

## Bio-nematicidal properties of some citrus peel extracts against the golden potato cyst nematode, *Globodera rostochiensis* (Wollenweber, 1923) behrens, 1975 (Tylenchida: Heteroderidae)

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### Abstract

The extracts of peels of kumquat, pomelo and bergamot showed significant bio-nematicidal effect against *Globodera rostochiensis* second-stage juveniles after 48h application. The bio-nematicidal activity was very low in all the extracts of fresh peels but was greatly increased in the extracts of stored pulped peels with 92.4 %, 94.8 % and 86.4 % mortality of nematodes for kumquat, pomelo and bergamot respectively. The data showed that essential oils from citrus peels may have been released in the extracts during storage of the pulped citrus peels. The egg hatch inhibition of the extracts from stored pulped citrus peels was 86.3 %, 93.3 % and 81.1 % for kumquat, pomelo and bergamot respectively. The reversibility tests revealed that the effect of citrus extracts on the hatch of eggs was not permanent. The hatching partially resumed after the removal of the citrus peel extracts but was still significantly lower than the control group. The infection of *Globodera rostochiensis* second-stage juveniles on potato seedling roots was significantly inhibited by the extracts of the cooler-stored pulped peels of kumquat, pomelo and bergamot. The results support the use of biologically derived preparations with almost no side effects, as an alternative to chemical preparations that have serious adverse effects on the environment and human health in agricultural pest control.

**Keywords:** fruit peel extract, *globodera rostochiensis*, nematode, bio-control

### Introduction

Nematodes or roundworms that feed on plants, called plant-parasitic nematodes (PPNs), are recognized as one of the greatest threats to crops throughout the world. They cause a loss of billion dollars of crop losses in the world each year. Nematodes alone or in combination with other soil microorganisms have been found to feed on almost every part of the plant including roots, stems, leaves, fruits and seeds (Perry and Moens, 2006; Karakas and Bolukbasi, 2019) <sup>[1, 2]</sup>.

Although PPNs may live within or on the plant parts, most nematodes, most nematodes inhabit the thin film of moisture around soil particles. The soil around small plant roots and root hairs is a particularly rich habitat for many kinds of nematodes. Nematodes which feed on plants may cause damage by themselves or they may join with other soil-borne organisms such as viruses, fungi and bacteria, to promote disease development. Most often, nematode feeding reduces the flow of water and nutrients into the plant, increasing the plant's susceptibility to other stress factors such as heat, water and nutritional deficiencies (Robinson, 2003; Zuckerman, 1971) <sup>[3, 4]</sup>.

Cyst nematodes cause a variety of plant diseases, mostly in the temperate regions of the world. Some species of cyst nematodes attack only a few plant species and are present over limited geographic areas, whereas others attack a large number of plant species and are widely distributed. The genera *Heterodera* and *Globodera* represent one of the largest groups of economically important PPNs. These nematodes are known by the common name "cyst nematodes" (Evans and Rowe, 1998) <sup>[5]</sup>.

Two species of PPNs, *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975 (the golden or yellow

potato cyst nematode) and *Globodera pallida* Stone, 1973 (the pale or white potato cyst nematode) are commonly referred to as "Potato Cyst Nematodes (PCNs)". Both species feed on and produce cysts on potato roots, thus causing substantial damage to potato crops. They also cause damage to tomatoes and eggplant and can also attack other members of the Solanaceae plant family, such as nightshade (Evans, 1977; Kort *et al.*, 1977; Van Riel and Mulder, 1998) <sup>[6, 7, 8]</sup>.

In the management against pests today, there is a global search for alternatives to chemical pesticides and as part of this process there are various efforts to test the use and efficacy of natural products for pest control and crop protection (Kokalis-Burelle and Rodríguez-Kábana, 2006) <sup>[9]</sup>.

Nematicides are a type of pesticide. These are synthetic chemicals for chemical control that have a lethal effect on nematodes. But these have many negative effects on the ecosystem (Kokalis-Burelle and Rodríguez-Kábana, 2006) <sup>[9]</sup>.

Bio-pesticides, also known as biological pesticides, are pesticides derived from natural materials such as animals, plants, bacteria, and certain minerals. Typically, bio-pesticides have unique modes of action and are considered reduced risk pesticides (D'Addabbo *et al.*, 2014) <sup>[10]</sup>.

Bio-pesticides products, compared to broad spectrum chemical pesticides, are usually more target specific and inherently less toxic which would cause less impact to other non-target species, such as other insects, birds and mammals, while application. In addition, bio-pesticides are often effective in low application rate and could decompose quickly in natural environment. This leads to lower exposures and largely avoid the pollution problems caused

by conventional pesticides. Besides, chance of pests developing resistance is much lowered (Sprag et al., 2004; D'Addabbo et al., 2014) [11, 10].

In this paper, the bio-nematicidal properties of peels of kumquat (*Fortunella margarita* Swing.), pomelo (*Citrus maxima* (Burm.) Merr.) and bergamot (*Citrus bergamia* Risso) were tested against the golden potato cyst nematode *G. rostochiensis*.

## Materials and Methods

### Preparation of nematode species

A population of *G. rostochiensis* was collected from the potato (*Solanum tuberosum* L.) fields of Ödemiş-İzmir, Turkey (38° 13' 8.4216" N - 27° 58' 18.3432" E) and cultured on potato seedlings grown in greenhouse. Cysts were dissected from roots and hatched in an incubator. Fresh second-stage juveniles (J2) were collected every day and kept at 16 ± 2 °C, and used within two or three days. The concentration of nematodes was adjusted to approximately 350 nematodes/ml for the *in vitro* tests and 700 nematodes /ml for the *in vivo* tests. Cysts were used directly after being dissected from the roots for the tests on inhibition of hatching.

### Preparation of citrus peel extracts

The citrus of kumquat (*F. margarita*), pomelo (*C. maxima*) and bergamot (*C. bergamia*) were obtained from a local supermarket of Antalya (36° 54' 29" N - 30° 41' 44" E). The peels of the citrus were weight and cut into small pieces (approx. 0.50 cm<sup>2</sup>). Distilled water was added to make 1:5 (w/v) dilution for each citrus peel. The mixture was homogenized in a blender at high speed for 2 minutes. One half of the homogenate was filtered with Whatman No.1 filter paper immediately, and another half was preserved in a glass jar and then stored in a cooler at +4 °C for one week before filtering, because the active compounds in the citrus peels may be released during the storage time.

### Extract-juvenile application

For this section, 10 ml of the filtrates and 0.4 ml of the nematode suspension were pipetted into a Petri dish (90 mm diam.) for testing. The Petri dishes were sealed with Parafilm M and incubated at 28 ± 1 °C for 24h and 48h. After the treatment, nematodes were counted under a dissecting microscope (DSS 10x and 20x). Nematodes that did not respond to touch were considered dead as paralyzed. Immobilized nematodes were transferred to distilled water and live and dead nematodes were counted after 24 hours to confirm the paralysis effect. The filtrates of fresh citrus peels and pulped peels stored in the +4 degree cooler were tested in the same way. Distilled water was used in place of the extracts for the control. There were three replicates for each application and the experiment was repeated twice.

### Extract-cyst application

The extracts were divided into 0.5 ml observation dishes with a diameter of 2 cm. Ten cysts were picked into the extract. Distilled water was used in place of the extracts for the control group. The observation dish was placed in a Petri dish (90 mm diam.) and sealed with Parafilm M and incubated at 28 ± 1 °C and observed after 24h and 72h. The number of nematodes hatched during the application periods was counted and the cysts were transferred at 24h and 72h after application from the citrus extracts into distilled water

and incubated again for 10 days at 28 ± 1 °C for the reversibility tests. The number of nematodes hatched in the control during the 24h and 72h application periods was counted and the cysts were transferred to other observation dishes to continue hatching. The number of nematodes hatched after the removal of the citrus extracts was counted. The percentage of inhibition of egg hatch by the citrus extracts was calculated by dividing the number of juveniles (Js) hatched in the extract during the application period by those in the control. The percentage of inhibition of egg hatch from the cysts after the removal of the citrus extracts was calculated by dividing the number of Js hatched after transferring from the citrus extracts into distilled water by those hatched in the control for the same amount of time. The citrus extracts of the fresh peels and the citrus extracts of the cooler-stored pulped peels were tested by same way. In each application, the number of repetitions was determined as three and the number of experiments as two.

### Infectivity test of citrus peel extracts

Nematode suspension (approx. 1000 nematodes/ml) was added to 200 g of sterile sands in a plastic pot (250 ml), followed by 40 ml of the extracts of citrus peels. Tap water was used in place of the citrus extracts for the control group. The plastic pots were then sealed with plastic bag and rubber band to prevent evaporation. They were incubated at 28 ± 1 °C in a growth chamber for three days. After the incubation, plastic bag was removed and the 7 day old potato seedlings were transplanted one for each pot. The plants were returned to the growth chamber at 28 ± 1 °C, 12h/12h photoperiod and kept for five days. After cleaning and staining plant tissues for detection of nematodes, the roots were specially stained with acid-fuchsin 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 pot X 100%. The citrus extracts of the fresh peels and the citrus extracts of the cooler-stored pulped peels were tested the same way. In each application, the number of repetitions was determined as three and the number of experiments as two.

Experimental findings were evaluated by one-way analysis of variance and applications means were compared with Duncan's multiple range test (DMRT) at the 95% level of confidence.

## Results

The extract of fresh peel of kumquat had very low level effect on *G. rostochiensis* second-stage juveniles (J2s) after 24h application, however, it palsied 95.6% of the nematodes after 48h exposure (Table 1). The extracts of fresh peel of pomelo and bergamot were moderately bio-nematicidal but were nemato-toxic after 24h exposure. The bio-nematicidal effect did not increase with time but nemato-toxic effect increased to above 85.0% after 48h exposure for pomelo and bergamot peels. The percentages of mobile nematodes were significantly lower than the control group in all the applications after 48h.

After storing the pulped peels in the cooler for one week, their extracts were highly bio-nematicidal (Table 1). There were 85.1% nematodes killed by the extract of kumquat peel and none of the nematodes were mobile in the application after 24h. After 48h exposure, 92.4% nematodes were dead. Similar result occurred with the extracts of pomelo and

bergamot peels. There were 94.8% and 86.4% mortality of *G. rostochiensis* J2s in the 48h applications of pomelo and bergamot peels, respectively.

**Table 1:** The effect of citrus peel extracts (fresh-pulped) on *Globodera rostochiensis* J2s

Extracts *	Nematodes (%) **					
	24h			48h		
	Dead	Palsied	Mobile	Dead	Palsied	Mobile
Fresh kumquat peel	1.0 <sup>e</sup>	1.0 <sup>e</sup>	98.0 <sup>a</sup>	1.8 <sup>e</sup>	95.6 <sup>a</sup>	2.6 <sup>e</sup>
Fresh pomelo peel	6.8 <sup>d</sup>	68.6 <sup>a</sup>	24.6 <sup>c</sup>	6.9 <sup>c</sup>	91.1 <sup>a</sup>	2.0 <sup>e</sup>
Fresh bergamot peel	4.2 <sup>d</sup>	56.3 <sup>b</sup>	39.5 <sup>b</sup>	5.6 <sup>d</sup>	85.3 <sup>b</sup>	9.1 <sup>d</sup>
Pulped kumquat peel	85.1 <sup>b</sup>	14.9 <sup>d</sup>	0.0 <sup>e</sup>	92.4 <sup>a</sup>	7.6 <sup>d</sup>	0.0 <sup>e</sup>
Pulped pomelo peel	89.4 <sup>a</sup>	10.6 <sup>e</sup>	0.0 <sup>e</sup>	94.8 <sup>a</sup>	5.2 <sup>e</sup>	0.0 <sup>e</sup>
Pulped bergamot peel	73.7 <sup>c</sup>	25.3 <sup>c</sup>	1.0 <sup>e</sup>	86.4 <sup>b</sup>	11.3 <sup>c</sup>	2.3 <sup>d</sup>
Control group	0.0 <sup>f</sup>	0.0 <sup>f</sup>	100.0 <sup>a</sup>	0.0 <sup>f</sup>	0.0 <sup>f</sup>	100.0 <sup>a</sup>

\*Dilution factor – Peel: Distilled water; fresh kumquat peel and pulped kumquat peel – fresh pomelo peel and pulped pomelo peel 1:3, fresh and pulped bergamot peel 1:5.

\*\*Means within each column with different letters are significantly different at  $P \leq 0.05$  according to DMRT.

There were significant but very low level of inhibition on the hatch of eggs from cysts by the extracts of fresh pomelo and bergamot peels after 24h application, and none by that

**Table 2:** The effect of citrus peel extracts (fresh-pulped) on the hatch of *Globodera rostochiensis* eggs

Extracts *	Hatching inhibition (%) **			
	24h		72h	
	Immersed ***	Transferred ****	Immersed	Transferred
Fresh kumquat peel	0.0 <sup>d</sup>	0.0 <sup>e</sup>	30.1 <sup>e</sup>	22.6 <sup>e</sup>
Fresh pomelo peel	4.1 <sup>c</sup>	2.3 <sup>d</sup>	37.7 <sup>d</sup>	27.5 <sup>d</sup>
Fresh bergamot peel	0.1 <sup>d</sup>	0.1 <sup>e</sup>	24.3 <sup>f</sup>	12.9 <sup>f</sup>
Pulped kumquat peel	75.5 <sup>a</sup>	58.6 <sup>b</sup>	86.8 <sup>b</sup>	80.2 <sup>b</sup>
Pulped pomelo peel	80.3 <sup>a</sup>	71.2 <sup>a</sup>	93.3 <sup>a</sup>	86.2 <sup>a</sup>
Pulped bergamot peel	64.7 <sup>b</sup>	43.3 <sup>c</sup>	81.1 <sup>c</sup>	73.6 <sup>c</sup>
Control group	0.0 <sup>d</sup>	0.0 <sup>e</sup>	0.0 <sup>g</sup>	0.0 <sup>g</sup>

\*Dilution factor – Peel: Distilled water; fresh kumquat peel and pulped kumquat peel – fresh pomelo peel and pulped pomelo peel 1:3, fresh and pulped bergamot peel 1:5. \*\*Means within each column with different letters are significantly different at  $P \leq 0.05$  according to DMRT.

\*\*\*Eggs hatched while the cysts were immersed in the citrus extracts. \*\*\*\*Eggs hatched after the cysts were transferred to distilled water from the citrus extracts after the application.

**Table 3:** The effect of citrus peel extracts (fresh-pulped) on the infection of *Globodera rostochiensis* J2s on potato seedling roots

Extracts *	Decrease in infection (%) **	
	Fresh	Pulped
Kumquat peel	4.1 <sup>b</sup>	94.6 <sup>a</sup>
Pomelo peel	13.3 <sup>a</sup>	88.7 <sup>a</sup>
Bergamot peel	6.6 <sup>b</sup>	78.4 <sup>b</sup>
Control group	0.0 <sup>c</sup>	0.0 <sup>c</sup>

\*Dilution factor – Peel: Distilled water; fresh kumquat peel and pulped kumquat peel – fresh pomelo peel and pulped pomelo peel 1:3, fresh and pulped bergamot peel 1:5.

\*\*Means within each column with different letters are significantly different at  $P \leq 0.05$  according to DMRT.

**Discussion**

The extracts of fresh peels of kumquat, pomelo and bergamot showed significant bio-nematicidal activity against *G. rostochiensis* J2s after 48h application. All the extracts of fresh citrus peels had very low bio-nematicidal activity but was greatly improved when pulped and stored in a cooler for one week. The difference in effectiveness between the two showed that allowing time for the active ingredients to dissolve in the extracts is important for efficacy. The same tendency occurred in the test for the

of kumquat peel (Table 2). The inhibition on the hatch of eggs from cysts increased to 37.7% and 24.3% after 72h exposure in the extracts of fresh pomelo and bergamot peels but the effects were reduced after the cysts were transferred into distilled water from the citrus extracts. Similar result occurred in the application with kumquat peel.

The extracts of cooler-stored pulped peels had more prominent inhibition effect on egg hatching (Table 2). The inhibition of hatch was 75.5% and 80.3% with kumquat peel and pomelo peel respectively. The extract of bergamot peel was less effective in inhibition the egg hatching with 64.7%. The maximum inhibition of hatch after 72h application was 93.3% with pomelo peel extract. After transferring the cysts into distilled water from the extracts, the effect on the inhibition of hatch was reduced but was still significantly higher than the control group.

When the citrus peel extracts were applied in the soil for 4 days, the infection of *G. rostochiensis* J2s on potato seedling roots was greatly inhibited by the citrus extracts of the cooler-stored pulped citrus peels from tested citrus (Table 3). The efficacy of citrus peel extracts were similar for kumquat and pomelo, while the efficacy of bergamot peel was significantly lower than that of kumquat and pomelo peels.

inhibition of egg hatch; only low inhibition level of hatch was detected with fresh citrus peel extracts but the efficacy was greatly increased in the extracts of stored pulped peels. Reversibility tests revealed that the inhibition effect of the extracts was not permanent. Scanning resumed partially after extraction of the extracts, but still significantly lower than control. Based on this information, heavy rainfall in the field can interfere with the action of fruit peels for nematode control.

Utilization of organic compounds for the control of plant-parasitic nematodes has been studied by many researchers. In researches for this purpose, garden waste compost was very effective for the control of *Paratrichodorus minor* and provided a convenient means of disposal of a common waste product from urban areas (McSorley and Gallaher, 1996) [12]. The use of agro-industrial wastes for nematode control has also been studied. Akhtar (1993) [13] reported that sugarcane trash was beneficial in nematode control. Nico *et al.*, (2004) [14] used compost dried mushrooms for potting mixes to manage *Meloidogyne* species and found that the modification with dried mushrooms reduced the final nematode population in the olive in the pot. Tiyagi and Alam (1995) [15] evaluated the efficacy of neem, castor and

mustard oilseed cakes against plant parasitic nematodes and found that the population of *Meloidogyne incognita* and *Rotylenchulus reniformis* decreased significantly with these treatments.

An important issue that needs to be addressed is that large amounts of agricultural industrial waste can become an environmental problem over time. The use of agro-industrial wastes for nematode control not only provides an alternative to chemical nematicides, but also helps the disposal of agro-industrial wastes. The citrus industry is one of the largest agricultural industries worldwide. Processing citrus juice generates an enormous amount of waste. The present findings showed that kumquat, pomelo, and bergamot peels could be used to control *G. rostochiensis*. This provides an alternative to chemical nematicides for organic farming. It can also aid in the disposal of citrus juice processing waste or non-marketable fruit in orchards.

Abolusoro *et al.*, (2010)<sup>[16]</sup> reported that the suppressive effect of sweet orange (*Citrus sinensis*) peel juicy extract on root-knot nematode (RKN).

Essential oils have been identified in the peels of citrus. Essential oils of plants have been shown to possess bio-nematicidal activity. Oka *et al.*, (2000)<sup>[17]</sup> reported that some of the essential oils extracted from different plant species inhibited mobility and hatching of root-knot nematodes. The essential oil of *Chrysanthemum coronarium* flower heads showed strong bio-nematicidal activity against RKNs. Park, *et al.*, (2005)<sup>[18]</sup> reported that essential oil from garlic was effective against the pine wood nematode, *Bursaphelenchus xylophilus*. Onion oil was also effective against pine wood nematode. The extracts from the stored pulped 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. Limonene was the main component of the essential oils of citrus. It has been reported to be effective against termite, beetles and fungi (Omran *et al.*, 2011; Almeida *et al.*, 2015)<sup>[19, 20]</sup>. No nematicidal activity of limonene has been tested. However, Duschatzky, *et al.*, (2004)<sup>[21]</sup> reported that essential oil isolated from lemon verbena, *Aloysia triphylla* killed the juveniles of the root-knot nematode *Meloidogyne* species and that limonene was one of the components of the essential oil. Therefore, it can be a research subject for future studies.

### Conclusion

Plants are a great source of nematicidal compounds that are components of secondary metabolism involved in plant defence mechanisms against abiotic and biotic agents. These nematicidal compounds can be used directly as plant extracts, phytochemical formulations, or organic modifications, or can be used as model compounds for the development of chemically synthesized derivatives. Herbal-derived nematicides, also called bio-nematicides can comply well with the principles of Integrated Pest Management (IPM), thanks to their safety to the environment, humans and animals, their selective mode of action and the absence of pest resistance. Another advantage of plant-based nematode management strategies is represented by their wide flexibility, as the wide variety of application modes allow adaptability to different crops, seasons and farming systems. In addition, they can be easily combined with other

control applications such as soil solarisation, bio-control agents as well as synthetic nematicides. Finally, some exploitation techniques of nematicidal plants such as green fertilizers or organic modifications can also result in an improvement in the physical and chemical properties of the soil. Numerous plant species have been reported to contain metabolites with nematicidal activity, and new nematicidal herbal-derived compounds have been discovered every year.

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