Int. J. Curr. Res. Biosci. Plant Biol. 2016, 3(4): 83-90

EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 (Online) • Volume 3 • Number 4 (April-2016)

Journal homepage: www.ijcrbp.com

Original Research Article

doi: http://dx.doi.org/10.20546/ijcrbp.2016.304.013

Role of Physical and Biochemical Parameters of Different Cluster Bean Varieties on the Guar Gum Content and Aphid Infestation

Pratyusha Sambangi[1] and Pathipati Usha Rani[2]*

[1]*Senior Research Fellow, Biology and Biotechnology Division, CSIR- Indian Institute of Chemical Technology, Tarnaka, Hyderabad – 500 007, Andhra Pradesh, India*

[2]*Senior Principal Scientist, Biology and Biotechnology Division, CSIR - Indian Institute of Chemical Technology, Taranaka, Hyderabad – 500 007, Andhra Pradesh, India*

Corresponding author.

Abstract

Cluster bean, *Cyamopsis tetragonoloba* L. is one of the most important and potential vegetable cum industrial crop grown for its tender pods as vegetable and for endospermic gum (30-35%). The present study was conducted in a net house using pot experiments on seven different cluster bean varietal plants namely HG365, JJ1, JG2, GL1031, REC1025, RGC963, and RGC936. In these guar plants the physical, biochemical and enzymatic parameters were studied in correlation to the guar gum content and *Aphis craccivora* K. infestation. The physical parameters like germination percentage, seedling vigour and vigour index were superior in RGC963, HG365, JJ1, REC1025 plant varieties resulting in less aphid infestation. In addition, the gum content, total soluble sugars and total protein content were high in guar varieties of RGC963, HG365, JJ1, REC1025 followed by JG2, GL1031, and RGC936 along with a reduced content of amino acids. Whereas, enhanced levels of primary metabolites and antioxidant enzymes such as peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) were observed in the aphid infested cluster bean plants in comparison to normal, uninfested plants in all the varieties. This study reveals the possible role of enhanced plant biochemicals like carbohydrates and phenols towards higher yield of guar gum and lower aphid infestation respectively. In future the present research can lead us to the development of cluster bean varietal plants with higher gum yield potential for desired resistance towards biotic stresses.

Article Info

Accepted: 01 April 2016
Available Online: 06 April 2016

Keywords

Anti-oxidant
Aphid infestation
Cyamopsis tetragonoloba
Gum content
Phenol content
Plant varieties

Introduction

Cluster bean, *Cyamposis tetragonoloba* L. or guar bean is an annual legume and belongs to the family Leguminaceae and subfamily Paplionaceae. It is cultivated mainly as a rainfed crop in arid and semi arid regions for vegetable, forage and green manure. India is one of the main producers of cluster bean accounting to 80% of total world production. It is also a well known cash crop (Pathak et al., 2010). Guar gum has emerged as the most important agro-chemical, which is non-toxic, eco-friendly and Generally Recognized as Safe (GRAS) by FDA. Seeds of cluster bean have large endosperm (35-42%), and contain galactomannan type of gum which forms a viscous gel and has diversified applications in sectors, viz. paper, food, cosmetics, mining, petroleum, well drilling, pharmaceuticals etc (Pathak et al., 2010; Senapati et al., 2006). The guar gum also has high demand in agriculture sector as water retainer, soil aggregate and anti-crusting agent. Due to its good binding property it is used as an ingredient in products like sauces and ice creams etc. The cluster bean is

cultivated in many parts of India, and only some of its varieties are used for the extraction of gum. Thus, among different varieties produced, the best varieties with higher yield of gum content are often preferred.

Cowpea aphid, *Aphis craccivora* infestation is recorded in considerable proportion in areas where guar is grown (Mohy-ud-din et al., 2009). It is a phloem-sap feeder well known to reduce growth and dry-mass yield of cluster bean. It also accounts for considerable yield losses in India. Due to the aphid feeding on cluster bean the plant growth rates are normally affected. This biotic stress also may induce various chemical, biochemical and anti-oxidative enzymatic changes as a defensive response. Therefore in the present study, attempts were made to elucidate the cluster bean plant chemical and enzymatic responses to the aphid feeding. Further correlation between the guar gum content and the infestation rates of aphids were also examined, as the higher contents of gum content in the guar plant may reduce the ease of aphid sucking on the plant tissue.

Meager information is available on the varietal variability of cluster bean plants and therefore the present study was undertaken to assess the extent of physical and biochemical parameters among seven varieties of guar and their association with qualitative trait *viz*., gum content and on the rates of aphid infestation. This study also aimed at understanding of defense strategies in the cluster bean plant under feeding stress caused by *A. craccivora*. The impact of herbivory on the plant metabolites and antioxidant enzymes was determined in both the infested and healthy cluster bean plants.

Materials and methods

Plants

Cluster bean *Cyamopsis tetragonoloba* L. seeds of seven varieties HG365, JJ1, JG2, GL1031, REC1025, RGC963, and RGC936 were procured from Agricultural Research Station, ANGRAU, Anantapur, Andhra Pradesh. The seeds were soaked in water for 10 h and incubated at 28°C for 24 hrs, and the germinated seeds were planted in 2 L pots. The plants were watered (500 ml) for every 2 days.

Insects

A colony of aphids, *Aphis craccivora* Koch, was maintained on cluster bean plants placed in isolation cages under controlled temperature conditions. To maintain the insect population, 3-week-old plants were continuously supplied to the aphid culture. Infestation of the plants was done by releasing apterous adults of *A. craccivora* on to the cluster bean plants of four week old and allowed to settle. After 24 hrs of release the number of feeding aphids on each plant was counted in order to calculate the rate of infestation.

Physical parameters

Germination percentage: In between the moistened germination filter papers twenty seeds in five replicates were placed and incubated in a germinator maintained at constant temperature of 30°C. Seeds with 0.5cm radical and plumule were considered as germinated (ISTA, 1993).

Seedling vigour: Seeds were planted between germination papers and kept in vigour stands maintained at 30°C. After seven days total root length and shoot length of all seedlings were noted.

Vigour index: Vigour index was calculated by taking the product of seedling vigour (root and shoot length) and germination percentage (Abdul-Baki and Anderson, 1973)

Biochemical parameters

Gum content: The gum content in seeds of cluster bean was estimated by the method of Das et al. (1977) and modified by Joshi (2004). Grounded seed sample (100mg) was weighed and transferred to a conical flask with 40mL of 0.01M $HgCl_2$ and autoclaved at 15 psi for 1 hr. The samples were cooled and the volume was made to 100mL with distilled water. From this extract 10mL was taken and centrifuged at 5000 rpm for 15 min, and 0.5mL of supernatant was taken in another centrifuge tube, to which 4.5mL ethyl alcohol was added to make 90% alcohol. The solution was kept overnight and centrifuged at 5000 rpm and supernatant was removed. Then the residue was dissolved in 0.01M $HgCl_2$ (5 mL) by boiling in the water bath for 1 hr, and volume was made to 5mL after cooling. From this 1mL of the extract was taken in a test tube and 2mL of 2% phenol was added. Finally, 5mL concentrated H_2SO_4 was added followed by shaking and cooling for 30 min and absorbance was noted at 490 nm. Standard and blank were run simultaneously and Standard curve was prepared using galactose: mannose in 1: 2 ratio.

Primary metabolites: Standard methods were executed to estimate the total phenolic content (Singleton and

Rossi, 1965), total soluble sugars (Dubois et al., 1956), amino acids (Moore and Stein, 1954) and total protein content (Lowry et al., 1951). A comparative estimation of primary metabolites was carried by taking the ethanol extract (1 mL) of both aphid infested and healthy cluster bean leaves of one month aged plant.

Enzymatic parameters

To estimate the biotic stress induced defense enzymes in the uninfested and aphid infested cluster bean plants the antioxidant enzyme estimations of catalase (CAT, EC 1.11.1.6), peroxidase (POD, EC 1.11.1.7), and superoxide dismutase (SOD, EC 1.15.1.1) were performed by following the standard methods of Aebi (1984), Kar and Mishra (1976), and Beyer and Fridovich (1987), respectively.

A comparative study between insect infested and healthy cluster bean plants was done and represented. All the enzyme activities were expressed as Units/gm FW of leaf. The plant leaf material (0.2 g) was homogenized with sodium phosphate buffer at pH 7.0 for CAT, POD and at pH 7.8 for SOD activities. The supernatant was used to measure the activity of the enzymes.

Statistical analysis

All the experiments were performed in 20 replicates and the values were represented as mean ± SE. The physical and biochemical parameters of seven cluster bean varieties were analyzed using Tukey's multiple comparison test at $P<0.05$. The differences in the activities between aphid-infested and normal non-infested plants (controls) were analyzed using paired t tests at $P<0.001$. All statistical analysis was performed using the software Origin (version 7.5).

Results

Physical parameters

Seeds of seven cluster bean varieties were evaluated for their germination and seedling vigour under controlled laboratory conditions. Among them RGC963, HG365, JJ1, REC1025 varieties showed better germination rates (Fig. 1A) with further significant root and shoot growths (Fig. 1B) compared to JG2, GL1031, RGC936. This result correlated with the vigour index of the cluster bean plants. Among all the varieties tested HG365 exhibited higher vigour index followed by REC1025, JJ1, RGC963, GL1031, RGC936 and JG2 (Fig. 2).

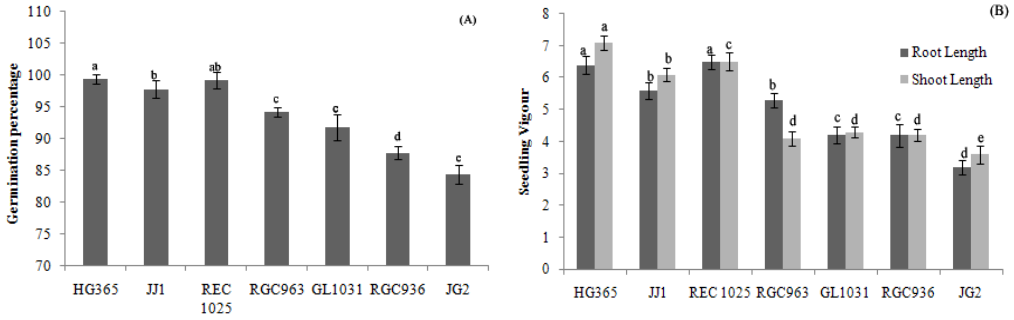

Fig. 1: Percentage of (A) germination and (B) seedling vigour of seven different cluster bean varieties. Values are mean of 20 replicates ± SE. Different letters next to the bars representing each compound indicate significant differences at $p<0.05$ by Tukey's multiple comparison test.

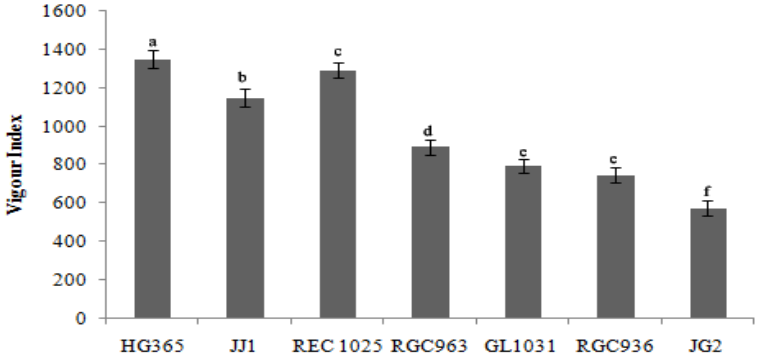

Fig. 2: Vigour Index of seven different varieties of cluster bean. Values are mean of 20 replicates ± SE. Different letters next to the bars representing each compound indicate significant differences at $p<0.05$ by Tukey's multiple comparison test.

Biochemical parameters

Experimental assessment of metabolite differences among varieties of cluster bean crops were analyzed for their influence over the yield of guar gum content and aphid infestation. The total soluble sugars or carbohydrates, total phenol content and total protein content were quantitatively higher in RGC963, HG365, JJ1, REC1025 guar varieties (Table 1). However, an elevated content of total amino acids was observed in the JG2, GL1031, RGC936 varieties. Further the guar gum content was analyzed from the seeds of all the cluster bean varieties and HG2 exhibited highest percentage of yield followed by REC1025 and JJ1 varieties (Fig. 3). Accordingly, a clear direct correlation between the plant metabolite contents and guar gum yield was observed in all the varieties. Abundance of aphids were monitored among the seven varieties of guar and higher incidence of infestation was observed in RGC936, followed by JG2 and GL1031 (Fig. 4).

Table 1. Carbohydrate and amino acid content in leaves of uninfested and aphid infested cluster bean varieties.

Varieties	Total carbohydrate content (mg/g FW)		Total amino acid content (µg/g FW)	
	Uninfested	Infested	Uninfested	Infested
HG365	1.695±0.013a	0.844±0.007b	728.79±4.14a	443.28±4.4b
JJ1	1.335±0.028a	0.740±0.002b	789.97±5.66a	432.26±5.2b
REC1025	1.37±0.039a	0.544±0.018b	465.00±7.5a	439.59±3.6b
RGC963	0.926±0.05a	0.626±0.005b	377.3±4.86a	346.3±5.4b
GL1031	0.624±0.03a	0.298±0.005b	389.88±5.78a	328.45±4.5b
RGC936	0.188±0.04a	0.142±0.004b	320.93±5.24a	274.14±6b
JG2	0.622±0.04a	0.482±0.004b	286.17±3.89a	240.98±4b

FW is the Fresh Weight. Values are mean of 20 replicates ± SE. Values followed by different letters in a row are statistically different (p<0.001, paired t-test).

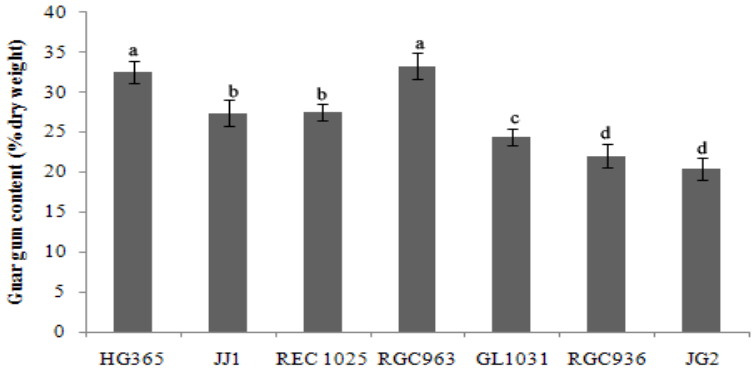

Fig. 3: Guar gum content in seeds of different cluster bean varieties**.** Values are mean of 20 replicates ± SE. Different letters next to the bars representing each compound indicate significant differences at p<0.05 by Tukey's multiple comparison test.

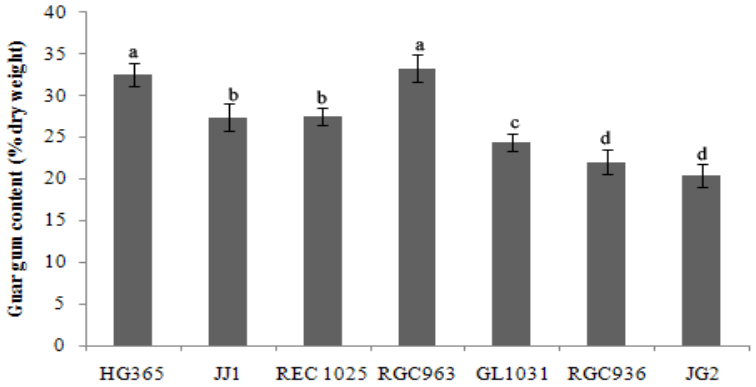

Fig. 4: Aphid infestation rate on different cluster bean varieties. Values are mean of 20 replicates ± SE. Different letters next to the bars representing each compound indicate significant differences at p<0.05 by Tukey's multiple comparison test.

Aphid infestation

Feeding of *A. carccivora* on cluster bean plants resulted in decreased total soluble sugars and amino acids with enhanced phenol content and proteins compared to healthy plants. Among all the varieties JG2, GL1031, RGC936 sugar concentrations were very drastically decreased followed by RGC963, HG365, JJ1, and REC1025 varieties. The total amino acids levels were also reduced in all the varieties due to the aphid population attack in comparison to healthy plants (Table 1). Whereas in all the guar varieties namely RGC963, HG365, JJ1, REC1025, JG2, GL1031, RGC936 the total phenol and total protein contents were elevated due to the aphid infestation (Table 2). In addition, the activities of defense-related enzymes were estimated in between the uninfested and aphid infested guar plants and an elevated enzyme activity of POD, SOD and CAT were significant in all seven varieties of aphid infested cluster bean when compared to the healthy plants (Fig. 5).

Table 2. Carbohydrate and amino acid content in leaves of uninfested and aphid infested cluster bean varieties.

Varieties	Total phenol content (µg/g FW)		Total protein content (µg/g FW)	
	Uninfested	Infested	Uninfested	Infested
HG365	656.89±6.4a	276.41±3.49b	116.83±5.3a	131.39±4.21b
JJ1	407.89±4.2a	266.50±4.69b	112.36±1.7a	144.82±3.2b
REC1025	524.86±4.8a	330.02±4.04b	102.34±5.1a	138.83±5.5b
RGC963	447.03±6.8a	185.03±3.56b	93.22±2.1a	113.34±5.5b
GL1031	364.77±3.3a	176.08±2.81b	68.44±3.3a	74.66±3.1b
RGC936	405.05±6.2a	133.94±2.96b	64.78±2.2a	69.66±2.9b
JG2	212.32±5.5a	120.33±5.12b	35.13±3a	35.06±2.8b

FW is the Fresh Weight. Values are mean of 20 replicates ± SE. Values followed by different letters in a row are statistically different (p<0.001, paired t-test).

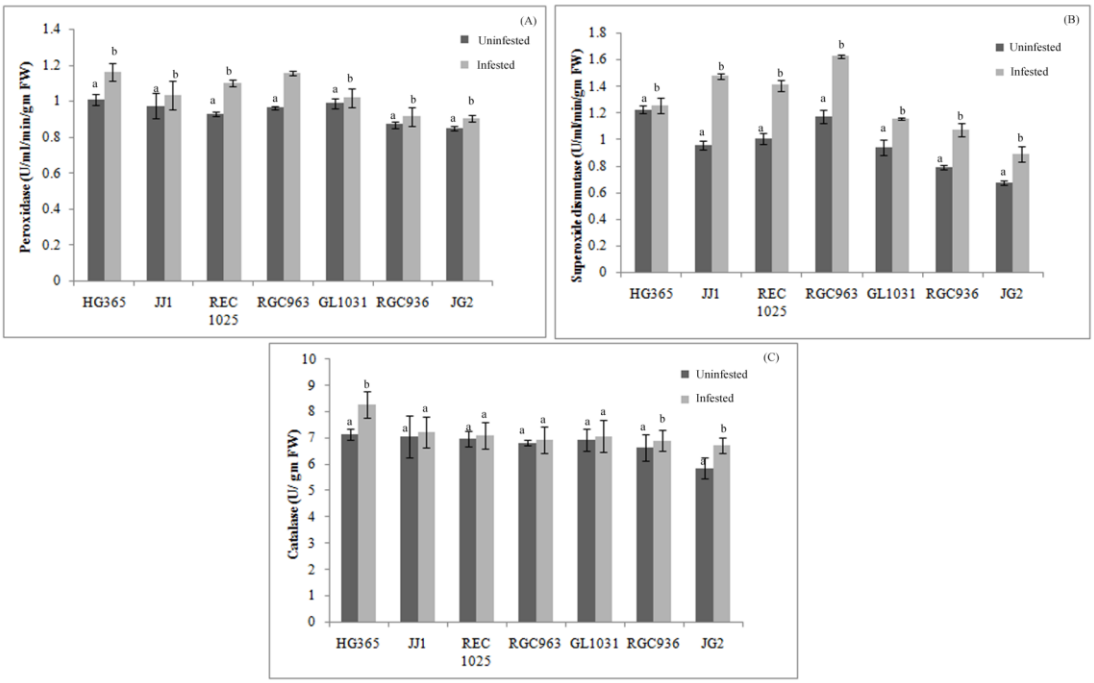

Fig. 5: Anti-oxidant enzyme activity of (A) peroxidase, (B) superoxide dismutase and (C) catalase in leaves between uninfested and aphid infested of cluster bean varieties. Values are mean of 20 replicates ± SE. Values followed by different letters next to bars are statistically different (p<0.001, paired t-test).

Discussion

Cluster bean is one of the most important vegetable and commercial crop of the world, with a global production of 7.5 to 10.0 lakh tons of guar every year. In order to contribute for better crop production, it is necessary to find out the effects of various physical and biochemical parameters of different guar varieties on its growth and

yield potential. Sometimes the row spacings can also influence the growth and yield of the guar varieties (Mahmood et al., 1988). From the present investigation it is revealed that among all seven varieties of guar tested RGC963, HG365, JJ1, and REC1025 were more efficient with better germination, vigour and sustainability, which could aid in increased crop yield.

The growth and development of plants is often affected by its primary metabolism (Rojas et al., 2014). In the present research it is observed that the primary metabolite contents such as total carbohydrates, proteins and phenols of different cluster bean varieties were directly in relation to the plant vigour. Among all the guar varieties, RGC963, HG365, JJ1, and REC1025 had better germination percentage and vigour and accordingly they resulted in significant contents of primary metabolites. Thus the regulation primary metabolism in the plant is essential for its yield and defense responses.

From literature it is evident that the physical parameters and chemical composition of guar seed content can affect its yield of gum content (Murwan et al., 2012). The higher content of carbohydrates in HG365, RGC963, REC1025, and JJ1 cluster bean varieties had resulted in their significant yield of guar gum content. This can further assists us in selection of guar varieties with better gum yield and in the production of guar plants with an upregulated carbohydrate metabolism.

The primary metabolism of plant can modulate its herbivore resistance (Schwachtje and Baldwin, 2008). The guar varieties of JG2, GL1031, and RGC936 were highly susceptible to the invasion of aphids, in comparison to other varietal plants. It is due to a quantitatively reduced metabolic rate in these JG2, GL1031, and RGC936 varieties that resulted in an increased rate of pest attack. Plant primary metabolites such as carbohydrates, amino acids and proteins provide essential nutrients to the insects (Howe and Schaller, 2008). So they are expected to serve important function in the interaction of plant with biotic/aphid environment.

The primary metabolites which are precursors for secondary metabolite production are important compounds of resistance in plants (Whittaker and Feeny, 1971; Bolton, 2009). The quantitative estimations of the total phenol content and total proteins in aphid-infested guar plants exhibited increased levels in comparison to the healthy normal plant of all the seven guar varieties. This alteration in phenolic metabolism due to herbivory in the infested plants was reported earlier. Enhanced phenol contents were observed in the *Rhopalosiphum padi* L infested winter wheat cultivars (Leszczynski, et al., 1985). Increased levels of gallic, chlorogenic, vanilic, caffeic, syringic and salicylic acids were found in the winter Triticale due to feeding of grain aphid, *Sitobion avenae* F (Chrzanowski and Leszczynski, 2008). This phenolic elevation in guar plant can be explained as a mechanism of defense that acts as a barrier to aphid feeding. Infested guar plant also expressed higher levels of proteins than normal plant. This enhancement of protein content in the plant due to infestation as a host resistance mechanism was also observed in *Ricinus communis*, *Eucalyptus globulus*, and *Manihot utilissima* infested by *Retithrips syriacus* (Ananthakrishnan et al., 1992). These enhanced phenols and proteins in guar plant can serve in defending themselves from further aphid feeding.

In comparison to healthy plants, the aphid populations resulted in declined levels of carbohydrate concentrations in the infested cluster bean plants. Similar trend of reduced sugar concentrations was observed in all the seven infested varietal plants. It can be a nutrition deficit condition maintained by the guar plant to avoid further feeding of the aphids. A significant reduction in the sugar levels was also observed in cabbage plant against phloem sucking aphid, *Brevicoryne brassicae L*. (Khattab, 2007). Also the effect of aphid infestation exhibited reduced carbohydrate contents in the *Brassica juncea* plant (Singh and Sinhal, 2011). Insect herbivory exhibited a decline in the amino acid contents of the infested guar plants. The levels of free amino acids were also reduced in the maize seedlings due to *Sitobion avenae* F. attack (Eleftherianos et al., 2006).

Plants are able to produce reactive oxygen species (ROS) under stress conditions that are involved in the plant defense (Liu et al., 2010). Higher levels of anti-oxidative enzymes such as SOD, CAT, and POD are significantly elevated in the cluster bean plants due to aphid feeding. These anti-oxidant enzymes are even altered in cluster bean plant under zinc stress (Vijayarengan, 2013). Through literature it is evident that aphid infestation enhances the biochemical and defense enzymes of different host plants (Sadek et al., 2013). The increase in SOD and POD explains the enhanced ROS due to herbivory. Similar results were observed in wheat lines infected by powdery mildew (Pal et al., 2013). These biochemicals and enzymes can play their respective roles to shield the plants from further aphid feeding.

Conclusion

In conclusion, a positive correlation was found between the physical/ biochemical parameters of guar plant towards the gum content and rates of aphid infestation. Among the seven guar varieties tested, RGC963, HG365, JJ1, and REC1025 were more desirable with vigour and better gum yield. The regulation of carbohydrate and phenolic metabolism in guar plants can aid in the development of short duration, fast growing, and high yielding varieties of cluster bean for gum and vegetable purposes. This study also assists in better understanding of phytochemical and anti-oxidant enzyme changes in aphid infested cluster bean plants which helps in development of varieties of guar with required tolerance/resistance towards biotic stresses.

Conflict of interest statement

Authors declare that they have no conflict of interest.

Acknowledgement

Authors express their gratitude to Dr. Chandrasekhar, Director, CSIR-Indian Institute of Chemical Technology, Hyderabad, India for the encouragement and facilities provided to carry out this work. We also thank Mrs. P. Radhika, Scientist, Agricultural Research Station, ANGRAU, Anantapur, Andhra Pradesh, for providing the plant material.

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How to cite this article:

Sambangi, P., Usha Rani, P., 2016. Role of physical and biochemical parameters of different cluster bean varieties on the guar gum content and aphid infestation. Int. J. Curr. Res. Biosci. Plant Biol. 3(4), 83-90. doi: http://dx.doi.org/10.20546/ijcrbp.2016.304.013