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Identification of Antifeedants in Bitter Gourd Leaves and their Effects on Feeding Behavior of Several Lepidopteran Species

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Abstract

Bitter gourd, *Momordica charantia*, was less palatable to 2 species of armyworms, *Spodoptera litura* and *Pseudaletia separata*, than 2 other cucurbitaceous plants. A methanol extract of *M. charantia* leaves inhibited feeding of the armyworm larvae. The 2 most active fractions obtained by silicagel chromatography were purified by HPLC. Momordicine II, triterpene mono-glucoside, was identified as an antifeedant compound from the most active of these fractions. The second active fraction led to the isolation of a new triterpene di-glucoside. Fresh leaves of *M. charantia* contained ca. 0.3% of momordicine II. Momordicine II showed a significant antifeedant effect on *P. separata* at the concentrations of 0.02, 0.1 and 0.5% in artificial diets. Momordicine II caused a significant feeding reduction in *S. litura* only at the highest concentration (0.5%) tested. The difference in the feeding response of the 2 armyworms to momordicine II may be related to the diversity in their host range. The author also examined whether stress applied to plant exerted an effect on insect's feeding preference. *M. charantia* is the host plant for the larvae of the pyralid moth, *Diaphania indica* but not for those of *P. separata*. Feeding response to UV-irradiated *M. charantia* leaves was compared between these 2 insects. *D. indica* preferred intact leaves, while *P. separata* preferred UV-irradiated leaves. These differences might be caused by the difference in the contents of antifeedants and feeding stimulants in the intact and UV-irradiated leaves.

Discipline: Agricultural chemicals
Additional key words: momordicine, triterpene glycoside, Cucurbitaceae, *Momordica charantia*, armyworm, *Spodoptera litura, Pseudaletia separata*, *Diaphania indica,* UV irradiation

Introduction

Bitter gourd, *Momordica charantia* , is widely cultivated in tropical Africa and Asia as a vegetable crop. The leaves of *M. charantia* are less attacked by insects than those of other cucurbitaceous crops. Repellent and/or antifeedant chemicals may play a major role in the unsuitability of non-host plants as food for insects. The analysis of these chemicals is important not only for understanding the ecological aspect of insect-plant relationships, but also for their potential in pest control. As chemicals in plants frequently depend on their environment[12], environmental stress applied to plants may also play a role in insect's host selection.

An armyworm, *Spodoptera litura* (Lepidoptera: Noctuidae), a well-known polyphagous insect, has often been used to evaluate antifeedants in plants[9]. A paddy armyworm, *Pseudaletia separata* (Lepidoptera: Noctuidae), feeds mostly on graminaceous plants and some other plant families. Its gregarious larvae are tolerant to unpalatable food[7]. In contrast, *Diaphania indica* larvae (Lepidoptera: Pyralidae) feed especially on *Gossypium* spp. and most Cucurbitaceae including *M. charantia* as host plants.

In the present paper, the author described the feeding response of 2 polyphagous armyworms to 3 cucurbitaceous plants, including *M. charantia,* and also determined whether chemicals in *M. charantia* might play a major role in the rejection of feeding. In addition, the author examined whether stress applied to plant may exert an effect on insect's host selection using 2 lepidopteran species, one (*D. indica*) which prefers *M. charantia* unlike the other (*P. separata*).

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Materials and methods

1) Plants

Fresh leaves of cucumber, *Cucumis sativus* L., pumpkin, *Cucurbita moschata* Duch., and bitter gourd, *Momordica charantia* L. were sampled from the plants grown in a greenhouse at the National Institute of Agrobiological Sciences, Tsukuba.

2) Insects

The armyworm, *Spodoptera litura* larvae were reared on an artificial diet[10] containing soybean instead of kidney bean. The paddy armyworm, *Pseudaletia separata* larvae were reared on the Insecta LF artificial diet (Nihon Nousan Kogyo Co.). *Diaphania indica* larvae were reared on cucumber leaves. These insects were reared under gregarious conditions at 25ºC, under a L16:D8 photoperiod. Third or fourth-instar larvae within a half-day after ecdysis were used in the bioassays.

3) Feeding response of S. litura *and* P. separata *to 3 cucurbitaceous plants*

Two bitter gourd leaf discs (1.5 cm in diameter) paired with either 2 cucumber or 2 pumpkin leaf discs were put on a moistened filter paper in a 9 cm plastic dish. Six third-instar larvae were released in the dish for 4 h. The extent of feeding was evaluated by determining the weight of the leaf consumed.

4) Fractionation of antifeedant extracts from M. charantia *leaves*

The freeze-dried powdered leaves of *M. charantia* were extracted with methanol. The fractionation procedure is shown in Fig. 1. Each fraction was assayed after every step. The active fraction was further fractionated and assayed repeatedly.

5) Bioassay of fractions from leaf extracts

Each fraction was dissolved in methanol (1 mg/100 μL) and applied (10 μL × 2) uniformly to both surfaces of cucumber leaf discs 1 cm in diameter, and air-dried. Leaf discs treated with methanol were used as a control. One test and one control leaf discs were kept on moistened filter paper in a 9-cm Petri dish. Five third-instar larvae were released on it for 12 h. The extent of feeding was evaluated by measuring the area of the leaf consumed. The fractions that significantly reduced feeding ($P<0.05$, *t*-test) were further fractionated to isolate the antifeedant components.

6) Feeding response of S. litura *and* P. separata *to isolated compounds (artificial diet)*

Each of the test compounds was added to the artificial diets at the concentrations of 0.02, 0.1, 0.5, and 2.5% (w/w). The artificial diet (150 mg) was placed in a plastic microtube (1.5 mL). A single third-instar larva was released in a tube for 12 h. The amount of feeding was evaluated by counting the number of fecal pellets.

7) Preparation of UV-irradiated M. charantia *leaves*

Fresh *M. charantia* leaves cut from the petioles were irradiated with UV light (254 nm) for 20 min and kept at room temperature for 1 to 7 days under dark conditions.

8) Feeding response of P. separata *and* D. indica *to UV-irradiated* M. charantia *leaves*

Two UV-irradiated *M. charantia* leaf discs (1.5 cm in diameter, kept for 4 days after UV irradiation) paired with 2 control leaf discs were put on a moistened filter paper in a 9 cm plastic dish. Six fourth-instar larvae of *P. separata* or *D. indica* were released in the Petri dish for 24 h (*P. separata*) or 6 h (*D. indica*). The extent of feeding was evaluated by measuring the area of the leaf consumed.

9) HPLC analysis of extracts of UV-irradiated M. charantia *leaf*

(1) Concentrations of momordicine II and its aglycon in leaf extracts

Intact (unirradiated) leaves and UV-irradiated leaves kept for 2, 4, and 7 days were freeze-dried and

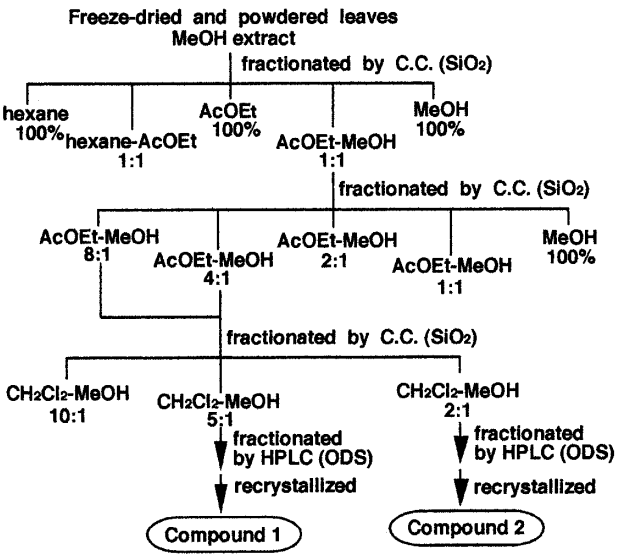

Fig. 1. Fractionation procedure of *M. charantia* leaf extracts

C.C.: Column chromatography.

extracted with methanol. HPLC was performed using a Cosmosil-5C_{18}-AR-II column (Nacalai Tesque, Inc.) with 80% methanol under a 0.8 mL/min flow rate at 30ºC (210 nm).

(2) Contents of sugars in leaves
Intact leaves and UV-irradiated leaves kept for 2 days were freeze-dried and extracted with 80% ethanol. The extracts were concentrated to dryness and 1 mL of distilled water was added to the residues, respectively. HPLC was performed with 75% acetonitrile under a 2 mL/min flow rate using a refractive index detector. Sugar concentrations of the samples were calculated based on the concentrations of standard sugars. The calculated sugar concentrations of the samples were converted to the contents in dried leaves.

Results and discussion

1) Feeding response of 2 polyphagous armyworms to 3 cucurbitaceous plants and bioassay for isolation of antifeedants in M. charantia

Among the 3 cucurbitaceous plants tested, *M. charantia* leaves were less fed by both *S. litura* (Fig. 2a) and *P. separata* (Fig. 2b) larvae. In particular, *M. charantia* leaves were not fed by *P. separata*, although some biting marks were observed. The observation that the larvae bit the *M. charantia* leaves but that sustained feeding never persisted suggests that some antifeedants in the *M. charantia* leaves caused the reduction of larval feeding rather than toxic or volatile repellent components. An antifeedant can be defined as a chemical that inhibits feeding but does not kill the insect directly; the insect may often remain on the treated plant material and possibly may die of starvation. Although cucurbitaceous plants contain secondary components such as cucurbitacins that protect against some insects[1)], both armyworms in this study were able to accept cucumber and pumpkin leaves, but not *M. charantia*. It was thus concluded that components present only in *M. charantia* leaves led to a different feeding response of armyworm larvae. Since a methanol extract of *M. charantia* leaves inhibited the feeding of the armyworm, the extract was fractionated and the fractions that significantly reduced feeding were further fractionated to isolate the antifeedant components.

2) Identification of antifeedants in M. charantia *and feeding response of* S. litura *and* P. separata *to isolated compounds*

The 2 most active fractions obtained by silicagel chromatography were purified by HPLC (Fig. 1). Each compound isolated was identified by NMR, MS, and IR spectra. Compound 1 was identified as momordicine II, a triterpene monoglycoside (Fig. 3)[11,13)]. Compound 3 obtained by enzymatic hydrolysis (β-D-glucosidase) of Compound 1 was identified as momordicine I, the aglycon of momordicine II[11, 13)]. Compound 2 contains one glycosyl group in addition to momordicine II and was identified as 7-O-β-glucopyranoside of momordicine II (Fig. 3)[13)]. Momordicine II and Compound 2 were present at levels of ca. 0.3 and 0.08% in fresh leaves of *M. charantia,* respectively.

Momordicine II (1) displayed a significant antifeedant effect on *P. separata* at concentrations of 0.02, 0.1 and 0.5% in artificial diets (Fig. 4b). The extent of the feeding reduction ranged from 45 to 60% at these con-

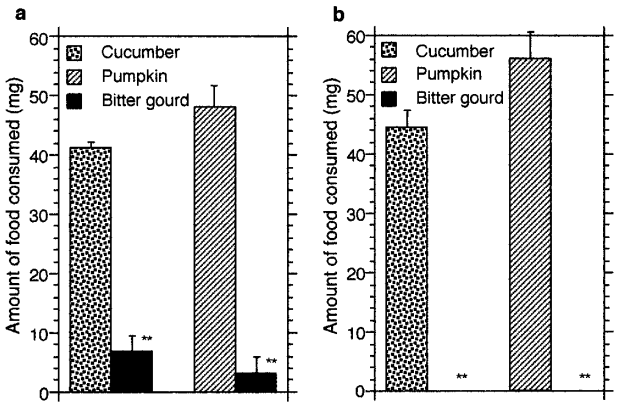

Fig. 2. Feeding response of *S. litura* and *P. separata* to 3 cucurbitaceous plants (two-choice test between bitter gourd and other cucurbitaceous plants)
a: *S. litura*, b: *P. separata*. Vertical bars indicate S. E. Each test was replicated 3 times.
**Significantly different at P<0.01 (*t*-test).

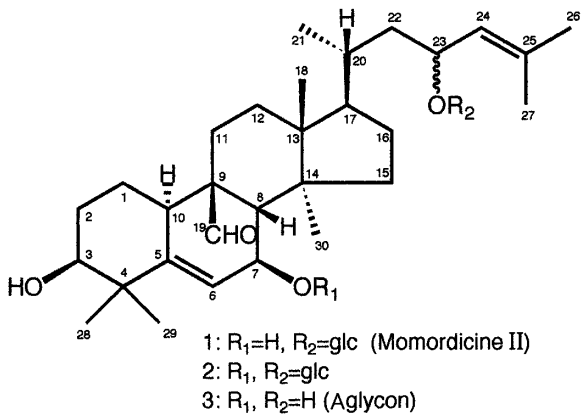

Fig. 3. Structure of momordicine II and its related compounds isolated from *M. charantia* leaves

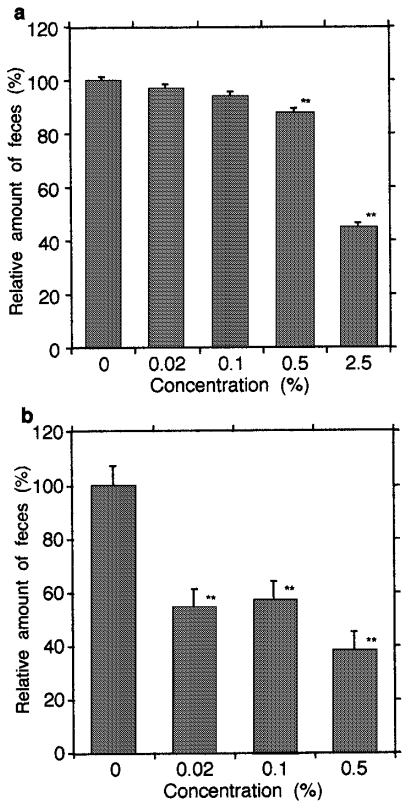

Fig. 4. Feeding response of *S. litura* and *P. separata* to Compound 1 (momordicine II) at 5 or 4 different concentrations

a: *S. litura*, b: *P. separata*. The number of fecal pellets was converted to a percentage based on the number of pellets from the control diet. Vertical bars indicate S. E. Each test was replicated 6 times.

**Significantly different at P<0.01 (*t*-test).

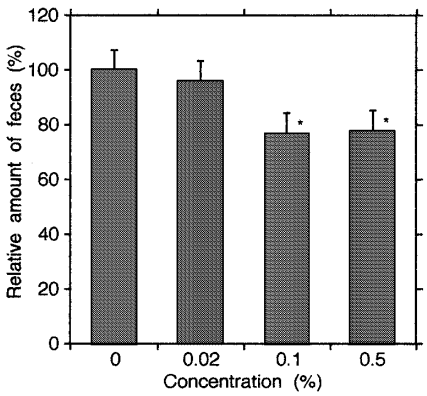

Fig. 5. Feeding response of *P. separata* to Compound 2 at 4 different concentrations

The number of fecal pellets was converted to a percentage based on the number of pellets from the control diet. Vertical bars indicate S. E. Each test was replicated 6 times.

*Significantly different at P<0.05 (*t*-test).

centrations. In the case of *S. litura*, it led to a significant feeding reduction at the highest 2 concentrations, 0.5 and 2.5%, respectively (Fig. 4a). Compound 2 caused a significant feeding reduction of 22% at the concentrations of 0.1 and 0.5% (Fig. 5), but no feeding reduction in *S. litura* at the 2.5% level.

P. separata appeared to be more sensitive to momordicine II than *S. litura*. The difference in the feeding response to *M. charantia* leaves of the 2 armyworms can be ascribed to differences in their sensitivity to momordicine II. Although the larvae of *P. separata* used in this study were gregarious-phase larvae that have been shown to exhibit a higher tolerance to some unpalatable foods than solitary-phase larvae[7], their feeding on *M. charantia* and on the momordicines was strongly inhibited. The lower sensitivity of *S. litura* to momordicine II can be correlated with its strong polyphagy. The fresh leaves of *M. charantia* which contained ca. 0.3% of momordicine II completely inhibited the feeding of *P. separata*, while 0.5% of isolated momordicine II in the artificial diet was necessary to cause 60% inhibition of feeding in the case of *P. separata*. These observations suggest that some other compounds also may be involved in the antifeedant activity of fresh leaves.

Kumar et al.[8] reported that *M. charantia* seed oil emulsion showed an antifeedant effect and insecticidal properties against the larvae of the mustard sawfly, *Athalia proxima*. Chandravadana & Pal[4] also reported that the cotyledons and leaves of *M. charantia* were unacceptable to red pumpkin beetles, *Aulacophora foveicollis,* and momordicine II was identified as a major antifeedant in the leaves[3]. The present study demonstrated that momordicine II displayed an antifeedant activity against 2 lepidopteran species with different polyphagy levels.

3) Influence of stress applied to plants on insect's feeding preference : Feeding response of P. separata *and* D. indica *to UV-irradiated* M. charantia *leaves*

Feeding response of *P. separata* and *D. indica* to UV-irradiated *M. charantia* leaves was investigated. *P. separata* larvae apparently preferred UV-irradiated leaves, while *D. indica* preferred intact leaves (Fig. 6). Changes in the leaf components must have occurred due to UV irradiation[5]. Therefore, HPLC analysis of UV-irradiated *M. charantia* leaf extracts was performed.

(1) Concentrations of momordicine II and its aglycon in leaf extracts

HPLC analysis of the extracts of the UV-irradiated leaves kept for 7 days after irradiation revealed that the concentration of momordicine II decreased while that of the aglycon of momordicine II (momordicine I) increased with time (Fig. 7). No significant differ-

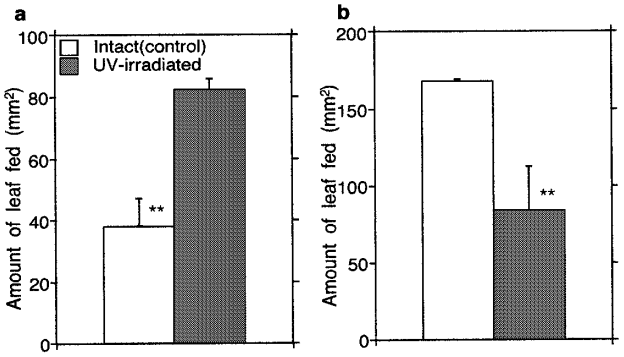

Fig. 6. Feeding response of 2 lepidopteran species, *P. separata* and *D. indica* to UV-irradiated *M. charantia* leaves

a: *P. separata,* b: *D. indica*. Vertical bars indicate S. E. Each test was replicated 3 times.
**Significantly different at P<0.01 (*t*-test).

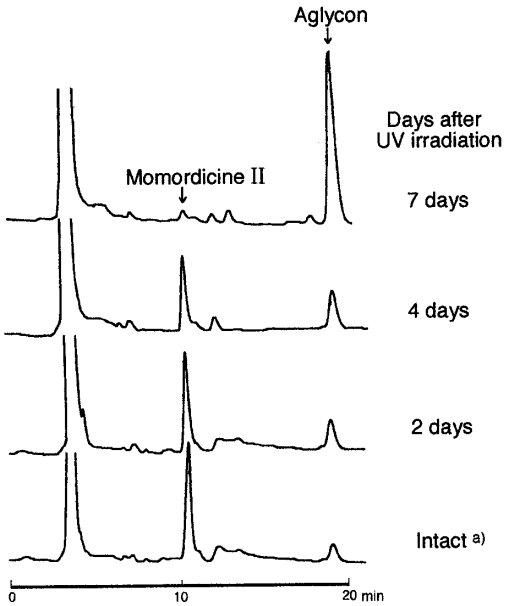

Fig. 7. HPLC profiles of methanol extracts of UV-irradiated *M. charantia* leaves

a): Methanol extracts of unirradiated leaves (kept at room temperature under dark conditions for 7 days).

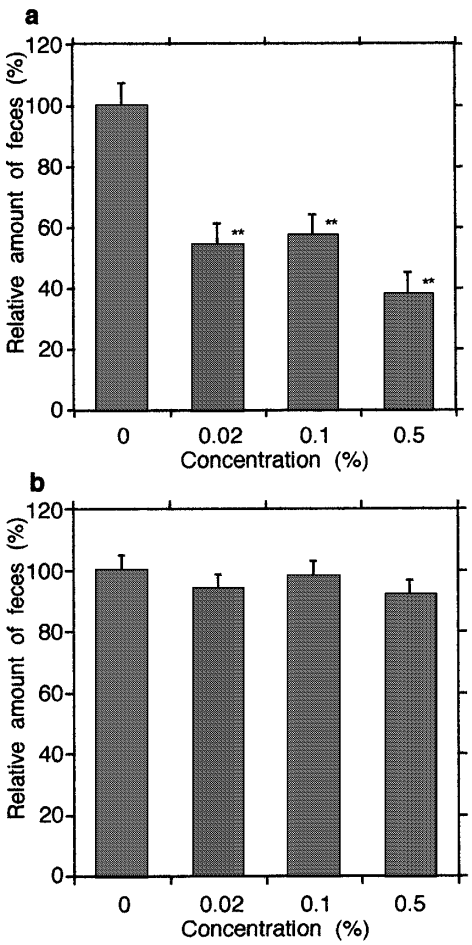

Fig. 8. Feeding response of *P. separata* to momordicine II and its aglycon at 4 different concentrations

a: Momordicine II, b: Aglycon.
The number of fecal pellets was converted to a percentage based on the number of pellets from the control diet. Vertical bars indicate S. E. Each test was replicated 6 times.
**Significantly different at P<0.01 (*t*-test).

ences in the concentrations of momordicine II or aglycon were observed among the intact (unirradiated) leaves kept for 7 days at room temperature under dark conditions. Therefore, the feeding response of *P. separata* to momordicine II and its aglycon was investigated. Momordicine II showed a significant antifeedant effect on *P. separata* at concentrations of 0.02, 0.1 and 0.5% in artificial diets, while aglycon did not cause any feeding reduction even at the concentration of 0.5% (Fig. 8). In the case of *D. indica*, there was no significant difference in the consumption between the addition of 2% momordicine II to the artificial diet and the absence of momordicine II in the artificial diet. Therefore, it was considered that momordicine II did not display any antifeedant or feeding stimulant activity toward the larvae, and that momordicine II did not affect larval feeding at any concentrations in the diet in this experiment.

(2) Contents of sugars in *M. charantia* leaves

Since momordicine II did not affect the feeding of *D. indica*, it was assumed that other substances may exert some effect on the feeding behavior of *D. indica*. Although feeding stimulants in *M. charantia* leaves for *D. indica* were not revealed, sugars, especially sucrose, are known to be feeding stimulants for many insects[2, 6]. Therefore, the contents of sugars in

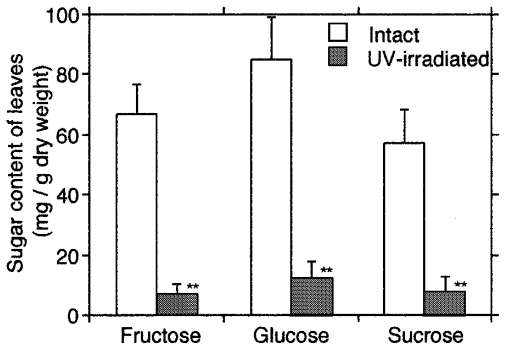

Fig. 9. Sugar contents of UV-irradiated *M. charantia* leaves

Vertical bars indicate S. E. Each analysis was replicated 3 times.

**Significantly different at P<0.01 (*t*-test).

the leaves were analyzed. Sugar contents (glucose, sucrose, and fructose) were all apparently low in the UV-irradiated leaves compared to those in the intact leaves (Fig. 9). *D. indica* larvae preferred leaves with higher contents of sugars to leaves with low contents. Although *D. indica* may not prefer UV-irradiated leaves because some antifeedants for *D. indica* may have been induced by UV irradiation, sugars in the leaves may be one of the potential feeding-controlling substances for *D. indica* larvae. In the case of *P. separata*, larval feeding was assumed to be affected by the antifeedant (momordicine II) rather than the feeding stimulant (sugars).

Conclusion

In this paper, emphasis was placed on the interaction mediated by chemicals related to plant feeding by herbivorous insects. Insects do not eat every plant they encounter, and this is true even for species that eat a very wide range of food plants. This study indicated that insect feeding is influenced by antifeedants and feeding stimulants, and that these chemicals from plants are potential chemicals for insect control. Plant components are likely to be influenced by environmental stresses because various metabolic changes are induced[5]. We showed that UV irradiation applied to plants influenced the feeding preference of insects, so that the application of some kinds of stresses to plants may enable to develop insect control methods. Since the stress we examined here is an extreme case, further investigations using various stresses should be conducted to obtain information about environmental stress-plant-herbivore interactions related to feeding.

References

1) Ananthakrishnan, T. N. & Raman, A. (1993): Chemical Ecology of Phytophagous Insects. International Science Publisher, New York, pp. 332.
2) Bartlet, E. (1996): Chemical cues to host-plant selection by insect pests of oilseed rape. *Agric. Zool. Rev.*, **7**, 89–116.
3) Chandravadana, M. V. (1987): Identification of triterpenoid antifeedant of red pumpkin beetles (*Aulacophora foveicollis*) from *Momordica charantia*. *J. Chem. Ecol.*, **13**, 1689–1694.
4) Chandravadana, M. V. & Pal, A. B. (1983): Triterpenoid antifeedant of *Aulacophora foveicollis* from *Momordica charantia*. *Curr. Sci.*, **52**, 87–88.
5) Chappell, J. & Hahlbrock, K. (1984): Transcription of plant defense genes in response to UV light or fungal elicitor. *Nature*, **311**, 76–78.
6) Hsaio, T. H. (1985): Feeding behavior. *In* Comprehensive Insect Physiology, Biochemistry and Pharmacology. Vol. 9. eds. Kerkut, G. A. & Gilbert, L. I., Pergamon Press, Oxford, 471–512.
7) Iwao, S. (1962): Studies on the phase variation and related phenomena in some Lepidopterous insects. *Mem. Coll. Agric., Kyoto Univ.,* **84**, 1–80.
8) Kumar, A. et al. (1979): Studies on the antifeeding and insecticidal properties of bitter gourd (*Momordica charantia* L.) against mustard sawfly *Athalia proxima* Klug. *Pesticides,* **13**, 9–11.
9) Wada, K. & Munakata, K. (1968): Naturally occurring insect control chemicals. *J. Agric. Food Chem.*, **16**, 471–474.
10) Wakamura, S. (1988): Rearing of the beet armyworm, *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae), on an artificial diet in the laboratory. *Jpn. J. Appl. Entomol. Zool.*, **32**, 329–331 [In Japanese with English summary].
11) Yasuda, M. et al. (1984): Structures of momordicines I, II, and III, the bitter principles in the leaves and vines of *Momordica charantia* L. *Chem. Pharm. Bull.*, **32**, 2044–2047.
12) Yasui, H. & Yazawa, M. (1995): Feeding response of silkworm, *Bombyx mori*, to compounds induced by UV irradiation of mulberry leaves. *Biosci. Biotech. Biochem.*, **59**, 1326–1327.
13) Yasui, H. et al. (1998): Antifeedants to armyworms, *Spodoptera litura* and *Pseudaletia separata*, from bitter gourd leaves, *Momordica charantia*. *J. Chem. Ecol.*, **24**, 803–813.