower concentrations were chosen in studies. Dot blot assay reveals *Citrullus colocynthis, Ipomoea hederacea, Dryptes roxburghii, Cassia absus* showed inhibition in all three concentrations used

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Table 1. Trypsin inhibitory activity in seeds μg/g of seed powder

Sr. No.	Sample	TIU/g of seedpowder ± SD	Protein contentμg/g ± SD in seeds
1	Pimpinella anisum	254.1 ± 3.21	0.388 ± 0.34
2	Citrullus colocynthis	54.86 ± 2.28	0.192 ± 0.21
3	Juniperus communis	28.9 ± 1.35	0.173± 0.32
4	Piper cubeba	180.63 ± 2.33	0.276 ± 0.27
5	Ipomoea hederacea Jacq.	112.68 ± 5.4	0.092 ± 0.87
6	Dryptes roxburghii	157.26 ± 3.2	0.276 ± 0.98
7	Apium leptophyllum	248.5 ± 2.34	0.432 ± 0.65
8	Allium cepa	259.05 ± 4.04	0.123 ± 0.12
9	Mesua Ferrea	387.2 ± 5.91	0.411 ± 0.85
10	Cassia absus Linn.	266.89 ± 2.33	0.289 ± 0.13
11	Piper longum	301.32 ± 4.23	0.432± 0.22
12	Piper nigrum	423.22 ± 2.21	0.494 ± 0.32
13	Piper cubeba	9.2 ± 0.32	0.97 ± 2.12
14	Mucuna pruriens	4.9 ± 3.44	0.671 ± 1.22
15	Lepidium iberis	30.21 ± 2.86	0.775± 2.21

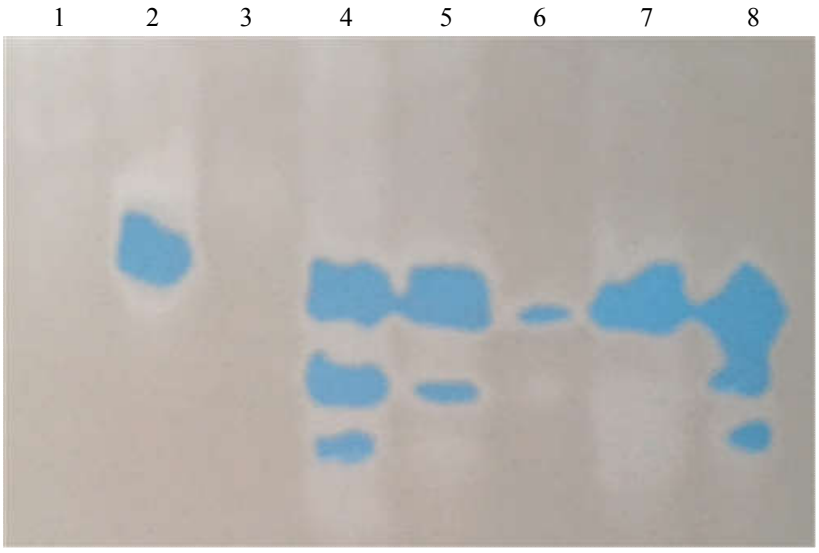

Figure 1. Response of the *Spodoptera* gut proteases to inhibitors fed in diet, hydrolysis shows no inhibition whereas no hydrolysis indicates inhibition. Legends to images are similar numbers in table 1. Lane 1 and Lane 3 shows all protease forms inhibited while lane 2, 4, 5, 8 one is not, and lane 6 has only one active protease

whereas *Lepidium iberis, Piper nigrum, Pimpinella anisum showed inhibition at 1:3 concentrations.* Spodoptera gut proteases when pre-incubated with different inhibitors to test enzyme inhibitory activity, amongst the samples tested *Piper cubeba, Mucuna pruriens* showed high inhibitory activity against the larval enzymes i.e. 56.84% and 56.59%. *Pimpinella anisum, Citrullus colocynthis, Juniperus communis, Piper cubeba, Apium leptophyllum, Cassia absus* Linn. Lowest activity was shown by *Ipomoea hederacea* Jacq. and *Dryptes roxburghii.*Trypsin inhibitory potential of different seeds was assayed in TIU/g of seed powder shown in table 1, *Mesua Ferrea* 387.2 ± 5.91 show highest inhibitory activity and *Mucuna pruriens* 4.9±3.44 showed lowest. Protein content in seeds revealed *Piper longum* 0.432±0.22 μg/g and *Lepidium iberis* 0.775±2.21μg/g.

Feeding trials

Insects maintained on artificial diet were analysed for growth with respect to weight gain and weight loss, inhibitors incorporated in the diet as shown in Table 1, have shown a mixed response in the growth of larvae. Though the inhibitors have a positive impact on digestive gut proteases of *Spodopterain vitro*, this effect was overcome in the *in viv*o trials.

It can be concluded that the herbivore has a capacity to digest, or bind irreversibly or shifts to another set of proteases in response to the inhibitors as shown in Fig 1. Such kind of adaptation points us to look for such protease inhibitors which can inhibit all the proteases at all the times to ensure 100% inhibition for the protection of crop plants 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 1 and Lane 3 shows all protease forms inhibited while lane 2, 4, 5, 8 one is not, and lane 6 has only one active protease.

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