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Effects of cranberry juice and pineapple juice on *Pratylenchus coffeae*

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ABSTRACT

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The nematodes killed by cranberry juice often had puffed cuticle similar to the blisters on human skin after been scalded by boiling water. There were 75.7 % *P. coffeae* with puffed cuticles after 96 h exposure to cranberry juice. None of the nematodes in the fructose or pineapple juice treatment had puffed cuticle. Cranberry juice was very effective in killing all the developmental stages of *P. coffeae*. The mortality rates were 100% for J2 and male, 97 % for female and 99 % for J3+J4 after 96 h exposure. Dilution of cranberry juice to 1:5 rendered it ineffective in killing *P. coffeae* but 71.5 % of nematodes were paralyzed after 96 h exposure. Cranberry juice remained very effective in killing *P. coffeae* after boiling for 2 minutes. The canned pineapple juice was completely ineffective in killing *P. coffeae*. However, hand-squeezed fresh pineapple juice caused high mortality of the nematodes. Boiling for 2 min rendered it completely ineffective. The cranberry juice and pineapple juice have different nematicidal principles as indicated by heat stability test and the difference in their ability to cause puffed cuticle. The infection of mung bean roots by *P. coffeae* was significantly reduced by cranberry juice and fresh pineapple juice in the growth chamber tests.

Key words: control, cranberry, juice, pineapple, *Pratylenchus*

INTRODUCTION

Chemical nematicides have been the major means of control for plant-parasitic nematodes, an important category of plant pests, to ensure good quality and abundance of crop production. However, pesticide residues in the vegetables, fruits, and cereals are a potential threat to our health. The pollution of ground water and the environment by pesticide residues is also of great concern. Therefore, alternative methods for the control of nematodes, such as biological control, crop rotation, green manure and biofumigation have gained increasing interests in recent years. Moreover, the search for natural nematicides has also been conducted by numerous researchers [7].

Marigolds (*Tagetes* spp.) are well known to produce nematicidal compounds [3] and *Tagetes patula* has been demonstrated to be an effective catch crop for long-term control of *Pratylenchus penetrans* in strawberry cultivation and the effect lasted three years, longer than the effect of soil fumigation [10]. Moreover, Pudasaini *et al.* [17] reported that culturing marigold for 105 days reduced *P. penetrans* populations at greater depths than soil fumigation. The extract of marigold was also effective in controlling *Meloidogyne incognita* [26]. Tsay, *et al.* [23] studied Asteraceae plants and found that *Gaillardia pulchella* was effective in the control of *M. incognita* and *Rotylenchulus reniformis*. *Brassica* residues were used to control the citrus nematode *Tylenchulus semipenetrans* [24].

Zasada and Ferris [27] found that *B. hirta* and *B. juncea* meal was effective against *M. javanica* and *T. semipenetrans*. Other vegetables, such as cabbage, onion, and radish, and spices, such as chili pepper and wasabi, have been reported to be effective against *M. javanica* [21, 22]. The leaves of *Magnolia grandiflora* L. was reported to be effective against the pine wood nematode *Bursaphelenchus xylophilus* [11]. A medicinal herb, wild feverfew, *Parthenium hysterophorus*, was reported to exhibit nematicidal activity against *M. incognita* [9]. The essential oil of *Chrysanthemum coronarium* flowerheads showed strong nematicidal activity against *M. artiellia* [16]. Moreover, *C. coronarium* was applied to the soil as a green manure to control *M. incognita* and *M. javanica* on tomato roots [4].

While extracts or amendments of various plants, including medicinal plants [21, 9], cover crops [10], vegetables [24, 21, 27], trees [11], and flowers [16] have been tested for their nematicidal activities, there has been no report on fruits and their activity against plant-parasitic nematodes, other than tomato fruits [21]. However, the effects of fruits on animal parasites have been documented. Papaya, pineapple, and fig all have been found to have anthelmintic activities [2, 14, 18, 20]. This paper reports the effects of cranberry juice and pineapple juice on the lesion nematode, *Pratylenchus coffeae* (Zimmermann) Filipjev & Schuurmans Stekhoven.

MATERIALS AND METHODS

Preparation of nematodes

The lesion nematode *P. coffeae* was originally collected from the experimental station of the National Taiwan University and cultured on excised tomato roots in Gamborg's B5 medium (GIBCOBRL, Life Technologies, Inc. Grand Island, NY, U.S.A.) following surface sterilization with H_2O_2 for 2 min. The nematodes from one-month old culture were extracted with modified Baermann funnels, collected daily and kept at 15℃. They were used within three days. The concentration of nematodes was adjusted to 200 nematodes/ml for the *in vitro* tests and 1000 nematodes/ml for the in vivo tests.

Source of juices

Cranberry juice (Cranberry Classic, 27% cranberry juice, Ocean Spray Australia Pty., Ltd.), canned pineapple juice (100% juice, Typhone, Taiwan), fresh pineapple fruit, and Fong Leng fructose (Fonen and Fonher Enterprise Co. Ltd., Taiwan, 75% Sugar (90% Fructose, 5% Glucose, and 5% other sugar)) were purchased from the local supermarket. Fructose (Sigma) was also used for comparison. Slices of the fresh pineapple fruit were put in a polyethylene bag and squeezed by hand to obtain fresh pineapple juice and used immediately for the experiments.

Effects of cranberry juice and pineapple juice on *P. coffeae*

Parallel lines, approximately 0.4 cm apart, were drawn with a fine blue marking pen at the bottom of Petri dishes to facilitate the counting of nematodes under dissecting microscope. The aliquots of 3 ml of cranberry juice and 2 ml of distilled water were added to small Petri dishes (5.7 diam.) and mixed well. Then 1 ml of nematode suspension was added to the Petri dish to make 1:1 dilution of the Cranberry Classic and mixed well (final concentration = 13.5% pure cranberry juice). The Petri dish was then sealed with Parafilm to avoid changing the concentration of the juice due to evaporation. Distilled water was used in place of the juice for the control. To make 1:5and 1:10 dilution of the Cranberry Classic (final concentration = 4.50 and 2.45 % pure cranberry juice, respectively), 1 ml and 0.5 ml of juice, respectively, 2 ml of nematode suspension and 3 ml of distilled water were loaded to the Petri dish. Canned pineapple juice (1:2 dilution) and hand-squeezed fresh pineapple juice (1:2 and 1:5 dilutions) were tested the same way as the cranberry juice. The sealed Petri dishes were then incubated at 28℃ for 24 h, 48 h, and 96 h. At each sampling time, the nematodes were counted under a dissecting microscope (Olympus SZ61). Those nematodes that were not moving after touching with a nematode pick were considered dead. Since there were many nematodes immobile but responded to touch in the dilution test of cranberry juice, the immobile nematodes were transferred to distilled water and the live and dead nematodes were counted after 24 h to determine % paralyzation. To examine the response of the different developmental stages of *P. coffeae*, male, female, and J2 were counted separately. The third- and fourth- stage juveniles were difficult to separate under a dissecting microscope and were combined in the counting.

In the preliminary test, the cuticle of some nematodes killed by cranberry juice was found to puff up at certain places. The nematodes with and without puffed cuticle were counted separately for comparison. Because fructose was added in the commercially made cranberry juice, Fong Leng Fructose and fructose (Sigma) were also tested to determine if fructose could cause puffed cuticle. All the processes were carried out in a lamella flow hood to avoid fungal contamination from the air. There were four replicates for each treatment. The experiment was repeated twice.

Heat stability test

Cranberry juice and hand-squeezed pineapple juice were heated in a microwave oven to boil for 2 min in a beaker covered with saran wrap. Distilled water was added to the boiled juices to maintain the original volume to keep the juices from been concentrated by evaporation. After cooling to room temperature, the boiled juices were tested as described above. Distilled water was used in place of the juices for the control. Boiled water was used as a second control to determine if boiling could cause suffocation of the nematodes due to loss of oxygen in the process of boiling. There were four replicates for each treatment. The experiment was repeated twice.

Protection of plant roots by the juices

Mung bean seeds were surface sterilized with 70% ethanol for 4 minutes and rinsed three times with tap water. The seeds were then sown in sterile sands in plastic cups and kept at 28℃ in a growth chamber with 16 h photoperiod. The seedlings were used five days after germination. The aliquot of 1 ml of nematode suspension was added to 150 g of sterile sands in a plastic cup (7 cm diam. at top, 4 cm diam. at bottom), along with 30 ml of cranberry juice (1:1, 1:2, and 1: 5 dilution, vol/vol) or fresh pineapple juice made in a blender at 1:2 or 1:5 dilution (w/v). Tap water was used in place of the juice for the control. The cups were then sealed with saran wrap and rubber band to prevent evaporation. They were incubated at 28℃ in a growth chamber for four days. After the incubation, saran wrap was removed and the mung bean seedlings were each transplanted to a cup. They were returned to the growth chamber and kept for three days. Then the roots were stained with acid-fuchsin[6]. The number of nematodes penetrated the roots were counted under a dissecting microscope. The % infection was calculated as the number of nematodes penetrating the roots/ the number of nematodes inoculated per cup × 100%. There were four replicates for each treatment, and experiment was repeated twice.

RESULTS

The nematodes killed by cranberry juice often had puffed cuticle (Fig. 1) similar to the blisters on human skin scalded by boiling water. The location of puffed cuticle was variable. The head, the middle of the body and the tail all have been observed to exhibit puffed cuticle. Usually the puff was on one side of the nematode body (Fig. 1A), however, some puff occurred at both sides of the body (Fig. 1B). The length of the puffed cuticle was also variable, some was short and some was longer than half of the length of the body. The body contents at the puffed location appeared to stick together and had a narrower appearance than the other places of the nematode body. The cuticles of nematodes treated with fructose (Sigma), Fong Leng fructose, and hand-squeezed fresh pineapple juice appeared normal (Fig. 1C and 1D). Different developmental stages of *P. coffeae* all had puffed cuticles (Table 1) but the male had the lowest percentages of nematodes with puffed cuticles. There were 75.7 % *P. coffeae* with puffed cuticles after 96 h exposure to cranberry juice. None of the nematodes in the fructose or pineapple juice treatment had puffed cuticle.

Cranberry juice was very effective in killing all stages of *P. coffeae* (Fig. 2). There were 88 % mortality for J2, J3+J4, and female nematodes, and 94% mortality for male nematodes after 24 h exposure. The mortality was 100% for J2 and male, 97 % for female and 99 % for J3+J4 after 96 h exposure. Since the sensitivity of different stages of *P. coffeae* to cranberry juice was very similar, data of different stages were pooled in the other experiments.

Dilution of cranberry juice to 1:5 rendered it ineffective in killing *P. coffeae* but 71.5 % of nematodes were paralyzed after 96 h exposure (Fig. 3). There were 30.7 % nematodes paralyzed at the dilution of 1:10 after 96 h exposure.

Cranberry juice remained very effective in killing *P. coffeae* (Fig. 4) after boiling for 2 minutes. Boiled water control showed that boiling did not cause suffocation of

the nematodes.

The canned pineapple juice was completely ineffective in killing *P. coffeae* (Table 2). However, hand-squeezed fresh pineapple juice caused high mortality of the nematodes. There were 89.5 % of the nematodes killed by hand-squeezed fresh pineapple juice after 96 h exposure. Dilution to 1:5 significantly lowered its effectiveness and boiling for 2 min rendered it completely ineffective (Table2).

The infection of mung bean roots by *P. coffeae* was significantly reduced by cranberry juice and fresh pineapple juice (Fig. 5). There was 100% reduction of infection in the cranberry juice (1:1 dilution) and fresh pineapple juice (1:2 dilution) treatment. The effectiveness was reduced by dilution of the juices.

DISCUSSION

The cranberry juice has been found to have medical value in preventing urinary tract infection and tooth decay [19]. The present findings showed that cranberry juice also possessed nematicidal activities against *P. coffeae*. It was very effective against all the developmental stages of *P. coffeae* (Fig. 2). Since different stages of *P. coffeae* exist in soil, it is important to have an understanding on the response of different stages to a control strategy. Although the mortality of males was significantly higher than the other stages after 24 h exposure, there was no significant difference between the mortality of different stages at 96 h after treatment.

The killing of *P. coffeae* by cranberry juice was often accompanied by puffed cuticle (Fig. 1). Although fructose was added in the commercial cranberry juice, the results showed that cranberry juice and not fructose was responsible for causing the symptom (Table 1). Puffed cuticle appeared in all developmental stages of *P. coffeae*. The percentages of nematodes with puffed cuticles were lowest in male and highest in J3+J4. It is possible that the cuticles of different developmental stages had certain difference which affected their response to cranberry juice. The appearance of dead nematodes was considered to be related to the nematicidal ingredients [13, 8]. Kong, *et al.* [13]

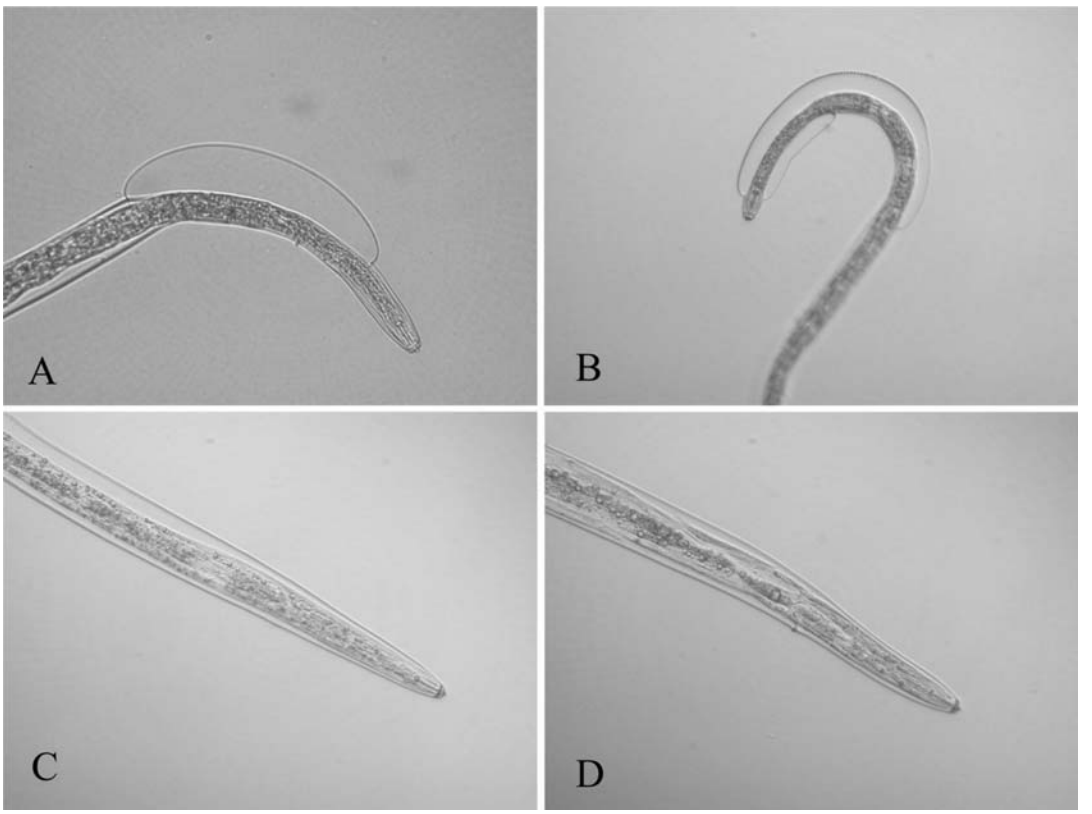

Fig. 1. Cuticle of *Pratylenchus coffeae*. (A) Puffed cuticle on one side of the body of adult female *Pratylenchus coffeae* treated with cranberry juice; (B) Puffed cuticle on both sides of the body of adult female *P. coffeae* treated with cranberry juice; (C) Cuticle of *P. coffeae* treated with fructose; (D) Cuticle of *P. coffeae* treated with fresh pineapple juice.

Table1. Percentages of *Pratylenchus coffeae* with puffed cuticle

Treatment	% Nematodes with puffed cuticle [1]					
	J2	J3+J4	Female	Male	Mixed stages	Control [2]
Cranberry 24 h	55.6 b	76.3 a	47.6 c	11.8 d	52.5	0.0
Cranberry 96 h	69.8 b	88.0 a	70.8 b	37.9 c	75.7	0.0
Fructose (Fong Leng)	0.0	0.0	0.0	0.0	0.0	0.0
Fructose (Sigma)	0.0	0.0	0.0	0.0	0.0	0.0

[1] Means within each row followed by different letters are significantly different at $P \leqq 0.05$ according to Duncan's multiple range test after arcsine square root transformation.

[2] Mixed stages

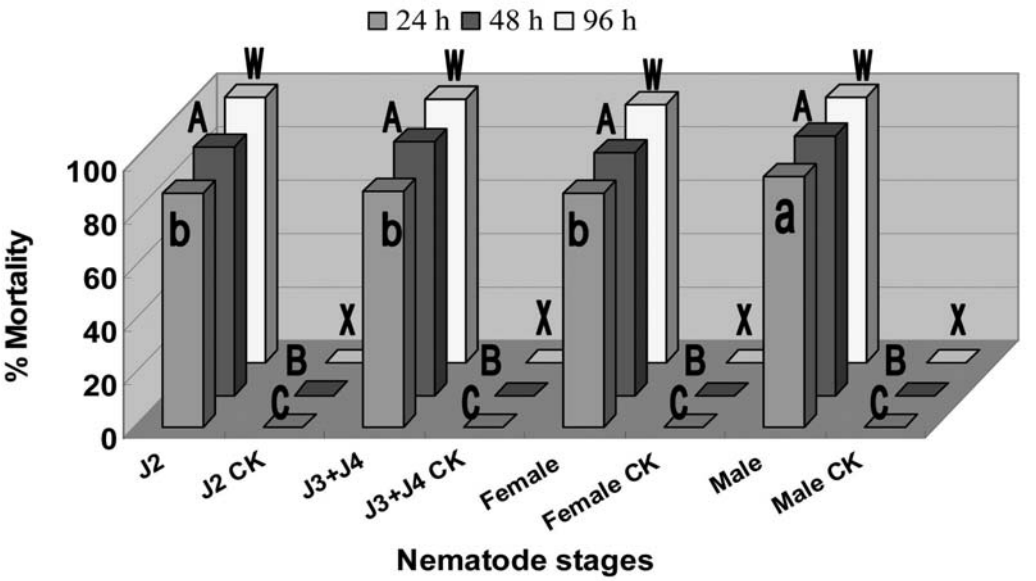

Fig. 2. Mortality of different developmental stages of *Pratylenchus coffeae* after exposure to cranberry juice (1:1 dilution) for 24, 48, and 96 hours. Bars with different letters within each exposure time are significantly different at $P \leqq 0.05$ according to Duncan's multiple range test after arcsine square root transformation.

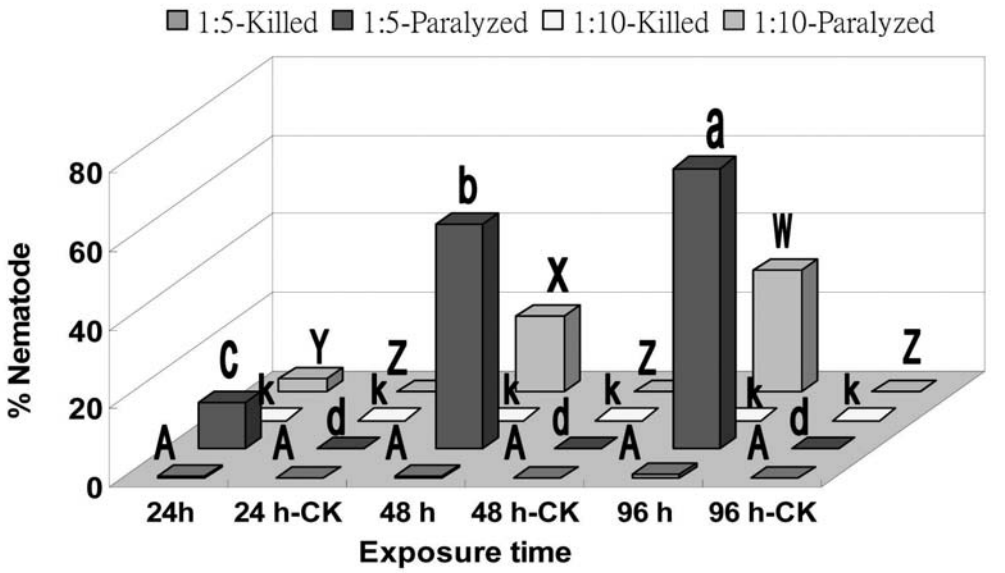

Fig. 3. Effect of cranberry juice diluted to 1:5 and 1:10 on mixed stages of *Pratylenchus coffeae*. Bars with different letters within each category are significantly different at $P \leqq 0.05$ according to Duncan's multiple range test after arcsine square root transformation.

reported that *B. xylophilus* killed in the treatment with muscle activity blocker levamisole hydrochloride appeared to be semicircular shape and those treated with morantal tatrate appeared to be coiled shape. Choi, *et al.*[8] reported that *B. xylophilus* killed by onion oil exhibited an extended, straight form. None of the above mentioned literatures reported puffed cuticles of nematodes. A high-molecular-weight constituent of cranberry juice was reported to inhibit the *Helicobacter pylori* adhesion to human gastric mucus[5]. It was also found to have inhibitory effect on influenza virus adhesion and infectivity[25]. The high-molecular-weight constituent responsible for the inhibition of cranberry juice to bacteria and virus has not been identified. It was only reported as NDM (Nondialysable material). Whether the nematicidal principle in cranberry juice responsible for causing the puffed cuticles was the same as NDM needs further studies.

The hand-squeezed fresh pineapple juice was also found to be highly effective in killing *P. coffeae* in this study (Table 2). Canned pineapple juice was not effective against *P. coffeae*. On the contrary, the nematodes were more active in canned pineapple juice than in the control. The reason for it is unknown. The nematicidal effects of pineapple juice on animal parasites was attributed to cysteine proteinases[20]. Whether the nematicidal effect of fresh pineapple juice on plant-parasitic nematodes discovered in the present studies is also due to cysteine proteinases needs further research. If it is the case, then it is understandable that pineapple juice lost its nematicidal effects after boiling (Table 2), since enzymes are often heat labile. The canned pineapple juice may have lost its effective ingredient in the canning process which involved heating of the juice.

Dilution of cranberry juice to 1:5 (vol/vol) rendered it ineffective in killing *P. coffeae* but the nematodes were paralyzed by the treatment (Fig. 3). The paralyzing effect remained at 1:10 dilution to a lesser degree. Paralyzation of nematodes has been reported to be useful in plant protection. Extract of *Crotalaria* leaves was found to cause paralyzation of the root-knot nematodes and protected tomato roots from infection by the nematodes[12].

In the heat stability test, boiled water control showed that boiling did not cause suffocation of the nematodes. It is possible that the oxygen carried in the nematode

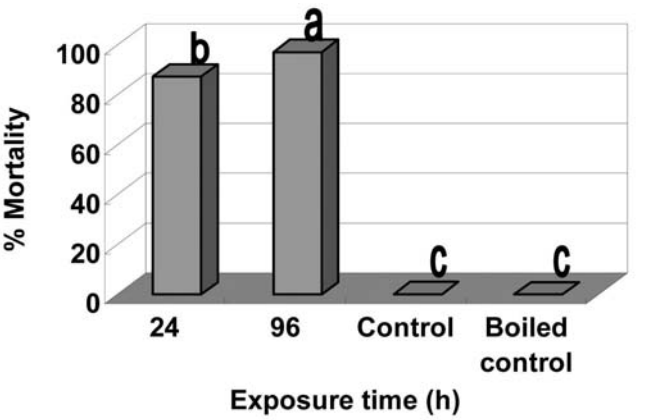

Fig. 4. Mortality of *Pratylenchus coffeae* treated with boiled cranberry juice. Bars with different letters are significantly different at $P \leqq 0.05$ according to Duncan's multiple range test after arcsine square root transformation.

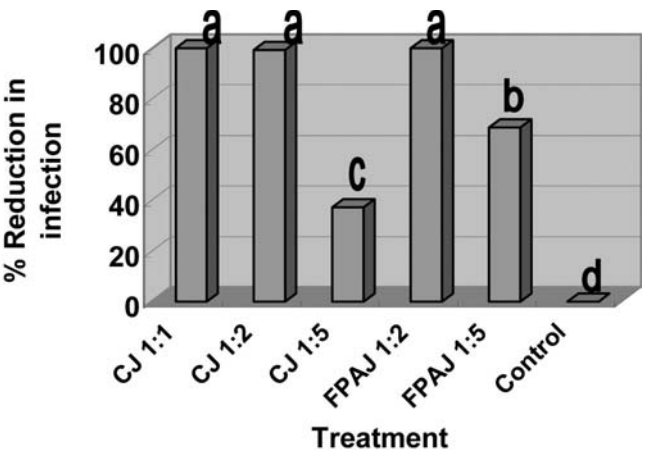

Fig. 5. Effect of cranberry juice (CJ 1:1, 1:2, 1:5 dilution) and fresh pineapple juice (FPAJ 1:2, 1:5 dilution) on the infection of *Pratylenchus coffeae* on mung bean roots. Bars with different letters are significantly different at $P \leqq 0.05$ according to Duncan's multiple range test after arcsine square root transformation.

Table2. Effect of pineapple juice on mixed stages of *Pratylenchus coffeae*

Treatment	% Mortality[1]	
	24 h	96 h
Canned pineapple juice	0.0 c	0.0 c
Fresh pineapple juice (1:2 dilution)	77.6 a	89.5 a
Fresh pineapple juice (1:5 dilution)	3.1 b	2.9 b
Boiled fresh pineapple juice (1:2 dilution)	0.0 c	0.0 c
Control	0.0 c	0.0 c

[1] Means within each column followed by different letters are significantly different at $P \leqq 0.05$ according to Duncan's multiple range test after arcsine square root transformation.

suspension was sufficient for the nematodes as the inoculum was always shaken vigorously in the flask and mixed with lots of air bubbles each time before sucking with a pipet. In addition, the water layer was not very deep in the Petri dish and some oxygen might have diffused from the air into the water. Boiling did not affect the nematicidal activities of cranberry juice against *P. coffeae* (Fig. 4), indicating that the nematicidal principle in cranberry juice was heat stable. On the contrary, boiling rendered the hand-squeezed pineapple juice completely ineffective in killing *P. coffeae* (Table2). The evidence showed that the nematicidal principle in hand-squeezed pineapple juice was heat labile. This is another indication that cranberry juice and pineapple juice have different nematicidal principles besides the difference in their ability to cause puffed cuticle.

Cranberry juice and fresh pineapple juice were not only effective against *P. coffeae in vitro* but also effective in protecting mungbean roots in soil. The findings presents additional example on the natural nematicidal principles existing in our daily diet besides garlic [21, 15], onion [21, 1, 8] and dikon radish [22]. The results of present studies are helpful to the search for alternative nematode control.

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摘 要

蔡碧雲. 2008. 蔓越莓汁與鳳梨汁對 *Pratylenchus coffeae* 之殺線蟲效果. 植病會刊 17 ：111-118.
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蔓越莓汁殺死之線蟲，其表皮常可見類似人類皮膚燙傷時之起泡現象。用蔓越莓汁處理 96 小時後 75.7 % *P. coffeae* 表皮有起泡現象。果糖或鳳梨汁處理之線蟲表皮皆無起泡。蔓越莓汁對不同齡期根腐線蟲皆有良好殺線蟲效果。處理四天後，二齡幼蟲及雄蟲致死率高達 100 %，雌成蟲致死率爲 97 %，三齡及四齡幼蟲致死率爲 99 %。蔓越莓汁稀釋 5 倍已失去殺線蟲效果，但仍有麻痺作用。處理 96 小時後有 71.5 % 之根腐線蟲被麻痺。煮沸兩分鐘對蔓越莓汁之殺線蟲效果沒有影響。罐裝鳳梨汁對根腐線蟲完全沒有殺線蟲效果。手榨新鮮鳳梨汁則對根腐線蟲有良好之殺線蟲效果。煮沸兩分鐘會使手榨新鮮鳳梨汁完全喪失殺線蟲效果。對熱穩定性之差異及對線蟲表皮造成起泡能力之差異顯示蔓越莓汁及新鮮鳳梨汁之殺線蟲成分爲不同物質。於生長箱環境下，蔓越莓汁及新鮮鳳梨汁施用於沙土中皆可顯著降低根腐線蟲侵入綠豆苗根部。

關鍵詞：蔓越莓、鳳梨、*Pratylenchus*、防治