

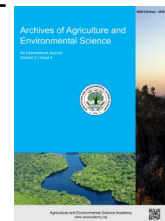


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ORIGINAL RESEARCH ARTICLE



## Qualitative detection of different enzymes from the gut and salivary glands of *Nezara viridula* Linn. using the chemical inference

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### ABSTRACT

Globally the crop insect pests damage the 30% of agriculture production due to insect spoil, and the situation is very awful in particularly developing countries such as India. There are large number of insect pest families are well known which induce the critical destroy to agriculture crops. Crop insect pest's harm is one of the significant features that ruin the agriculture crop productivity, although the large scale utilization of chemical pesticides. The successfulness of the pesticides depends upon the interaction of chemicals with the physiology of the insect of the pests. In India, the *Nezara viridula* Linn, is one of the critical insect because of its destructive feeding on major crop which is the utmost source of economic deprivation in agriculture. The enzymes present in the gut and salivary glands of the *N. Viridula* play key role in the feeding behaviour. Presently very little is investigated about the gut and salivary glands of the *N. Viridula* at minute level. We first time investigated the different enzymes existing in the gut and salivary gland using the chemical inference. This research work is carried out to analysis the enzymes for digestive and feeding behaviour of *N. Viridula* which will assist the biologists to manage the challenges are created by their resistance to the traditional chemical insecticides used for prevention of *N. Viridula* (a type of southern green stink bug) inhabitants and high reproductive amplitude. The extensive statistics of invertase enzymes, maltase, alpha galactosidase, amylase, esterase, proteinase and polypeptidase enzymes for the *N. Viridula* in salivary glands and gut inspired by this research imparts supplementary resource for further understanding of the biology of this Pentatomidae (Heteroptera) species.

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### INTRODUCTION

Insects are the wide range of dissimilar species alive creatures on planet. They are found in the various diverse regions such as forest, open Ocean, swamps, deserts, pools of crude petroleum etc. The large quantity or billions in number may survive in large area of land on earth. There are millions of species have been investigated, identified and studied. In spite of these large numbers of insect species up until now identified. Among, the entire organisms on earth, the insect pests are the main consumers of crops and plants. Therefore, these insect pests are mainly

responsible for the crop spoiling and disease transmission and possess the significant influence on the economic loss and health of human being. It is analysed that the almost 30% of the annual crop production is demolish by the insects in world (Sasser and Freckman 1987). The main obstacle of insect pests in developing countries rises day by day. In India crop losses is almost the 15.7% in present time and also gives the yearly economic loss about the US 36 billion dollar (Dhaliwal *et al.*, 2015). From the last three decades the large amount of chemical pesticides were used in both developed and developing countries.



**Figure 1.** Morphological features of *Nezara viridula* Linn.

These chemical pesticides are implemented to control pests along with augmented the crop yield in modern agriculture which involved the various chemicals such as herbicides, insecticides and fungicides. It is well established that the efficiency rate of pesticides depend upon the control of pests such as algae, bacteria, insects, rodents, or nematodes in agriculture. The categorizations of chemical pesticides primary established on their nature, employ and physiological effects (Lushchaka *et al.*, 2018). On the basis of targeted insect pests the pesticides are categorised as fungicides, herbicides, and insecticides. Therefore, it is significant to investigate the physiology of insect pests. The digestive system of insect is main part for the feeding of plants. The insect herbivore is the major crop damage which causes the economic loss in agriculture in India (Nicol and Sasser, 2011). *Nezara viridula* Linn. Species belong to the family of Pentatomidae (Heteroptera), (Panizzi *et al.*, 1997). *N. Viridula* (Figure 1) is a connection with the Phytophagous stink bugs (Heteroptera: Pentatomidae) which are primary pests of numerous crops and nourishing predominantly on the immature fruits and seeds of more than 100 plant species in 30 various families (Todd *et al.*, 1989).

In the course of feeding, they instigate their stylets to abolish the cells' contents. This causing the destruction which includes drop and/or malformation of seeds and fruits. A number of these plants include the costly and profitable significance crops such as cotton, soybean, and corn (Tillman *et al.*, 2009). *Nezara viridula* Linn nourishes by a piercing-sucking procedure via digestion initiation with the extra-oral secretion of saliva with digestive enzymes like trypsin and chymotrypsin into plant tissues. The studying of the enzymes naturally existing in the digestive region of pests is basically predominant to understand the features. It provides the initial intention for physiological and chemical inspection of the insect's digestive processes. There are lots of understanding acquire such that the how many types of foodstuffs that can be digested and also by products during digestion. All organisms involve the proteases that hydrolyze peptide bonds in order to preserving the systemic homeostasis and for its normal growth and development



**Figure 2.** Living of *N. viridula* on the host plant of bajra.

(Otín, 2008). There is only report on the *N. Viridula* parasitizing on vegetable crop of *Vigna unguiculata* (Linn.) Walp (Ewunkem *et al.*, 2020). They worked on the features of histopathology and pathogenicity of phaeohyphomycosis of *Nezara viridula* caused by *C. Lunata* (Singh *et al.*, 1991). Since there are no reports on detection of different enzymes in the gut and salivary glands of *N. Viridula* were found in the literature. It is a crop feeding insect of economic importance, being particularly fatal the crops such as cotton, bajra (Figure 2 *N. viridula* on host plant of bajra), soybean, and corn in India. The digestive enzymes and alimentary gut are typical of those of most *N. viridula*. Here in this research article, we investigate the digestive enzymes in alimentary canal and gut of the *Nezara viridula* Linn. The present paper reports a sequence of qualitative detection of a few of the digestive enzymes present in the alimentary gut of the *N. viridula*.

## MATERIALS AND METHODS

### Qualitative detection of enzymes

Qualitative detection of enzymes, the extracts were prepared as described by (Swingle, 1925; Thomson *et al.*, 1932; Farris *et al.*, 2016, Banik *et al.*, 2018).

### Preparation of extract

The insect was anesthetized dissected in Ringer's solution for preparing the enzyme extract. The alimentary canal after dissection was subjected to washing in distilled water. The gut and the salivary glands were placed at 20°C in distilled water with few toluene drops to stop fungal growth. The various parts of the gut were placed in the mortar and crushed with the pestle into pulp which was subjected to centrifugation and the homogenate substance leaves supernatant liquid above, which was placed in labelled micro-tubes which is subjected to testing individually. The micro-tubes were covered at the top with surgical cotton. As per the test of each enzyme, the micro tube was incubated and subjected to various enzyme detection.

## RESULTS AND DISCUSSION

The saliva of *N. viridula* included the large variety of digestive enzymes which either induce or inhibit plant defence (Will *et al.*, 2013). The important function of *N. iridula* saliva was likely to play a key role in crop-insect interactions. The digestive enzymes of insect physiology on which strangely negligible research has been done so far, in view of the economic importance of the food of insects. Since many limitations of the various enzymes were undoubtedly ingested stink bug during normal feeding on crops and were also discharged by the micro-organisms present in the digestive tract. The alimentary canal of stink bug (*N. viridula*) was straight and unbranched, and the mid-gut has no appendages or various histological portions (Figure 3). The salivary glands and the gut were the only organs which may be anticipate the detailed of true digestive enzymes.

It was previously observed that there is no fluid in the gut which is not enough to moisten a filter paper, and certainly not sufficient to collect in a capillary tube. After a number of different methods have been tried and the following procedure or tests were adopted to study the variety of digestive enzymes in salivary glands (Table 1 (a-b); Figure 4) and gut of the *N. viridula*:

### Invertase enzymes

For the test of invertase the above homogenate extract was placed in microtube and two drops of three percent solution of sucrose was added to it and the mixture is incubated for 48 hours. After these following tests were carried out.

### Fluckiger's test

For this test small quantity of powdered copper tartrate and one drop of 20% NaOH was placed on a slide till the copper salt was dissolved. In this one drop of incubated gut extract was added. Precipitate appears on heating. This indicated the presence of invertase enzymes.

### Barfoed's test

In this test, the incubated mixture was added to Barfoed's reagent and then it was boiled for one minute and allowed to cool. Brick red colour appeared which confirmed the presence of invertase enzyme.

**Inference:** Maximum activity of invertase has been observed in midgut III of *N. viridula*. In midgut I, II, IV and showed the weak activity while absent in salivary gland and hind gut of *N. viridula*.

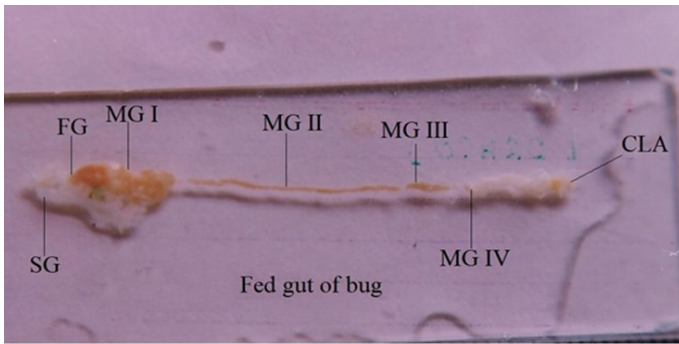
**Table 1 (a).** Summary of tests results for enzymes in the digestive tract and salivary gland of *N. viridula*.

Incubation period in hours	Salivary gland	Mid get regions				Hindgut regions
		I	II	III	IV	
48	-	I	II	III	IV	-
48	-	+	+	±±	+	±
24-48	±	+	±	+	±	±
36	+	+	±	+	±	±
96	+	+	+	+	±	±
96	-	±	±	-	-	-
48	-	-	+	+	-	-
24-48	-	-	±	±	-	-
96-120	±	±	±	-	-	-
48-120	-	-	-	-	-	-

Symbols: ++ = strong action, + = weak action, ± = Present in traces or very weak, - = Absent

**Table 1 (b).** Continued.....

Enzymes	Substrate	Test
INVERTASE	5% Sucrose solution	Fluckiger's test Barfoed'sTest
MALTASE	15% Maltaose solution	Barfoed'sTest
ALPHAGALACTOSIDASE	5% Raffionse solution	Fluckiger's test Barfoed'sTest
AMYLASE	0.5% Lactose Solution	KI + I test Fehling's test
BETAGALACTOSIDASE	5% Lactose solution	Fehling's test
LIPASE	0.5ml. Olive emulsion	Chlorophenol +0.1 N Na <sub>2</sub> CO <sub>3</sub>
ESTERASE	1% Emulsion of Ethyl acetate	1% Na <sub>2</sub> CO <sub>3</sub> + One drop Phenol red
PROTEINASE	1% Alkaline casein solution	1% Acetic acid (drop by drop)
POLYPEPTIDASE	1% Peptone Solution	1% Peptone Solution



SG- Salivary Gland, FG- Fore gut, MG-Mid gut, CLA- Cloacal aperture

**Figure 3.** Fed gut of bug.

### Maltase

In this test, 3% maltose solution was used as a substrate in micro-tube. In each micro-tube containing substrate, few drops of toluene were added and then it was incubated for 1-2 days. Its presence was detected and confirmed by Barfoed's test.

**Inference:** This enzyme showed very weak action in salivary gland, midgut II, III, IV and hind gut while weak action in midgut I of *N. viridula*.

### Alpha galactosidase

For the detection of Alpha galactosidase, Raffinose was used as substrate. The extracts of gut and salivary glands were incubated with 5% Raffinose solution (substrate) for 36 hours. It was then tested frofluckiger's and Barfoed's reagent for reducing sugars.

**Inference:** It showed weak activity in salivary glands, midgut I, II and III while IV midgut and hindgut of *N. viridula* in traces.

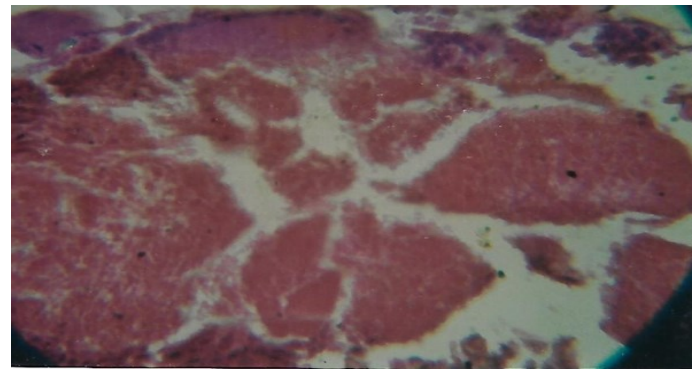
### Amylase

Absence or presence of amylase enzymes was detected and confirmed as under:

### Potassium iodide-iodine test

The homogenate extracts of different regions of the gut and salivary glands were taken in the micro-tubes separately in which few drops of 1% soluble starch solution was added. Simultaneously, few drops of extract in a separate micro-tube were also taken as a control in which the extract with substrate was boiled on water-bath for about 30 minutes to eliminate the presence of enzymes. After 24 and 48 hours, one drop of mixture was taken out and one drop of potassium iodide-iodine solution was added to it. If blue colour appeared, it would indicate that starch is not yet hydrolysed.

Solution which did not showed the blue colour with potassium iodide-iodine solution was tested for the presence of maltase which appeared as a decomposed product by picramic acid test. In this, four drops of incubated solution, one drop of 10% sodium hydroxide and 2 drops of saturated aqueous picric acid was placed in a micro-tube, and placed in an oven at 60°C. The yellow colour of picric acid changed gradually into reddish brown picramic acid, which indicated that amylase enzyme was present.



**Figure 4:** T.S. of salivary gland (Fed) of *N. viridula*.

### Fehling's solution test

The above test for reducing sugar can be confirmed by Fehling's solution test. Fehling's solution was added in it and then allowed to cool. A reddish-brown precipitate appeared in the cold solution which shows reducing sugars were present.

**Inference:** In salivary glands, mid gut I, II and III weak activity was detected while in mid gut IV and hindgut of *N. viridula* very weak activity was seen.

### Beta galactosidase

In this test, 5% solution of lactose was used as substrate. For this, mixture was incubated for 48 to 96 hours and the activity of this enzyme was noted by performing the Fehling's test.

**Inference:** It was present in mid gut I and II in traces and absent in the rest part including the salivary glands.

### Amylase

Lipase splinted fat into fatty acid and glycerol. Olive emulsion was used as a substrate for the detection of this enzyme, 0.5 ml. Of this olive emulsion was incubated with an equal volume of gut extract, then added few drops of chlorophenol red and a few drops of 0.1 N solution of sodium carbonate in this mixture so that the colour of the mixture turns pinkish. After in incubation of 48 hours, pink colour changed to yellow showed the presence of this enzyme.

**Inference:** The midgut II and III shows weak activity of this enzyme.

### Esterase

For the detection of Esterase, 1% emulsion of ethyl acetate was used as substrate with 1% solution of sodium carbonate. Mixture was incubated for 24 to 48 hours. Before incubation, one drop of phenol red was added to this mixture. Colour changes from pink to yellow showing the presence of Esterase.

**Inference:** A trace of esterase enzymes was detected in midgut II and III of *N. viridula*. It was absent in rest parts.



### Proteinase

For the detection of this enzyme, 1% alkaline casein solution was used as substrate. The incubation period varies from 3 to 5 days. After incubation, proteinase activity was tested by adding 1% acetic acid drop by drop. If white precipitate did not appeared, it indicated the positive test.

**Inference:** Very weak action of proteinase enzyme was recorded in salivary gland, midgut I and II of *N. Viridula* while in rest parts, it was absent.

### Polypeptidase

The activity of this enzyme has been in the presence of 1% peptone solution. The substrate and the extract were incubated for 2 to 5 days. After incubation, the reaction mixture was tested by Burette test. It was confirmed by the presence of violet or pink colour.

**Inference:** No trace of this enzyme was recorded in the gut of *N. viridula*.

### Conclusion

In conclusion, the objective for this study was to chemically characterize the digestive enzymes from the salivary gland and gut of *N. viridula*. We have been identified the major enzymes like invertase enzymes, maltase, alpha galactosidase, amylase, esterase, proteinase and polypeptidase produced by the salivary gland and gut of *N. viridula*. The broad examination of *N. Viridula* (a type of southern green stink bug) digestive enzymes furnished here may anticipate the guide for novel control approaches intending the digestive enzymes for management of multiple stink bug of Pentatomidae (Heteroptera) species, and point out the common enzymatic challenges faced by biologists in evolution for stink bug control for the development of chemical insecticides and pesticides.

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### Conflicts of Interest

The author declares there are no conflicts of interest.

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