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Biochemical basis of resistance in *Vigna mungo* (L.) Hepper against *Callosobruchus chinensis* Linnaeus (1758) and *Callosobruchus maculatus* Fabricius (1755) (Coleoptera: Bruchidae)

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Abstract
The present study investigated the biochemical factors related to resistance and susceptibility of *Callosobruchus chinensis* (L.) and *Callosobruchus maculatus* (F.) in response to twenty-five different genotypes of *Vigna mungo* (L.) Hepper. Results revealed that, out of twenty-five cultivars of *V. mungo* differential resistant to *C. maculatus* was observed. It was found that the cultivar IC-436511 was more susceptible because the life cycle of the bruchid completed only in 31.00±1.00 days, maximum 87.00±1.63 eggs were laid and 49.67±4.04 adult emerged successfully with the growth index of 1.61. Whereas, cultivars DKV-116 was least susceptible to the attack of *C. maculatus* since pest completed its life cycle in 34.33±1.15 days with 91.33±6.65 eggs and adult emerged 33.67±2.52 with growth index of 0.98. From the present studies, it has been concluded that blackgram cultivars are completely resistant to the attack of *C. chinensis* but most susceptible to *C. maculatus*.

Keywords: *Vigna mungo* (l.), Coleoptera, *C. maculatus*

Introduction
In traditional diets all over the world, legumes are a key component and a valuable food source. These are widely grown and consumed in various regions and are excellent sources of proteins, complex carbohydrates and fairly good sources of minerals, vitamins, and polyunsaturated fatty acids. Besides major pulses such as chickpea, pigeon pea, green gram, black gram, lentil, and peas other legumes are cultivated for multifarious uses in marginal lands mainly in remote areas and have tremendous scope to become an important component of the agricultural system because of their being intrinsic resilient and associated with important useful traits. Blackgram, *Vigna mungo* (L.) Hepper, a grain legume from the genus *Vigna* and subgenus Ceratotropis, is widely grown in South and Southeast Asia. This crop is cultivated in India, Pakistan, China, Myanmar, Bangladesh, Thailand, the Philippines, Malaysia, Kenya, Malawi, and the United States. Blackgram is an important source of dietary protein, particularly in the Indian Subcontinent, which is primarily vegetarian. In India, these crops are grown on a 6.62 million hectare area, yielding 3.40 million tonnes of grain in 2011-12 (DAC, 2013) [6]. The bruchid species *Callosobruchus* (Coleoptera: Bruchidae) reduces the quantity and quality of legumes stored in tropical and subtropical regions around the world. The bruchid species *Callosobruchus* (Coleoptera: Bruchidae) reduces the quantity and quality of legumes stored in tropical and subtropical regions around the world. The most common bruchids in Asia are *C. chinensis* and *C. maculatus*, which cause significant damage to Vigna seeds. Infestation begins in the field and spreads to storage facilities, where it can destroy seeds within six months (Srinivasan *et al*., 2010). The seed thus loses viability, and the grain becomes unfit for human consumption. Aside from the high cost and pesticide residue issues, current pest management methods do not provide the expected levels of control. Developing bruchid resistance in improved blackgram (urdbean) cultivars is a cost-effective strategy for reducing severe postharvest losses.

Materials and Methods
1. Source of Sample collection
The infested mungbean and soybean seeds were gathered from Palampur and Dharamshala Tehsils in Kangra District, Himachal Pradesh. After adult emergence from infested sample, cultures were initiated and subletted in disposable Petridishes (90×15mm) with host seeds under controlled conditions of temperature 25ºC and relative humidity 70.0% in a Biological Oxygen Demand incubator (DB-2025, Decibel) with three replicas each. Adults that emerged were recognized as *C. chinensis* and *C. maculatus* using the dichotomous key devised by Arora (1977) [2], Johnson (1990) [10], and Kingsolver (2004) [11].

2. Sources of experimental legume cultivars
For biochemical analysis, samples of twenty-five genotypes of *V. mungo* were used, including DKV-116, HIM-MASH, IC-261182, IC-281975, IC-281981, IC-281989, IC-281993, IC-281995, IC-282004, IC-335331, IC-343936, IC-343967, IC-382811, IC-383412, IC-398983, IC-426769, IC-436511, IC-436524, IC-436597, IC-436604, IC-436628, IC-436632, IC-436647, IC-436659, IC-436675 were collected from Indian Institute of Pulse Research, Kanpur, U.P. and ICAR-NBPGR, Krishi Bhawan, New Delhi.

3. Biochemical basis of bruchids resistance in different genotypes of *V. mungo*
All the different cultivars were homogenized and analyzed for nutritional components *viz.* Carbohydrates, proteins, and lipids and anti-nutritional components like phenols, tannins and flavonoids.

3.1 Preparation of homogenate for biochemical analysis
The seed of all the cultivars of *V. mungo* were grinded separately into a fine powder with the help of an electric grinder. The homogenates were prepared in cold PBS

(Phosphate buffer saline, pH 7.2) with the help of a homogenizer (RQT-127A) by mixing 1gm (w/w) of seed samples with PBS and then transferred to the homogenizer. Homogenization was carried out at 2000 rpm for 10 minutes.

3.2 Protein estimation
The concentration of protein was estimated spectrophotometrically by a slightly modified method of Lowry *et al.* (1951) [13] using bovine serum albumin (BSA) as standard (Banyal and Kumar, 1994) [3].

3.3 Carbohydrates determination
Carbohydrates were determined by the Anthrone method by using a spectrophotometer**.** The chemicals required were anthrone, concentrated sulphuric acid and glucose.

3.4 Lipid determination
Lipids were determined according to the method of Ganai *et al*. (2005) [8].

3.5 Phenol contents
Total phenol contents were estimated according to the methods of Goldstein and Swain (1963) [9].

3.6 Tannin contents
Total tannin contents were determined according to the method of Price *et al*. (1978) [18].

3.7 Flavonoid contents
Total flavonoid contents were calculated according to the method of Ordonez *et al*. (2006) [16].

Data analysis
All experiments were conducted in triplicates. The data were analyzed using the ANOVA test. Duncan's multiple range test was also employed to assess the significance of treatment mean differences.

Results and Discussion
The current investigations focused on the genotypes of *Vigna mungo* in relation to their susceptibility and resistance to the bruchids, *Callosobruchus chinensis* and *C. maculatus*. This relative resistance was caused by physical and biochemical variables in the seed, such as nutritional and anti-nutritional components. According to the findings, the nutritional profile was not the primary cause of relative resistance or susceptibility to bruchid in legumes; nevertheless, anti-nutritional content of legumes was found to be responsible for relative resistance against bruchid species. In the current study, proteins content in different cultivars were different and highest amount of protein contents found in cultivar IC-398983 (29.85±0.00mg/ml) and lowest in IC-436597 (14.46±0.00mg/ml) in black gram. Chakraborty and Sahni (1972) and Umarao and Verma (2003) reported that least susceptible varieties have low protein contents while highly susceptible varieties having higher protein contents.

The carbohydrate contents in twenty-five cultivars of *V. mungo* varied from 11.62mg/ml (IC-398983) to 26.61mg/ml(IC-436675). Lowest carbohydrates in IC-398983 resulted moderate fecundity of 74.00±2.94 eggs with development period of 31.33±3.21 days by *C. maculatus.* Similarly, the highest carbohydrate content in cultivar IC-436675 resulted fecundity of 84.33±3.40 eggs and development period of 33.00±0.00 days. Present results showed that the lowest and highest carbohydrate contents did not play any role in decreasing the fecundity and developmental period of *C. maculatus.*

The protein profiles of *V. mungo* and *V. radiata* cultivars varied both at qualitative and quantitative levels in all cultivars. The current results revealed the similarity of molecular weights in protein bands among different varieties might be due to genetic relation among them. Total 29 different bands were observed in black gram cultivars.

In black gram cultivars band number 8 and 9 of molecular weight 60.71 and 62.70kDa were present only in DKV-116 and IC-398983 respectively. Cultivar, DKV-116 was more resistant to *C. maculatus* since pest completed its life cycle in 34 days and laid least number of eggs and minimum adult emerged with low growth index. Natarajan *et al.* (2013) define trypsin inhibitors as defense-related proteins composed of cysteine-rich protease inhibitors with molecular weights ranging from 8-16 kDa. These protease inhibitors have a role in plant protection against pests and diseases.

The resistant variety of black gram contained significantly different levels of inhibitors including anti-nutritional compounds and secondary metabolites, such biochemical traits affected the metabolic activity of bruchids (Sarikarin *et al*., 1999, Appleby and Credland, 2003, Lattanzio *et al.*, 2005 and Somta *et al*., 2007) [1, 12, 19].

Tannins are a potent feeding deterrent that confers resistance against insects and illnesses, making them the most significant defense category among secondary metabolites.

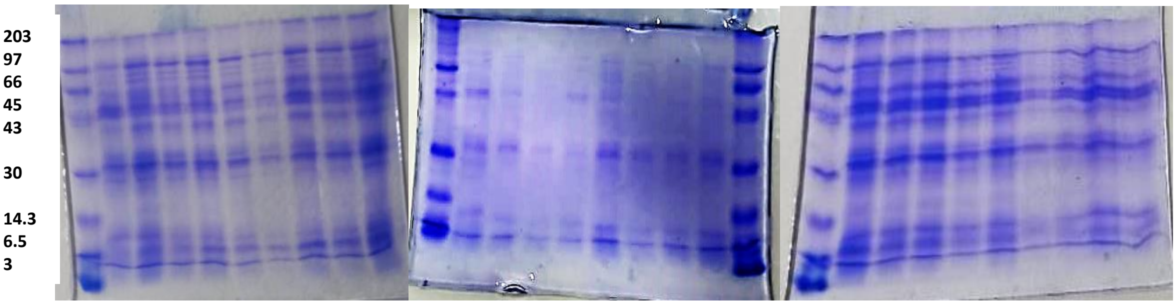
Tannins restrict insect growth by generating midgut lesions (War *et al*., 2012) [24], which is most likely owing to the semiquinone and quinone free radicals produced, whenever tannins oxidize (Barbehenn and Constabel, 2011). Furthermore, because of their bitter flavor, tannins inhibit insects from feeding (War *et al*., 2012) [24]. Tannins have a strong negative impact on insects that consume plants. They affect insect growth and development by adhering to proteins, limiting food absorption and producing midgut lesions (Dubey *et al*., 2016 and Martemyanov *et al.*, 2006) [7, 14]. Tannins are mouth-puckeringly bitter polyphenols that are efficient insect pest feed deterrents (Dubey *et al.*, 2016) [7]. The present studies found that the highest amount of tannin contents found in cultivar IC-426769 (6.56±0.00 mg/gm) and lowest in DKV-116 (1.22±0.00 mg/gm) cultivar of black gram.

The lowest amount of tannin contents found in most susceptible genotype of black gram was DKV-116 (1.22±0.00 mg/gm). Price *et al.*, 2019 observed that tannic acid is not an effective insecticide at 0, 1 and 3 concentrations. It showed potential as a future herbicide since it had no effect on the expansion of aphid populations and adversely affected plant growth at a concentration of 3% when aphids were present.

In black gram, genotype IC-398983 had the highest flavonoid content (1.74±0.01 mg/gm), while genotype IC-261182 had the lowest (0.6±0.00 mg/gm).

The highest total phenolic contents (6.56±0.00 mg/gm) found in cultivar IC-426769. Similar findings were observed by Singh *et al*. (2018) [20], 139 distinct cultivars of four pulse crops showed significant genotypic diversity for both antioxidant activity (AOA) and total phenolic content (TPC). Among the four primary pulse crops that were assessed, the cultivars of black gram showed the highest mean phenolic content (7.01 mg GAE/g), followed by lentils (3.46 mg GAE/g), pigeon peas (3.32 mg GAE/g), and chickpeas (2.30 mg GAE/g).

From the present studies, it has been concluded that blackgram cultivars are completely resistant to the attack of *C. chinensis* but most susceptible to *C. maculatus*. Seed resistance against insect infestation may be due to physical attributes such as hard and lignified seed coat, cell wall and biochemical factors such as the presence of secondary compounds and protein (Caswell, 1961) [4]. Some protein components in legumes seeds have potential antibiosis factors that could affect predation (Osborn *et.al*., 1988) [17]. The amount of total phenol contents in resistant cultivars was higher than in least resistant cultivars and connected with total number of eggs laid and adult emergence, therefore significantly and positively correlated with total development period in both pests.



(a) 1 2 3 4 5 6 7 8 9 10 **(b) 1 2 3 4 5 6 7 8 9** **(c) 1 2 3 4 5 6 7 8 9 10**

Plate 9: SDS-PAGE of different cultivars of *V. mungo.* (a) lane 1=Protein marker (b) lane 1=Protein marker (c) lane 1=Protein marker Lane 2= DKV-116 Lane 2= IC-335331 Lane 2= IC-436524 Lane 3= HIM-MASH Lane 3= IC-343936 Lane 3= IC-436597 Lane 4= IC-261182 Lane 4= IC-343967 Lane 4= IC-436604 Lane 5=IC-281975 Lane 5= IC-382811 Lane 5= IC-436628 Lane 6= IC-281981 Lane 6= IC-383412 Lane 6= IC-436632 Lane 7= IC-281989 Lane 7= IC-398983 Lane 7= IC-436647 Lane 8= IC-281993 Lane 8= IC-426769 Lane 8= IC-436659 Lane 9= IC-281995 Lane 9= IC-436511 Lane 9= IC-436675 Lane 10= IC-282004

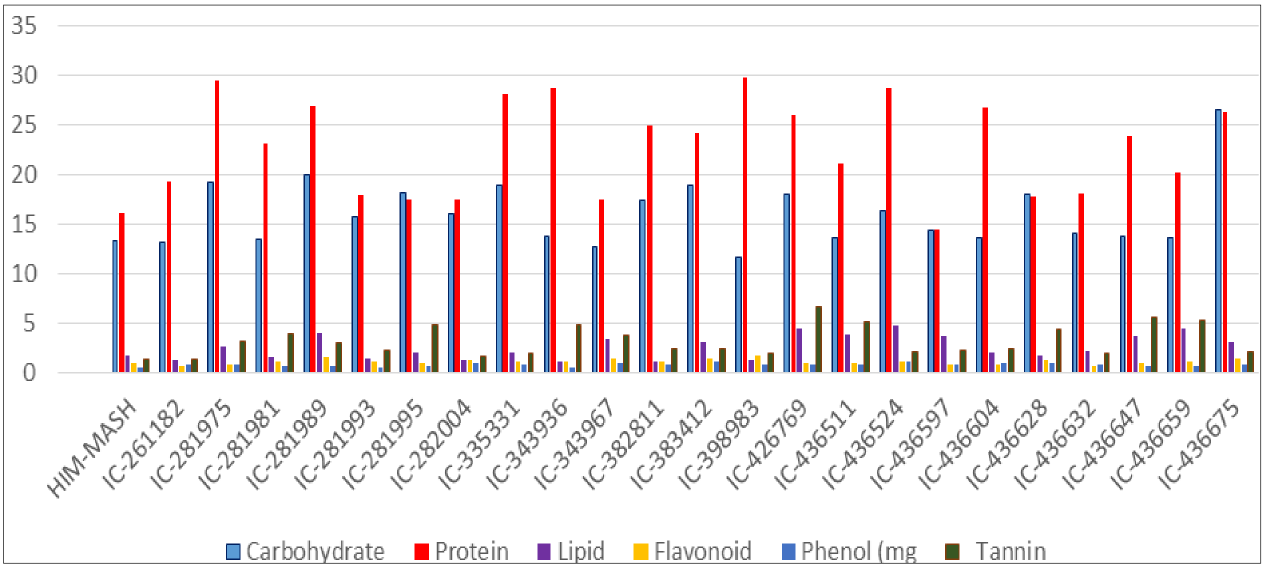


Fig 1: Histogram showing biochemical composition of different genotypes of *V. mungo*

Table 1: Molecular weight (KDa) of protein bands in different cultivars of *V. mungo.*

Cultivars	marker	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	203	203	203	203	203	203	203	203	203	203									203	203	203					
2											103.64	103.64														
3	97		97	97	97	97	97	97	97	97			97				97		97	97	97	97	97	97	97	
4														92.44		92.67										
5																					85.31					
6														72.67		72.79								72.81		
7	66	66	66.03	66	66	66.03	66.26	66	66	66																
8																62.7										
9		60.71																								
10																58.89										
11															58.25									57.58		
12														56.48			56.48									
13												48.34												41.87		
14	45										45		45.16	45		45.19										
15	43			43											43	43			43	43			43		43	43
16		41.15	41.15		41.15													41.87								
17				39			39.49	39.49	39.49	39.49					39.14	39.14										
18		31.65	31.65		31.65	30.80		30.80	30.80	30.80																
19	30						30								30	30										
20		26.92	27.16	27.16	26.92		26	26.38	26.38	26.38		26.92	26.92	26.92					26	26	25.65					
21																						22.65	23		23.26	23.26
22		21.15	21.25	21.25	21.15	21.15	20.32	20.73	20.73	20.73	20.37	20.75		20.37				21.26						21.15		
23		19.13	19.13										20								20					
24							18.29																			
25	14										14.50	14			14.3	14.3										
26		10.1	10.1	10.1			10	10.1	10.1	10.1					9.65			10.42								
27					8.6														8.73	8	8.23					
28		7.39	7.93			7.93																	7.31		7.31	7.31
29	6.5			6.5		6.1	6	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6	6.5	6.5	6.5	6.5	6.5	6	6.44	6.5	6.44	6.44
30	3.5																									

Table 2: Biochemical composition of different genotypes of *V. mungo.*

Sr. No.	Genotype	Carbohydrate mg/ml	Protein mg/ml	Lipid mg/ml	Flavonoid (mg QE/gm)	Phenol (mg GAE/gm)	Tannin (mg CE/gm)
1	DKV-116	16.61±0.00^{bcd}	18.02±0.00^{bcd}	2.5±0.00^{defgh}	0.86±0.00^{bcde}	0.72±0.00^{defgh}	1.22±0.00^{a}
2	HIM-MASH	13.33±0.00^{a}	16.10±0.00^{ab}	1.7±0.02^{abcde}	0.93±0.00^{def}	0.41±0.00^{a}	1.27±0.00^{b}
3	IC-261182	13.09±0.00^{ab}	19.35±0.00^{bcd}	1.3±0.00^{abc}	0.6±0.00^{a}	0.79±0.00^{efgh}	1.32±0.00^{ab}
4	IC-281975	19.20±0.00^{de}	29.51±0.02^{k}	2.6±0.00^{efgh}	0.78±0.00^{bc}	0.73±0.00^{defgh}	3.09±0.00^{f}
5	IC-281981	13.51±0.00^{ab}	23.08±0.01^{efg}	1.5±0.00^{abcde}	1.04±0.00^{hi}	0.67±0.00^{cdef}	3.83±0.00^{g}
6	IC-281989	19.94±0.00^{e}	26.96±0.02^{hijk}	3.9±0.00^{ijk}	1.58±0.00^{m}	0.56±0.00^{bc}	3.02±0.00^{f}
7	IC-281993	15.69±0.00^{bcd}	17.93±0.03^{abc}	1.4±0.00^{abcd}	1.07±0.00^{hij}	0.49±0.00^{ab}	2.22±0.00^{de}
8	IC-281995	18.24±0.00^{cde}	17.44±0.01^{abc}	2.0±0.00^{cdefg}	0.94±0.00^{efg}	0.66±0.00^{cde}	4.84±0.00^{hi}
9	IC-282004	16.12±0.00^{bcd}	17.44±0.01^{abc}	1.2±0.00^{abc}	1.18±0.00^{ij}	0.86±0.00^{hij}	1.58±0.00^{abc}
10	IC-335331	18.87±0.00^{cde}	28.21±0.01^{ijk}	2.0±0.00^{abcdef}	1.13±0.00^{ij}	0.84±0.02^{ghi}	1.9±0.00^{cde}
11	IC-343936	13.77±0.00^{a}	28.70±0.01^{jk}	1.0±0.00^{a}	1.02±0.00^{gh}	0.52±0.00^{ab}	4.84±0.00^{ij}
12	IC-343967	12.66±0.00^{a}	17.48±0.03^{abc}	3.3±0.00^{ghij}	1.35±0.00^{kl}	0.88±0.00^{ij}	3.72±0.00^{g}
13	IC-382811	17.41±0.00^{cde}	25.02±0.04^{ghi}	1.0±0.00^{a}	1.14±0.00^{j}	0.76±0.00^{efghi}	2.38±0.00^{e}
14	IC-383412	18.96±0.00^{de}	24.26±0.01^{fgh}	3.0±0.00^{fghi}	1.39±0.00^{l}	1.02±0.00^{kl}	2.33±0.00^{de}
15	IC-398983	11.62±0.01^{a}	29.85±0.00^{k}	1.2±0.00^{ab}	1.74±0.01^{m}	0.8±0.00^{efghi}	1.95±0.00^{cde}
16	IC-426769	18.04±0.00^{bcd}	26.07±0.00^{ghijk}	4.4±0.00^{jk}	0.98±0.00^{fgh}	0.81±0.00^{fghi}	6.56±0.00^{l}
17	IC-436511	13.63±0.00^{ab}	21.12±0.01^{def}	3.8±0.00^{ijk}	0.87±0.00^{cde}	0.75±0.00^{efgh}	5.15±0.25^{ijk}
18	IC-436524	16.42±0.00^{bcd}	28.77±0.00^{jk}	4.8±0.00^{k}	1.0±0.00^{fgh}	1.09±0.00^{l}	2.06±0.00^{cde}
19	IC-436597	14.42±0.00^{abc}	14.46±0.00^{a}	3.6±0.00^{ijk}	0.83±0.00^{bcd}	0.82±0.00^{ghi}	2.27±0.00^{de}
20	IC-436604	13.62±0.00^{ab}	26.74±0.00^{ghijk}	2.0±0.00^{abcdef}	0.77±0.00^{b}	0.98±0.00^{ijk}	2.33±0.00^{e}
21	IC-436628	17.96±0.00^{cd}	17.75±0.01^{abcd}	1.7±0.00^{abcde}	1.28±0.00^{k}	0.89±0.00^{jkl}	4.31±0.01^{h}
22	IC-436632	14.09±0.00^{abc}	18.16±0.01^{bcd}	2.2±0.00^{bcdefg}	0.62±0.00^{a}	0.81±0.00^{defg}	1.9±0.00^{bcd}
23	IC-436647	13.71±0.00^{ab}	23.89±0.01^{efg}	3.7±0.00^{ijk}	0.87±0.00^{bcde}	0.61±0.00^{bcd}	5.56±0.00^{k}
24	IC-436659	13.60±0.00^{ab}	20.18±0.00^{cde}	4.4±0.00^{jk}	1.02±0.01^{fgh}	0.66±0.00^{cde}	5.24±0.00^{jk}
25	IC-436675	26.61±0.00^{f}	26.29±0.01^{ghij}	3.0±0.00^{ijk}	1.38±0.00^{l}	0.74±0.00^{efghi}	2.06±0.00^{cde}

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