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# Evaluation of bio-chemical constituents in green gram associated with resistance to *Callosobruchus maculatus during* storage

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**Abstract**
The pulse beetle, *Callosobruchus maculatus* (Fabricius) is an important limiting biotic factor in the storage of green gram. The present study was carried out to study the biochemical constituents imparting resistance/susceptibility to pulse beetle *Callosobruchus maculatus* Fabricius in green gram varieties under ambient conditions. The 9 promising varieties of green gram obtained from Oilseed Research Station, Jalgaon and evaluated for biochemical and physical parameters under storage conditions at Seed Testing Research Unit, MPKV, Rahuri. After storage period the seed were evaluated for biochemical parameters in which results showed that the protein content in seeds of different green gram varieties ranged from 20.23 to 22.32 percent. The highest per cent protein content was found in Kopargoan (22.32%). The less per cent protein content was observed in PM-302-46 (20.23 %). The highest per cent carbohydrate content was found in Kopargoan (62.23 %), followed by PM-302-46 (61.82%). The less carbohydrate content was observed in BM-4 (56.34 %). The grain infestation on number and weight basis ranged between 12.33 – 21.40 and 78.36– 85.14 per cent, respectively. The minimum grain infestation on number and weight basis was noticed in the variety BM-2003-1 (12.33 %) and (79.18 %) which was found statistically at par with BM-4 (15.33%) and (79.88%) and BM-2002-1(16.00 %) and (78.36 %). Under correlation between biochemical parameters and grain infestation by pulse beetle, it was observed that protein, carbohydrate, fat and moisture content indicated positively and non significant correlation with orientation of *C. maculatus* adults towards seeds of green gram.

**Keywords:** Green gram, pulse beetle, protein, varieties, grain infestation

## Introduction
Pulses play an important role in Indian Agriculture, not only to increase the soil fertility but also in providing proteinaceous grains and nutritive fodder. Pulses contain at least two to three times more protein and the amount of lysine as compared to the proteins of cereal grains. Pulses have been considered as poor man's meat for the people who cannot afford animal protein. Pulses have been referred to as unique jewels of Indian crop husbandry. In pulses, green gram is an important pulse crop in India and is the main source of protein. Green gram is mostly consumed in the form of whole grain, dal. In green gram minerals, proteins, CHO and vitamins besides, a good source of iodine and rich essential amino acids like lysine, tyrosine, cystine and arginine (Anonymous, 2015) [2]. Green gram suffers losses both qualitative and quantitative from the attack of storage pest. It is infested by the multivoltine bruchids species *Callosobruchus chinensis* and *C. maculatus* commonly known as pulse beetle. Among the several important insect pests of stored grains, bruchids i.e. pulse beetles, *Callosobruchus chinensis* (Linnaeus), *Callosobruchus maculatus* (Fabricius), *Callosobruchus analis* (Fabricius) and *Callosobruchus phaseoli* (Gall) (Coleoptera : Bruchidae) are commonly occurring and considered to be the most serious insect pests of stored pulses *viz*., Arhar, black gram, green gram, horse gram, bengal gram, peas, cowpea, soybean, mothbean, etc ( Raina, 1970) [17]. Pulse beetles do not attack all kinds of pulses to the same extent and the average per cent damage has been found to be highest in green gram (55.4%) followed by black gram (35.3%), pigeon pea (22.1%), cowpea (16.8%), gram (11.1%) and pea (8.8%) (Bhaduria and Jakhmola, 2006) [3].

Developing countries are adopting the use of resistant grain varieties to control stored grain weevils as a popular alternative to the use of chemicals (Garib, 2004) [9]. Development of improved varieties through genetic methods plays a major role in protecting the seeds from post harvest damage (Karthikeyan *et al*., 2008) [12]. Screening of resistant cultivars is a major component of IPM and is a continuous process.

Various workers (Manohar and Yadava, 1990, Chavan *et al*., 1997a and 1997b, Umrao and Verma, 2002 and Jha *et al*., 2008) [14, 6, 7, 21, 11] have reported varietal preference of different species of bruchids. So keeping all these issues for protecting the green gram from attack of pulse beetle and what is damage causes to the seeds the present investigation was taken for the study.

## Material and methods

The present studies on effect of pulse beetle on green gram were carried out during *Kharif* season at laboratory of Department of Agricultural Entomology, Post Graduate Institute and Seed Technology Research Unit, Mahatma Phule Krishi Vidyapeeth, Rahuri-413 722, Dist. Ahmednagar (M.S.) under the ambient condition. The seeds of 9 varieties of green gram *viz* ; Vaibhav, Kopergaon, BM-2002-1, Uttkarsha, BPMR-145, Green gold BM-4, BM-2003-1 and PM-302-46 obtained from Oilseed Research Station, Jalgaon were dried in bright sunlight for three days to bring down moisture content to less <10 per cent for conducting this study.

The initial culture of pulse beetle, *Callosobruchus macutalus* (Fabricius) was obtained from the storage godown of Central Store, Seed Cell and Seed Technology Research Unit, MPKV, Rahuri. The identification key of *Callosobruchus* spp. given by Raina (1970) [17] was used. Initial culture, healthy seed of green gram was kept in to 32×22.5 cm size cylindrical jar and 10 pairs of adult beetles were isolated and released into jar. The top of the jar was covered with muslin cloth secured firmly by rubber band. After emergence of new adults, the beetles were introduced into green gram seed kept in a series of cylindrical jars for building up a homogenous population. The sample of each variety (10 seed) was weighed out with the help of mono pan micro analytical balance. The samples were kept in plastic containers and 5 pair of adults *C. maculatus* was released in each of them. The containers were covered with the help of muslin cloth, fastened with rubber bands and kept at room temperature.

Newly emerged beetles were removed daily from the 25$^{th}$ day after the release of beetles and counted for further period of 15 days till there was no further emergence of beetles. Finally the samples were weighed with the help of same balance after removing hatched and unhatched eggs along with dough and the per cent of loss in weight of different varieties was worked out after adjusting any increase or decrease in the weight of the control. Percent weight loss was calculated using the formula given by Adams (1976) [1]. The number of grains infested by *C. maculatus* was counted and per cent infestation was worked out on the basis of grains with characteristics holes made by beetles. The damage was computed by subtracting the sound grains from the already counted total number of grains.

Moisture content of grains was determined by following Hot Air Oven method (Chalam *et al*., 1967) [4]. Biochemical constituents *viz*., protein, carbohydrate, fat and ash was analyzed on the equipment *i.e.* NIR-Spectrometer available at the Department of Agricultural Botany, PGI, MPKV, Rahuri. The observations on development period, growth index, per cent grain infestation, per cent weight loss of each genotype correlated with the biochemical constituents' *viz.,* protein, CHO, fat, ash and moisture content by applying simple correlation coefficient method.

## Results and Discussion:

### Infestation of pulse beetle in green gram

The results of the reaction of pulse beetle on biochemical contents in seeds of different green gram varieties are presented in Table 1. The protein content in seeds of different green gram varieties ranged from 20.23 to 22.32 percent. The highest per cent protein content was found in Kopargoan (22.32%). The less per cent protein content was observed in PM-302-46 (20.23 %). The carbohydrate content ranged from 56.34 to 62.23 per cent in seeds of different green gram varieties. The highest per cent carbohydrate content was found in Kopargoan (62.23 %), followed by PM-302-46 (61.82%). The less carbohydrate content was observed in BM-4 (56.34 %). The fat per cent in seeds of different green gram varieties ranged from 0.89 to 1.33 per cent. The highest per cent fat was found in BPMR-145 (1.33). The less per cent fat was observed in PM-302-46 (0.89 %). The ash per cent ranged from 2.20 to 3.71 percent in seeds of different varieties. Highest ash content was recorded in BM-4 (3.71 %) followed by BM-2002-1 (3.19%). The less ash content recorded in Uttakarsha (2.20 %) followed by PM-302-46 (2.23 %). The moisture content in seeds of different varieties recorded in between the range of 8.67 to 9.32 per cent. The highest moisture content recorded in Green gold (9.32 %). The less moisture content was recorded in BM-2003-1 (8.67 %). The findings of present investigations are in conformity with the results reported by Sing *et al.* (1995) [20] who reported fecundity and index susceptibility were comparatively lesser for the varieties with characteristics of high protein content. Sing and Sharma (2001) [19] observed that longest period for incubation can be attributed to the variety containing high amount of protein.

The data on per cent grain infestation caused by pulse beetle, *C. maculatus* adults on different green gram varieties are presented in Table 2. The grain infestation on number and weight basis ranged between 12.33 – 21.40 and 78.36– 85.14 per cent, respectively. The minimum grain infestation on number and weight basis was noticed in the variety BM-2003-1 (12.33 %) and (79.18 %) which was found statistically at par with BM-4 (15.33%) and (79.88%) and BM-2002-1(16.00 %) and (78.36 %). The maximum grain infestation on number basis was exhibited in Vaibhav (21.40 %) and it was at par with Kopargoan (20.17 %), PM-302-46(20.33 %), Uttakarsha (19.36 %) and Green gold (19.12 %). While PM-302-46 variety recorded significantly maximum per cent damage of grain against rest of variety. PM-302-46 was found to be highly susceptible variety to *C. maculatus* on number and weight basis. The statistical analysis of the data in Table 2 indicated that most of the varieties tested suffered weight loss due to feeding of *C. maculatus*. The significant difference was found in percent grain weight loss on weight basis which ranged from 15.33 to 21.00 percent. The minimum per cent grain weight loss of (15.33 %) recorded in the variety BM-4 and it was found statistically at par with BM-2002-1 (16.20 %), Uttkarsha (16.24%) and BM-2003-1 (16.61%) and BPMR-145 (16.91%). The maximum grain weight loss 21.00 % was observed in PM-302-46 which was found statistically at par with Green gold (19.67%).These present findings are supported with the results recorded by Patnaik and Samalo (1987) [16], Dasbak *et al.* (2009) [8] Mukherjee *et al.* (1970) [15] and Khokhar and Singh (1987) [13] who reported the seed infestation of pigeon pea genotypes by *C. maculatus* in the range of 7.0 to 28.7%, 24.7 to 38.5%, 32.64 and 5.2 to 88.7 per cent, respectively.

The results on development period indicated that the significantly less development period was observed in the variety BM-2002-1 (26.04 days) followed by BM-2003-1(27.31 days). The highest development period was noted in the variety Kopargaon (31.24 days) followed by Vaibhav (30.22 days), Green gold (29.51 days), PM-302-46 (29.42

days), BM-4 (29.37 days), Uttkarsha (29.23 days) and BPMR-145 (29.17 days) (Table 3). The growth index ranged from 2.74 to 3.06 in different varieties of green gram. PM-302-46 proved to be most nutritious to *C. maculatus* recording high growth index of 3.06. The least nutritious variety was BM-4 which recorded 2.74 growth index and it was at par with BM-2003-1(2.78), Kopargaon (2.79) and BPMR-145 (2.80) (Table 3). The results of present investigations are in agreement with the results reported Wadnerkar *et al*. (1978) [24] who reported that the varieties having maximum growth index are more susceptible for pulse beetle in arhar and gram. Ghokhale (1973) observed the growth index of *C. maculatus* in the range of 2.67 – 3.14 on Bengal gram and Vishwamitra *et al.* (2015) also observed the growth index of *C. chinensis* in between 0.00 to 6.00 in different varieties of pigeon pea.

The results on correlation of biochemical constituents to pulse infestation are presented in Table 4. It was observed that protein, carbohydrate, fat and moisture content indicated positively and non significant correlation with orientation of *C. maculatus* adults towards seeds of green gram. Whereas, ash content was noticed strong significantly (r= -0.824) negative association with the adults oriented towards the seeds. Also the protein, carbohydrate, fat content in seed were found significant and positively correlated with per cent weight loss (r=0.686, 0.834 and 0.872, respectively), whereas, ash content was significant and negatively correlated with per cent grain weight loss (r=-0.885). These present findings are in agreement with the results reported by following earlier workers. Venugopal *et al.* (2000) [22] reported strongly confirmed positive correlation of protein to *C. maculatus* infestation. Shams Fawki *et al.* (2012) [18] indicated a negative correlation between the seed total protein content and susceptibility index. Vishwamitra *et al.* (2015) observed that the chemical parameters like high ash of test varieties were detrimental to the growth and development of test insect while protein content of the test varieties favored the successful development of bruchids and high infestation. Chandel and Bhaduria (2015) [3] reported the infestation and losses were found to be positively associated with the test weight and moisture content.

**Table 1:** Effect of pulse beetle on biochemical attributes in green gram varieties

| Sr. No. | Varieties | Protein Content (%) | Carbohydrate (%) | Fat (%) | Ash (%) | Moisture (%) |
|---|---|---|---|---|---|---|
| 1 | Vaibhav | 21.23 | 60.50 | 0.94 | 2.46 | 9.05 |
| 2 | Kopargaon | 22.32 | 62.23 | 1.00 | 2.33 | 9.27 |
| 3 | BM-2002-1 | 20.24 | 60.37 | 1.06 | 3.19 | 8.90 |
| 4 | Uttakarsha | 20.83 | 62 | 0.95 | 2.20 | 8.87 |
| 5 | BPMR-145 | 20.34 | 60.21 | 1.33 | 2.30 | 8.85 |
| 6 | Green gold | 21.97 | 61.27 | 1.13 | 2.82 | 9.32 |
| 7 | BM-4 | 20.62 | 56.34 | 1.21 | 3.71 | 9.12 |
| 8 | BM-2003-1 | 21.81 | 60.09 | 1.06 | 2.53 | 8.67 |
| 9 | PM-302-46 | 20.23 | 61.82 | 0.89 | 2.23 | 9.07 |
| | Mean | 21.07 | 60.54 | 1.06 | 2.64 | 9.01 |

**Table 2:** Growth index, development period and average no. of adults oriented in pulse beetles in green gram varieties

| Sr. No. | Variety | Development period (Days) | Growth index | Average no. of adults oriented | Category |
|---|---|---|---|---|---|
| 1 | Vaibhav | 30.22 | 2.94 | 7.00 (2.73) | MR |
| 2 | Kopargaon | 31.24 | 2.79 | 7.00 (2.75) | MR |
| 3 | BM-2002-1 | 26.04 | 2.95 | 5.00 (2.34) | MR |
| 4 | Uttkarsha | 29.23 | 2.91 | 4.50 (2.23) | MR |
| 5 | BPMR-145 | 29.17 | 2.80 | 4.25 (2.18) | MR |
| 6 | Green gold | 29.51 | 2.86 | 6.00 (2.56) | MR |
| 7 | BM-4 | 29.37 | 2.74 | 4.25 (2.17) | MR |
| 8 | BM-2003-1 | pp27.31 | 2.78 | 4.00 (2.12) | MR |
| 9 | PM-302-46 | 29.42 | 3.06 | 6.00 (2.57) | MR |
| | Mean | 29.87 | 2.06 | 5.33 | |

**Table 3:** Grain infestation caused by pulse beetle in different varieties of green gram

| Sr. No. | Variety | % Grain infestation (No. basis) | % Damage of grain (Wt. basis) | % Grain weight loss (Wt. basis) |
|---|---|---|---|---|
| 1 | Vaibhav | 21.40 (27.52) | 84.12 (66.52) | 18.92 (25.78) |
| 2 | Kopargaon | 20.17 (26.68) | 84.35 (66.70) | 18.08 (25.17) |
| 3 | BM-2002-1 | 16.00 (23.57) | 78.36 (62.28) | 16.20 (23.73) |
| 4 | Uttakarsha | 19.36 (26.08) | 81.12 (64.24) | 16.24 (23.76) |
| 5 | BPMR-145 | 15.49 (23.14) | 83.75 (66.23) | 16.91 (24.28) |
| 6 | Green gold | 19.12 (25.90) | 85.06 (67.27) | 19.67 (26.30) |
| 7 | BM-4 | 15.33 (23.04) | 79.88 (63.35) | 15.33 (23.04) |
| 8 | BM-2003-1 | 12.33 (20.41) | 79.18 (62.86) | 16.61 (24.04) |
| 9 | PM-302-46 | 20.33 (28.80) | 85.14 (67.33) | 21.00 (27.27) |
| SE ± | | 1.31 | 0.21 | 0.48 |
| CD at 5% | | 3.89 | 0.62 | 1.42 |

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