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Water Stress-induced Plant Metabolism and its Effect on the Insect Pest. I. *Momordica charantia* cv. Korola vs *Epilachna dodecastigma*

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Epilachna dodecastigma, a polyphagous pest, when cultured on water-stressed and unstressed leaves of *Momordica charantia* cv. Korola, showed variations in both biochemical and biological parameters. Proline, free amino acid (FAA) and total steroid content increased while total protein, carbohydrate, ascorbic acid and relative water content (RWC) decreased in the water-stressed leaves. Body weight, body length, total protein, carbohydrate and ascorbic acid contents were lower, whereas total free amino acids, proline and cholesterol contents were higher in the insects fed water-stressed leaves. The larval and pupal durations increased in insects cultured with stressed leaves. The increased duration and decreased body weight of *E. dodecastigma* could presumably be due to higher accumulation of sesquiterpenoid-like compounds, or poorer food quality in stressed leaves or low food-water intake by insects grown on stressed leaves.

Key Words: *Momordica charantia* **cv. Korola,** *Epilachna dodecastigma*, **Water-stress, Sesquiterpenoid-like compound, Insect development**

Introduction

Various changes and alterations in plant metabolism occur during water stress (Levitt 1972, Hsiao 1973, 1976, Mukherjee & Choudhuri 1981 a,b). A direct correlation between host-plant metabolism and life history and growth potential of the insect pest has been reported by many workers (Basu 1944, Kapil 1967, Pandey et al. 1968, Sharma & Sharma 1975). The type of food is known to play an important role on the growth and bionomics of pest population (Lal & Mukherjee 1978, Choudhuri et al. 1983). Whether changes of certain specific macromolecules of the host plant do affect the changes in

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metabolism and morpho-bionomics of the insect pest is still not established. Effect of some of important biochemical changes in leaves of water stressed *Momordica charantia* cv. Korola on the macromolecules and bionomic pattern of its pest, *Epilachna dodecastigma*, was studied by us.

Materials and Methods

Healthy seeds of Korola (*Momordica charantia* cv. Korola) were surface-sterilized with 0.1% $HgCl_2$ for 2 min, washed well in running water and germinated. Seedlings were maintained in 17.3 cm pot and watered (250 ml) at intervals of 24 hr. Five-day-old seedlings were subjected to stress treatment by withholding water for 10 days. The controls were supplied water regularly up to the desired age. On the 15th day, the leaves of the stressed and control plants were detached and used separately (at intervals of 2 hr) as the food for culture of *Epilachna* in the laboratory at 25° ± 0.5°C, 70% RH and 12 hr photoperiod, with intensity of light $= 100\mu E/m^2$

The changes in total protein (Lowry et al. 1951), carbohydrate (Mc Cready et al. 1950), free amino acids (Dwivedi et al. 1979), proline (Bates et al. 1973), total steroid (Stadtman 1957), cholesterol (Zaltikis et al. 1953), ascorbic acid (Oser 1979) and relative water content (Weatherley 1950) were analysed in stressed and unstressed leaves and also in insect pest during different post-embryonic developmental changes. The fat bodies and haemolymph were collected from larva/pupa of different instars. Haemolymph was collected from a large number of larvae/pupae (in each case) separately in graduated glass capillery tubes and stored in a polythene centrifuge tube with a few crystals of phenylthiourea (to inhibit the tyrosinase activity) under 0°C±0.5°C in a refrigerator for subsequent analyses. The age range of the sacrificed larvae (from 1st instar to pupa) was 70, 50, 80, 30 and 60 hr after their moult, respectively.

Sesquiterpenoid-like compounds were estimated following Mizrahi et al. (1970). For this, 30 g leaf samples from both stressed and unstressed plants were collected separately, immediately frozen and then extracted three times with 70% cold methanol. The extract was evaporated to 15% of the original volume and the *p*H was adjusted to 8.5 with 1 M $NaHCO_3$. The solution was partitioned with petroleum ether and then with ethylacetate. The aqueous fraction was adjusted to *p*H 2.7 with 1 M HCl and again partitioned twice with ethyl acetate. The ethyl acetate phases were pooled together, evaporated to dryness at 40°C, and the residue dissolved in 3 ml of methanol and stored in a deep-freeze for analysis.

The sesquiterpenoid-like substances in the plant extract were separated by thin-layer chromatography (TLC). A portion of 200 μl of the methanol extract was streaked on 20× 20 cm plates coated with silica gel G having marker spots of authentic (±) abscisic acid (Sigma Chemical Company, USA). The spots were developed three times repeatedly with isopropanol : ammonia : water (10:1:1 v/v). Sesquiterpenoid-like compounds were isolated from the comparable spots and the activity was determined by wheat coleoptile growth suppression test (Mizrahi et al. 1970).

Some aspects of the post-embryonic development, like the number of instars, feeding habit, mean larval and pupal duration and the length and weight, were taken daily. The larval and pupal weights were taken in a precision balance and the lengths measured by a micrometer scale under the binocular microscope. To minimize error, the data were taken from seven separate generations. In other cases, observations were taken from six separate generations of *E. dodecastigma* in the laboratory. Data were processed statistically by students 't' test and duncans Multiple

Range Test. Biochemical estimations, were based on 15 replications.

Results

The duration of each larval instars was more when fed on stressed leaves than when fed on unstressed leaves with the exception of pre-pupa and adult stages (table 1). However, the body weight and length were higher during all the post-embryonic developmental stages in insects reared on unstressed leaves than those reared on the stressed ones (table 1)

Table 2 includes data on relative water content (RWC), total protein, carbohydrate, free amino acids (FAA), ascorbic acid, proline and total steroid concentration of stressed and unstressed leaves of the host plant. The RWC of leaves decreased in stressed leaves, varying from 96.5% to 78.0%. There was a significant decline in the concentration of total protein, carbohydrate, ascorbic acid and rise in the concentration of total steroid, FAA and proline (table 2).

Table 1 *Effects of fresh and stressed leaf* Momordica charantia *cv. Korola as food on the development of* Epilachna dodecastigma

Insects fed on fresh leaves			Post-embryonic developmental stages	Insects fed on stressed leaves		
Length (mm)	Wet weight (mg)	Duration (hr)		Duration	Wet weight (mg)	Length (mm)
2.3 (1.8–2.5)	0.5 (0.2–0.8)	78.5 (58–85)	1st Instar	81.3 (69–96)	0.4 (0.2–0.7)	2.1 (1.6–2.37)
3.0 (2.5–3.6)	1.9 (0.9–3.5)	60.9 (58–63)	2nd Instar	65.0 (60–74)	1.4 (0.7–2.8)	2.4 (2.4–3.4)
5.3 (4.0–6.5)	7.17 (3.6–10)	71.0 (62–75)	3rd Instar	80.0 (72–96)	5.8 (3.0–8.2)	4.9 (3.7–6.3)
8.1 (6.8–10.0)	27.5 (10.6–47.0)	88.2 (80–92)	4th Instar	100.00 (92–108)	19.0 (8.6–36)	7.3 (7.3–8.4)
8.6 (7.8–10.0)	40.7 (37–42)	63.1 (30–37)	Pre-pupa	37.2 (32–42)	32.0 (29–35)	7.7 (7.3–8.4)
7.4 (7.2–7.5)	31.5 (28.5–35)	78.2 (60–83)	Pupa	86.0 (80–96)	23.5 (17.5–28)	6.3 (5.8–6.9)
4.5 (4.0–4.9)	38.5 (35.5–42)	245.5 (220–265)	Adult	180 (150–190)	21.25 (18.5–24)	3.5 (3.0–3.8)

125 mg unstressed/stressed leaves as food/10 insect larvae/adult
Data are mean of 7 seperate replications

Table 2 *Changes of different macromolecules in the fresh and water-stressed leaves (15 days) of* Momordica charantia *cv. Korola*

Treatment	RWC (%)	Protein (mg)	Total carbohydrate (mg)	Free amino acid (mg)	Ascorbic acid (μg)	Proline (μg)	Total steroid (mg)
Unstressed	96.5	36.50	41.6	1.42	86.28	80.33	0.25
	(2.32)	(2.5)	(1.2)	(0.08)	(8.85)	(5.25)	(0.02)
Stressed	78.0**	24.3**	18.4**	3.68**	65.31*	820.24**	0.61**
	(1.05)	(1.12)	(0.7)	(0.54)	(6.28)	(16.29)	(0.12)

Contents were represented as unit/100 mg dry weight; number within the parentheses indicates the ± 1 SE n=15; *=Significant at $P < 0.05$; **=Significant at $P < 0.01$ in comparison to unstressed leaves; RWC=Relative water content

Table 3 *Changes of sesquiterpenoid compound in the leaves of* Momordica charantia *cv. Korola after and before water stress. Contents represent as the ng/gm dry and fresh weight*

Treatment	Total sesquiterpenoid compound (Abscisic acid-like compounds)	
	Per g fresh weight	Per g dry weight
Normal	449.25 (16.20)	5615.63 (120.44)
Stressed leaves	1347.75 (55.66)	11231.26 (482.21)

Number within parentheses indicates the ± 1 SE, where n=15

It is evident from (table 3) that sesquiterpenoid-like compounds significantly increased (about 2–3 fold) in the stressed leaves over unstressed control leaves.

Biochemical studies on the levels of different macromolecules in both haemolymph and fat-body from different post-embryonic developmental stages, cultured with stressed and non-stressed leaves separately (tables 4 & 5) reveal that the total protein, carbohydrate, FAA, protein, cholesterol and ascorbic acid concentration in both haemolymph and fat-body increased in different developmental stages in both the cases. But

Table 4 *Changes of macromolecules in haemolymph of* Epilachna dedecastigma *during the post-embryonic developmental stages cultured by stressed and unstressed leaves separately*

Developmental stages	Protein (μg/0.1 ml)	Carbohydrate (μg/mg protein)	Free amino acid (μg/mg protein	Ascorbic acids (μg/mg protein)	Proline (μg/mg protein)	Cholesterol (μg/mg protein)	Fed with the leaves US=Unstressed S=Stressed
	540 (18.0)	374 (50.0)	275 (67.0)	15.25 (2.20)	15.20 (1.90)	525 (98.0)	(US)
1st	320 (12.0)	175 (16.2)	495 (36.50)	9.92 (0.50)	80.90 (3.36)	685 (51.0)	(S)
	595 (10.20)	388 (5.75)	295 (2.70)	15.95 (10.70)	15.85 (1.85)	535 (15.20)	(US)
2nd	329 (7.10)	170 (4.20)	525 (6.70)	9.90 (2.35)	88.90 (5.31)	695 (20.21)	S
	625 (23.0)	420 (18.0)	350 (15.21)	21.22 (3.35)	16.68 (2.75)	585 (21.25)	US
3rd	385 (19.0)	185 (9.92)	585 (24.20)	9.21 (0.88)	105.34 (6.66)	720 (27.27)	S
	770 (31.20)	485 (23.30)	359 (16.2)	17.33 (3.10)	77.20 (2.75)	630 (28.2)	US
4th	395 (17.20)	205 (15.60)	580 (27.31)	9.05 (1.02)	165.0 (14.90)	750 (36.0)	S
	625 (16.0)	315 (12.59)	480 (13.52)	19.22 (2.22)	27.30 (2.77)	785 (15.39)	US
Pupa	370 (33.33)	201 (9.99)	625 (17.77)	12.31 (1.3)	206.37 (17.32)	975 (27.25)	S
	885 (24.64)	525 (15.34)	398 (11.42)	16.31 (3.4)	13.21 (1.28)	565 (35.70)	US
Adult	425 (12.33)	222 (12.22)	530 (15.80)	9.70 (1.94)	76.07 (7.78)	635) (21.44)	S

Number within parentheses indicates the ± 1 SE, where n = 15

Table 5 *Fluctuation of some macromolecules in the fat-body of* E. dodecastigma *during the post-embryonic developmental stages cultured with stressed and unstressed leaves of* Monordica charantia *cv. Korola*

Developmental stage	Protein (μg/mg dry weight)	Carbohydrate (μg/mg protein)	Proline (μg/mg protein)	Cholesterol (μg/mg protein)	Ascorbic acid (μg/mg protein)	Fed with the leaves US=Unstressed S=Stressed
	1325 (36.22)	520 (70.21)	60.28 (8.34)	440 (38.60)	27.27 (3.34)	US
First larva	725 (22.00)	366 (50.22)	180.27 (12.82)	590 (32.30)	20.27 (2.22)	S
	1864 (33.88)	569 (22.50)	90.34 (8.23)	492 (11.18)	28.20 (4.20)	US
Second larva	835 (21.22)	380 (13.00)	190.37 (18.29)	610 (43.99)	18.80 (1.62)	S
	1904 (21.50)	723 (26.22)	130.12 (10.38)	515 (20.56)	28.51 (3.38)	US
Third larva	860 (19.30)	388 (11.55)	210.40 (26.00)	640 (27.74)	25.00 (4.15)	S
	1965 (59.34)	737 (27.00)	156.10 (18.30)	565 (32.22)	29.20 (2.00)	US
Fourth larva	955 (7.59)	392 (9.34)	255.60 (20.23)	645 (16.00)	20.00 (2.60)	S
	1830 (29.00)	592 (15.22)	235.30 (16.16)	740 (43.23)	30.62 (5.62)	US
Pupa	840 (10.45)	350 (8.66)	420.60 (25.68)	900 (33.21)	21.52 (1.59)	S
	1885 (33.44)	620 (17.00)	188.2 (10.38)	580 (20.34)	25.21 (4.18)	US
Adult	820 (28.04)	425 (12.00)	385.16 (15.23)	600 (31.20)	4.18 (2.12)	S

Number within parentheses indicates the ± SE, where n = 15

the concentration of different macromolecules in each developmental stage varied in two cultures. The haemolymph and fat-body from the cultures with the stressed leaves had the higher quantity of total protein, carbohydrate and ascorbic acid and lower quantity of proline, FAA and cholesterol than those from the cultures with stressed leaves (tables 4 & 5).

Discussion

Katiyar et al. (1975) and Lal and Mukherji (1978) observed a correlation between the food and the feeder. It was also documented that polyphagous insect pests affect the metabolism of different plant species on which they live, and in turn, their life-cycle also gets modified. The present study shows that water stress treatment of the plant significantly affected the water status of the plant, which, in turn, led to a number of secondary changes at the subcellular level, such as decrease in the content of protein, total carbohydrate and ascorbic acid and an increase in proline and other free amino acids contents. A significant increase in total steroid content was also recorded.

There are some reports which reveal that

both qualitative and quantitative changes of food can alter the biology and physiology of the insect pest. While, both fat-body and haemolymph of *E. dodecastigma* instars fed on unstressed leaves (control) showed higher contents of protein, carbohydrate and ascorbic acid over those for stressed leaves; the contents of proline, free amino acids and cholesterol were lower in the former case. Thus, the biochemical alterations of the insects seem directly dependent upon the qualitative and quantitative changes of leaves fed to the insects. The loss in body weight and body length in insects cultured on stressed leaves, also supports this view point.

Edney (1957) reported that the performed water of the food was the main source of water for body's metabolic processes in phytophagous insects, particularly so during their larval stages. Thus, the longer larval pupal duration and decreased body weight and body length (stressed vs unstressed leaves) appear to be related to lower food-water intake.

It has been amply demonstrated that sesquiterpenoid compounds (both cyclic and acyclic) act as the analogue of juvenile hormone in insects (Novak 1975, Gilbert 1980). The data (table 3) showed 2–3 fold increase in the total sesquiterpenoid compounds in stressed leaves and it can be argued that the intake of sesquiterpene-like compounds with food by the insects cultured with stressed leaves might break the endogenous titre of the hormone thereby lengthening the larval pupal duration (Gilbert & King 1973, Riddiford 1979).

All the results referred to above seem, thus, to suggest that the physiological stress, particularly water-stress, can alter the metabolic events of the host-plant which ultimately may affect the biology and the physiology of the insect pest.

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