Jackfruit seed and areca nut extracts inhibit gut protease activity of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae)

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ABSTRACT

Proteinase inhibitors (PIs) are small proteins found in many seeds and other parts of plants. These PIs inhibit the gut proteases of many insects. Transgenic plants expressing foreign PIs have been produced for enhanced levels of insect resistance. *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae) popularly known as swarming caterpillar or paddy armyworm is a sporadic pest of *Oryza sativa*. In this study we screened several plant seeds to identify extracts containing PIs against *S. mauritia* gut protease. For this, seeds were soaked and homogenized in bicarbonate buffer, pH 9.0 and centrifuged at 10,000 rpm for 10 minutes. Supernatant (inhibitor) stored at -20ºC until use. Alimentary canal of several 6th instar *S. mauritia* larvae were pooled and homogenized in bicarbonate buffer, pH 9.0 and centrifuged at 10,000 rpm at 4ºC for 10 minutes. Supernatant (enzyme) was frozen at -20ºC until use. The protease activity of gut extract (10µl of crude gut extract) was assessed by incubating for 1 hour at room temperature with 25 µl of 2% casein in a total volume of 60 µl in bicarbonate buffer, pH 9.0. Inhibition was assessed by incubating with 25 µl of seed extract. Reaction was terminated by adding TCA and the amino acid released in the supernatant was estimated by Lowry's method. We found that *Spodoptera mauritia* gut extract proteases cleaved casein and this proteolytic activity was inhibited by aqueous extracts of jackfruit seed (*Artocarpus heterophyllus*) and extract from areca nut (*Areca catechu* L.) up to 78% and 62 % respectively.

Keywords: proteinase inhibitors, *Spodoptera mauritia*, insect gut protease, jackfruit seed, areca nut.

Proteinase inhibitors (PIs) are proteins or peptides which inhibit the activity of proteolytic enzymes. In general, they are small defense-related proteins present in seeds and induced in certain plant tissues by herbivory or wounding [1]. PIs commonly are present specifically in storage organs and their synthesis can be induced systemically or locally by cell damage [2]. Plant proteases have been implicated in the regulation of defense, embryogenesis, cell fate, cuticle formation and general cellular housekeeping [3]. For cellular homeostasis plants must regulate protease activity and PIs serve as a means of protease regulation by directly binding to catalytic sites and blocking activity [4]. In addition to regulation of endogenous proteinases particularly during the dormancy of seeds PIs act as protective agents against insect or microbial predators [5]. Proteinase inhibitors are grouped into families according to their specificity. PIs are classified into serine-, cysteine-, aspartate- and metallo-proteinase inhibitors [6]. Of these, the most abundant is serine PIs that are present in seeds, leaves and tubers of several plants [7].

Depending on the pH of the gut, different classes of proteases are used by different insects. The gut pH of Lepidopterans varies from 8 to 11 and they utilize mostly serine endopeptidases for digestion. It is well established that specific serine PIs can reduce the growth rate and survival of

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Lepidopteran insects [8,9]. The inhibition of proteases present in insect guts or secreted by microorganisms, lead to a reduction in the availability of amino acids necessary for the growth and development in insects [10]. Two purified PIs from soybean when fed to third instar larvae of the melon fruit fly *Bactrocera cucurbitae* (Coquillett) delayed developmental period and decreased the percentage of pupation and adult emergence [11]. Transgenic plants expressing foreign PIs have been produced for enhanced levels of insect resistance. Thus the study of proteases and PIs offers tremendous potential in developing better pest control strategies. *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae) popularly known as swarming caterpillar or paddy armyworm is a sporadic pest of *Oryza sativa*. In this study, we screened several plant seeds and found that extracts from jackfruit seed (*Artocarpus heterophyllus*) and areca nut (*Areca catechu* L.) inhibited the protease activity of gut extract of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae).

Casein was obtained from Nice Chemicals Pvt. Ltd., Cochin. All other chemicals used were of analytical grade. The adult moths of the insect were collected at night using fluorescent light traps. They were kept in glass beakers covered with muslin cloth and fed with a dilute solution of honey. They were allowed to mate and lay eggs. Larvae hatched out after 3-4 days and were reared in plastic troughs at room temperature, fed with fresh leaves of the grass *Ischaemum aristatum.* The total larval period was found to range from 17-19 days and consisted of 6 larval instars. Intestine of several 6^{th} instar larvae were dissected out and weighed. Then homogenized with 1ml of sodium bicarbonate buffer (pH=9) per gm of tissue. The homogenate was centrifuged at 10,000 rpm at 4ºC for 10 minutes (Eltek refrigerated centrifuge RC 4100 D). The soluble protein recovered from the supernatant was stored at 4ºC until use. Seeds were collected from Botanical Garden, University of Calicut and neighbouring places and were soaked overnight in bicarbonate buffer (pH=9), 2.5 ml per gm seed, and ground in mortar for few minutes. The homogenate was centrifuged at 10,000 rpm at 4ºC for 10 minutes (Eltek refrigerated centrifuge RC 4100 D). The soluble proteins recovered from the supernatant were used for protease inhibition assay.

The total protease activity was assessed by incubating 10µl (100 µg) of crude gut extract with 25 µl of 2% casein and made up to a volume of 60 µlwith bicarbonate buffer, pH 9.0. The mixture was incubated for 1 hour at room temperature. To assess the inhibition, 25 µl of seed extract was pre-incubated with gut extract for 15 minutes and then added to the assay mixture containing casein. Inhibitor (seed extract) together with casein was incubated for 1 hour to assess the proteolytic activity of the seed extract alone (inhibitor control). A control without enzyme was also included. At the end of the incubation enzyme extract and inhibitor (seed extract) was added to tubes such that all the tubes have gut extract and seed extract. To stop the reaction the mixture was diluted to 1 ml and then 0.5 ml of 10% trichloro acetic acid added. The tubes were centrifuged at 10,000 rpm at 4ºC for 10 minutes (Eltek refrigerated centrifuge RC 4100 D). From the supernatant 0.5 ml was diluted to 1 ml with 0.1N sodium hydroxide and the concentration of amino acid in the supernatant measured by Lowry's method [12]. All assays were done in triplicate and percentage of inhibition calculated by taking the activity in presence of the enzyme extract as 100%. The absorbance of the seed extract alone was subtracted from the absorbance in presence of the inhibitor to account for the amino acid released by the proteolytic activity of the seed extract alone. Statistical analysis was performed with R-package.

S. mauritia gut extract proteases cleaved casein and this proteolytic activity was inhibited by aqueous extracts of jackfruit seed and extract from areca nut. The inhibition was up to 77.69% for jackfruit seed extract and up to 61.61 % for areca nut (Table 1). An inhibitor which inhibits vertebrate trypsin/chymotrypsin activity was reported from jackfruit seed extract by Bhut and Pattabiraman [13]. It is likely that the same inhibitor may also be involved in the inhibition of *S. mauritia* gut protease. Areca nut aqueous extract also inhibited the activity of *S. mauritia* gut protease. An HIV protease inhibitor was reported from methanol extract of areca nut [14].

Table 1. Inhibition of *Spodoptera mauritia* gut protease activity by aqueous extracts of jackfruit seed and areca nut.

Name of extract	Protein concentration of seed extract in the incubation mixture (µg/µl) (±SD)	% Inhibition of caseinolytic activity (±SD)
Jackfruit seed	5.51 ± 0.18	77.69 ± 1.41
Areca nut	2.95 ± 0.06	61.61 ± 1.20

Ethanolic (90%) aqueous extract of areca nut inhibited porcine pancreatic elastase and human leukocyte elastase [15]. As the proteinase inhibitors from jackfruit seed and areca nut inhibit *S. mauritia* gut protease to great extent, purification and characterization of these protease inhibitors will be helpful in designing better insect control strategies including development of transgenic plants over expressing the protease inhibitor.

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