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Morphological and biochemical basis of tolerance to bruchid, *Caryedon serratus* (Olivier) in groundnut pods

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Abstract
Groundnut genotypes were screened for their relative tolerance to bruchid, *Caryedon serratus* (Olivier). Morphological characters and biochemical constituents of pods were studied to understand their contribution towards susceptibility to the bruchid. The genotypes *viz.,* Narayani and K 9 were found to be less preferred by the bruchids for oviposition (20.33 and 21.0 eggs/100 g pods respectively) and resulted in emergence of significantly lower number of adults (13.67 and 14.33 adults respectively). Whereas, the highly susceptible genotype, ICGV 350 that possessed smooth reticulation received 47.33 eggs/100 g pods and resulted in emergence of 40.33 adults. Major contribution for tolerance to groundnut bruchid was found with the pod reticulation, wherein the less susceptible genotypes possessed prominent reticulation. The biochemical analysis also revealed that these genotypes possessed higher amounts of proteins (25.77 and 24.53%), ash (3.80 and 3.80%), total phenols (254.16 and 313.46 mg/g), pod surface phenols (268.14 and 317.97 mg/g) and lower total soluble sugars (3.55 and 3.84%) respectively when compared to susceptible genotypes. Thus, resistance to groundnut bruchid is a complex mechanism governed by both morphological and biochemical characters of pods.

Keywords: Groundnut pods, morphological characters, biochemical constituents, bruchid tolerance

1. Introduction
Groundnut is an important oil seed crop in India. Groundnut bruchid, *Caryedon serratus* (Olivier) (Bruchidae: Coleoptera) has become a major threat to pods and kernels which are stored for different purposes (Kapadia, 1995) [1]. The problems associated with synthetic insecticides and fumigants necessitated the need for alternate measures for the protection of stored produce. Host plant resistance plays a significant role in insect pest management (Painter, 1951) [2].
Though many workers had reported the biology and damage potential of bruchid on groundnut, limited information is available regarding the resistance of groundnut cultivars to groundnut bruchid. Moreover, crop improvement is a continuous programme. The groundnut cultivars with moderate reticulation on pods were less preferred by *C. serratus* for egg laying (Devi and Rao, 2000) [3]. Keeping this in view, an investigation was undertaken to identify the morphological and biochemical characters of groundnut pods responsible for tolerance to the bruchid.

2. Material and Methods
A total of nineteen groundnut genotypes (released and pre-released) *viz*., Abhaya, Dharani, Narayani, TPT 4, TCGS 1157, TCGS 1342, TCGS 1343 and TCGS 1273 from Regional Agricultural Research Station, Tirupathi; Harithandra, K 6, K9, K 1501, K1535 and K 1454 from Agricultural Research Station, Kadiri and ICGV 03057, ICGV 91114, ICGV 00350, ICGV 87846 and ICGV 93468 from ICRISAT, Hyderabad were screened at Post Harvest Engineering
Technology Centre, Bapatla, Guntur district, Andhra Pradesh during 2015-16. Healthy groundnut pods (100 g) of each genotype were taken in a plastic container (1000 ml capacity). Three pairs of newly emerged *C. serratus* beetles were released into each jar. Three replications were maintained for each treatment. The adult beetles were removed after 10 days from the jars and the number of eggs laid by the bruchid females on pods of different genotypes was counted.

Later the jars were kept for observation under laboratory conditions till the emergence of adults. The adults of *C. serratus* that emerged from different treatments were noted daily and removed from the respective jars. Counting was continued till they cease to emerge. Data was pooled to get the total number of adults emerged from each genotype and the weight loss was also calculated and expressed in percentage.

To understand the basis of resistance, physical parameters such as pod size (length - width), weight (g), pod shell thickness (mm) were recorded. Length, width of pods, pod shell thickness was measured using digital Vernier calipers and expressed in millimeters (mm). Width and pod shell thickness was taken at two points (seed center points) and average was worked out. Pod weight of each genotype was taken individually with the help of analytical balance and expressed in grams. Observations of all the parameters were taken for 20 pods of each genotype. Pod reticulation was characterized based on descriptors in the peanut IBPGR and ICRISAT manual, 1992 [4]. All the selected genotypes were analysed for their biochemical constituents *viz.*, total soluble sugars, proteins, total phenol content and ash content (total minerals) responsible for susceptibility or tolerance to *C. serratus*. As the insect continues to feed on kernels and grow inside the pod, kernels were taken for analysis purpose. The data collected pertaining to different parameters was subjected to statistical analysis of variance by Completely Randomized Design (CRD), with three replications for the test of significance.

3. Results and Discussion

The results indicated that the insect preference for oviposition varied in different genotypes (Table 1). The number of eggs laid by *C. serratus* was the lowest in groundnut genotype, Narayani (20.33) of which the pods had prominent reticulations on their surface. Higher numbers of eggs were recorded in TCGS 1157 (28.67), TCGS 1342 (33.33) and TCGS 1343 (40.67), may be due to the presence of three seeded pods. Similarly, lowest number of eggs was recorded in K 9 (21.0) which had small sized pods compared to other genotypes. The number of adults emerged was the least in Narayani (13.67) and was highest in ICGV 00350 (40.33) which also received maximum oviposition (47.33).

3.1 Morphological characters of groundnut pods

3.1.1 Length and width: Among the different genotypes pod length and width were maximum in ICGV 87846 (33.05 mm x 13.39 mm). While the genotype, K 9 had minimum pod length (22.18 mm) and the genotype, Abhaya measured minimum pod width (10.73 mm) (Table 1). Preference to bigger sized pods by *C. serratus* for oviposition was also observed by Haritha *et al.* (1999) [5] and Prasad *et al.* (2012) [6].

3.1.2 Shell thickness: The pod shell thickness of different groundnut genotypes was ranged between 0.63 and 1.02 (mm), with the minimum in ICGV 93468 (0.63 mm) followed by Dharani (0.70 mm). Maximum pod shell thickness was noticed in K 1454 (1.02 mm).

3.1.3 Weight: The pod weight was highest in genotype, ICGV 87846 (1.37 g) followed by ICGV 00350 (1.19 g). The lowest pod weight was recorded in genotype K 9 (0.83 g). Though the pods of ICGV 00350 had thicker shell (0.86 mm), their pod weight was also higher (1.19 g) due to which more food material supply was ensured for the developing insects rendering the genotype susceptible. Female pulse bruchid, *Callosobruchus maculatus* preferred fresh chickpea seeds having more quantity of resources for egg laying as they ensure the nutrition of her offspring (Anamika and Jayalaxmi, 2016 [7]).

3.1.4 Reticulation: The less susceptible genotypes; Narayani, K 9 and ICGV 87846 possessed prominent to moderate reticulations while the susceptible genotypes; Dharani, K 1535 and ICGV 03057 had smooth or less reticulation on the pods. The results indicated that the pods with prominent reticulation were less preferred by the groundnut bruchid for oviposition as compared to smooth or less reticulated pods. The groundnut cultivars; TCGS 61, TMV 2 and TPT 3, which had moderate reticulation (Devi and Rao, 2000 [3]) and 1CGS 76 with prominent reticulation (Haritha *et al.*, 1999 [5]) on pods were less preferred by *C. serratus* for egg laying.

3.2 Biochemical constituents of groundnut pods:

Chemical compounds present in plant parts affect the behaviour of phytophagous insects in various ways. The selected genotypes of groundnut were analysed for the biochemical constituents such as proteins, total soluble sugars, ash (total minerals), phenols and pod surface phenols by following standard methods of estimation which may influence growth and development of insect.

3.2.1 Proteins: Higher per cent of protein was observed in less susceptible genotypes *viz.,* Narayani, K 9 and ICGV 87846 (25.77, 24.53 and 21.45 per cent respectively), whereas it was less in susceptible genotypes *viz.*, Dharani, K 1535 and ICGV 00350 (23.96, 23.03 and 18.5 per cent respectively) (Table 2). Similarly, Sharma and Thakur 2014 [8] also recorded that highly resistant soybean genotypes possessed high amounts of proteins than cowpea and chickpea genotypes that are susceptible to pulse bruchid.

3.2.2 Total soluble sugars (TSS): The highest TSS 7.36 was noticed in genotype, ICGV 00350 (7.36%) followed by K 1535 and Dharani with 4.41 and 4.18 per cent respectively. The per cent TSS noticed was lower in less susceptible genotypes *viz.,* Narayani, K 9 and ICGV 87846 (3.55, 3.84, 4.13 respectively). The observation of higher TSS content in susceptible genotypes is in agreement with Halder and Srinivasan 2007 [9] who recorded higher amounts of total sugars in pod borer susceptible urdbean cultivar.

3.2.3 Ash: The data pertaining to ash content in selected groundnut genotypes revealed that higher per cent of ash was noticed in less susceptible genotypes compared to highly susceptible genotypes. High levels of ash content in cowpea varieties conferred high resistance to *C. maculatus* infestation (Mogbo *et al.,* 2014 [10]). Hameed and Qayyum 1984 [11] concluded that wheat varieties with high ash and protein contents were less prone to *Sitophilus oryzae* insect attack during storage.

3.2.4 Phenols: The less susceptible genotypes had higher phenol content compared to highly susceptible genotypes. The genotype, K 9 (313.46 mg/100 g) recorded the highest phenol content compared to its counterpart genotype, K 1535 (236.57 mg/100 g) which was highly susceptible to *C. serratus*. The genotypes, Narayani and Dharani recorded the phenols at 254.16 and 192.16 mg/100 g respectively. The genotypes, ICGV 87846 and ICGV 00350 were on par with each other and recorded phenol contents at 181.33 and 172.13 mg/100 g respectively. These findings gain support from Halder and Srinivasan 2007 [9]; Sharma and Thakur 2014 [8] who stated that phenols inhibit the action of trypsin enzyme in insects and were responsible to form complex with food nutrients, thus rendering them less soluble or less susceptible to degradation and absorption.

3.2.5 Pod surface phenols: The genotypes, K 9 and ICGV 87846 contained higher amounts of phenols (317.97 and 303.45 mg/100 g respectively) in their pod surfaces due to which they were less preferred for oviposition by groundnut bruchids. The other susceptible genotypes *viz.*, K 1535 and ICGV 00350 were on par with each other and had 292.5 and 294.2 mg/100 g of phenols respectively in their pod surfaces. Walker *et al.* 2014[12] reported significant effects ranging from stimulatory to deterrence due to phenolic acids on oviposition by *Pieris rapae* on crucifers.

Table 1: Relative susceptibility of groundnut pods of different genotypes to *Caryedon serratus* and their morphological characters.

S. No.	Genotype	Oviposition*	Adult Emergence**	Morphological characters of pods				
		Eggs (No.)/100 g	(No.)	Length (mm)	Width (mm)	Weight (g)	Shell Thickness (mm)	Reticulation
1	TCGS 1273	23.00 (4.78)^{a}	17.33 (4.14)^{abc}	27.55^{efg}	13.34^{fg}	1.01^{fg}	0.89^{cd}	Moderate
2	TCGS 1157	28.67 (5.35)^{b}	22.33 (4.71)^{cde}	32.96^{ij}	13.17^{fg}	1.13^{cdef}	0.85^{def}	Smooth
3	TCGS 1342	33.33 (5.77)^{bcd}	27.00 (5.19)^{efgh}	30.43^{h}	11.68^{c}	1.23^{abcd}	0.87^{cde}	Smooth
4	TCGS 1343	40.67 (6.37)^{ef}	33.33 (5.77)^{hijk}	34.97^{k}	12.37^{d}	1.14^{cdef}	0.77^{gh}	Smooth
5	TPT 4	21.67 (4.65)^{a}	16.33 (4.03)^{ab}	27.02^{cdefg}	11.78^{c}	0.72^{i}	0.79^{fg}	Smooth
6	Abhaya	22.67 (4.75)^{a}	17.33 (4.14)^{abc}	27.03^{cdefg}	10.73^{a}	0.93^{gh}	0.77^{gh}	Smooth
7	Narayani	20.33 (4.50)^{a}	13.67 (3.69)^{a}	26.63^{cdef}	11.39^{bc}	1.05^{efg}	0.80^{efg}	Prominent
8	Dharani	44.00 (6.63)^{fg}	39.00 (6.24)^{jk}	26.63^{cdef}	12.36^{d}	0.94^{gh}	0.70^{hi}	Smooth
9	K 6	42.00 (6.48)^{efg}	33.67 (5.80)^{hijk}	27.17^{defg}	10.78^{a}	1.27^{abc}	0.81^{efg}	Moderate
10	Harithandra	32.33 (5.68)^{bc}	25.33 (5.02)^{def}	24.82^{b}	11.81^{c}	1.07^{efg}	0.82^{defg}	Smooth
11	K 9	21.00 (4.57)^{a}	14.33 (3.77)^{a}	22.18^{a}	10.77^{a}	0.83^{hi}	0.85^{def}	Prominent
12	K 1501	32.33 (5.68)^{bc}	26.00 (5.08)^{defg}	26.31^{bcde}	13.25^{fg}	1.35^{a}	0.84^{defg}	Smooth
13	K 1535	41.00 (6.40)^{efg}	36.00 (5.99)^{ijk}	28.43^{g}	12.81^{def}	1.07^{efg}	0.80^{efg}	Smooth
14	K 1454	31.67 (5.62)^{bc}	25.67 (5.06)^{def}	28.49^{g}	11.34^{bc}	1.27^{abc}	1.02^{a}	Smooth
15	ICGV 91114	39.33 (6.27)^{def}	31.67 (5.63)^{fghi}	25.59^{bcd}	12.56^{de}	1.08^{defg}	0.94^{bc}	Smooth
16	ICGV 00350	47.33 (6.88)^{g}	40.33 (6.35)^{k}	31.20^{hi}	10.94^{ab}	1.19^{bcde}	0.86^{cdef}	Smooth
17	ICGV 93468	42.00 (6.48)^{efg}	32.33 (5.67)^{ghij}	28.14^{fg}	12.98^{efg}	1.30^{ab}	0.63^{i}	Prominent
18	ICGV 03057	37.00 (6.08)^{cde}	29.00 (5.37)^{fgh}	25.36^{bc}	12.47^{de}	0.98^{fgh}	0.98^{ab}	Smooth
19	ICGV 87846	30.33 (5.50)^{b}	21.00 (4.58)^{bcd}	33.05^{j}	13.39^{g}	1.37^{a}	0.82^{defg}	Moderate
	SEm±	0.18	0.21	0.64	0.19	0.06	0.03	-
	CD (0.05)	0.5	0.6	1.78	0.54	0.15	0.08	-

Values in parentheses are transformed (*square root, ** angular) values.
In each column values with similar alphabet do not vary significantly at P=0.05.

Table 2: Contents of Biochemical constituents of groundnuts of certain genotypes.

S. No	Genotype	Reaction to bruchid	Proteins (%)	Total Soluble Sugars (%)	Ash (%)	Phenols (mg/100g)	Phenols in pod surface (mg/100g)
1	Narayani	Less Susceptible	25.77^{a}	3.55^{d}	3.80^{a}	254.16^{b}	268.14^{b}
2	Dharani	Highly susceptible	23.69^{ab}	4.18^{bc}	3.50^{b}	192.70^{c}	198.13^{c}
3	K 9	Less Susceptible	24.53^{b}	3.84^{cd}	3.80^{a}	313.46^{a}	317.97^{a}
4	K 1535	Highly susceptible	23.03^{bc}	4.41^{b}	3.49^{b}	236.57^{b}	292.50^{ab}
5	ICGV 87846	Less Susceptible	21.45^{c}	4.13^{bc}	3.87^{a}	181.33^{c}	303.45^{a}
6	ICGV 350	Highly susceptible	18.50^{d}	7.36^{a}	3.80^{a}	172.13^{c}	294.20^{ab}
	SEm±	-	0.53	0.15	0.09	6.97	10.26
	CD (0.05)	-	1.64	0.47	0.29	21.47	31.62

In each column values with similar alphabet do not vary significantly at P=0.05

4. Conclusion
Although, groundnut pods are rich in carbohydrates and proteins, their acceptability and utilization by the groundnut bruchid species may be limited due to the presence of high levels of phenols. Higher content of total sugars and lower amount of phenols were observed in groundnut genotypes susceptible to leaf miner. Higher amounts of phenols in groundnut genotypes contributed for field level resistance to insect infestation, while total sugars showed positive relationship with insect infestation.

Overall, the groundnut genotypes having less pod weight and size, thick pod shell and prominent reticulation coupled with more contents of proteins, ash, phenols and pod surface phenols and lesser amounts of total soluble sugars showed resistance reaction against *C. serratus*. It is also evident that no single physical factor or biochemical constituent of groundnuts is responsible for imparting tolerance/susceptible reaction against the bruchid pest. However, they can contribute synergistically when both factors are combined with in a genotype. Hence, the groundnut genotypes may be genetically improved in terms of storage insect tolerance by having a balance of these traits without affecting yield, oil per cent, shelling per cent and other organoleptic qualities.

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