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【Research report】

番茄素對小菜蛾幼蟲之拒食效應【研究報告】

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Abstract

摘要

番茄素對多種鱗翅目幼蟲之拒食作用已有諸多發現，惟仍未有其對小菜蛾作用之報告。本試驗即希望找出番茄素對小菜蛾是否為拒食劑之證明。在小菜蛾幼蟲對番茄素處理葉片之行為反應試驗中，發現並無明顯之偏好差異，顯示番茄素不具揮發性的忌避作用，而取食後之反應，在非選擇性試驗中，發現經番茄素處理的取食面積減少為對照組之1/41~1/3，而選擇性試驗中亦減為對照組之1/41~1/6，其番茄素對小菜蛾幼蟲之生長發育與存活率則具明顯抑制之影響。一般而言，番茄素對小菜蛾之拒食作用隨處理幼蟲之齡期增加而遞減，隨處理濃度之增加而增加。至於番茄素溶液之效力持續試驗，以每天取食葉面積而言，隨噴灑後時間之增加而略為遞增。以20％處理為例至少可持續七天，仍具輕度抑效果，其取食葉面積減少為對照組之1/4。若就處理組與對照組之取食葉面積之比值（FR）而言，選擇性試驗示高於非選擇性試驗之結果。此可能由於有選擇性時，幼蟲偏向於取食未經處理之葉片的緣故。

Key words:

關鍵詞: 番茄素、小菜蛾、拒食劑、抑制活性指數。

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Antifeeding Effects of α-Tomatine on Larvae of the Diamondback Moth (*Plutella xylostella* L.)

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ABSTRACT

α–Tomatine's effect on the diamondback moth (DBM) was tested using cabbage leaves. Behavioral preference tests revealed that tomatine dose not have a repellent action on DBM larvae, as larvae feeding on tomatine treated leaves only showed a decrease in feeding area (1/41～1/3 of the control), similar to those with a choice (1/41 and 1/6 of the control). In addition, α–tomatine was demonstrated to have an inhibiting effect on the growth and survival rate of DBM. All of these effects became less prominent as larvae matured and as more dilute concentration of α–tomatine were used.

Feeding increased over time, however, the effect of residual α–tomatine was still found to exhibit weak inhibitory activity, keeping the fed leaf area to 1/4 that of the control. While feeding ratios in the choice test were apparently higher than those of the no–choice test, this may be attributed to the larvae preference for control as opposed to test leaves.

Key words: Tomatine, diamondback moth (*Plutella xylostella*), antifeedant, inhibitory activity index.

番茄素對小菜蛾幼蟲之拒食效應

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摘　要

番茄素對多種鱗翅目幼蟲之拒食作用已有諸多發現，惟仍未有其對小菜蛾作用之報告。本試驗即希望找出番茄素對小菜蛾是否為拒食劑之證明。在小菜蛾幼蟲對番茄素處理葉片之行為反應試驗中，發現並無明顯之偏好差異，顯示番茄素不具揮發性的忌避作用，而取食後之反應，在非選擇性試驗中，發現經番茄素處理的取食面積減少為對照組之1/41～1/3，而選擇性試驗中亦減為對照組之1/41～1/6，其番茄素對小菜蛾幼蟲之生長發育與存活率則具明顯抑制之影響。一般而言，番茄素對小菜蛾之拒食作用隨處理幼蟲之齡期增加而遞減，隨處理濃度之增加而增加。至於番茄素溶液之效力持續試驗，以每日取食葉面積而言，隨噴灑後時間之增加而略為遞增。以0.2%處理為例至少可持續七天，仍具輕度抑制效果，其取食葉面積減少為對照組之1/4。若就處理組與對照組之取食葉面積之比值(FR)而言，選擇性試驗顯示高於非選擇性試驗之結果。此可能由於有選擇性時，幼蟲偏向於取食未經處理之葉片的緣故。

關鍵詞：番茄素、小菜蛾、拒食劑、抑制活性指數。

Introduction

Due to their high resistance to many kinds of insecticides, the diamondback moth (=DBM, *Plutella xylostella* L.) is the most destructive insect pest of cruciferous vegetables in the world. Many methods have been developed to control DBM, including Buranday and Raros (1975) and Talekar *et al.* (1984), who tried intercropping tomatos with cabbage, with a resulting reduction in damage to cabbage. It is possible that tomato plants have a repellent effect on DBM. Unfortunately, this culture system caused difficulties in the management and harvest of cabbage, and therefore is not considered practical.

Subsequently, a steroid glycoalkaloid -tomatine isolated from tomato plants, (*Lycopersicon pimpinellifolium*) and many plants of *Solanum* species. (Fontaine *et al.*, 1948) was studied using such insects as: *Empoasca fabae* (Dahlman and Hibbs (1967)); *Melanoplus bivittatus* (Harley and Thorsteinson (1967)); *Aedes aegypti* (Harley (1967)); *Leptinotarsa decemlineata* (Sinden *et al.*, (1978)); *Heliothis zea* (Juvik *et al.*, (1982)); *Myzus persicae* (Qin and Ke (1983)); *Choristoneura fumiferana* (Bentley *et al.*, (1984)); *Ceratitis capitata* (Chan and Tam (1985)); *Spodoptera littoralis* (Dhillon (1986)); *Earias insulana* (Weissenberg *et al.*, (1986)); *Spodoptera exigua* (Bloem *et al.*, (1989)). The readers attention is especially drawn to Roddik's review (1974) of α-tomatine's inhibitory effect on the larval growth of several insects.

The present work is concerned with the development of a new method of controlling DBM using α-tomatine. The antifeeding effect of α-tomatine on DBM larvae is also evaluated.

Materials and Methods

Field collected larvae were reared with fresh cabbage under room temperature conditions, and their progenies were used for the experiments. Crystalline α-tomatine (Sigma Chemical Co.) was dissolved with 50% ethyl alcohol in order to prepare 0.1, 0.2 and 0.4% solutions.

1. Behavioral preference of larvae

Two cabbage leaflets, (one untreated and the other dipped in 0.2% tomatine solution for 30 seconds), were placed in a petri dish (ϕ =11 cm). A single 3rd instar larva that had been starved for half a day was released at the central point (2.5 cm from either leaf edge). Each time a larva was observed to remain on a leaflet for 5 minutes, the event was recorded. Each test was repeated 30 times; each replication involving a single new larva for which a new leaflet and petri dish were prepared.

2. Feeding response by larvae

1. No-chioce test

A piece of cabbage leaflet was dipped into α-tomatine solution for 30 seconds and the excess solution allowed to evaporate before being placed into a glass tube (ϕ =2.5×10 cm) along with a single DBM instar larva, and covered with a cotton cloth. The control was a water dipped leaflet. Test tubes were kept at the same laboratory conditions of room temperature and humidity. 30 larvae were used for each treatment involving a different concentration of α-tomatine. The fed area on each leaflet (changed daily) was measured with graph paper (1 mm×1 mm). The feeding ratio (FR) was measured by Munakata's method (1970); FR=(T/C) 100%, where T=Treated leaflet fed area, and C=Control leaflet fed area.

The inhibitory activity index (IAI) was evaluated using a strong (IAI=0~20% FR), slight (IAI=20.1~50% FR) and none (IAI>50% FR) criterion.

In addition, moulting, growth and pupation of tested larvae were recorded from which percent pupated, accumulative mortality, total fed area and adult emergence were calculated. Emergent female adults were mated with males originating from the same treatment, and the resulting number of deposited eggs counted.

2. Choice test

Two treated and two untreated cabbage leaves (having an area of about 7 cm^2 each), were placed in a petri dish (ϕ =16 cm) along with a single 3rd instar larva (see Fig. 1). The fed area and FR etc., were measured daily till larva died or pupated. Each treatment was replicated with 20 larvae.

3. Effectiveness of α-tomatine over time

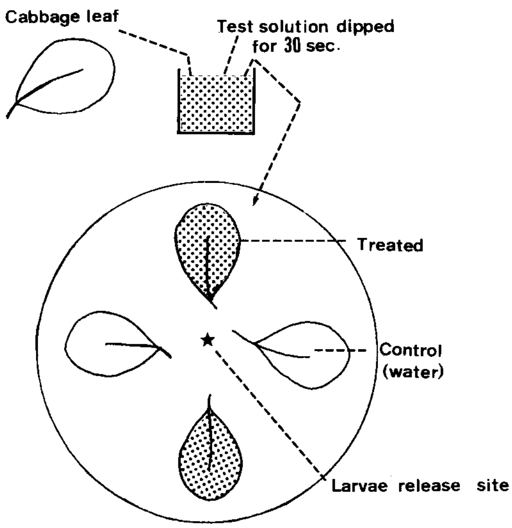

Fig 1. Procedure for the estimation of fed leaf area by DBM larvae. (choice test).

A 0.2% α-tomatine solution was sprayed on field planted cabbage seedlings. 1, 3, 5 and 7 days after the spraying, a single 3rd instar larva was raised on a treated leaflet under laboratory conditions till larva died or pupated. The feeding response to the treated leaflet was observed in a manner similar to that of the previous experiments. Each treatment was replicated 4 times using 20 larvae.

Results and Discussion

1. Behavioral preference of larvae

As shown in Table 1, about 53% of the tested larvae went directly to the untreated leaf (→C), while 47% first moved to the treated leaf (→T, →T(F)→C and →T(NF)→C). Evidently, tomatine sprayed leaves do not repel DBM larvae. Among the 47% choosing treated leaf, only 10% remained (→T), the others moving to the control leaf after either a short feeding period (→T(F)→C) or without feeding at all (→T(NF)→C). This suggests that the repellent action of tomatine affects the contact sense of larvae. It should be noted that no larvae changed from an untreated leaf to a treated one (→C→T).

2. Feeding response of larvae

The feeding data obtained from the no-choice test is given in Table 2. In the case of 1st instar larvae being reared with 0.4、0.2 and 0.1% tomatine treated leaves until the 4th instar stage, the daily leaf area intake throughout the testing was 1.1、1.5 and 6.5 mm^2, respectively. According to the Inhibitory Activity Index (IAI), these figures are within the range of strong inhibition (20%). While the 2nd

Table 1. Preference of DBM 3rd instar larvae for tomatine treated (0.2%) vs. untreated cabbage leaf

Pathway of larvae[1)]	Time required[2)] $\bar{x} \pm SD$ (min.)	Percentage of larvae (%)
→T	16.0±3.5	10.0%
→T(F)→C	45.0±7.1	6.7%
→T(NF)→C	25.1±9.4	30.0%
→C	21.3±8.3	53.3%
→C→T	–	0.0%

1) →T: moved directly towards treated leaf; →T(F)→C: towards treated leaf at first, but then moved to untreated leaf after feeding for a short time; →T(NF)→C: towards treated leaf at first, but then moved to untreated leaf without feeding; →C: moved directly towards untreated leaf; →C→T: moved to treated leaf from untreated leaf.

2) Each time a larva remained at a leaflet for five minutes, the event was observed and recorded.

Table 2. Effect of α-tomatine concentration on the feeding of DBM larvae (no-choice test)

Treatment	Larval instar							
	I–IV		II–IV		III–IV		IV	
	FLA[1)]	FR[2)]	FLA	FR	FLA	FR	FLA	FR
0.4% tomatine	1.1	2.9	2.5	3.3	2.3	2.4	6.6	9.4
(IAI[3)])		++		++		++		++
0.2% tomatine	1.5	3.9	3.6	4.8	5.1	5.4	14.6	20.7
(IAI)		++		++		++		+
0.1% tomatine	6.5	16.8	4.9	6.6	11.0	11.7	28.2	40.1
(IAI)		++		++		++		+
50% EtoH	4.8	12.5	65.7	87.3	91.2	97.4	62.8	89.2
(IAI)		++		–		–		–
Control	38.8	–	75.3	–	93.7	–	70.4	–

1) Fed leaf area: mm^2 / day / larva

2) FR (Feeding Ratio) : $FR = \frac{\text{fed area on treated leaf}}{\text{fed area on control leaf}} \times 100(\%)$

3) Inhibitory Activity Index (IAI) : ++ : Strong 0~20% of FR; + : Slight 20.1~50% of FR; – : None > 50% of FR

and 3rd instar larvae tested all showed strong IAI, the 4th instar larvae seemed less sensitive to the inhibiting action of tomatine. 4th instar larvae did not exhibit strong IAI until presented with leaves treated with 0.4% solution. Furthermore, a solution of 50% EtOH is scarcely deters DBM larvae, strong inhibition being exhibited only by lst instar larvae.

The influence of α–tomatine on the growth and survival of DBM larvae is given in Table 3. Of the larvae fed on 0.1% solution treated leaves since the lst instar stage, only 20% grew to adulthood, while of those fed on leaves treated with higher concentrations, none managed to reach even the 3rd instar stage. A similar tendency was also observed in those test groups beginning with 2nd and 3rd instar larvae. This trend was even more striking when 4th larvae were fed with 0.4% solution treated leaves, in which case around half of the tested larvae pupaed, showing higher pupation rated than those fed on 0.1% or 0.2% tomatine solution. Although EtOH treated leaves were highly toxic to lst~2nd instar larvae, the toxicity effect was negligible after the larvae grew to the 3rd instar stage.

The treated larvae accumulated mortality trends are shown in Fig.2. It is apparent that in order to obtain a high mortality rate, treatment must be effected before the 3rd instar stage is reached.

Table 3. Influence of α-tomatine on the survival, pupation and emergence of DBM larvae (no-choice test)

Larval instar	Treatment	% Survival in following larval stage			% Pupation	% Adult emergence
		II	III	IV		
	0.4% tomatine	0.0	–	–	–	–
	0.2% tomatine	6.3	0.0	–	–	–
I-IV	0.1% tomatine	20.0	20.0	20.0	20.0	20.0
	50% EtOH	31.6	21.1	15.8	15.8	15.8
	Control(water)	100.0	90.0	90.0	90.0	90.0
	0.4% tomatine		5.9	0.0	–	–
	0.2% tomatine		11.1	5.6	0.0	–
II-IV	0.1% tomatine		33.3	0.0	–	–
	50% EtOH		87.5	87.5	87.5	87.5
	Control(water)		100.0	100.0	100.0	100.0
	0.4% tomatine			40.0	0.0	–
	0.2% tomatine			23.5	0.0	–
III-IV	0.1% tomatine			36.8	10.5	5.3
	50% EtOH			89.5	89.5	89.5
	Control(water)			100.0	100.0	100.0
	0.4% tomatine				55.0	40.0
	0.2% tomatine				40.0	40.0
IV	0.1% tomatine				70.0	70.0
	50% EtOH				88.9	88.9
	Control(water)				100.0	100.0

The ovipositional potential was evaluated from the number of eggs deposited within 3 days by a female which had copulated with a male from the same treated group. As shown in Table 4, none of the pre-3rd instar larvae reared with 0.2 or 0.4% tomatine treated cabbage reached adulthood. For the same concentrations, only 40% of the 4th instar larvae reached adulthood, and their

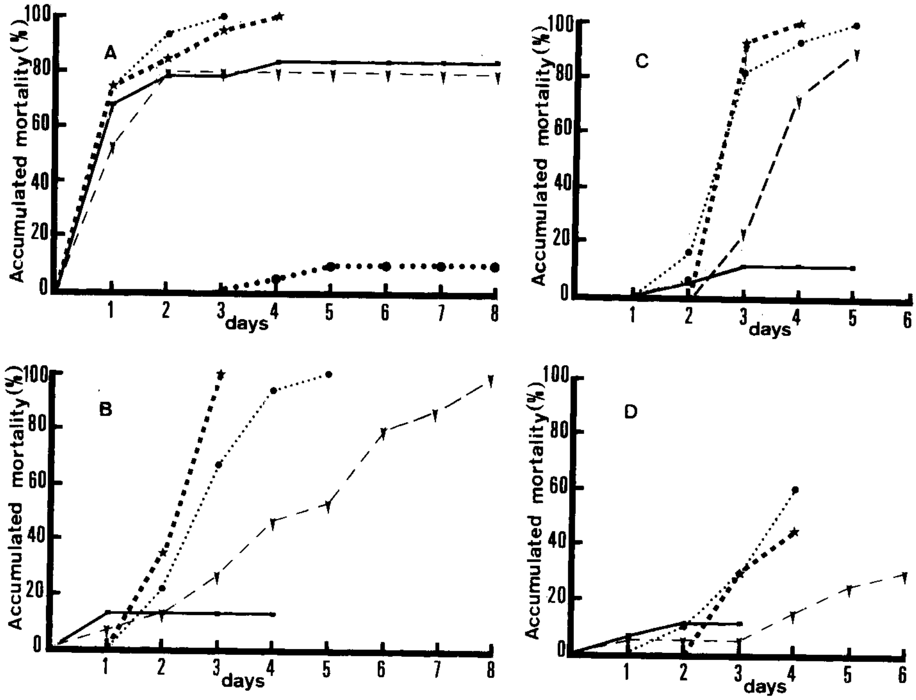

Fig 2. Accumulated mortality of DBM larvae fed with different dosages of tomatine treated cabbage starting from various instar larval stages. ▼------▼: 0.1% tomatine; ●·········●: 0.2% tomatine; ★■■■★: 0.4% tomatine; ■———■: 0.5% EtOH; ● • • • ●: Control; A: Fed from the first instar larval stage; B: Fed from the 2nd instar larval stage; C: Fed from the 3rd instar larval stage; D: Fed from the 4th instar larval stage.

Table 4. The oviposition of DBM fed with tomatine in their larval stage

Treatment	Larval instar reared with tomatine leaf							
	I−IV		II−IV		III−IV		IV	
	%A[1)]	E[2)]	%A	E	%A	E	%A	E
0.4% tomatine	0.0%	−	0.0%	−	0.0%	−	40.0%	15.0
0.2% tomatine	0.0%	−	0.0%	−	0.0%	−	40.0%	13.3
0.1% tomatine	20.0%	11.5	0.0%	−	5.3%	−	70.0%	24.8
50% EtOH	15.8%	−	87.5%	64.6	89.5%	66.5	88.9%	50.0
Control (water)	90.0%	27.0	100.0%	66.8	100.0%	95.2	100.0%	49.1

1) %A: Percent adult emergence

2) E: No. of eggs / ♀ / 3 days; I−IV: fed from the first instar larval stage; II−IV: fed from the 2nd instar larval stage; III−IV: fed from the 3rd instar larval stage; IV: fed from the 4th instar larval stage.

ovipositional potential was very low, less than 1/3 that of the control individuals (Table 4).

The results of the 0.1% tomatine treated groups are especially noteworthy. If the treatment began with 4th instar stage larvae, 70% of the tested larvae emerged as adults, and the ovipositional potential was approximately half that of the control. On the other hand, when the treatment began from the 3rd instar stage, only a few adults emerged which subsequently failed to deposit eggs. It is worth noting that of the 20% reared with treated leaves from their lst instar stage that reached adulthood, all deposited eggs. This fact implies the existence of tomatine tolerant DBM individuals, but further study is required in order to verify this observation.

Note that in the choice test, although the total fed area on those leaves treated with 0.2% tomatine solution was greater than that on 0.1% solution treated leaves, tomatine treated cabbage strongly inhibited the feeding action of DBM to between 1/6 and 1/41 of the control (Table 5).

3. Effectiveness of α-tomatine over time

In the no-choice test, 3rd instar larvae intake of a cabbage leaf sprayed 24 hours previously with 0.2% tomatine solution was 17.7 mm^2, or only 19.3% of the control (91.5 mm^2). The larval feeding amount in survival durations increased over time after spraying, with the result that leaf intake after 3, 5 and 7 days of spraying corresponded to 20.0, 21.1 and 21.4 mm^2, repectively. Feeding ratios here range between 21~24%, indicating slight inhibition (Table 6). In order to avoid over-estimation of the effective duration of tomatine, the cabbage leaves were replaced daily with a fresh leaf that had been sprayed 7 days previously. The total fed area until pupation for the sprayed cabbage was estimated to be 85.5 mm^2, while that of control was 274.4 mm^2. Note that after 7 days' rearing, larvae mortality was over 89%. This result points out that the effective duration of 0.2% tomatine solution is at least 7 days.

The feeding ratios in the choice test are obviously higher than those of the no-choice test (Table 7), but this fact is most likely attributed to larval preference for feeding on untreated leaves. The feeding ratio is highest for those larvae fed with leaves that had been sprayed 7 days previously, but the feeding index indicates slight inhibition for all leaflets.

Acknowledgment

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Table 5. Effect of tomatine on the feeding of DBM 3rd instar larvae (choice test)

Treatment	Total fed leaf area (mm^2 / day / larva)	FR[2)]	IAI[3)]
0.4% tomatine	4.6± 3.6**[1)]	2.4	++
Control	190.0± 20.3		
0.2% tomatine	22.7± 18.5**	4.4	++
Control	513.9±143.7		
0.1% tomatine	12.2± 7.8**	16.4	++
Control	74.4± 8.5		

1) : Means are significantly different (by t-test) at a significance level of 1%.

2) FR (Feeding Ratio) : $FR=\frac{\text{fed area on treated leaf}}{\text{fed area on control leaf}}\times 100(\%)$

3) Inhibitory Activity Index (IAI) : ++ : Strong 0~20% of FR; + : Slight 20.1~50% of FR; − : None > 50% of FR

Table 6. The daily amount of 0.2% tomatine treated cabbage eaten by DBM 3rd instar larvae over time (no-choice test)

Days after treatment	Fed leaf area (mm^2/larva/day)	Feeding ratio[2]	IAI[3]
1	17.7 b[1]	19.3	++
3	20.0 b	21.9	+
5	21.1 b	23.0	+
7	21.4 b	23.4	+
Control(water)	91.5 a	–	–

1) indicates that those figures denoted by the same letter are not significantly different (as calculated by Duncan's New Multiple Range Test) at a significance level of 5%.

2) FR (Feeding Ratio) : $FR=\frac{\text{fed area on treated leaf}}{\text{fed area on control leaf}}\times 100(\%)$

3) Inhibitory Activity Index (IAI) : ++ : Strong 0~20% of FR; + : Slight 20.1~50% of FR; – : None > 50% of FR

Table 7. The daily amount of 0.2% tomatine treated cabbage eaten by DBM 3rd instar larvae over time (choice test)

Days after treatment	Fed leaf area (mm^2/larva/day)	Feeding ratio[2]	IAI[3]
1	9.9 **[1]	24.1	+
Control	41.0		
3	11.8 **	23.3	+
Control	50.7		
5	29.9 **	20.9	+
Control	143.1		
7	39.6 **	36.5	+
Control	108.6		

1) Means are significantly different (by t-test) at a significance level of 1%.

2) FR (Feeding Ratio) : $FR=\frac{\text{fed area on treated leaf}}{\text{fed area on control leaf}}\times 100(\%)$

3) Inhibitory Activity Index (IAI) : ++ : Strong 0~20% of FR; + : Slight 20.1~50% of FR; – : None > 50% of FR

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