



E-ISSN: 2320-7078

P-ISSN: 2349-6800

[www.entomoljournal.com](http://www.entomoljournal.com)

JEZS 2021; 9(1): 2212-2214

© 2021 JEZS

Received: 29-10-2020

Accepted: 06-01-2021

**Bavita Yadav**

Department of Plant Molecular  
Biology & Genetic Engineering,  
Acharya Narendra Deva  
University of Agriculture &  
Technology, Kumarganj,  
Ayodhya, Uttar Pradesh, India

**Pratibha Yadav**

Department of Plant Molecular  
Biology & Genetic Engineering,  
Acharya Narendra Deva  
University of Agriculture &  
Technology, Kumarganj,  
Ayodhya, Uttar Pradesh, India

**Sumant Pratap Singh**

Department of Plant Molecular  
Biology & Genetic Engineering,  
Acharya Narendra Deva  
University of Agriculture &  
Technology, Kumarganj,  
Ayodhya, Uttar Pradesh, India

**Nawaz Ahmad Khan**

Department of Plant Molecular  
Biology & Genetic Engineering,  
Acharya Narendra Deva  
University of Agriculture &  
Technology, Kumarganj,  
Ayodhya, Uttar Pradesh, India

## Insect bioassay test using pods protein of pigeon pea against pod borer (*Helicoverpa armigera*)

**Bavita Yadav, Pratibha Yadav, Sumant Pratap Singh and Nawaz Ahmad Khan**

### Abstract

Insects are responsible for major crop losses in pigeon pea. Here we have conducted an experiment in laboratory to test pigeon pea pod proteins activity against *H. armigera*. The total protein was extracted from 40 g pods of nine cultivated pigeon pea genotypes and one wild type species *Cajanus scarabaeoides* IC15683. Proteins were precipitated at 80% ASF and followed by dialysis. Total protein was concentrated at a range of 1mg/ml. Method of insects rearing and the bioassay of insect were examined on the neonatal larvae of *H. armigera* in laboratory (25±2 °C) by feeding the protein extract incorporated in chickpea based artificial diet. 100µl of protein samples were uniformly coated on the surface of diet and allowed to get absorbed. Two insect larvae were released in each plates and their average weight was measured after 2, 4 and 6 days intervals. Mortality and percent growth retardation was calculated in comparison to control. Wild species showed growth retardation in larvae as compared to the rest genotypes. It has been observed that Protein extracts of cultivated pigeon pea genotypes did not showed any insecticidal activity or growth retardation in insect larvae while *Cajanus scarabaeoides* IC15683 showed retardation in growth as compared with cultivated genotype. Thus the result of the present investigation indicated that deep study of wild type pods protein will be helpful in future for developing insect resistance plant from natural plant proteins.

**Keywords:** Insect, pigeon pea, *Cajanus scarabaeoides*

### Introduction

Pigeon pea (*Cajanus cajan* (L.) Millspaugh), belonging to the genus *Cajanus* and family Fabaceae, is one of the most important legume crops of India. It is an integral component of the livelihood of marginal resource poor farmers apart from being a key source of dietary protein for the vegetarian population (Rani *et al.*, 2018)<sup>[7]</sup>. Continuous efforts have been made in pigeon pea towards crop improvement, stagnant and unstable productivity are the characteristic features of this crop due to its inability to alleviate various biotic and abiotic factors (Choudhary *et al.*, 2013)<sup>[3]</sup>. Among the various biotic stresses, insect pests are responsible for major yield loss. Amongst various insect pests *Helicoverpa armigera* is the most devastating (Rani *et al.*, 2018; Ramu *et al.*, 2012)<sup>[7, 6]</sup>. *Helicoverpa armigera*, a polyphagous pest, causes significant yield losses in many agriculturally important crops like cotton, chickpea, pigeon pea, corn, maize, tomato, okra, sorghum, pearl millet, sunflower, and groundnut (Volpicella *et al.*, 2003)<sup>[11]</sup>. Studies have been done for plants resistance to insects in this context to use insect-feeding bioassays to examine the efficiency of plant synthesizes various proteinaceous compounds such as plant defense proteins against an insect attack. Wild relatives of pigeon pea have great potential of resistance against insect attack. So performing a bioassay of *H. armigera* challenged *C. scarabaeoides* and *C. cajan* can provide insights response at the biochemical level. In the present study, we attempt the insect bioassay against protein of cultivated pigeon pea, *C. cajan* and resistant wild relative of pigeon pea, *C. scarabaeoides* on basis of toxicity and larval growth retardation.

### Materials and methods

#### Plant material

Pods of ten pigeon pea genotypes were collected freshly from student instructional farm, ANDUA&T, Kumarganj Ayodhya. Plant materials were stored at -80°C until use.

**Corresponding Author:****Bavita Yadav**

Department of Plant Molecular  
Biology & Genetic Engineering,  
Acharya Narendra Deva  
University of Agriculture &  
Technology, Kumarganj,  
Ayodhya, Uttar Pradesh, India

### Protein extraction

Total soluble proteins were extracted separately from immature pods of ten pigeon pea genotypes; the extraction was carried out at 4 °C. The samples were chopped into small pieces and then grind using liquid nitrogen into mortar and pestle. Sodium phosphate buffer was added in grinded sample. After thawing, samples were homogenized and incubated for 1 hr. Samples were filtered, then spin 5000 rpm for 20 min to collect homogenous supernatant.

### Protein precipitation

Supernatant volume was measured and weighed proper ammonium sulphate amount for each sample. Proteins were precipitated by adding 80% ammonium sulphate with continuous stirring, then centrifuged at 12000rpm for 15 min. Taken pellets and re-suspended in 20mM Tris buffer (pH 8.0) in minimum volume.

### De-salting

Salt from re-suspended protein samples were removed by dialyzing against 20mM Tris buffer pH8.0 (buffer changes 3 times at 8 h intervals). Dialyzed proteins were concentrated on 5kDa molecular cut off (MWCO) filter. Concentrated protein samples were finally clarified by centrifugation at 18000 rpm for 20 min and supernatant were collected.

### Protein quantification

Concentration of protein were measured by protein assay reagent (Bio-Rad) based on Bradford method (Bradford 1976). Protein estimation involved the addition of assay reagent to protein samples, incubated for 5 min and measurement of absorbance at 595 nm. Bovine serum albumin served as standard. Quality of protein samples was examined on denaturing polyacrylamide gel.

### Insect culture

Neonatal larvae were obtained from IIPR Kanpur and maintained in our laboratory in chickpea based artificial diet.

### Insect Bioassay

Insect bioassay was performed to examine the toxicity of protein extracts. Insect were fed with protein samples mixed in artificial diet. Method of insects rearing and the bioassay of insect were examined on the neonatal larvae of *H. armigera* by feeding the protein extract incorporated in chickpea based artificial diet (Armes *et al.*, 1992) [1].

1. Larval diet was prepared and poured in insect rearing tray and kept for solidification.
2. After proper solidification, the diet was cut into small pieces and transferred into 30 Petri plates.
3. 100µl of protein samples containing 100 µg protein concentrations were uniformly coated on the surface of diet and allowed to get absorbed.
4. Two insect larvae were released in each plates and their average weight was measured after 2, 4 and 6 days.
5. Mortality and percent growth retardation was calculated in comparison to control.

### Result and Discussion

Total soluble proteins were extracted separately from the pods of ten pigeon pea genotypes. The nature of protein and have toxic is not known, but this approach could be useful for control of polyphagous *H. armigera* in Indian Agriculture. Based on laboratory studied on *M. anisopliae* Gopalkrishnan and Narayanan (1989) reported 80 to 100% mortality of ammonium sulphate fraction protein.



Fig 1: Images showing culture of *Helicoverpa armigera*



Fig 2: Pod borer feeding on protein contained artificial diet

Rhodes and Milton (1998) [8] have reported Ammonium Sulfate is the most common salt used for precipitating proteins due to its stability in cold solutions. Ammonium Sulfate fractionation is widely used in the first stage of protein purification to remove non protein molecules. The protein concentrations of extracts were in the range of 1.2 to 1.5 mg/ml. The extracted proteins were used for insect bioassay.

The protein extracts were tested on *H. armigera* at concentration of 250µg/ml for insecticidal activity. The bioassay results are shown in table 1. The results suggest that the larvae reared on the *Cajanus scarabaeoides* pod protein consumed relatively less food and showed significantly lower larval weight as compared to cultivated genotypes. Protein extracts of wild species caused growth retardation to *H.*

*armigera*. Extracts of rest of the species did not showed insecticidal activity or growth retardation against insect.

**Table 1:** Result of bioassay test by total protein content

| S. No. | Proteins extract (genotype) | Toxicity of the protein extract |           | Weight (mg) |
|--------|-----------------------------|---------------------------------|-----------|-------------|
|        |                             | Growth retardation              | Mortality |             |
| 1.     | NDA-1                       | Not occurred                    | Nil       | 0.479       |
| 2.     | NDA-2                       | Not occurred                    | Nil       | 0.438       |
| 3.     | BAHAR                       | Not occurred                    | Nil       | 0.451       |
| 4.     | NDA-14-6                    | Not occurred                    | Nil       | 0.444       |
| 5.     | NDA-13-6                    | Not occurred                    | Nil       | 0.439       |
| 6.     | NDA-3                       | Not occurred                    | Nil       | 0.439       |
| 7.     | UPAS-120                    | Not occurred                    | Nil       | 0.431       |
| 8.     | MAL-6                       | Not occurred                    | Nil       | 0.469       |
| 9.     | MAL-13                      | Not occurred                    | Nil       | 0.463       |
| 10.    | Wild                        | Occured                         | Nil       | 0.339       |

Toxic principle active against *H. armigera* was not detected in the protein extracts from any of the pigeon pea species tested in the study. Total soluble protein were higher in the pods of cultivated pigeon pea than in the wild relatives, and this may be one of the factors leading to greater feeding by *H. armigera* larvae on the pods of cultivated pigeon pea compared to the wild pigeon peas. Thus wild accessions of pigeon pea are used as a resistance donors to *H. armigera* (Romeis *et al.*, 1999; Sharma *et al.*, 2001) <sup>[9, 10]</sup>. MacFoy *et al.*, (1983) <sup>[5]</sup> recorded high concentrations of sugars and amino acids in the cowpea cultivar Vita-1, which is susceptible to spotted pod borer, *Maruca testulalis* (Geyer).

### Conclusion

Pigeon pea is largely cultivated in various states of India and provide dietary protein to major population. Production of this crop is mainly destroyed by pod borer (*H. armigera*). Wild accession of pigeon pea exhibiting high level of resistance to *H. armigera*. So it may be possible to identify resistant protein (gene) into wild accession and incorporate in cultivated genotype against insect pests.

### Acknowledgement

I thank to department of plant molecular biology and genetic engineering, ANDUA&T, Kumarganj Ayodhya for providing necessary facility and also thankful to Indian Institute of pulse research, Kanpur for providing neonate larvae of *H. armigera* so that I could proceed with the experiment.

### References

- Armes NJ, Bond GS, Cooter RJ. The Laboratory Culture and Development of *Helicoverpa armigera*. Bulletin No. 57. Natural Resources Institute, Chatham, UK 1992.
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry 1976;72(1-2):248-254
- Choudhary AK, Raje RS, Datta S, Sultana R, Ontagodi T. Conventional and molecular approaches towards genetic improvement in pigeon pea for insects resistance. American Journal of Plant Science 2013;4:372-385
- Gopalakrishnan C, Narayanan K. Occurrence of two entomofungal pathogens *Metarhizium anisopliae* (Metschnikoff) Sorokin var. minor Tulloch and *N. omuraea* riley; (Farlow) Samson on *Heliothis armigera* Hubner (Noctuidae: Lepidoptera). J. Bio. Cont. 1988;

3(1):50-52.

- MacFoy CA, Dabrowski ZT, Okech S. Studies on the legume pod borer *Maruca testulalis* (Geyer)—VI. Cowpea resistance to oviposition and larval feeding. Insect Sci Appl 1983;1(2):147-152.
- Ramu SV, Rohini S, Keshavareddy G, Gowri NM, Shanmugam NB, Kumar ARV et al. Expression of a synthetic cry1AcF gene in transgenic Pigeon pea confers resistance to *Helicoverpa armigera*. Journal of Entomology. 2012;136:675-687
- Rani DS, Kumar SP, Venkatesh MN, Sri CNS, Kumar KA Bio efficacy of insecticides against gram pod borer, *Helicoverpa armigera* in Redgram. Journal of Entomology and Zoological Studies 2018;6:3173-3176.
- Rhodes M, Milton J. Lectin methods and protocols. New Jersey, Humana Press 1998.
- Romeis J, Shanower TG, Peter AJ. Trichomes on pigeon pea (*Cajanus cajan*) and two wild *Cajanus* spp. Crop Science 1999;39:564-569.
- Sharma HC, Green PWC, Stevenson PC, Simmonds MSJ. What makes it tasty for the pest? Identification of *Helicoverpa armigera* (Hubner) feeding stimulants and location of their production on the pod-surface of pigeon pea [*Cajanus cajan* (L.) Millsp.]. Competitive Research Facility Project R7029C. Final Technical Report. Department for International Development, London, UK 2001.
- Volpicella M, Ceci LR, Cordewener J. "Properties of purified gut trypsin from *Helicoverpa zea*, adapted to proteinase inhibitors. European Journal of Biochemistry. 2003; 270(1):10-19.
- Khan Y, Yuan C, Roy M, Khan Z, Yaqub Khan M, Hsu PC, Sharma R, Srivastava H. Insects as a source of food for human hunger: a glimpse of hope for the future. International Journal of Food Science and Nutrition. 2020;5:132-7.