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Effect of peels of lemon, orange, and grapefruit against *Meloidogyne incognita*

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ABSTRACT

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The extracts of fresh peels of lemon, orange, and grapefruit showed significant nematostatic effect against *M. incognita* second stage juveniles after 48 h treatment. The nemticidal activity was very low in all the extracts of fresh peels but was greatly enhanced in the extracts of stored pulpified peels with 90.8 %, 93.5 %, and 85.0 % mortality of nematodes for lemon, orange, and grapefruit, respectively. The data indicated the possibility of essential oils from the citrus peels might have released in the extracts during storage of the pulpified peels. The egg hatch inhibition of the extracts from stored pulpified peels was 85.7 %, 91.0 %, and 78.3 % for lemon, orange, and grapefruit, respectively. The reversibility tests revealed that the effect of extracts on the hatch of eggs was not permanent. The hatching partially resumed after the removal of the extracts but was still significantly lower than the control. The infection of *M. incognita* second-stage juveniles on mung bean roots was significantly inhibited by the extracts of the refrigerator-stored pulpified peels of lemon, orange, and grapefruit. The findings provide an alternative to chemical nematicides for organic farming and help the disposal of citrus juice processing waste as well as the fallen fruits in the orchards in the typhoon season.

Key words: grapefruit, hatch, lemon, *Meloidogyne*, nematicidal, orange, peels

INTRODUCTION

The root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood is an important plant-parasitic nematode in Taiwan. Its control has been relied mainly on chemical nematicides. As organic agriculture is gaining popularity, alternative control tactics need to be developed because chemical pesticides are not acceptable in organic agriculture. Alternative of nematode control is a subject pursued by many researchers in recent years. Among the various possibilities, the search of natural nemticides from plant materials has caught the attention of many researchers [1, 11, 13, 16, 18, 21, 22, 23]. Many plants have been tested for their nematicidal activities. Tsay, *et al.* [22] reported that the extract from roots of *Gaillardia pulchella* was effective for the control of *M. incognita* and *Rotylenchulus reniformis*. Tariq [16], *et al.* reported that stem and leaves of *Avicennia marina* (Forsk.) Vierh. were effective for controlling *M. javanica* in both mash bean and okra. Leaf extracts of noxious weeds *Solanum xanthocarpum* and *Argemone maxicana* were found to be effective for the management of *M. incognita*, *R. reniformis* and *Tylenchorhynchus brassicae* infesting tomato and chilli plants [18]. In addition, leaf extracts of *Argemone mexicana* L. (Papaveraceae), a tropical annual weed, caused juvenile

mortality of *M. javanica* and suppressed gall formation on tomato roots [13]. Tsai [22] reported that extract of petiole of *Raphanus acanthiformis* was effective against *M. javanica*. Pérez [11] found that flowerheads of *Chrysanthemum coronarium* were effective against *M. artiellia*. Zia, *et al.* [24] reported that soil amendments with powdered seeds of *Trigonella foenum graecum* (fenugreek) was effective for soil suppressiveness against *M. javanica*. Additionally, Agbenin, *et al.* [1] used neem seed powder for the control of root-knot nematode on tomato.

While different parts of the plant has been examined, including roots [23], stem [16], leaves [13, 16, 18], flowers [11], and seeds [1, 24], there has been no report on the nemticidal activity of peels of fruits. Since nematicidal priciples exist in so many plants and in various parts of the plants, the existence of nematicidal priciples in the peels of fruits can not be ruled out without experiments. In this paper, the nematicidal activities of peels of lemon, orange, and grapefruit were tested against the root-knot nematode *M. incognita*. Citrus peels are the major part of the juice processing waste. The results of this research can provide alternative nematode control tactic for the organic farming and also help disposing the agro-industrial waste as well as the fallen fruits in the orchards during the typhoon season.

MATERIALS AND METHODS

Preparation of nematodes

A population of *M. incognita* was originally collected from the experimental station of the National Taiwan University and cultured on mung bean (*Vigna radiata* (L.) Wikzek) seedlings grown in Seed-Pack Growth Pouch (Mega International of Minneapolis). Egg masses were dissected from roots and hatched in a hatching chamber. The hatching chamber was made of a small Petri dish with lid (5.4 cm in diam.) containing a thin layer of distilled water over two layers of facial tissue supported by a compressed-styrofoam disposable sauce dish carved with many rectangular holes to serve as a screen. Fresh second-stage juveniles were collected every day and kept at 15℃, and used within three days. The concentration of nematodes was adjusted to approximately 500 nematodes/ml for the *in vitro* tests and 1000 nematodes/ml for the *in vivo* tests. Egg masses were used directly after being dissected from the roots for the tests on inhibition of egg hatch.

Preparation of fruit peel extracts

The fruits of lemon *Citrus limon* (L.) Burm., orange *Citrus sinensis* (L.) Osbeck, and grapefruit *Citrus paradisi* Macf. were purchased from the local supermarket. The peels of the fruits were weighed and cut into small pieces (approximately 0.25 cm^2). Distilled water was added to make 1:3 (w/v) dilution for the lemon and orange peels and 1:5 dilution for the grapefruit peel. The mixture was homogenized in a food blender (Type HR2810/A, Philips, Mexico) at high speed for 2 min. One half of the homogenate was filtered with filter paper (Whatman No. 1) immediately, and another half was stored in a beaker and sealed with Saran wrap and a rubber band and stored in refrigerator for one week before filtering, because the active ingredients in the peels may be released during the storage time.

Effect of extracts on the juveniles

The aliquots of 6 ml of the filtrates and 0.2 ml of nematode suspension were pipeptted into a small Petri dish (5.4 cm diam.) for the test. The Petri dishes were sealed with Parafilm and incubated at 28℃ for 24h and 48 h. After the treatment, nematodes were counted under a dissecting microscope (Olympus SZH). Those nematodes that had no response to touching were considered dead and those that responded were counted as paralyzed. The immobile nematodes were transferred to distilled water and the live and dead nematodes were counted after 24 h to confirm the paralyzation effect. The filtrates of the fresh peels and the refrigerator-stored pulpified peels were tested the same way. Distilled water was used in place of the extracts for the control. There were four replicates for each treatment, and the experiment was repeated twice.

Effect of extracts on egg hatch

The aliquot of 0.3 ml of extracts was loaded into each BPI dish (Bureau of Plant Industry, 1.8 cm inner diam.). Ten egg masses were picked into the extract. Distilled water was used in place of the extracts for the control. The BPI dish was placed in a small Petri dish (5.7 diam.) and sealed with parafilm, and incubated at 28℃ and observed after 24 h and 72 h. The number of nematodes hatched

during the treatment periods was counted and the egg masses were transferred at 24 h and 72 h after treatment from the extracts into distilled water and incubated again for 7 days at 28℃ for the reversibility tests. The number of nematodes hatched in the control during the 24 h and 72 h treatment periods was counted and the egg masses were transferred to other BPI dishes to continue hatching. The number of nematodes hatched after the removal of the extracts was counted. The percentage of inhibition of egg hatch by the extracts was calculated by dividing the number of juveniles hatched in the extract during the treatment period by those in the control. The percentage of inhibition of egg hatch after the removal of the extracts was calculated by dividing the number of juveniles hatched after transferring from the extracts into distilled water by those hatched in the control for the same amount of time. The extracts of the fresh peels and the extracts of the refrigerator-stored pulpified peels were tested the same way. There were four replicates for each treatment. The experiment was repeated twice.

Effect of extracts on infectivity

The aliquot of 1 ml of nematode suspension (1000 nematodes/ml) was added to 150 g of sterile sands in a plastic cup (175 ml), followed by 30 ml of the extracts of fruit peels. Tap water was used in place of the extracts 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 two days. After the incubation, saran wrap was removed and the 5 day old mung bean seedlings were transplanted one for each cup. The plants were returned to the growth chamber at 28℃, 16h photoperiod and kept for three days. The roots were stained with acid-fuchsin [3] and the number of nematodes penetrated the roots were counted. The percentage of infection was calculated as the number of nematodes penetrated the roots/ the number of nematodes inoculated per cup X 100%. The extracts of the fresh peels and the extracts of the refrigerator-stored pulpified peels were tested the same way. There were four replicates for each treatment. The experiment was repeated twice.

Arcsine square root transformation was performed for percentages of all the above experiments before statistical analysis. One-way analysis of variance was carried out with SAS 9.1 software (SAS Institute, Cary, NC, U.S.A.) and treatment means were compared with Duncan's multiple range test at the 95% level of confidence. In the repeated experiment, the data were combined with the first one because the same trend was observed in the two experiments.

RESULTS

The extract of fresh peel of lemon had no effect on *M. incognita* second-stage juveniles after 24 h treatment, however, it paralyzed 90.2 % of the nematodes after 48 h exposure (Table 1). The extracts of fresh peel of orange and grapefruit were slightly nematicidal but were nematostatic after 24h exposure. The nematicidal effect did not increase with time but the nematostatic effect increased to above 80 % after 48 h exposure for orange and grapefruit peels. The percentages of active nematodes were significantly lower than the control in all the

Table1. The effect of extracts of fresh and refrigerator-stored pulpified citrus peels on *Meloidogyne incognita* second-stage juveniles

Treatment[1]	% Nematodes[2]					
	24 h			48 h		
	Dead	Paralyzed	Active	Dead	Paralyzed	Active
Lemon peel	0.0 f	0.0 f	100 a	1.0 e	90.2 a	8.8 c
Orange peel	5.0 d	63.3 a	31.7 c	5.9 c	87.0 a	7.1 c
Grapefruit peel	2.7 e	52.9 b	44.4 b	3.8 d	80.4 b	15.8 b
Stored lemon peel	82.0 b	18.0 d	0.0 e	90.8 a	9.2 d	0.0 e
Stored orange peel	87.1 a	12.9 e	0.0 e	93.5 a	6.5 e	0.0 e
Stored grapefruit peel	70.3 c	27.7 c	2.0 d	85.0 b	13.7 c	1.3 d
Control	0.0 f	0.0 f	100 a	0.2 f	0.0 f	99.8 a

[1] Dilution factor-Peel: distilled water; lemon peel, stored lemon peel, orange peel, and stored orange peel 1:3, grapefruit peel and stored grapefruit peel 1:5.

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

Table 2. The effect of extracts of fresh and refrigerator-stored pulpified citrus peels on the hatch of *Meloidogyne incognita* egg masses

Treatment[1]	% Inhibition of hatch[2]			
	24 h		72 h	
	Immersed[3]	Transferred[4]	Immersed	Transferred
Lemon peel	0.0 d	0.0 e	27.5 e	19.2 e
Orange peel	3.5 c	1.9 d	33.0 d	24.7 d
Grapefruit peel	0.0 d	0.0 e	20.9 f	11.3 f
Stored lemon peel	72.8 a	57.1 b	85.7 b	79.0 b
Stored orange peel	76.3 a	65.0 a	91.0 a	84.2 a
Stored grapefruit peel	60.9 b	39.5 c	78.3 c	69.5 c
Control	0.0 d	0.0 e	0.0 g	0.0 g

[1] Dilution factor-Peel: distilled water; lemon peel, stored lemon peel, orange peel, and stored orange peel 1:3, grapefruit peel and stored grapefruit peel 1:5.
[2] Means within each column with different letters are significantly different at $P \leqq 0.05$ according to Duncan's multiple range test after arcsine square root transformation.
[3] Eggs hatched while the egg masses were immersed in the extracts.
[4] Eggs hatched after the egg masses were transferred to distilled water from the extracts after the treatment.

treatments after 48 h.

After storing the pulpified peels in the refrigerator for one week, their extracts were highly nemticidal (Table 1). There were 82 % nematodes killed by the extract of lemon peel and none of the nematodes were active in the treatment after 24 h. After 48 h exposure, 90.8 % nematodes were dead. Similar situation occurred with the extracts of orange and grapefruit peels. There were 93.5 % and 85 % mortality of *M. incognita* second-stage juveniles in the 48 h treatments of orange and grapefruit peels, respectively.

There were significant but very low level of inhibition on the hatch of eggs from egg masses by the extract of fresh orange peel after 24 h treatment and none by that of lemon and grapefruit peels (Table 2). The inhibition on the hatch of eggs from egg masses increased to 33% after 72 h exposure in the extract of fresh orange peel but the effect was reduced after the egg masses were transferred into distilled water from the extract. Similar situation occurred in the treatments with lemon and grapefruit peels.

The extracts of refrigerator-stored pulpified fruit peels had more pronounced inhibition effect on egg hatching (Table 2). The inhibition of hatch was 72.8 % and 76.3 % with lemon peel and orange peel, respectively. The extract of grapefruit peel (1:5 dilution) was less effective to inhibit the egg hatching. The highest inhibition of hatch after 72 h treatment was 91% with orange peel extract. After transferring the egg masses into distilled water from the extracts, the effect on the inhibition of hatch was reduced but was still significantly higher than the control.

Table 3. The effect of extracts from fresh and refrigerator-stored pulpified citrus peels on the infection of *Meloidogyne incognita* second-stage juveniles on mung bean roots

Treatment[1]	% Reduction in infection[2]	
	Fresh	Stored
Lemon peel	3.2 b	93.3 a
Orange peel	11.7 a	91.5 a
Grapefruit peel	5.9 b	80.0 b
Control	0.0 c	0.0 c

[1] Dilution factor-Peel: distilled water; lemon peel, stored lemon peel, orange peel, and stored orange peel 1:3, grapefruit peel and stored grapefruit peel 1:5.
[2] Means within each column with different letters are significantly different at $P \leqq 0.05$ according to Duncan's multiple range test after arcsine square root transformation.

When the extracts of fruit peels were applied in the soil for 2 days, the infection of *M. incognita* second-stage juveniles on mung bean roots was greatly inhibited by the extracts of the refrigerator-stored pulpified peels from tested fruits (Table 3). The efficacy of extracts from lemon and orange peels were similar, while the efficacy of grapefruit peel was significantly lower than that of lemon and orange peels.

DISCUSSION

The extracts of fresh peels of lemon, orange, and grapefruit showed significant nematostatic activity against

M. incognita second stage juveniles after 48 h treatment (Table 1). All the extracts of fresh peels had very low nematicidal activity but was greatly enhanced when pulpified and stored in refrigerator for one week (Table 1). The difference on the effectiveness between the two showed that allowing time for the active ingredients to dissolve into the extracts was important for the effectiveness. The same trend occurred in the tests for the inhibition of egg hatch; only low inhibition level of hatch was observed with fresh peels extracts but the efficacy was greatly increased in the extracts of stored pulpified peels (Table 2). The reversibility tests revealed that the inhibition effect of extracts was not permanent, the hatching partially resumed after the removal of the extracts but was still significantly lower than the control. Based on this finding, heavy rainfall in field may interfere with the effect of the fruit peels for nematode control. However, it is the same way with the chemical nematicides which are also affected by heavy rainfall.

Utilization of organic amendments for the control of plant-parasitic nematodes has been studied by many researchers [2, 7, 8, 15, 17]. Chitin amendment was used to control *Heterodera avenae* and *Tylenchulus semipenetrans* [15]. It was also used in combination with biocontol agents for soybean cyst nematode *H. glycines* [17]. Yard waste compost was very effective for the control of *Paratrichodorus minor* and provided a convenient means for disposal of a common waste product from urban areas [7]. Utilization of agro-industrial wastes for nematode control has also been studied. Akhtar [2] reported that sugarcane trash was beneficial in nematode control. Nico, *et al.* [8] used composted dry cork for potting mixtures to manage *Meloidogyne* spp. and found that amendment with dry cork reduced the final nematode population by 87.9% in olive in the pot. Tiyagi and Alam [19] evaluated the efficiency of oil-seed cakes of neem (*Azadirachta indica*), castor (*Ricinus communis*), and mustard (*Brassica campestris*) against plant-parasitic nematodes and found that the population of *M. incognita* and *R. reniformis* were significantly reduced by these treatments. A several-fold improvement was observed in plant growth parameters on mung bean, and the residual effects of oil-seed cakes were also noted in the subsequent crop, chickpea, in the next growing season. Agro-industrial wastes in large quantity could become an environmental problem. The use of agro-industrial wastes for nematode control not only provides alternatives to chemical nematicides but also help disposing the agro-industrial wastes. The citrus industry is one of the major agro-industries worldwide. The processing of citrus juice produces tremendous amount of waste. The present findings showed that peels of lemon, orange, and grapefruit could be used for the control of *M. incognita*. This provides an alternative to chemical nematicides for organic farming. It can also help the disposal of citrus juice processing wastes or non-marketable fruits in the orchards.

Essential oils have been identified in the peels of lemon, orange, and grapefruit [6, 20]. Essential oils of plants have been shown to possess nematicidal acivity [4, 9, 10, 11]. Oka *et al.* [9] reported that 12 of the essential oils extracted from25 plant species inhibited mobility and hatching of *M. javanica*. The essential oil of *Chrysanthemum coronarium* flowerheads showed strong nematicidal activity against *Meloidogyne artiellia* [11]. Park, *et al.* [10] reported that essential oil from garlic (*Allium sativum*) was effective against the pine wood nematode, *Bursaphelenchus xylophilus*. Onion (*Allium cepa*) oil was also effective against *B. xylophilus* [4]. The extracts from the stored pulpified fruit peels was more effective than the extract from fresh peels in killing nematodes and inhibiting hatch of eggs. The data indicated that certain ingredient was dissolved in the extracts from the peels during storage and made them more effective. The filtrate of the stored pulpified lemon peel was light green in color instead of light yellowish green as in the fresh one. One possibility is that part of the essential oils in the fruit peels might have dissolved in the filtrates during the storage. The color change of the filtrate further supported this speculation.

Limonene was the main component of the essential oils of lemon, grapefruit [6] and sweet orange [20]. It has been reported to be effective against termite [12], beetles [21], and fungi [14]. No nematicidal activity of limonene *per se* has been tested. However, Duschatzky, *et al.* [5] reported that essential oil isolated from *Aloysia triphylla* killed more than 80% of the juveniles of the root-knot nematode *Meloidogyne* species and that limonene was one of the components (12.7%) of the essential oil. Whether the nematicidal effect of lemon, orange, and grapefruit peels in the present findings was due to limonene needs further study.

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摘　要

蔡碧雲. 2008. 檸檬皮、橘子皮、及葡萄柚皮對 *Meloidogyne incognita* 之影響. 植病會刊 17: 195-201. (台北市 國立台灣大學植物病理與微生物學系；電子郵件：bieyntm@ntu.edu.tw；傳眞：+886-2-2363-6490)

新鮮之檸檬皮、橘子皮、及葡萄柚皮之抽出液對 *Meloidogyne incognita* 之二齡幼蟲有顯著麻痺作用，但致死效果很低。將果皮打成漿狀後貯存於冰箱一星期可大幅提升其抽出液之殺線蟲效果。貯存後之檸檬、橘子、及葡萄柚皮漿之抽出液的殺線蟲效果分別爲 90.8 %、93.5 % 及 85.0 %。實驗數據顯示檸檬皮、橘子皮、及葡萄柚皮所含之精油可能在貯存期間溶解至抽出液中。貯存後之檸檬、橘子、及葡萄柚皮漿之抽出液對卵塊孵化之抑制率分別爲 85.7 %、91.0 % 及 78.3 %。將卵塊自抽出液中移到蒸餾水後，卵可繼續孵化，但總孵化率仍較對照組低。*M. incognita* 對綠豆苗根部之侵入顯著受到貯存後之檸檬、橘子、及葡萄柚皮漿抽出液之抑制。本研究結果可供有機農業用於對線蟲之防治，並且有助於果汁製造業廢棄物之循環利用及颱風季節柑桔園落果之再利用。

關鍵詞：檸檬皮、橘子皮、葡萄柚皮、抽出液、殺線蟲、孵化、*Meloidogyne*