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Action of Tannic Acid on Consumption and Utilisation of Food by *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae)

MOHAMMED A. J. AL-IZZI* and SADIKA K. AL-MALIKY

Entomology Department, Agriculture and Biology Research Center, PO Box 765, Baghdad, Iraq

(Accepted 7 June 1996)

Abstract—Tannic acid as 1.5% of an artificial diet extended the larval developmental period of *Ectomyelois ceratoniae* three times and reduced the development rate by one fourth. The larvae consumed twice as much tannic acid diet as control diet without increasing in weight. They assimilated some tannic acid but eliminated most in the faeces. These results were considered in relation to tannins as allelochemicals in pomegranate fruits. Copyright © 1996 Published by Elsevier Science Ltd

Key words—Ectomyelois ceratoniae, tannic acid, artificial diet

INTRODUCTION

Larvae of the pomegranate fruit moth (*Ectomyelois ceratoniae*) develop on immature and mature pomegranate fruit which contain a considerable amount of tannic acid ranging from 85 to 168 μg/g fresh weight of tissue (Al-Izzi and Al-Maliky, 1986). A diet based on pomegranate fruit tissues reduced growth and development of *E. ceratoniae* larvae (Al-Izzi, 1986) and the effect was attributed to the presence of tannin in the fruit and pericarp (Okuda *et al.*, 1980; Al-Izzi *et al.*, unpublished data).

Tannins are defined as high molecular weight polyphenolic compounds that can form insoluble complexes by cross-linkages with protein (Goodwin and Mercer, 1980; Jones and Morgan, 1977). Tannins have anti-nutritional effects (Chan *et al.*, 1978) due to this interaction with protein in the gut (Martin-Tunguy *et al.*, 1977). Moreover, tannins can cause direct toxic effects on the gut (Kreber and Einhellig, 1972) as well as growth retardation as a result of protein deficiency (Jambunathan and Mertze, 1973). Cotton tannins suppress larval growth and development of *Heliothis* spp. (Waiss *et al.*, 1981). The effect of oak leaf tannin on caterpillars of *Operophtera brumata* has been studied; larvae grow slowly and avoid some of the plant defences (Feeny, 1968, 1970).

Field larvae grown on pomegranate fruits contained a higher quantity of tannic acid (TA) (in chemical studies, the tannin content of a sample is expressed in terms of tannic acid) than those reared on an artificial diet (Al-Izzi and Al-Maliky, 1986). The quantification of tannic acid in insect stages was necessary to interpret its effect on insect growth and development. This is concerned with the dietary effects of tannic acid on insects through its formulation in artificial larval diets, and correlates with the effect on digestibility, larval feeding behaviour and production of faeces.

*Author for correspondence.

Larval feeding behaviour can be explained by the elevated percentage of tannins in tissues of the calyx (85 μg/g fresh weight), pericarp (118 μg/g fresh weight) and stigma (168 μg/g fresh weight) compared with the low level of tannin in artificial diet (1.5%).

The objective of this investigation was to determine the effects of tannic acid incorporated into the artificial diet on larval growth and development of *E. ceratoniae*.

MATERIALS AND METHODS

The larvae of *E. ceratoniae* were obtained from a laboratory colony fed on artificial diet for more than three years (Al-Izzi *et al.*, 1987). The test insects were maintained at 29 ± 0.2°C, 65 ± 10% r.h., with a photoperiod of 16.8 (L:D). Tannin (Riedel-De Haen Ag-Seelze-Hannover) was coated on the diet ingredients in concentrations from 0.5 to 2.0% of fresh weight. Tannin was not added to the control diet.

Effect of tannic acid on larval growth and development

Newly hatched larvae were transferred singly to the vials (70 × 25 mm diameter) containing either the control diet or the tannic acid diet with one of the tannic acid concentrations 0.5, 1.0, 1.5, and 2.0%. The treatment with 1.5% tannic acid was used for spectrophotometric studies. Vials were plugged with sterilised non-absorbent cotton, covered with aluminium foil and numbered. Daily observations of larval growth and development were recorded for each individual during the entire development period.

Efficiency of utilisation

To understand how tannin affected larval weight, measurements were made of the efficiency of utilisation of the control diet and the diet with 1.5% tannin. Food utilisation was measured by using a group of *ca.* 160 newly hatched larvae which were fed individually for their entire larval feeding period. At the end of larval feeding period, the larvae, faeces, and uneaten diet were weighed separately to the nearest 0.1 mg. Since the media lost weight by evaporation during the feeding period, the amount eaten was corrected for evaporation determined from 50 aliquots of each of the control and tannic acid diet media. The two diet media were weighed and left under the same conditions to obtain a linear regression of weight against time as a function of the initial weight.

Tannin analysis

The condensed tannin content of larvae, diet media and faeces was determined according to the procedure of Polles *et al.* (1981) with simple modification. Both sexes of *E. ceratoniae* larvae, pupae, and adults from both media (control and tannic acid diet) were examined using 100 mg aliquots of the control and tannic acid diet media, and faecal pellets from each diet. Insect stages, diet samples, or faecal pellets were macerated separately by hand pestle and mortar and washed with 2 × 5 ml of 5% HCl in 1-butanol. Washes were combined in a 100 ml beaker. The beaker was immersed in a water bath at 80°C for 1 h then filtered through a porous disk funnel No. 2 and re-extracted twice. The combined filtrate was evaporated to dryness under vacuum, re-dissolved in butanolic-HCl (5%) and transferred to a 10 ml volumetric flask. More than four samples of diet or faecal pellets, and 15–20 insects of each stage and sex were analysed.

A tannic acid standard was prepared by dissolving 100 mg in 10 ml of 5% butanolic-HCl (v/v). A volumetric flask (10 ml) and micro-pipette (250 μl) were used to prepare the concentrations of 0, 62.5, 125, 187.5, 250, 312.5 and 375 μg tannin per 10 ml solvent. During the experiments, the standards and samples were sealed at 4°C until the time of spectrophotometer reading. Light and air cause insignificant effects during the first 2 h (Rosenblatt and Peluso, 1941).

An ultraviolet absorption curve for tannin was recorded between 265 and 275 nm (Owades *et al.*, 1958) using a Shimadzu UV-visible recording spectrophotometer Model UV-240 which showed a peak at 275 nm with the standard aliquot. Quantitative determinations of tannin in samples at 275 nm were automatically calculated by constructed absorbance versus concentration. The least squares method found the concentration (C) from $C = K.\ \text{abs.} + B$.

Larval feeding behaviour can be explained by the elevated percentage of tannins in tissues of the calyx (85 μg/g fresh weight), pericarp (118 μg/g fresh weight) and stigma (168 μg/g fresh weight) compared with the low level of tannin in artificial diet (1.5%).

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Table 1. Effect of supplemental tannic acid on development of *Ectomyelois ceratoniae*

Tannic acid conc. (g/100 g diet)	Larval period days (mean ± SD*)
0.0	16.3 ± 3.7 a
0.5	32.9 ± 8.4 b
1.0	44.5 ± 9.7 c
1.5	46.5 ± 12.9 c

* Means within column followed by the same letter are not significantly different ($P > 0.05$; Duncan's multiple range test).

RESULTS AND DISCUSSION

Larval development

At all tannic acid concentrations the larvae of *E. ceratoniae* ingested more media, and the larval developmental period was significantly extended from 16 d in the control diet to 47 d for the highest tannic acid diet (Table 1). Pupal periods ranged from 6.5 to 8 d with the control diet. Effects of tannic acid diets on pupal period, however, were insignificant although smaller pupae were produced at concentrations of 1.5 and 2.0% of tannic acid. Optimal larval growth and development on the control diet was recorded previously (Al-Izzi *et al.*, 1987) but the experiments reported here, where the artificial diet contained a low level of tannic acid compared with high tannic acid level diets, had negative effects on digestibility of crude protein and therefore affected larval growth and development, and larval mortality increased with increasing tannic acid concentration. Further mortality occurred during the development of pupae to adults.

There was no significant difference in the weight of control diet media consumed by either male or female. The male and female control larvae consumed 422 ± 140 mg ($n = 25$) and 650 ± 190 mg ($n = 22$) of wet media, respectively, while those on 1.5% tannic acid diet consumed 825 ± 281 mg ($n = 26$) and 943 ± 288 mg ($n = 22$) which is *ca.* 2 times and 1.5 times the amount of wet control diet media consumed by larvae of the same sex.

Analyses of both control and 1.5% tannic acid diet media revealed that the amount of tannic acid taken during larval feeding on 1.5% tannic acid diet was 39 ± 1.2 mg and 44 ± 1.3 mg for the male and female, respectively. These quantities were larger than those of males and females fed on the control diet which were 13 ± 0.4 mg and 20 ± 0.8 mg, respectively. The higher consumption of medium with the 1.5% tannic acid diet consistently agreed with the higher amount of tannic acid ingested, causing reduction in the synthesis of protein, and increasing simultaneously the level of faeces production, as well as tannic acid in faecal pellets. It is reasonable, therefore, to propose that the production of high quantities of faecal pellets is a significant result of tannic acid in insect food. The weight gained by the insect stages were negatively correlated with the amount of tannic acid diet consumed, which corresponds with the effect of tannic acid on digestibility. For this reason, larger amounts of tannic acid were eaten to meet growth requirements. The larvae from the 1.5% tannic acid diet were smaller in size. Body weight and tannin content decreased markedly during development to pupa and adult (Table 2). The increase of tannin content per mg of body tissues was directly associated with the quantity of tannin in the food consumed. Larvae from the control media ingested less food but weighed 1.2 to 1.3 times more

Table 2. Tannic acid (TA) content in *Ectomyelois ceratoniae* stages fed a control or 1.5% tannic acid diet (mean ± SD*)

Diet	No. of larvae (sex)	Larva		Pupa		Adult	
		Wt (mg)	TA-content (μg)	Wt (mg)	TA-content (μg)	Wt (mg)	TA-content (μg)
Control	15 (♂)	37 ± 3 b	251 ± 90 a (6.7)	31 ± 3 ab	243 ± 73 a (7.8)	17 ± 2 a	232 ± 91 a (13.6)
Control	17 (♀)	57 ± 3 d	290 ± 65 ab (5.1)	50 ± 6 c	355 ± 70 ab (7.1)	27 ± 2 b	281 ± 55 ab (10.4)
Tannic acid diet	18 (♂)	31 ± 3 a	225 ± 64 a (7.3)	29 ± 3 a	240 ± 71 a (8.3)	17 ± 2 a	246 ± 41 a (14.5)
Tannic acid diet	17 (♀)	43 ± 3 c	310 ± 33 b (7.2)	41 ± 6 bc	456 ± 75 b (11.1)	20 ± 2 a	368 ± 24 b (18.4)

Numbers in parentheses represent tannic acid content per 1 mg weight of insect.
* Means within columns followed by the same letter are not significantly different ($P > 0.05$; Duncan's multiple range test).

Table 3. Effect of 1.5% supplemental tannic acid in the diet of *Ectomyelois ceratoniae* larvae on the final tannin content of the larvae, the dry weight of their faeces and the tannin content of the faeces (mean ± SD*)

Diet	No. of larvae	Tannic acid content of larvae (mg)	Faeces	
			Dry wt (mg)	% Tannic acid
Control	19 ♂	15 ± 6 a	119 ± 49 a	12 ± 1 a
Control	18 ♀	23 ± 9 a	188 ± 52 ab	12 ± 1 a
1.5% TA-diet	16 ♂	41 ± 12 b	203 ± 72 bc	20 ± 2 b
1.5% TA-diet	15 ♀	60 ± 17 c	299 ± 54 c	20 ± 2 b

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than those from the 1.5% tannic acid diet. Insect weight reduction was proportional to the level of tannin in the media consumed. This corresponded with the results of Maxwell *et al.* (1967) on boll weevil. High tannin contents in insects from the 1.5% tannic acid diet indicated low utilisation of tannin for larval growth with the increase in rate of intake of the tannic acid diet by *E. ceratoniae* larvae to meet the nutritional requirements for larval growth. Insufficient feeding and nutritional imbalances of the tannic acid diet probably were due to complexes of the tannin with protein (Jones and Morgan, 1977). The complexes of tannin with protein cause nutritional deficiency due to lower nitrogen assimilation, therefore reducing the uptake of nitrogen from ingested food (Vohra *et al.*, 1966; Moseley and Griffiths, 1979).

Field observations showed that full-grown larvae produce large quantities of faeces and a possible correlation between larval nutrition and faecal pellets production was hypothesised. The larvae clear their gut contents before pupation and an examination of larval faeces showed significant amounts of tannic acid pass through the gut without being assimilated although some of it was found in body tissue. Product from gut evacuation was higher in larvae from tannic acid diets than from artificial diets (Table 3). Tannin in larval faeces reflects the overall ability of the larvae to utilise nutrients including proteins and to discharge as much as possible of the tannin complexes as a waste product. The tannin in larval faeces from the artificial diet was due to the tannic acid content of wheat and soybean flour. Bernays (1978) reported that the defence mechanism against dietary tannins was the high pH of the insect gut which would inhibit complexing of tannin with protein. Dietary studies with *Tribolium confusum* and acridid insects showed that faecal pellets contained metabolic waste and undigested food (Bhattacharya and Waldbauer, 1972; Bernays, 1978). Larvae of *E. ceratoniae* feed mostly on the calyx and then tunnel into the pericarp to feed on its inner layer. The pericarp of mature fruits contained 26 μg tannic acid per gram fresh weight (Al-Izzi and Al-Maliky, 1986) and provided a better habitat for the larvae in the orchard and during fruit storage after harvest. Gravid females which emerged from the harvested and stored fruits increased infestation during storage by laying eggs inside the calyx with the newly hatched larvae feeding inside the fruits. Faeces of these larvae contained more tannic acid (Al-Izzi and Al-Maliky, 1986). The discharge of tannin through the gut with the other waste products has been reported by Goldstein and Swain (1965). Such larval feeding behaviour and excretion of waste products will decrease the value of the stored fruit. This study allows interpretation of the effect of secondary compounds on digestibility and their role in food selection by gravid females, and demonstrates how the feeding behaviour of newly hatched larvae depends on acquisition of essential nutrients from the fruit tissues. In conclusion, much of the tannin-containing diet as well as pomegranate fruit tissues are consumed by larvae of *E. ceratoniae* but not assimilated, or are not metabolised completely and pass through the gut without change. Kumar and Singh (1984) reviewed the adverse effects of tannins on ruminant nutrition and found nutritional problems associated with a high quantity of tannins in feeds and fodders. Larvae of pomegranate fruit moth, however, feed on fruits containing variable amounts of tannic acid (85 to 168 μg fresh weight) and can survive, develop and reproduce under field conditions. Calyx tissue was found to be the most preferred site for female oviposition, but larval feeding behaviour was related to the quantity of tannic acid in tissues. The adverse effect of tannic acid upon the insect can afford protection from insect feeding, but needs further investigation to be relevant in plant breeding programs because of the similarities of tannins in plants and a correspondence between insect digestive systems.

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tannin content

s	
% Tannic acid	
12 ± 1	a
12 ± 1	a
20 ± 2	b
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