Proc. Indian natn. Sci. Acad. **B58** No.6, pp.371-376 (1992)

Cucurbitacins and some other Biochemicals in Relation to Cucurbits Susceptibility to Red Pumpkin Beetle

P K MEHTA* and G S SANDHU
Department of Entomology, Punjab Agricultural University, Ludhiana 141 004

(Received on 20 March 1991; after revision 28 August 1991;
Accepted on 23 April 1992)

Nine different cucurbits were studied for cucurbitacins content and other biochemical parameters in relation to host susceptibility to red pumpkin beetle, *Aulacophora foveicollis* (Lucas). The cucurbitacin (Cuc) content varied from 0.177 mg/g in sponge gourd to 0.29 mg/g in watermelon. Insect injury ratings after 48 hrs exposures ranged between 1 to 2.65. Cucurbitacins content was significantly correlated with insect injury ratings. The coefficient of correlation between insect injury and total sugars, phenols, orthodihydroxy phenols flavanoids and total free amino acids were also positive but was of a lower order. This indicated that cucurbitacins are more important in cucurbits susceptibility to red pumpkin beetle than other biochemicals. Topical application of pure Cucs B and E on comparatively non-preferred cultivar resulted in increased leaf injury ratings thereby confirming the primary role of Cucs as feeding stimulants and arrestant for red pumpkin.

Key Words: Cucurbitacins, Red pumpkin beetle, Feeding stimulants

Introduction

Differential attack by red pumpkin beetle *Aulacophora foveicollis* (Lucas), on different cucurbits is well documented (Sandhu & Bhalla 1974, Morey 1975). Differences in damage to the seedlings of cucurbits, by adult red pumpkin beetles is correlated with the quantity of tetracyclic triterpenoids, called 'cucurbitacins' (Vashishta & Choudhary 1971). Cucurbitacins (bitter principles of the family Cucurbitaceae (characterized by Enslin 1954), have been implicated in resistance to cucumber, squash and watermelon to this leaf feeding pest.

Cucurbitacins (Cucs) and their glycosides are distributed in foliage and fruits are known to act as specific attractants (kairomones) and feeding stimulants for Western spotted cucumber beetle (*Diabrotica undecimpunctata undecimpunatata* Mannerheim, the spotted cucumber beetle, *D.u. howardii* Barber, the banded cucumber beetle, *D. balteata* Laconte and promote compulsive feeding (Chambliss & Jones 1966, Sharma & Hall 1973, Howe et al. 1976, Metcalf et al. 1980, Metcalf & Lampman 1989). Both attract and repel the insects (Dacosta & Jones 1971).

In this paper the role of Cucs, total sugars, phenols, orthodihydroxy phenols, flavanoids and total free amino acids in relation to host plant susceptibility to red pumpkin beetle is discussed.

Materials and Methods

Seeds of nine cucurbits were hand dibbed in separate seed beds (150 × 50 cm) for their cultivars and covered with a field cage (170 × 70 cm) to confine the beetles. Beetles were field collected and starved for 4 hrs before

* Department of Entomology, HPKV, Palampur 176 062

releasing into the field cages @ 3 beetles per plant. The assessment of injury ratings on cotyledonary first and second leaves was made by adopting 1-5 visual rating scale as standardised by Grewal (1981) where, 1 = healthy leaf; 2 = approx. 1/4 leaf area damaged; 3 = approx. 1/2 leaf area damaged; 4 = approx. 3/4 leaf area damaged and 5 = approx. more than 3/4 leaf area damaged.

A 5 g fresh sample from cotyledonary, first and second leaves of each cultivar were collected and Cucs extractions were made using the procedure of Sharma and Hall (1971 a,b). Quantitative estimation of Cucs was made using Gilford UV spectrophotometer and the optical density was measured at 230 μ. A standard curve was prepared using pure Cuc B.

Total sugars, phenols, orthodihydroxy phenols, flavonoids and total free amino acids were estimated on dry weight basis. Samples were collected from cotyledonary first and second leaves on each cultivar, washed with distilled water, oven dried at 60°C and finally powdered and stored under room conditions. Methods of Loewus (1952), Swain and Hillis (1959), Nair and Vaidyanathan (1964), Balboa et al. (1974) and Lee and Takahashi (1966) were used for total sugars, total phenols, orthodihydroxy phenols, flavonoids content and total free amino acids, respectively.

In order to impart host susceptibility to red pumpkin beetle in the seedlings of comparatively a non-preferred cultivar, sponge gourd, eight seedlings of this species were grown in pots placed in screen house. Soon after emergence five plants per pot were retained and solutions containing 0.1 and 0.05 per cent of Cucs B and E in methanol were topically applied on fully expanded cotyledons. The seedlings for control set were treated with methanol only. These were subjected to insect pressure @ 2 beetles per plant and covered with split cages to confine beetles. Leaf injury by red pumpkin beetle was visually rated on 1-5 scale after 24, 36 and 48 hrs of confinement.

Results and Discussion

Maximum Cucs were recorded in watermelon (0.29 mg/g) (table 1) closely followed by muskmelon, bitter gourd, cucumber and roundmelon (Cucs content 0.28, 0.27, 0.25 and 0.27 mg/g, respectively). Sponge gourd contained the minimum Cucs content of 0.177 mg/g. Maximum insect injury was recorded in watermelon (2.65), followed by muskmelon (2.46), roundmelon (2.33). Minimum leaf damage was observed in bottle gourd (1.15) and sponge gourd (1.18). Despite high Cucs content (0.28 mg/g) absence of any leaf injury on bitter gourd indicated that it might be possessing strong feeding deterrent(s) and/or inhibitors making the effect of cucurbitacins.

The present results are in confirmity with those of Sharma and Hall (1971a) who found the Cucs content in the cotyledonary stages of *Citrullus colocynthis*, *C. lanatus*, *Cucumis melo*, *Lagenaria siceraria* and *Luffa acntangula* to be 0.27, 0.25, 0.15, 0.13 and 0.42 mg/g, respectively. Vashishta and Choudhary (1971) detected 0.51, 0.25 and 0.28 mg/g Cucs in resistant cultivars of muskmelon, bottle gourd and watermelon, respectively. The Cucs content of susceptible cultivars was 0.96, 0.51 and 0.28 mg/g, respectively.

In the present studies, it was observed that bottle gourd and sponge gourd revealed minimum insect injury and were comparatively low in Cucs, hence were categorised as "Resistant". Watermelon, roundmelon and muskmelon revealed maximum insecst injury and being high in Cucs were categories as 'Susceptible'. But it was intriguing to note that bitter gourd inspite of having high Cucs content was not preferred for feeding by red pumpkin beetle.

Biochemical Constituents

Muskmelon possesses highest total sugar

(9.20 mg/g) while it was much less in bitter gourd, bottle gourd, sponge gourd and watermelon (table 1). Sharma and Hall (1971a) also found low total sugars 2.9, 3.8, 4.4 and 0.9 mg/g in cotyledons of *L. siceraria, C. colocynthis, C. lanatus* and *C. melo*, respectively.

Bitter gourd revealed the lowest amino acid content (7.46 mg/g) whereas, in resistant cucurbits i.e. bottle gourd and sponge gourd, it is 7.99 and 7.58 mg/g, respectively (table 1).

The amount of total phenols in bottle gourd, cucumber, muskmelon and roundmelon was statistically equivalent. Orthodihydroxy phenols varied from 0.54 mg/g to 0.94 mg/g in bitter gourd and watermelon, respectively. The tested cultivars did not differ substantially in the orthodihydroxy phenols content. The amount of total flavonoids was highest in muskmelon (Bangan) and muskmelon (1.79 and 1.78 mg/g, respectively), while bitter gourd and bottle gourd revealed total flavonoid content of 0.78 and 0.96 mg/g, respectively.

When pure cucurbitacins (B&E) were topically applied on the cotyledons of the non preferred cultivars, the leaf injury ratings progressively increased with increase in the concentration of cucurbitacins. Maximum injury (3.09) was recorded when cotyledons were treated with Cuc B (0.1 per cent) (table 2) and minimum (2.60) with Cuc E (0.05) per cent. The mean leaf-injury grades of cotyledons treated with Cuc B 0.05, E 0.1 and E 0.05 per cent were statistically at par with each other. But all the treated cotyledons exhibited higher insect injury ratings as compared to that of untreated control (1.68).

The insect injury ratings were significantly correlated with the Cucs content of different cucurbits (0.66). The total sugars, total free amino acids, total phenols showed a non-significant positive correlation with insect injury ratings (0.54, 0.50, 0.30, respectively). The orthodihydroxy phenols and flavonoid content of plants also exhibited a positive nonsignificant correlation (0.53 and 0.50 respectively) with leaf injury caused by red pumpkin beetles. So the statistically significant positive coefficient of correlation between Cuc content and red pumpkin beetle feeding at initial plant growth stages may be ascribed

Table 1 *Biochemical constituents* of different cucurbits in relation to insect injury*

Cucurbit cultivar	Total sugars	Total free amino acids	Total phenols	Orthodihydroxy phenols	Total flavonoids	Cucurbitacin content	Insect injury
Bitter gourd	5.57a	7.46a	1.60a	0.55a	0.78a	0.276de	1.00a
Bottle gourd	3.97a	7.99ab	2.05bc	0.58a	1.21bc	0.203ab	1.15a
Cucumber	8.97cd	9.19ab	1.95bc	0.64abc	1.13bc	0.250cd	1.98b
Muskmelon(B)	6.83bc	13.54d	2.82d	0.66abc	1.79d	0.210b	1.58ab
Muskmelon	9.20d	11.54d	2.00c	0.62ab	1.78d	0.280c	2.46bc
Roundmelon	8.15cd	9.83bc	2.18c	0.66abc	1.21bc	0.277de	2.33bc
Small gourd	7.30bcd	9.96bc	2.30c	0.61ab	1.42cd	0.223bc	1.90ab
Sponge gourd	3.92a	7.58a	1.85ab	0.74c	0.96ab	0.177a	1.18a
Watermelon	4.58a	9.87bc	2.23c	0.94d	1.37c	0.290e	2.65c

* Average of three replicates (mg/g dry wt basis)

Figures followed by the same letters in a column (verticle) are statistically non-significant (Duncan's Multiple Range test)

Table 2 *Effect of topical application of different cucurbitacins on seedlings of "sponge gourd" at cotyledonary stage through caged red pumpkin beetle response*

Cucurbitacins applied (%)	Average leaf injury grades after* feeding exposure of			Mean
	24 h	36 h	48 h	
B 0.1	2.43	3.10	3.75	3.09
B 0.05	2.00	2.80	3.13	2.64
E 0.1	2.45	2.85	3.05	2.78
E 0.05	2.23	2.68	2.88	2.60
Untreated control	1.50	1.70	1.85	1.68
CD (P = 0.05)	—	—	—	0.26

* Average of four replicates

due to the presence of Cuc B and/or Cuc E in large amounts. Red pumpkin beetle was also strongly attracted to pure Cuc E (Sinha & Krishna 1969). But on contrary, Cucs are also known to act as deterrants for many insects including leaf beetles *Phyllotrala nenorum* (Nielson 1978, Metcalf et al. 1980).

Thorsteinson (1960), House (1965), Kennedy (1965) and Dadd (1970) stressed the importance of sapid nutrients such as sugars, amino acids, etc. which have been shown to act as gustating indicators of suitable host plant. Fraenkel (1959), however, considered that the "secondary plant substances e.g. glucosides, terpenoids, alkaloids etc. constituted the main defence arsenal of plant. The present results clearly indicated that Cucs (secondary plant substances) played a major role in host plant selection of red pumpkin beetle by acting as freeding stimulants, however, the minor contribution of orthodihydroxy phenols and flavonoids cannot be ruled out. Fraenkel (1969) also concluded that host selection was governed by the presence or and absence of secondary plant substances and the qualitative and quantitative nutrients can only play a minor role.

Cucs (secondary plant substances) played dominant role in the host plant susceptibility to red pumpkin beetle and behaved as 'Kairomones' promoting host selection and compulsive feeding by red pumpkin beetle even on non-preferred plant types.

Acknowledgement

We acknowledge the courtesy of Dr P R Enslin, National Chemical Research Laboratory, Pretoria, South Africa and Dr D Lavie of Rehovoth, Isreal for providing pure cucurbitacins one of author (PKM) is thankful to CSIR, New Delhi (India) for financial assistance as JRF and SRF.

References

Balboa S I, Zake A Y and Fishney A M 1974 Total flavonoids and rutin content of different organs of *Saphora japonica*. L.; *J. Assoc. Anal. Chem.* **57** 752-755

Chambliss O L and Jones C M 1966 Cucurbitacins: specific insect attractant in cucurbitaceae; *Science* **153** 1392-1393

Da Costa C P and Jones C M 1971 Cucumber beetle resistance and mite susceptibility controlled by the bitter gene in *Cucumis satevus* L.; *Science* **197** 1145-1146

Dadd R H 1970 Arthropod nutrition; *Chem. Zool.* **5** 35

Enslin P R 1954 Bitter principles of cucurbitaceae. I. observations on the chemistry of Cuc A; *J. Sci. Fd. Agric.* **6** 410-416

Fraenkel G 1959 The raison dietra of secondary plant substances; *Science* **129** 1466

—— 1969 Evaluation of our thoughts on secondary plant substances; *Ent. Exp. Appl.* **12** 473-486

Grewal S S 1981 *Studies on Resistance in Cucurbits to Red Pumpkin Beetle, Aulacophora foveicollis* (Lucus) Ph.D. (unpubl) dissertation submitted to PAU, Ludhiana

House H L 1965 Insect Nutrition; in *The Physiology of Insects* ed. K Rockstein **2** pp.769-813 (New York: Academic Press)

Howe W L, Sanborn J R and Rhodes A M 1976 Western root worm adults and spotted cucumber beetle associations with cucurbits and cucurbitacins; *Env. Entomol.* **5** 1043-1048

Kennedy J S 1965 Mechanism of host plant selection; *Ann. Appl. Biol.* **56** 317-322

Lee Y P and Takahashi T 1966 An improved colorimetric determination of amino acids with the use of ninhydrin; *Analyt. Biochem.* **14** 71-77

Loewus F A 1952 Improvement in anthrone method for determination of carbohydrate; *Analyt. Chem.* **24** 219

Metcalf R L, Metcalf R A and Rhodes A M 1980 Cucurbitacins as Kairomones for Diabroticite beetles; *Proc. natl. Acad. Sci.* (USA) **77** 3769-3772

— — and Lampman R L 1989 The chemical ecology of diabroticites and cucurbitaceae; *Experientia* **45** 240-247

Morey R J 1975 Feeding preference and development of *Aulacophora* spp. on various cucurbitaceous hosts and phytotoxicity of some modern insecticides to cucurbits. M.Sc. (unpublished) thesis submitted to JNKVV, Jabalpur

Nair P M and Vaidyanathan C S 1964 A colorimetric method for the determination of pyrocatechol and related substances; *Analyt. Biochem.* **7** 315-321

Nielson J K 1978 Host plant discrimination within Cruciferae: feeding response of four leaf beetles (Coleoptra, chrysomelidae) to gluconsinolates, Cucurbitacins and Cardenolides; *Ent. Expt. Appl.* **24** 41-54

Sandhu G S and Bhalla J S 1974 Red pumpkin beetle an important pest of cucurbits; *Punjab Hort. J.* **14** 70-74

Sharma G C and Hall C V 1971a Influence of cucurbitacins, sugars, fatty acids on cucurbit susceptibility to spotted cucumber beetle; *J. Amer. Soc. Hort. Sci.* **96** 675

— — and — — 1971b Cucurbitacin B and total sugar inheritance in *Cucurbits peopo* L. related to spotted cucumber beetle feeding; *J. Amer. Soc. Hort. Sci.* **96** 750-754

— — and — — 1973 Relative attractance of spotted cucumber beetle to fifteen species of cucurbitaceae; *Env. Entomol.* **2** 154-156

Sinha A K and Krishna S S 1969 Feeding of *Aulacophora foveicollis* on cucurbitacin: *J. Econ. Ent.* **62** 512-513

Swain T and Hillis W E 1959 The phenolic constituents of *Prunus domestica*. The quantitative analysis of Phenolic constituent; *J. Sci. Fd. Agric.* **10** 63-68

Thorsteinson A J 1960 Host selection of phytophagous insects: *Ann. Rev. Ent.* **5** 193-218

Vashistha R N and Choudhary B 1971 Studies on resistance to red pumpkin beetle, *Aulacophora foreivollis* (Lucas) in muskmelon, bottle gourd and watermelon; *Prog. Hort.* **2** 47-58