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Biochemical traits of cucumber genotypes in relation to leaf infestation by *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae)

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Abstract
The present study was carried out in cucumber, *Cucumis sativus* L. to identify the resistant sources for red pumpkin beetle during 2009 and 2010. The study of red pumpkin beetle resistance or tolerance was carried out in open field conditions without spraying any insecticide. Plants genotypes possess different biochemical properties, which resultantly induce in them different mechanisms of resistance. This mechanism enables the plants to avoid, tolerate or recover from the effects caused by insect pest. The results of the present studies revealed that there were significant variation in tested cucumber genotypes for percentage leaf infestation and beetle density per plant. Nepal Local was found least susceptible to *Aulacophora foveicollis* followed by Sikkim Cucumber, IC-355960, and IC-429994 whereas; Khira Paprola was most susceptible followed by Mohini-5300 and KCU-006 over check Khira-90. There was a significant positive correlation of per cent leaf infestation and number of beetles per plant with cucurbitacin content, non-significant correlation with total sugars, total free amino acids and total phenols.

Keywords: *Cucumis sativus*, *Aulacophora foveicollis*, biochemical basis resistance, mechanism

Introduction
Red pumpkin beetle, *Aulacophora foveicollis* (Lucas) is a widely distributed polyphagous pest of cucurbit crops in India [3]. This beetle has also been reported from Greece, south Europe, Algeria, Cyprus, Aden, Iraq, Sri Lanka, Nepal, and Burma [13]. The beetle is considered as one of the serious problems particularly at seedling stage in the commercial cultivation of the cucurbits. Among the cucurbits, cucumber is the most preferred by this pest while bitter gourd and sponge gourd are also equally preferred [10, 14]. Cucurbitacins widely distributed in cucurbits, play a major role in host acceptance. Glucoside a triterpenoid of bitter gourd however acts as a feeding deterrent [4]. The beetles start feeding just after the germination and retard the growth of the seedlings due to severe foliar damage. Both the grubs and adults cause damage, the grubs which live underground are destructive to the roots. The grubs enter into the roots, and underground stem or sometimes the fruits touching the ground. The underground portion starts rotting due to attack of saprophytic fungi at the injury site and the fruits become unfit for human consumption. The adult beetles are mainly responsible for damaging the plants above the ground, attacking leaves, flowers, fruits and causing complete defoliation and sometimes the entire crop requires resowing. The beetle resumes its activity in March and remains in the field till October. The peak period of activity is from April to June while the population starts declining from September onwards [20, 3]. A successful breeding program for pest resistance depends upon the sound knowledge on genetics of resistance. Breeding for resistance has been very successful in reducing damage caused by many pests, whereas the use of chemicals can create hazards to human health and produce undesirable side effects on non-target insects, animals and plants. Hence, it is desirable to develop genotypes resistant to red pumpkin beetle in order to step up the production and productivity in this important cucurbit crop. Efforts to develop pest resistant varieties have met with little success and there is still limited understanding of insect- host relationship. Hence there is an urgent need to develop cucumber varieties tolerant and/or resistant to this devastating pest as a component of integrated pest management.

Materials and Methods
Twelve varieties/genotypes *viz*., Khira Paprola, Sikkim Cucumber, Mohini-5300, Nepal Local, KCU-006, IC-355960, IC-469811, IC-479979, IC-430026, IC-4698, IC-429994 and check

Khira-90 were evaluated for their relative susceptibility against the *A. foveicollis* under natural infestation conditions. The cucumber varieties/genotypes for the present investigation were procured from the Department of Vegetable Science and Floriculture, College of Agriculture, CSKHPKV, Palampur and NBPGR, Regional Station, Bhowali Niglat, Nainital, Uttrakhand.

Sowing of crop

The seeds of each cucumber variety/genotype were sown in polythene bags containing mixture of soil, sand and FYM and raised under polyhouse conditions at Krishi Vigyan Kender, at Bara (CSKHPKV, Palampur). The seedlings were transplanted in the field during third week of February at farmer's field, Marhun, Bara (Hamirpur) in 2009 and 2010. The experiment was laid out in Randomized Block Design with plot size of 3.5 x 3.0 meter. The row to row and plant to plant distance was maintained as 1.5 meter and 60 cm, respectively. All the recommended agronomic practices were carried out, except the pest management practices to screen the varietal susceptibility of tested cucumber varieties/genotypes against red pumpkin beetle.

Screening for resistance to red pumpkin beetle, *A. foveicollis*: The seedlings were transplanted at 2-3 leaf stage and were kept under constant supervision for appearance of the pest.

The observations were recorded on the basis of number of beetles and per cent leaf infestation per plant at weekly intervals on ten randomly selected plants from each variety/genotype. The population of adult beetles was recorded on visual basis in the morning hours when beetles were less active.

In the beginning (seedling stage) all the leaves were observed for infestation. Whereas, at later crop growth stage, leaves were selected randomly from terminal, middle and lower portion of each plant. The observations were recorded on the basis of mean number of adult beetles as well as per cent leaf infestation per plant at different growing stages i.e. at seedling stage, 4-5 leaf stage, pre-vining, vining. Pre-flowering, flowering and fruit setting stages.

The genotypes were finally categorized on the basis of per cent leaf infestation (average of 2009 and 2010) according to [16] with slight modifications (Table 1).

$$\text{Per cent leaf infestation} = \frac{\text{No. of infested leaves}}{\text{Total no. of leaves}} \text{ X } 100$$

Table 1: Categorization of cucumber genotypes on the basis leaf infestation by *A. foveicollis*

Infestation (%)	Category	Genotype
0.0	Immune	_
< 25	Resistant	Nepal Local, Sikkim Cucumber, IC-355960, IC-469811, IC-479979, IC-4698, IC-429994
25-50	Moderately resistant	KCU-006, Mohini-5300, IC-430026, Check Khira-90
50-75	Susceptible	Khira Paprola
>75	Highly Susceptible	_

Analysis of biochemical traits

The healthy leaves of cucumber varieties/genotypes were cleaned and freed of any extraneous substances and dried in an electric oven at 55±5 ºC at each growing stage. The dried samples were ground in a grinder and stored in polythene air tight bags. These samples were properly labeled and kept at room temperature for further analysis. The total cucurbitacin content was estimated as per the procedure followed by Kumar [11] and sugars content was determined by the method of Dubois *et al*. [7]. The total phenols were estimated by the method suggested by Makkar *et al*. [12] and total free amino acids were estimated by the method given by Jayaraman [9]. Correlation coefficients were worked out to assess the relationship between biochemical components and pest incidence. The data was subjected to analysis by using CPCS-1 software and SPSS.

Results

Data pertaining to various biochemical constituents *viz*., cucurbitacins, total sugars, total free amino acids and total phenols in leaves of screened genotypes of cucumber and their correlation with mean number of beetles and per cent leaf infestation per plant is presented in Table 2.

Table 2: Biochemical compounds and relative susceptibility of cucumber genotypes in relation to *Aulacophora foveicollis* infestation at Bara (Hamirpur)

Genotype	MNBPP#	Leaf infestation## (%)	Cucurbitacins (mg/g)	Total sugars (mg/g)	Total amino acids (mg/g)	Total phenols (mg/g)
KCU-006	1.83 (1.68)	42.12 (40.45)	0.39	6.47	4.38	6.83
Mohini-5300	1.96 (1.72)	43.60 (41.31)	0.37	5.87	5.35	5.40
Nepal Local	0.63 (1.28)	10.18 (18.50)	0.10	4.60	3.50	10.33
K hira Paprola	2.18 (1.78)	55.41 (48.09)	0.47	3.56	3.68	7.91
Sikkim Cucumber	0.69 (1.30)	12.15 (20.33)	0.12	3.35	2.03	8.66
IC-355960	1.02 (1.42)	13.59 (21.62)	0.19	2.87	1.58	11.50
IC-469811	1.31 (1.52)	18.32 (25.32)	0.25	5.82	4.00	4.60
IC-479979	1.07 (1.44)	16.41 (23.88)	0.23	2.03	2.24	8.50
IC-430026	1.58 (1.61)	28.90 (32.48)	0.31	1.22	1.32	12.91
IC-4698	0.66 (1.29)	14.67 (22.51)	0.21	5.23	4.05	6.74
IC-429994	0.50 (1.23)	9.50 (17.74)	0.16	5.39	7.43	6.23
Check Khira-90	1.31 (1.52)	28.18 (30.75)	0.28	3.07	7.78	10.67
CD (P=0.05)	0.51	3.42	0.19	0.42	1.63	0.77

Figures in the parentheses are square root transformed values

Figures in the parentheses are arc sine transformed values

MNBPP = Mean number of beetles per plant

Cucurbitacins: The cucurbitacin content in leaves of evaluated genotypes ranged from 0.10 to 47 mg/g with values significantly lower in less susceptible genotypes and higher in more susceptible genotypes. Maximum cucurbitacins content was recorded in Khira Paprola (0.47 mg/g) which showed significantly high beetle density and leaf infestation (2.18 beetles and 55.41%/plant, respectively). The genotype KCU-006 and Mohini-5300 also recorded relatively high cucurbitacins content 0.39 and 0.37 mg/g resulting in high beetle density and per cent leaf infestation of 1.83 and 1.96 and 42.12 and 43.60 per plant, respectively. These were followed by IC-430026 (0.31 mg/g) which was at par with check Khira-90. However, resistant genotypes contained low cucurbitacins content as Nepal Local and Sikkim Cucumber having lowest content of cucurbitacins 0.10 and 0.12 mg/g resulting significantly less beetle incidence and leaf infestation. Mean beetle density and per cent leaf infestation per plant exhibited strong and positive correlation with cucurbitacins content in leaves of different genotypes (r = +0.956 with beetle density and +0.965 with leaf infestation) (Table 3).

Total sugars: It can be revealed from the data that total sugar content of 4.60 and 3.35 mg/g in cucumber genotypes *viz*., Nepal Local and Sikkim Cucumber showed resistance to *A. foveicollis* as compared to moderately resistant genotypes KCU-006 and Mohini-5300 (6.47 and 5.87 mg/g), but were statistically at par with susceptible genotype Khira Paprola (3.56 mg/g). The genotype IC-479979 and IC-430026 contained lower total sugars (2.03 and 1.22 mg/g) which also showed moderate resistance.

The total sugar content in remaining genotypes was mostly statistically at par with each other. The mean beetle and per cent leaf infestation per plant was found to be positively correlated with total sugar content, but the relationship was non-significant (Table 3).

Table 3: Correlation co-efficient between *A. foveicollis* incidence and biochemical compounds.

	Cucurbitacins (mg/g)	Total sugars (mg/g)	Total Amino acids (mg/g)	Total phenols (mg/g)
Mean no. of beetles per plant	+0.956**	+0.034	-0.008	-0.099
Per cent leaf infestation	+0.965**	+0.119	+0.127	-0.151

** Significant at 1% level of significance

Total free amino acids: The total free amino acid content in the leaves of tested cucumber genotypes varied from 1.32 to 7.78 mg/g. Maximum total free amino acid content was recorded in IC-429994 and check Khira-90 (7.43 and 7.78 mg/g) which showed significant variation in their level of susceptibility and fall in resistant and moderately resistant category, respectively. The moderately genotype KCU-006 and Mohini-5300 contained comparatively higher total free amino acids (4.38 and 5.35 mg/g) than resistant genotype. However, the moderately resistant genotype IC-430026 registered lowest free amino acid content (1.32 mg/g) and was at par with IC-355960 and Sikkim Cucumber. The mean number of beetles per plant showed non-significant negative correlation with total free amino acids, but per cent leaf infestation showed non-significant positive correlation with free amino acids (Table 3).

Total phenols: The total phenols in different cucumber genotypes were in the range of 4.60 to 12.91 mg/g. The highest total phenol content was observed in moderately resistant genotypes IC-430026 (12.91mg/g) followed by check Khira-90 (10.67mg/g), IC-355960 (11.50 mg/g) and resistant genotype Nepal Local (10.33 mg/g). The susceptible genotype Khira Paprola contained total phenols of 7.91 mg/g which was higher than some moderately resistant genotypes like Mohini-5300, KCU-006 and resistant genotypes IC-469811, IC-4698 and IC-429994. It seemed that total phenol content had no visible effect either on susceptibility or resistance of tested genotypes to the pest. There was also week negative correlation of mean number of beetle and per cent leaf infestation per plant with total phenols (Table 3).

Discussion

During the present study, the total content of biochemical traits like total sugars, total free amino acids, total phenols and cucurbitacin content varied significantly among the screened cucumber genotypes. Numerous studies have shown that the genotypes of the same species could significantly differ in their resistance to insect pests [17, 18] and it is caused by biophysical and/or biochemical compounds of plants [8].

In the present study on biochemical characterization of cucumber genotypes in relation to beetle incidence and leaf infestation by *A. foveicollis* indicated that the cucurbitacin content in leaves of tested genotypes ranged from 0.10 to 0.47 mg/g, being high in susceptible and moderately susceptible genotypes than resistant genotypes. The higher cucurbitacin content in cucurbits associated with susceptibility to *A. foveicollis* [21] and induced the preference for feeding by this pest which acts as feeding stimulant and plays an important role in host acceptance [15, 6]. The cucurbitacins also played an important role in host plant selection by the three cucurbitaceous feeding beetle species *viz*., *Aulacophora indica*, *A. lewisii* and *A. nigripennis* [1]. The highest mean number of beetle and per cent leaf infestation was observed in genotypes having high cucurbitacins and thus also acts as attractant to *A. foveicollis*. The cucurbitacins acted as strong attractant to field populations of the spotted cucumber beetle, *Diabrotica undecimpunctata* [2] and cucurbitacin B to stripped cucumber beetle, *Acalymma vittata*, spotted cucumber beetle, *D. undecimpunctata* and banded cucumber beetle, *D. balteata* [5]. The mean number of beetles and per cent leaf infestation showed highly positive correlation with cucurbitacin content. This is in conformity of Sharma and Hall [19] who also reported positive correlation between cucurbitacin concentration and feeding by spotted cucumber beetle.

The amount of total sugars was quite variable within the cucumber genotypes the less susceptible genotypes registered minimum sugar content varying from 2.03 to 5.82 mg/g. The susceptible genotype Khira Paprola also registered low total sugar content (3.59 mg/g). The mean number of beetles and per cent leaf infestation exhibited non-significant positive correlation with total sugars. There was also positive correlation between total sugars and feeding by spotted cucumber beetle [19].

The genotypes KCU-006, Mohini-5300 and check Khira-90 which proved to be moderately resistant to *A. foveicollis* recorded maximum total free amino acids content (4.38, 5.35 and 7.78 mg/g, respectively). However, maximum total free amino acid content (7.43 mg/g) was also recorded in resistant

genotype IC-429994. The remaining moderately resistant and resistant genotypes recorded low total free amino acid content. However, susceptible genotype Khira Paprola and one of the moderately resistant genotype IC- 430026 also registered low free amino acid content. There was a weak and non-significant positive correlation of beetle incidence and leaf infestation with total free amino acids. Similarly there was also no consistent trend in the level of total phenols among the different screened cucumber genotypes in relation to level of beetle density and leaf infestation. The pest also showed weak and non-significant correlation of mean number of beetles and per cent leaf infestation with total sugars. These results are in line with the findings of Benepal and Hall [2] who also observed non-consistent trend in the total free amino acid content among the comparatively resistant and susceptible plants of *Cucurbita foetidissima* and *Cucumis melo*. The earlier experiments also indicated that *A. foveicollis* exhibited no correlation with quantity of total phenols, free amino acids and non-reducing sugars [6].

Conclusions

It can be concluded from the present studies that the cucurbitacins are more important than other biochemical constituents discussed here to play a major role in host susceptibility to *A. foveicollis*. Nepal Local, which was identified as source of resistance for red pumpkin beetle can be used in IPM program for *A. foveicollis* and breeding programs of cucumber.

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