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Feeding Stimulants in Solanum viarum Dunal for Tomato Fruit Borer (Helicoverpa armigera Hübner)【Research report】

野生茄子 (Solanum viarum) 刺激番茄夜蛾取食之物質【研究報告】

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Abstract

Solanum viarum, a wild solanaceous plant, was heavily infested by tomato fruit borer (TFB), Helicoverpa armigera. S. viarum was consistently preferred by TFB over its natural host, tomato. Hence, we aimed to exploit the presence of feeding stimulants in S. viarum. Pure rice flour diet was prepared with an aqueous leaf extract of S. viarum, steam distillate (SD) of S. viarum, hexane fraction of SD of S. viarum, water fraction of SD of S. viarum and aqueous leaf extract of tomato. The purpose for halving the SD into a hexane fraction and water fraction was to determine whether the feeding stimulants, if any, are hydrophilic or lipophilic. In our experiments, there was very little larval feeding on the pure rice flour diet (check) and 60% died within a week. On the diet fortified with S. viarum aqueous extract, only 20% fed and survived up to four weeks. The larval duration was 25.60 days on diet fortified with the hexane fraction of SD. The percent pupation (70.00) and pupal weight (0.139 g) were significantly different on the diet fortified with the water fraction of SD. The adult emergence was only 20% where the larvae were fed with S. viarum extracts.

摘要

Solanum viarum是野生茄科作物之一，但卻也遭受番茄夜蛾嚴重危害，S. viarum 對番茄夜蛾雌蟲具強烈誘引產卵能力，但無論是初齡或其他較大幼蟲最終皆死亡，無法完成幼蟲發育。因此本試驗之目的將確認S. viarum是否含有取食刺激物質，在試驗中，我們利用純米人工飼料為對照組，並利用純米人工飼料分別加入S. viarum葉片汁液水溶液、S. viarum蒸餾液、以己烷萃取S. viarum蒸餾液後之溶劑溶液、以己烷萃取S. viarum蒸餾液後之水溶性溶液及番茄葉片汁液水溶液等五種人工飼料，餵食番茄夜蛾幼蟲。將S. viarum蒸餾液經己烷萃取，再分離成溶劑溶液及水溶性溶液兩部分，是為了瞭解是否有刺激取食物質存在於水性溶液或溶劑溶液中。結果顯示，所有幼蟲皆只取食極少的純米人工飼料，且60% 的幼蟲在一星期內死亡，若將S. viarum葉片汁液水溶液加入人工飼料中，則只有20% 幼蟲會取食且存活至四星期，但若加入S. viarum之蒸餾液，則幼蟲開始有取食行為，而含蒸餾液經己烷萃取後之溶劑溶液之人工飼料，可提供幼蟲發育，且幼蟲期平均為25.6天、化蛹率為70%、蛹平均重約0.139克，此結果與經己烷萃取S. viarum蒸餾液之水溶性溶液作比較，有明顯差異，但是成蟲羽化率只有20%。

Key words: Solanum viarum, tomato fruit borer, feeding stimulant, larvae growth
關鍵詞: Solanum viarum、番茄夜蛾、取食刺激物質、幼蟲生長

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Feeding Stimulants in *Solanum viarum* Dunal for Tomato Fruit Borer (*Helicoverpa armigera* Hübner)

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ABSTRACT

Solanum viarum, a wild solanaceous plant, was heavily infested by tomato fruit borer (TFB), *Helicoverpa armigera*. *S*. *viarum* was consistently preferred by TFB over its natural host, tomato. Hence, we aimed to exploit the presence of feeding stimulants in *S*. *viarum*. Pure rice flour diet was prepared with an aqueous leaf extract of *S*. *viarum*, steam distillate (SD) of *S*. *viarum*, hexane fraction of SD of *S*. *viarum*, water fraction of SD of *S*. *viarum* and aqueous leaf extract of tomato. The purpose for halving the SD into a hexane fraction and water fraction was to determine whether the feeding stimulants, if any, are hydrophilic or lipophilic. In our experiments, there was very little larval feeding on the pure rice flour diet (check) and 60% died within a week. On the diet fortified with *S*. *viarum* aqueous extract, only 20% fed and survived up to four weeks. The larval duration was 25.60 days on diet fortified with the hexane fraction of SD. The percent pupation (70.00) and pupal weight (0.139 g) were significantly different on the diet fortified with the water fraction of SD. The adult emergence was only 20% where the larvae were fed with *S*. *viarum* extracts.

Key words: *Solanum viarum*, tomato fruit borer, feeding stimulant, larvae growth

Introduction

Tomato fruit borer (TFB), *Helicoverpa armigera* Hübner (Lepidoptera : Noctuidae) is a highly mobile polyphagous pest of economic importance on many agricultural and horticultural crops, in 39 families (Reed and Pawar, 1982; Torres-Villa *et al*., 1996). In India, it is a predominant pest on cotton, tomato, pigeon pea and chickpea. Indiscriminate use of chemical insecticides triggered the insect to develop resistance to insecticides, caused a resurgence of sucking pests, and increased chemical residues in the environment. Hence, alternative methods aimed at exploiting the feeding and reproductive behaviour, as well as chemical ecology, of

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this insect have become more important.

Tropical soda apple, *Solanum viarum* Dunal, is an economically important medicinal plant with a rich source of solasodine, an alkaloid used in the synthesis of steroid hormones for treating cancer, Addison's disease, rheumatic arthritis, and for producing contraceptives (Chandra and Srivastava, 1978; Hendique, 1986; Satyabrata *et al*., 2000; Nayak and Patil, 2001). This plant, native to South America (Nee, 1991), has spread to other geographical regions including Central America, the Caribbean, India, China, Africa (Chandra and Srivastava, 1978; Coile, 1993; Muraleedharan *et al*., 1999), and South Florida (Mullahey *et al*., 1993). In spring 1998, heavy infestation of *S. viarum* foliage by TFB was noticed in Taiwan. *S. viarum* was consistently preferred by TFB over its natural host, tomato, in the field. This opened the possibility of using this wild medicinal plant as a trap crop. Also *S. viarum* has never been reported to be a host of TFB (Talekar *et al*., 1999; AVRDC, 1999).

Research has indicated that *S. viarum* was overwhelmingly preferred for oviposition compared to tomato (Liljana, 2001). Field experiments at AVRDC, Taiwan revealed that, on average, more than nine times as many eggs were laid on *S. viarum* than on tomato (AVRDC, 1999). Microwave-assisted hexane extracts of *S. viarum* leaves containing n-alkanes, and hexane extracts of volatiles collected from *S. viarum* plants, attracted *H. armigera* adults for oviposition (Srinivasan, 2003). Although *S. viarum* was highly attractive to ovipositing females and young larvae, they failed to develop, resulting in death.

Our objective was to investigate the potential for using *S. viarum* as a trap crop to control TFB on tomato. Specifically, we made an attempt to confirm the presence of feeding stimulants in *S. viarum* which sustain TFB feeding.

Materials and methods

Insect material

Larvae of *H. armigera* were reared on a meridic diet of a polyphagous insect, *Spodoptera exigua* Hübner (Bio-Serve French Town, NJ, USA; Product No. F 9219 B). All insects were reared in controlled conditions under a 14:10 h (L:D) photoperiod cycle at 27±1ºC and 70±10% RH. After hatching, larvae were placed in polystyrene cups (22 cm × 15 cm × 4 cm) with the diet until the second or early third instar. They were then individually reared to pupation in clear plastic cups (4.5 cm high and 4 cm wide) with lids. On pupation, they were sexed and placed in acrylic cylinders (30 cm long and 15 cm diameter) and held for adult emergence. Emerged adults were used for producing further generations, from which the larvae were used for larval bioassays.

Plant material

Tropical soda apple (*S. viarum*) and tomato (*Lycopersicon esculentum*) were grown under greenhouse conditions. The seeds were sown in seedling trays, filled with equal mixture of vermiculite (South Sea Vermiculite and Perlite Co., The Netherlands) and peat moss–growing media (Know–You Seed Co., Taiwan). After six weeks the seedlings were transplanted to plastic pots of 18 cm diameter and 17 cm in height. The pots were watered daily. Every 10 days, a foliar fertilizer of Nitrofoska (N:P:K:Mg = 20:19:15:0.5, BASF, Germany) was applied at the rate of 3 g/100 ml water by spraying uniformly over the plants.

Extraction of feeding stimulants

The initial field observations - *viz*., the overwhelming preference of gravid TFB females to lay more eggs on *S. viarum* plants than on its natural host plant, tomato, and voracious feeding by the TFB larvae on *S. viarum* -- revealed

that some ovipositional attractants and feeding stimulants are likely present in *S. viarum* plants. However, in later stages the insect failed to pupate and died due to unknown factors. The aqueous leaf extract was prepared using *S. viarum* leaves and tomato leaves by grinding them with distilled water in a blender. The aqueous leaf extracts were believed to contain all compounds as in the natural leaf chemistry. In order to isolate the active feeding stimulants, steam distillates (SD) were extracted in this experiment.

Two hundred grams of *S. viarum* leaves were ground with 500 ml of distilled water, which was transferred to a 5 litre round bottom flask of a SD apparatus. Steam was then passed through the slurry and 400 ml of SD representing 200 g of *S. viarum* leaves was collected. Two hundred milliliter of SD was taken out as whole SD. The remaining 200 ml was extracted with an equal volume of hexane two times to find whether the feeding stimulants in SD could be extracted in the hexane, and the combined hexane fractions were concentrated *in vacuo* to 10 ml. The remaining water layer of the hexane extracted SD was also kept in refrigerator until used.

The following diets were prepared by adding various steam distillation fractions to the rice flour, as it never sustains feeding by TFB (Table 1). The *S. viarum* extracts (aqueous and SD) and tomato aqueous extracts were added to the rice flour diet at the rate of 10% wet weight of the diet.

Each diet was distributed in 5.5 cm diameter plastic cups whose height was 7.5 cm. The diets were cut into pieces and placed in small rearing cups. Twenty five neonate larvae were released into each cup and there were four cups for each diet. Therefore, there were four replicates in a diet, containing 100 larvae *in toto*. The larvae were observed daily and used diets were replaced with fresh one. Observations were made on the larval duration, percent pupation, pupal weight, pupal duration and percent adult emergence and the data were statistically analyzed using one way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) for mean comparisons.

Results and Discussion

Pure rice flour diet was used for this experiment because it never initiates TFB feeding and it does not sustain normal feeding, survival, growth and development of TFB larvae. Results of the test on suitability of *S. viarum* for TFB larval development indicated that *S. viarum* was inferior to the normal host plant, tomato, in terms of feeding and growth and development. Larval growth and development and adult emergence were higher on the diet incorporated with an aqueous leaf extract of tomato.

As we speculated, most of the larvae did not initiate feeding on the pure rice flour diet, which served as check and all the larvae died (Table 2). Almost 60 percent of the larvae died within a week. However, 15 percent of the larvae survived up to three weeks with little feeding on the surface of the diet after a prolonged initial starvation. On the rice flour diet fortified with aqueous extract of *S. viarum*, 60 percent larvae survived up to two weeks. However, 20 percent of the larvae could feed and survive up to four weeks. There was a significant difference in larval duration ($F_{(5,18)} = 769.5$, $P < 0.0001$), which was greatest (25.60 days) on the rice flour diet fortified with the hexane fraction of SD of *S. viarum*. This was followed by diet fortified with whole SD of *S. viarum* (25.10 days), diet fortified with water fraction of SD of *S. viarum* (24.30 days) and diet fortified with aqueous leaf extract of tomato (22.20 days).

The percent pupation and pupal

Table 1. Treatments included in the study

Treatment	Diet ingredients
T_1	Rice flour fortified with *S. viarum* aqueous leaf extract
T_2	Rice flour fortified with whole SD of *S. viarum* leaves
T_3	Rice flour fortified with hexane fraction of SD of *S. viarum* leaves
T_4	Rice flour fortified with water fraction of SD of *S. viarum* leaves
T_5	Rice flour fortified with tomato aqueous leaf extract
T_6	Rice flour diet (Check)

Table 2. Growth and development of *H. armigera* on artificial diet fortified with *S. viarum* leaf steam distillate (SD), aqueous extract and tomato leaf aqueous extract

Rice flour diet fortified with	Larval period (days)	Percent pupation	Pupal weight (g)	Pupal period (days)	Percent adult emergence
Aqueous leaf extract of *S. viarum*	0.00a[1)]	0.00a	0.00a	0.00a	0.00a
SD of *S. viarum*	25.10d	40.00b	0.189c	14.80b	20.00b
Hexane fraction of SD of *S. viarum*	25.60e	35.00b	0.193c	14.20b	20.00b
Water fraction of SD of *S. viarum*	24.30c	70.00c	0.139b	14.00b	20.00b
Aqueous leaf extract of Tomato	22.20b	40.00b	0.194c	13.90b	30.00c
Rice diet (Check)	0.00a	0.00a	0.00a	0.00a	0.00a
LSD	0.48	11.32	0.03	1.52	4.72

[1)] Means followed by same letter(s) are not significantly different by DMRT (p=0.05)

weight were significantly different (70.00 and 0.139 g respectively) on the rice flour diet fortified with the water fraction of SD of *S. viarum* and they were on par in all other treatments, except the diet fortified with the aqueous leaf extract of *S. viarum* and pure rice flour diet. The percent pupation ranged from 0 to 70.00 percent ($F_{(5,18)}$ = 22.67, P < 0.0001) and pupal weight ranged from 0.139 to 0.194 g ($F_{(5,18)}$ = 34.29, P < 0.0001) (Table 2). The pupae were small, abnormal and had lower weights on the diet fortified with the water fraction of SD of *S. viarum* (Fig. 1). There were no significant differences in the pupal period among the *S. viarum* SD extracts, which ranged between 13.90 and 14.80 days. The percent adult emergence was significantly higher (30.00) on the diet fortified with aqueous leaf extract of tomato, and in all other diets fortified with *S. viarum* SD extracts, it averaged 20 percent; no adult emergence was observed in the diet fortified with aqueous leaf extract of *S. viarum* and the pure rice flour diet ($F_{(5,18)}$ = 27, P < 0.0001). Normal adults were seen on diet fortified with aqueous leaf extract of tomato and on diet fortified with whole SD of *S. viarum*; on other diets the adults were abnormal. For example, they were unable to emerge completely from pupae on the diet fortified with hexane and the water fraction of SD of *S. viarum* (Fig. 2).

The larvae could initiate feeding on diets fortified with *S. viarum* SD, which was indicative of the presence of feeding stimulants. Those present in the SD of *S. viarum* could also be extracted in hexane, as the growth parameters were similar to the diet fortified with whole SD. This could be supported by the previous findings (Singh and Mullick, 2002),

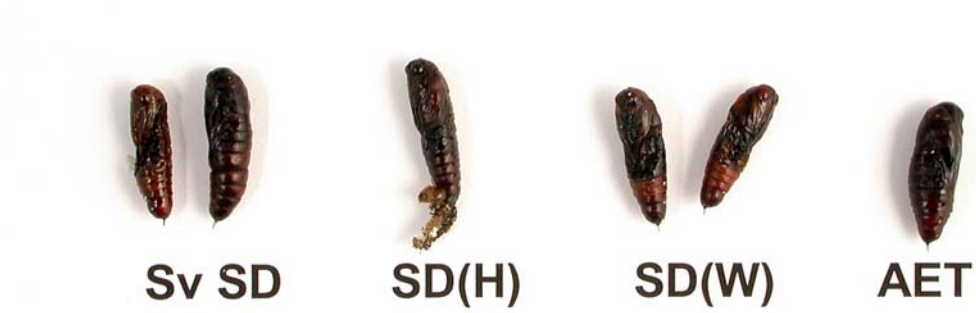

Fig. 1. Small and malformed pupae of TFB fed on rice flour diets fortified with tomato and *S. viarum* extracts.

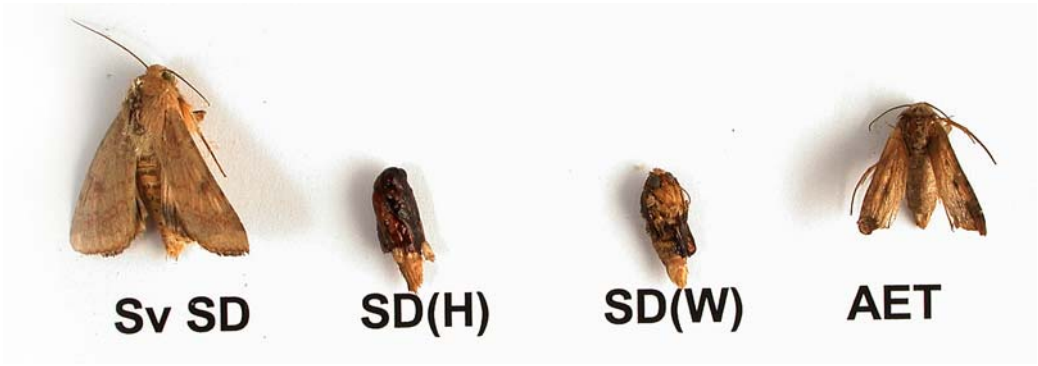

Fig. 2. Small and malformed pupae of TFB fed on rice flour diets fortified with tomato and *S. viarum* extracts. SvSD – *Solanum viarum* whole steam distillate; SD(H) – Hexane fraction of the *S. viarum* steam distillate; SD(W) – Water fraction of the *S. viarum* steam distillate; AET – Aqueous extract of the tomato leaves.

which reported that hexane extract from the foliage of some leguminous plants elicited higher orientational responses of TFB larvae compared to the methanolic extracts of same leaves. However, when the hexane fraction of SD of *S. viarum* was incorporated into the diet, it significantly extended the larval period, though there were no significant differences in percent pupation, pupal weight, pupal period and percent adult emergence. Adults were smaller, malformed, and unable to emerge completely from pupae. Hence, it is hypothesized that the qualitative constituents in *S. viarum* SD are not adequate for sustaining normal growth and development of the larvae. However, further studies are needed.

Most of the larvae could not feed on the diet prepared with crude *S. viarum* aqueous leaf extract. This may be due to the presence of some toxic allelochemicals in *S. viarum* leaves as they contain large quantities of glycoalkaloids (Chandra and Srivastava, 1978; Satyabrata *et al.*, 2000; Nayak and Patil, 2001). It has already been proven that *S. viarum* possess glandular trichomes which exude sesquiterpene hydrocarbons and methyl ketones (Gandolfo, 2000; Liljana, 2001) that are toxic to TFB. The present study provides clues concerning mechanisms underlying the extensive feeding by TFB larvae on *S. viarum*. However, it appears that undisclosed compounds in the plants

impede survival, growth and development of TFB larvae as evidenced on diets fortified with crude aqueous extracts of *S. viarum* leaves. Hence, further studies need to be made to exploit *S. viarum* as a trap crop for managing TFB.

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野生茄子 (*Solanum viarum*) 刺激番茄夜蛾取食之物質

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摘　要

Solanum viarum 是野生茄科作物之一，但卻也遭受番茄夜蛾嚴重危害，*S. viarum* 對番茄夜蛾雌蟲具強烈誘引產卵能力，但無論是初齡或其他較大幼蟲最終皆死亡，無法完成幼蟲發育。因此本試驗之目的將確認 *S. viarum* 是否含有取食刺激物質，在試驗中，我們利用純米人工飼料爲對照組，並利用純米人工飼料分別加入 *S. viarum* 葉片汁液水溶液、*S. viarum* 蒸餾液、以己烷萃取 *S. viarum* 蒸餾液後之溶劑溶液、以己烷萃取 *S. viarum* 蒸餾液後之水溶性溶液及番茄葉片汁液水溶液等五種人工飼料，餵食番茄夜蛾幼蟲。將 *S. viarum* 蒸餾液經己烷萃取，再分離成溶劑溶液及水溶性溶液兩部分，是爲了瞭解是否有刺激取食物質存在於水性溶液或溶劑溶液中。結果顯示，所有幼蟲皆只取食極少的純米人工飼料，且 60% 的幼蟲在一星期內死亡，若將 *S. viarum* 葉片汁液水溶液加入人工飼料中，則只有 20% 幼蟲會取食且存活至四星期，但若加入 *S. viarum* 之蒸餾液，則幼蟲開始有取食行爲，而含蒸餾液經己烷萃取後之溶劑溶液之人工飼料，可提供幼蟲發育，且幼蟲期平均爲 25.6 天、化蛹率爲 70%、蛹平均重約 0.139 克，此結果與經己烷萃取 *S. viarum* 蒸餾液之水溶性溶液作比較，有明顯差異，但是成蟲羽化率只有 20%。

關鍵詞：*Solanum viarum*、番茄夜蛾、取食刺激物質、幼蟲生長。