International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2026;10(1): 114-124
www.biochemjournal.com
Received: 06-10-2025
Accepted: 11-11-2025

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Correlation of biochemical traits with resistance to pod borer infestation in pigeonpea

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DOI: https://www.doi.org/10.33545/26174693.2026.v10.i1b.6878

Abstract
The present investigation was carried out to determine the biochemical traits associated with resistance to pod borers in various pigeon pea (*Cajanus cajan* L.) genotypes. Field experiments were conducted at the Research cum Instructional Farm, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, during the *Kharif* seasons of 2023-24 and 2024-25, while complementary biochemical analyses were performed in the Biochemical Laboratory, Department of Entomology, IGKV, Raipur. Significant variations were observed among genotypes in the biochemical constituents of pods and their association with pod borer damage. Total phenol content in the pods of genotypes exhibited a strong negative correlation (r = -0.670) and chlorophyll content (r = -742) with the percentage of pod damage, indicating its potential role in imparting biochemical resistance. In contrast, protein content, total soluble sugars, and reducing sugars were positively and significantly correlated with pod damage (protein: r = 0.782, total soluble sugars: r = 0.808, reducing sugars: r = 0.657,). The results suggest that higher phenolic and chlorophyll content enhances resistance, whereas elevated protein and sugar levels increases susceptibility to pod borers. These findings provide a biochemical basis for identifying and developing pigeonpea genotypes resistant to pod borer infestation.

Keywords: Biochemical, characters, pigeonpea, genotypes, pod

Introduction

Pigeonpea, [*Cajanus cajan* (L.) Millspaugh] (2n = 22) is an important pulse crop mainly grown under rain-fed condition s in the tropics and subtropics. In Asia, Africa and Latin America, pigeon-pea seeds have become the main source of protein especially for the vegetarians. Guandul in Latin America, Gungo peas in the Anglo Caribbean and Congo pea in Sub Saharan Africa are the names given to the pigeonpea (Carney and Rosomoff, 2009) [11]. In India, it is known by a variety of names including arhar, red gram, tur and tomarapayaru (Anonymous,). Pigeon-pea appears to have originated in Peninsular India, with its nearest wild relative (*Cajanus cajanifolia*) growing in tropical deciduous woodlands (Van der Maeson). Pigeonpea is grown in over 25 tropical and subtropical nations, either as a stand-alone crop or in combination with cereals such as sorghum and maize, or other legumes such as peanuts. Pigeonpea is the most adaptable edible legume, with a wide range of use including food, feed, fodder and fuel. Grain comes in a variety of forms, including green seeds as vegetables and reconstituted dried split seeds. It is rich source of protein and fulfils a major protein requirement of vegetarian population of the country. It is a major source of dietary protein in South Asian vegetarian civilizations. Pigeon-pea provides 50% of all protein ingested by Indians in some areas (Ryan *et al.*, 1984) [12]. The food value of pigeon-pea is due to its protein content (22.3%) along with iron, iodine and essential amino acids like lysine, cystine arginine, vitamins and minerals. It has better quality of fiber, 7g/100g of seeds (Kandhare, 2014) [13]."

A wide range of constraints (abiotic and biotic) are responsible for reduction in productivity of the country's pigeonpea. Among them biotic factors which include insect pests are most notable threat to the crop's potential yield. The havoc caused by insect pests is critical (Mishra *et al.*, 2012) [14]. Though pigeonpea is inundated by more than 250 species of insect pests belongings to 8 orders and 61 families that appear at variousgrowth stages of the crop in India, only a few of these cause consistent and significant damage to the crop (Lateef and Reed, 1990 and Gopali *et al.*, 2010) [15, 16]. Worldwide, over 30 species of Lepidoptera feed on pods and seeds of pigeonpea (Shanower *et al.*, 1999) [17].

The pod borer complex, which comprises *Helicoverpa armigera*, *Etiella zinckenella* and *Maruca vitrata*, has been identified as a significant threat to pigeonpea crop causing significant losses in grain yield ranging from 30 to 100 percent by targeting the reproductive sections of the plant.

The insect pod borer complex has a significant impact on the production of most pulses. Among these the *H. armigera* (Hübner) is the major pest in most parts of the country and it has attained the key pest prominence due to its direct attack on fruiting bodies, voracious feeding habits, high mobility, fecundity, multivoltine and overlapping generation with facultative diapauses, nocturnal "bahaviour, migration, host selection and propensity for acquiring resistance against insecticides (Satpute and Sarode, 1995) [18]. It accounts for 90- 95% of total damage. A single larva can damage 25-30 pods of gram in its life time. It feeds on tender shoots and young seeds. It makes holes in pods and inserts half of its body inside the pod to eat developing seeds "(Ojha *et al*., 2017) [19].

Materials and Methods

Biochemical characters conferring resistance against pod borers in pigeonpea genotypes during kharif 2023-2024 and 2024-2025. For the study of biochemical parameters *viz*.,total nitrogen and total protein contents, total phenols, total soluble sugars, reducing sugar and chlorophyll were estimated on three randomly selected samples from each genotypes (in total 22 genotypes) and data were correlated with the damage of pod borers."The procedures adopted for the estimation of biochemical parameters are described as under:

1. **Total nitrogen content:** Nitrogen in plant sample was determined by employing KELPLUS Digestion and Distillation systems as described by Subbiah and Asija (1956).
2. **Total protein content:** Total protein content was estimated by "Nitrogen-Protein (N:P) conversion factor". Firstly total nitrogen content of each genotype was analyzed by KELPLUS unit by Subbiahand Asija (1956) and then the total nitrogen content was multiplied with Nitrogen-Protein (N: P) conversion factor '6.25'.

Total protein content = Total nitrogen × 6.25

3. **Total phenols:** The total phenols present in pods of twenty two pigeonpea genotypes were estimated as per the method developed by Sadasivam and Manickam (1996) [20].
4. **Total soluble sugar (TSS):** The concentrations of total soluble sugar (TSS) were determined by Anthrone method of Dubois *et. al.* 1951 [21].
5. **Reducing sugar:** For the estimation of reducing sugar, the dinitro salicyclic acid method (Miller, 1972) [22] was used.
6. **Cholorophyll concentration (mg^{-1}) Measurement** Chlorophyll extraction protocol given by Arnon, D.I. 1949 was followed.

Results and Discussion

Estimation of biochemical parameters

1 Nitrogen and protein content (%)

The nitrogen content obtained ranged from 2.18 to 3.19 percent in the pods of different pigeonpea genotypes. Considerable differences were recorded in the nitrogen content among all the genotypes of pigeonpea tested for the resistance to pod borers. Maximum nitrogen content was recorded in highly susceptible genotype RP5-2016-45 of 3.19% followed by RP5-2016-31, RP5-2016-25 and RP5-2016-16 of 3.13%, 2.80% and 2.74% respectively, whereas least nitrogen per cent was recorded in RP5-2017-16 as 2.18% followed by RP5-2016-72, RP5-2016-62 and RP5-2016-44 of 2.24%, 2.32% and 2.35%respectively.(Table 1).

Similarly, the protein content obtained ranged from 14.00 to 19.95 percent in the pods of pigeonpea genotypes. Considerable differences in the protein per cent were observed among all the genotypes of pigeonpea tested for the resistance to pod borers. Maximum protein per cent was recorded in highly susceptible genotype RP5-2016-45 as 19.95% followed by RP5-2016-31, RP5-2016-25 and RP5-2016-16 as 19.60, 17.50 and 17.15% respectively, whereas least protein per cent was recorded in RP5-2016-72 as 14.00% followed by RP5-2017-33, RP5-2016-62 and RP5-2016-44 as 14.35%, 14.52% and 14.70% respectively. (Table 1).

Correlation analysis of nitrogen and protein per cent with per cent pod damage caused by pod borers conferred positively significant with r value 0.781* and 0.782* (Table 2). This indicated s that with the increase in nitrogen and protein per cent, there will be an increase in infestation level too.

The present findings are in coordination with Parre *et al*., (2018) [6] who had reported that the protein content showed positive correlation with percent of pod borer damage (0.8035) indicating that genotypes with more protein content are more susceptible to *Helicoverpa* infestation. Similarly, Mamta *et al*., (2023) [4] who also reported that analysis of protein per cent and per cent pod damage caused by pod borers depicted positively and highly significant correlation with r value 0.914*. This indicates that with increase in protein per cent, there will be an increase in the infestation level.

2. Total phenol (mg/g)

The phenol content showed significant variation among different genotypes of pigeonpea. The total phenol content of different genotypes varied from 2.41 - 4.16mg/g, in the pods of pigeonpea genotypes (Table 1). Maximum phenol content was estimated in RP5-2016-72 as 4.16 mg/g followed by RP5-2017-16, RP5-2017-21 and RP5-2017-7 as 4.14, 4.06 and 4.04 mg/g respectively, whereas minimum phenol content was observed in RP5-2016-31 of 2.41 mg/g followed by RP5-2016-45, RP5-2016-44 and RP5-2016-42 of 2.48, 2.70, 2.77 mg/g respectively.

Correlation studies between phenolic content and total per cent pod damage by pod borers showed highly significant but negative correlation with r value (-0.670*) which clearly shows that high phenol content exhibits critical role in offering resistance to pod borers in field condition (Table 2). The current findings are in accordance with earlier researchers such as, Rashmi *et.al*., (2020) [7] and Tyagi *et al*., (2021) [10] who reported that the correlation between the pod damage and phenol content in pods of different genotypes was negative andsignificant, indicating that increase in phenol content resulted in less pod damage. The present results are in total agreement with the findings of Sahoo and Patnaik (2002) [8], Anantharaju and Muthiah (2008) [1], Sharma *et al*., (2009) [9], Bommesha *et al*., (2012)

[2] and Jagtap *et al*., (2012) [3] who had reported that low protein and sugar content and high phenol content in pod coats and seeds were responsible for the resistance of pigeonpea varieties against pod borers. These present results are also in accordance with the findings of Vageesh Pandey *et al*., (2011) [5] and Mamta *et al*., (2023) [4] stating that the genotypes with more phenol content suffered less pod and grain damage by pod fly.

3. Total soluble sugar (TSS) (mg/g)

Considerable differences in the TSS among all the tested genotypes of pigeonpea were depicted for the resistance to pod borers and varied from 4.88 to 11.99 mg/g, (Table 1). Maximum total soluble sugar content was measured in RP5-2016-31 as 11.99 mg/g followed by RP5-2016-45, RP5-2016-25 and RP5-2016-16 as 11.12, 9.35 and 8.62 mg/g. respectively, whereas minimum total soluble sugar content was observed in RP5-2016-72 as 4.88 mg/g followed by RP5-2017-16, RP5-2016-42 and RP5-2016-44 as 5.21, 5.61, 5.81 mg/g respectively.

Correlation studies between total soluble sugar content and pod damage by pod borers showed highly significant but positive association with r value (0.808*) which clearly shows that high total soluble sugar content exhibits critical role in offering susceptibility to pod borers in field condition (Table 2).

The present findings are in coordination with Parre *et al*., (2018) [6] who reported that the Total sugars (0.8045) i.e., reducing and non-reducing sugars showed positive association with the percent of pod borer damage indicating that genotypes having more sugars are highly preferred by *Helicoverpa* species.The present results are in agreement with the findings of Mamta *et al*., (2023) [4].

4. Reducing sugar (mg/g)

Considerable differences in the presence of reducing sugar content were revealed among all the tested genotypes of pigeonpea for the resistance to pod borers and the values varied from 0.43 to 1.24mg/g, as presented in (Table 1). Maximum reducing sugar content was estimated in RP5-2016-45 as 1.24 mg/g followed by RP5-2016-31, RP5-2016-16 and RP5-2016-25 as 1.15, 1.11 and 1.03 mg/g respectively, whereas minimum reducing sugar content was observed in RP5-2016-72 as 0.43 mg/g followed by RP5-2017-16, RP5-2016-44 and RP5-2017-33 as 0.45, 0.46, 0.46 mg/g respectively (Table 1).

Correlation studies between reducing sugar content and total per cent pod damage by pod borers showed significant but positive correlation with r value (0.657*) which clearly shows that high reducing sugar content exhibit critical role in offering resistance to pod borers in field condition (Table 2).

The present findings are in match with Parre *et al*., (2018) [6] who reported that the total sugars (0.8045) i.e., reducing and non-reducing sugars showed positive association with the percent pod borer damage indicating that genotypes having more sugars are highly preferred by *Helicoverpa*species. Similar findings were also reported by Siva Kumar *et al*., (2015) [23] who documented that the correlation between the reducing sugars and pod damage due to pod fly was positive and significant, which indicated that increase in reducing sugar increased the incidence of pest infestation.

5. Chlorophyll content (mg/g)

Significant differences in the chlorophyll content among all the tested genotypes of pigeonpea for resistance to pod borers and varied from 0.14 to 1.11mg/g, as presented in (Table 1). Maximum chlorophyll content was measured in RP5-2016-72 as 1.11 mg/g followed by RP5-2017-16, RP5-2016-44 and RP5-2016-42 as 1.01, 0.91 and 0.78 mg/g. respectively, whereas minimumchlorophyll content was observed in RP5-2016-45 as 0.14 mg/g followed by RP5-2016-31, RP5-2016-25 and RP5-2016-17 as 0.20, 0.30, 0.33 mg/g respectively.

Correlation studies between chlorophyll content and pod damage by pod borers showed highly significant but negative association with r value (-0.742*) which clearly shows that high chlorophyll content exhibits critical role in attracting pod borers and susceptibility in field condition (Table 2).

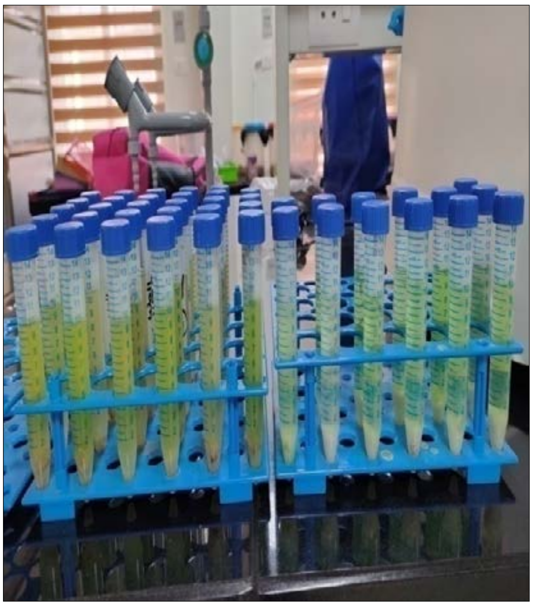
Chlorophyll a and chlorophyll b contents were also estimated separately. Chl. (a) content varied from 0.10 to 0.39mg/g whereas, Chl. (b) content varied from 0.12 to 0.72mg/g, as presented in (Table 1).

These findings are in agreement with those of Patel *et al*., (2015) [24], who reported that higher chlorophyll levels in pigeonpea pods could attract more oviposition by *H. armigera*, possibly due to greener pods acting as visual cues or offering a better nutritional substrate. Similarly, Rani and Srivastava (2010) [25] suggested that insect herbivores tend to prefer plant parts with higher chlorophyll content due to enhanced palatability and nitrogen content associated with photosynthetically active tissues. Further, Kumari *et al*., (2017) [26] reported that in pigeonpea and chickpea, varieties with darker green foliage were more prone to insect pest attacks, particularly by *H. armigera* and *Spodoptera litura*. This observation aligns and agrees with the present results, where genotypes with lower chlorophyll content (such as RP5-2016-45 and RP5-2016-31) exhibited lower pod damage, indicating a potential role of chlorophyll content as a marker trait for tolerance or resistance.

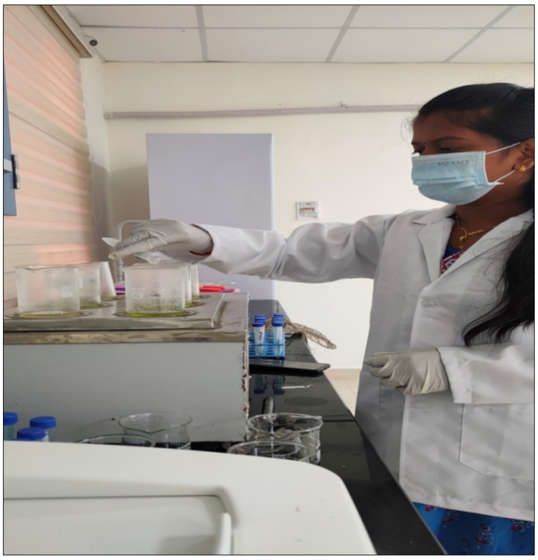
A. 1 gram of pod

B. Crushing of pod sample with mortar and pestle

C. Centrifugation



D. Centrifuged samples

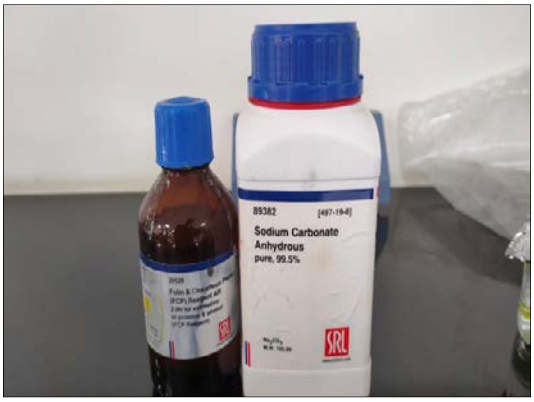


E. Evaporating supernatent by Water bath

F. Aliquots (Stock solution of genotypes)

G. Spectrophotometer Analysis

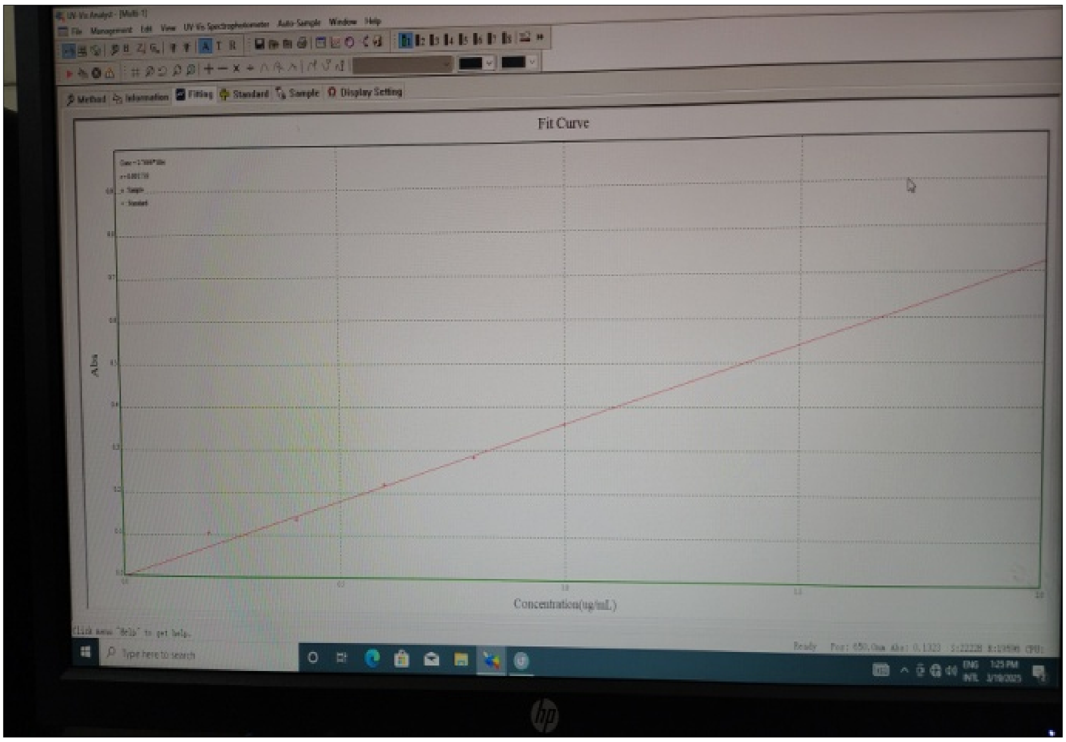
Fig 1: Preparation of a standard stock solution for the estimation of biochemical parameters



A. Chemicals for standard preparation

B. Mixing the chemicals

C. Evaporate standard by boiling water exactly for one minute

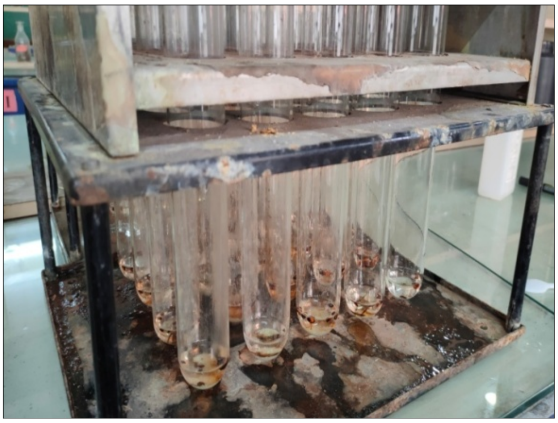


D. Standard curve prepared by using spectrophotometer

Fig 2: Estimation of Phenol of pigeonpea genotypes by using Spectrophotometer

A. Chemicals for Nitrogen estimation

B. Chemical preparation



C. Preparation for Digestion of the sample

D. Distillation process

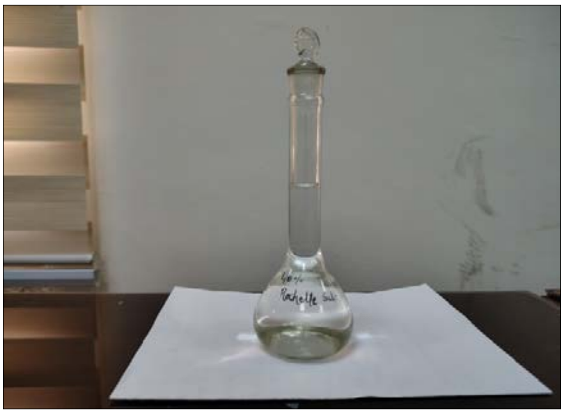

E. Chemicals after distillation F. Titration

Fig 3: Estimation of Nitrogen content by Kjeldahl method

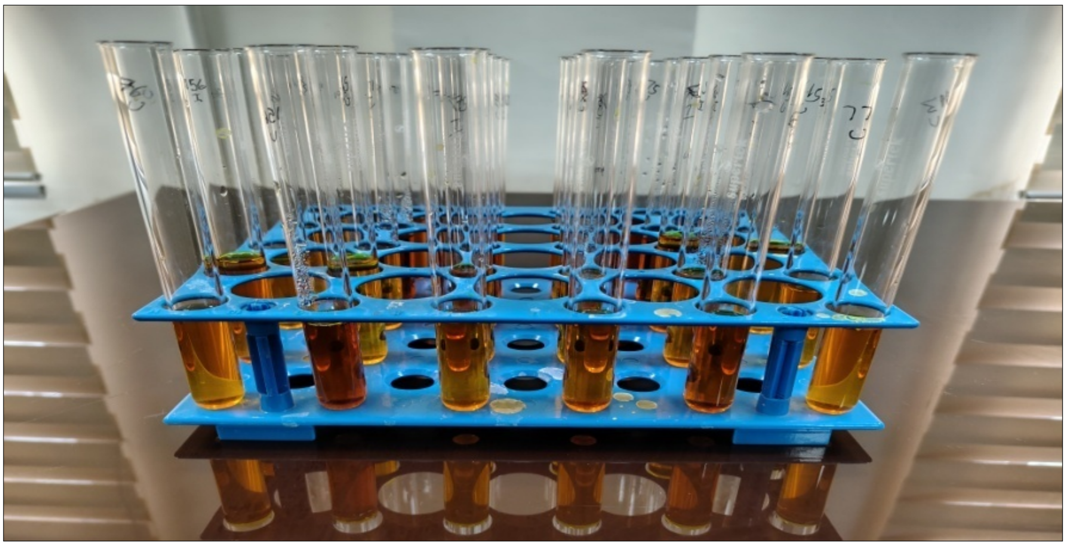

A. Chemical preparation B. Standard preparation


C. Evaporating by water bath D. Prepare standard curve using by Spectrophotometer

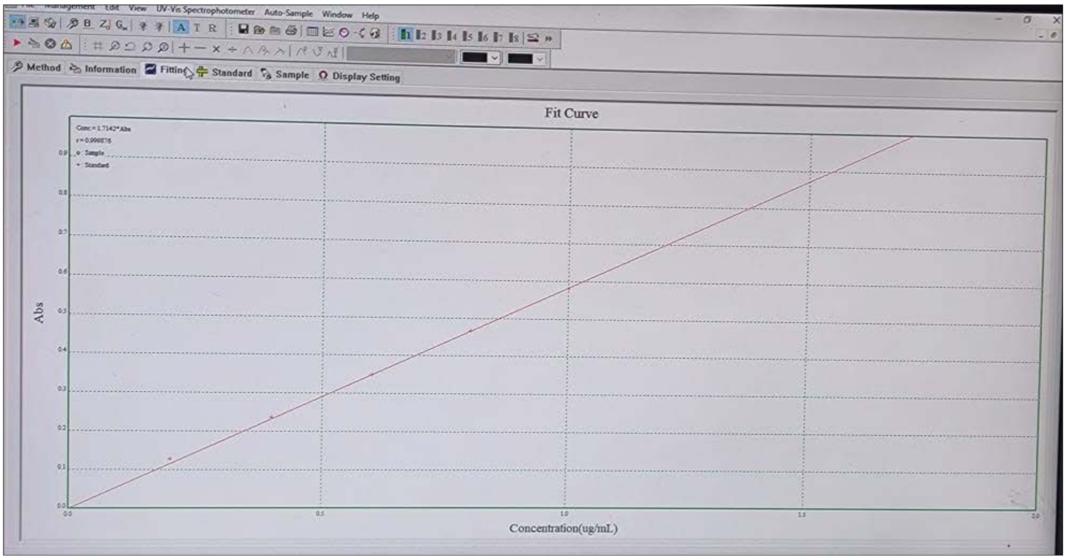
Fig 4: Estimation of total soluble sugar in pigeonpea genotypes by using Spectrophotometer



A. Preparation of DNS reagent B. Rochelle Salt

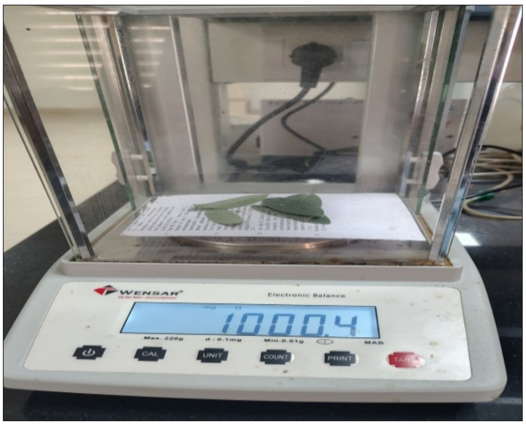


C. Preparation of sample



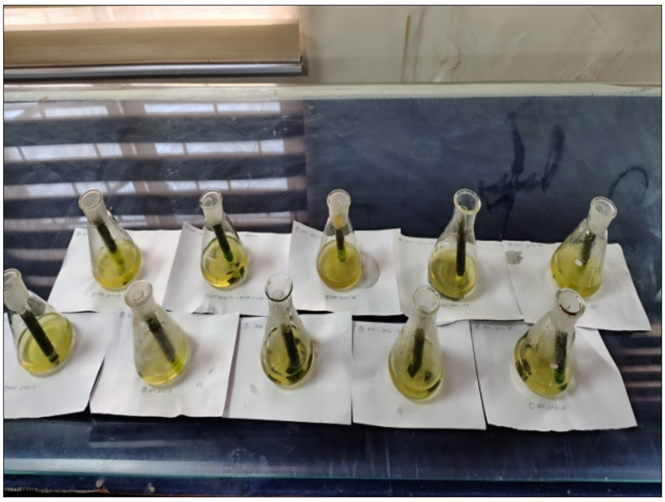
D. Prepare standard curve using by Spectrophotometer

Fig 5: Estimation of reducing sugar in pigeonpea genotypes by using Spectrophotometer

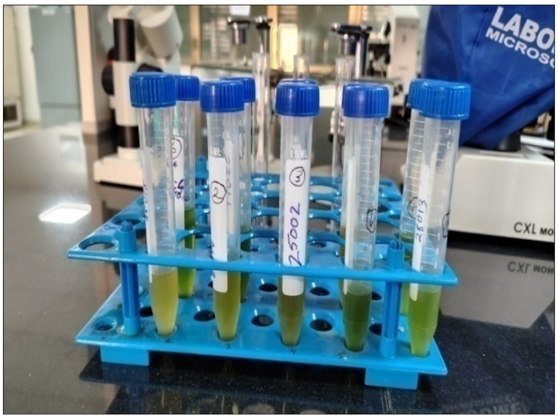


A. Weighing of leaves (1 gm)

B. Conical flask on microwave

C. Weighted leaves cut into small pieces D. Put the test tube into the conical flask




E. Collected liquid on Eppendorf tube F. Measured on spectrophotometer at 645 and 663 wavelength

Fig 6: Estimation of chlorophyll in leaves of pigeonpea genotypes by using Spectrophotometer

Table 1: Biochemical contents of pigeonpea genotypes against total per cent pod damage by pod borers during *Kharif* 2023-24 and 2024-25

S. No	Genotypes	Total % pod damage	Nitrogen content (%)	Protein content (%)	Total phenol (mg/g)	Total soluble sugar (mg/g)	Reducing sugar (mg/g)	Chlorophyll content (mg/g)		
								Chl. (a)	Chl. (b)	Total Chl.
1	RP5-2016-1	19.90	2.57	16.1	3.78	7.17	0.56	0.14	0.25	0.39
2	RP5-2016-3	17.69	2.54	15.92	3.69	6.63	0.64	0.22	0.37	0.6
3	RP5-2016-16	21.43	2.74	17.15	3.32	8.62	1.11	0.12	0.21	0.33
4	RP5-2016-17	18.09	2.54	15.92	3.52	7.55	0.62	0.27	0.43	0.7
5	RP5-2016-25	20.56	2.8	17.5	3.35	9.35	1.03	0.11	0.19	0.3
6	RP5-2016-31	25.96	3.13	19.6	2.41	11.99	1.15	0.07	0.12	0.2
7	RP5-2016-37	18.10	2.49	15.57	3.69	6.57	0.6	0.25	0.29	0.55
8	RP5-2016-42	14.96	2.4	15.05	2.77	5.61	0.49	0.3	0.47	0.78
9	RP5-2016-44	16.40	2.35	14.7	2.7	5.81	0.46	0.34	0.58	0.91
10	RP5-2016-45	24.30	3.19	19.95	2.48	11.12	1.24	0.06	0.08	0.14
11	RP5-2016-50	19.93	2.57	16.1	3.523	6.86	0.67	0.14	0.18	0.33
12	RP5-2016-53	17.69	2.52	15.75	3.69	6.77	0.63	0.13	0.19	0.33
13	RP5-2016-62	15.53	2.32	14.52	3.99	6.37	0.51	0.34	0.43	0.78
14	RP5-2016-72	13.93	2.24	14	4.16	4.88	0.43	0.39	0.72	1.11
15	RP5-2017-1	17.83	2.49	15.57	3.68	7.23	0.58	0.1	0.17	0.27
16	RP5-2017-7	16.36	2.4	15.05	4.04	6.57	0.5	0.3	0.36	0.66
17	RP5-2017-13	18.93	2.52	15.75	3.52	7.81	0.56	0.14	0.23	0.37
18	RP5-2017-16	14.16	2.18	13.65	4.14	5.21	0.45	0.36	0.64	1.01
19	RP5-2017-21	15.96	2.35	14.7	4.06	7.01	0.49	0.28	0.4	0.68
20	RP5-2017-28	18.33	2.54	15.92	3.46	7.52	0.55	0.2	0.28	0.48
21	RP5-2017-33	15.79	2.29	14.35	3.98	7.26	0.46	0.25	0.39	0.64
22	RP5-2014-34 (RC)	15.99	2.38	14.87	3.96	7.1	0.48	0.27	0.42	0.7

(*RC= Resistant check)

Table 2: Correlation coefficient between biochemical parameters of different pigeonpea genotypes and percent pod damage by pod borers

S. No.	Biochemical characters	Correlation coefficient (r) of pigeonpea genotypes
1	Nitrogen content	0.781*
2	Protein content	0.782*
3	Total phenol	-0.670*
4	Total soluble sugar	0.808*
5	Reducing sugar	0.657*
6	Chlorophyll content	-0.742*

*Significant at 5%
Table value: (r) = 0.423

Conclusion

Maximum nitrogen per cent was recorded in RP5-2016-45 (3.19%) while least was recorded in RP5-2017-16 (2.18%). Correlation of nitrogen per cent and per cent pod damage was positively significant (r = 0.781*).
Highest protein per cent was recorded in RP5-2016-45 (19.95%) while. least in RP5-2017-16 (13.65%). Correlation analysis of protein per cent and damage caused by pod borers was positively significant (r = 0.782*).
Highest phenol content was determined in genotype RP5-2016-72 (4.16 mg/g), whereas lowest was observed in RP5-2016-31 (2.41 mg/g). Correlation studies between phenolic content and pod damage by pod borers showed significant negative association. (r = -0.670*)
Maximum TSS was recorded in susceptible genotype RP5-2016-31 (11.99 mg/g), whereas the least was recorded in moderately resistant genotype RP5-2016-72 (4.88 mg/g). Correlation analysis of TSS and per cent pod damage by pod borers was positively significant. (r= 0.808*).
Highest reducing sugar was recorded in genotype RP5-2016-45 (1.24 mg/g), whereas the least in genotype RP5-2016-72 (0.43 mg/g). Correlation analysis of reducing sugar and per cent pod damage caused by pod borers was positively significant. (r= 0.657*).Highest chlorophyll content was recorded in genotype RP5-2016-72(1.11 mg/g), whereas least chlorophyll content was recorded in genotype RP5-2016-45 (0.14 mg/g).Significant negative association (r= -0.742*) was found with chlorophyll content of different genotypes and per cent pod damage by pod borers.

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