# EVALUATION OF ROSELLE (*HIBISCUS SABDARIFFA*) LEAF AND POMEGRANATE (*PUNICA GRANATUM*) FRUIT RIND FOR ACTIVITY AGAINST *MELOIDOGYNE INCOGNITA*

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## ABSTRACT

Meyer, S. L., F., K. R. Chauhan, and M. H. MacDonald. 2016. Evaluation of roselle (*Hibiscus sabdariffa*) leaf and pomegranate (*Punica granatum*) fruit rind for activity against *Meloidogyne incognita*. Nematropica 46:85-96.

Pomegranate (*Punica granatum*) fruit and roselle (*Hibiscus sabdariffa*) leaves have been used in traditional medicine, including as anthelmintics. Methanolic extracts from these plants were investigated for activity against the southern root-knot nematode (RKN), *Meloidogyne incognita*. Dried, ground powders were prepared from pomegranate fruit rinds and from roselle leaves. In *in vitro* assays, methanolic extracts from both powders, dried and dissolved in water, inhibited egg hatch and viability of second-stage juveniles (J2). Some of the effect of these extracts on J2 was nematostatic, depending on the extract concentration. The pomegranate extract (pH 3.4 to 3.8) was effective at the tested concentrations of 0.45% to 1.8% (volume extract per volume water), reducing egg hatch up to 93.9%, and killing more than 30% of the J2. Roselle extract concentrations of 4.5% and higher (pH 2.8 to 3.6) suppressed egg hatch by up to 97.4%, and 100% J2 mortality occurred in concentrations of 22.5% and 45.0% extract. Pomegranate and roselle extracts with pH adjusted to higher values (5.9 and 5.0, respectively) were also active against RKN. Pomegranate extract, pH 5.9, resulted in 96.5% to 99.7% egg hatch suppression and 100% J2 mortality at concentrations of 22.5% to 33.8%. Pomegranate fruit rind powder was also tested as a soil amendment in greenhouse trials, and was phytotoxic to cucumber seedlings at application rates of 5.0% and 10.0% (weight dried pomegranate rind/weight dried soil), resulting in plant death. Shoot heights and fresh weights were reduced at 0.25%, 0.5%, and 1.0% powder application rates, and root fresh weights also tended to be lower at these concentrations. The numbers of galls per root system and galls per g root fresh weight were suppressed in the 1.0% application rate. Amendment with pomegranate fruit rind powder resulted in rapid growth of *Rhizopus* sp. and *Aspergillus* sp. on the soil. Suppression of RKN egg hatch and J2 activity by extracts from pomegranate fruit rinds and roselle leaves indicate that these plant-derived products are potential candidates for future studies of nematode-antagonistic compounds.

*Key words:* gongura, *Hibiscus sabdariffa*, management, *Meloidogyne incognita*, natural products, plant extracts, pomegranate, *Punica granatum*, root-knot nematode, roselle, soil amendments.

## RESUMEN

Meyer, S. L., F., K. R. Chauhan, y M. H. MacDonald. 2016. Evaluación de la actividad frente a *Meloidogyne incognita* de las hojas de la rosa de jamaica (*Hibiscus sabdariffa*) y de la cáscara de la granada (*Punica granatum*). Nematropica 46:85-96.

El fruto del granado (*Punica granatum*) y las hojas de la rosa de jamaica (*Hibiscus sabdariffa*) han tenido un uso tradicional en medicina como antihelmínticos. Se investigó la actividad de los extractos metanólicos de estas plantas frente al nematodo formador de agallas en las raíces, *Meloidogyne incognita*. Se prepararon triturados secos de cáscara de granada y hojas de rosa de jamaica. En ensayos *in vitro*, los extractos metanólicos de ambos triturados, disueltos en agua, inhibieron la eclosión de los huevos y la viabilidad de los juveniles de segundo estadio (J2). Dependiendo de la concentración del extractos, el efecto sobre los J2 fue en ocasiones nematostático. El extracto de granada (pH 3.4 a 3.8) fue efectivo a las concentraciones de 0.45% a 1.8% (volumen de extracto por volumen de agua), reduciendo la eclosión de los huevos hasta 93.9%, y matando más del 30% de los J2.

El extracto de rosa de Jamaica a concentraciones mayores del 4.5% (pH 2.8 a 3.6) suprimió la eclosión de los huevos hasta 97.4%, y ocasionó una mortalidad del 100% de los J2 a concentraciones del 22.5% y 45.0% del extracto. Extractos de granada y rosa de Jamaica con pH ajustados a valores mayores (5.9 y 5.0, respectivamente) también fueron activos frente a *M. incognita*. El extracto de granada, pH 5.9, suprimió la eclosión de los huevos en 96.5% a 99.7% y causó una mortalidad de los J2 a concentraciones de 22.5% a 33.8%. El triturado de cáscara de granada también fue probado como enmienda del suelo en ensayos en invernadero, y resultó fitotóxico para plantones de pepino a dosis de aplicación del 5.0% y 10.0% (peso seco del triturado de cáscara de granada/peso seco del suelo), ocasionando muerte de la planta. La longitud y los pesos frescos de las plantas se redujeron a concentraciones de triturado del 0.25%, 0.5%, y 1.0%, y el peso fresco de las raíces también mostró una tendencia a ser menor a estas concentraciones. El número de agallas por sistema radical y por g de raíz se redujo a dosis de aplicación del 1.0%. Las enmiendas con triturados de cáscara de granada ocasionaron crecimientos rápidos de *Rhizopus* sp. y *Aspergillus* sp. en el suelo. Las reducciones en la eclosión de huevos y actividad de los J2 del nematodo formador de agallas en las raíces debidas a los extractos de cáscara de granada y hojas de rosa de Jamaica indican que estos productos naturales obtenidos de plantas son candidatos potenciales para futuros estudios de compuestos antagonistas de nematodos.

*Palabras claves*: Rosa de jamaica, *Hibiscus sabdariffa*, manejo, *Meloidogyne incognita*, productos naturales, extractos de plantas, granado, *Punica granatum*, nematodo formador de agallas en las raíces, enmiendas al suelo.

## INTRODUCTION

Deregistration of synthetic nematicides has led to a need for alternative means of managing plant-parasitic nematodes. Biological products, including plant-derived compounds and soil amendments, are therefore being investigated for nematicidal activity. In particular, plants that have demonstrated anthelmintic activity, or that are known to produce nematotoxic chemicals, are potential sources for biologically based management agents that could be applied or modified for use against phytopathogenic nematodes.

Pomegranate (*Punica granatum* L.) is a candidate plant for such products. Water extracts of pomegranate fruit powder reduced motility and viability of the plant-parasitic nematodes *Meloidogyne incognita* (Kofoid & White) Chitwood and *Helicotylenchus dihystera* (Cobb) Sher, and inhibited egg hatch of root-knot nematodes (RKN) (Korayem *et al.*, 1993). Crushed peels applied as a soil amendment suppressed *Meloidogyne javanica* (Treub) Chitwood on tomato (*Solanum lycopersicum* L.) (Ismail, 2015). Pomegranate has also been used in traditional medicine as a cure for intestinal worms (Ismail *et al.*, 2012), and an alcohol extract from pomegranate peels reduced strongyle egg counts from female goats, although peel powder was not effective (Boonmasawai *et al.*, 2013). Although the compounds toxic to plant-parasitic nematodes have not been identified, pomegranate fruits contain 124 phytochemicals, with polyphenols as the primary components (Viuda-Martos *et al.*, 2010; Akhtar *et al.*, 2015; García-Villalba *et al.*, 2015). Many phenolic substances are nematicidal or act as nematode repellants or inhibitors of motility (Ohri and Pannu, 2010; Ntalli and Caboni, 2012). Approximately 48 phenolics, including tannins, and flavonoids such as anthocyanins, have been found in the peel and other fruit parts (Viuda-Martos *et al.*, 2010; Ismail *et al.*, 2012; Akhtar *et al.*, 2015). Pomegranate husk and peel (the outer skin and mesocarp), which are waste byproducts of the pomegranate juice industry, contain higher levels of polyphenols, particularly ellagitannins, than the seeds, and are therefore potential sources of compounds that might act as biological nematicides (Seeram *et al.*, 2005; Akhtar *et al.*, 2015; García-Villalba *et al.*, 2015).

Another plant used in herbal medicine and in the pharmaceutical industry is roselle (*Hibiscus sabdariffa* L.). Roselle, also known as gongura, is grown for fiber, human and animal food, teas, and oil (Da-Costa-Rocha *et al.*, 2014; Sindi *et al.*, 2014). Like pomegranate fruits, the leaves contain ellagic acid, phenolic acids, flavonoids, anthocyanins, and at least ten different polyphenols, along with many other compounds (Da-Costa-Rocha *et al.*, 2014; Zhen *et al.*, 2016). The main compounds identified in leaf extracts were flavonol glycosides (Zhen *et al.*, 2016), and glycosides can also be lethal to plant-parasitic nematodes (Ohri and Pannu, 2010; Ntali and Caboni, 2012). Ellagic acid and gallic acids, which are found in both roselle and pomegranate, have been tested for activity against nematodes. Ellagic acid was toxic to the animal-parasitic nematode *Haemonchus contortus* (Rudolphi) Cobb and both acids were toxic to *Caenorhabditis elegans* (Maupas) (Ndjonka *et al.*, 2013; Mondal *et al.*,

2015). However, fractions from the plant *Hagenia abyssinica* (Bruce) J. F. Gmel. that contained ellagic acid derivatives were not active against *C. elegans* or against four species of trematodes (Thomsen *et al.*, 2012). These results indicate that activity may vary with nematode taxon, testing condition, amounts of the acid or ellagic acid vs. derivatives.

In laboratory assays, ethanolic leaf extract of roselle reduced motility in the microfilariae (mf) and female adult stages of *Brugia malayi* Brug, the nematode that causes lymphatic filariasis in humans (Saxena *et al.*, 2011). The greatest activity was from an n-butanol insoluble fraction, which had high concentrations of anthocyanin-glycosides. Additionally, the leaf extract killed 30% or more of the adult worms in the Mongolian gerbil *Meriones unguiculatus* Milne-Edwards and the southern multimammate mouse *Mastomys coucha* Smith, with less (*M. coucha*) or no effect (*M. unguiculatus*) on mf viability (Saxena *et al.*, 2011). To our knowledge, roselle leaves have not been tested for management of plant-parasitic nematodes. However, many varieties of roselle are resistant to RKN, including *Meloidogyne arenari*a (Neal) Chitwood, *M. incognita*, and *M. javanica* (Wilson and Menzel, 1964; Adeniji, 1970; Minton *et al.*, 1970; Vawdrey and Stirling, 1992), and resistance of plants to pathogens may include constitutive or induced production of chemical defenses (Kaplan *et al.*, 2008; Rasmann and Agrawal, 2008; Wurst *et al.*, 2010; Baetz and Martinoia, 2014). Some of these natural compounds might have nematicidal activity.

The presence of nematicidal chemicals in pomegranate husk and peel and roselle leaves indicates that these plants are potential sources of biologically based products or amendments that could be useful for nematode management. Reports of nematode suppression with pomegranate powders heighten this possibility. Therefore, these plant parts were selected for our study. The goals of this research were to determine whether: i) extracts from pomegranate rinds or from roselle leaves would inhibit RKN egg hatch and (or) be lethal to J2, and ii) dried pomegranate rinds, applied as a soil amendment, could suppress RKN populations on cucumber seedlings.

## MATERIALS AND METHODS

### *Preparation of powdered plant material and extracts*

Pomegranate fruits (cv. Wonderful) were purchased from a local grocery store (Shoppers Food Warehouse, College Park, MD). Pomegranate rinds (the outer red skins (exocarp) with a thin layer of attached white mesocarp) were peeled from the fresh fruits, and the seeds and large pieces of mesocarp were discarded. Roselle seeds were purchased from Seeds of India, LLC (Marlboro, NJ, USA). The roselle plants were grown at the Beltsville Agriculture Research Center, North Farm fields, during June-October, 2014. The plants were watered through drip irrigation and no fertilizers were applied. Fresh leaves were harvested after 50 days and air-dried for 120 to 150 hr in a 3.7 m × 3.7 m room with a relative humidity of 20 to 23% and temperature of 22 to 25°C. The pomegranate rinds were dried at room temperature, and then placed in an oven and dried at 121°C for one week. Dried samples of both plant materials were ground with a mechanical grinder to produce a powder that would pass through a 0.002- to 0.005-µm mesh. Both types of powder were stored in plastic bags at 2 to 4°C until use.

To make extracts, 50 g of powder was suspended in 500 ml of HPLC grade methanol (97.5% purity, Sigma–Aldrich, St. Louis, MO) and extracted for 48 hr by mechanical stirring in a 1-L flask at 25°C. The content of the mixture was filtered through analytical filter paper and the residue was rinsed twice with 25 ml of methanol. The combined filtrate was concentrated at reduced pressure with a rotary evaporator (30 to 40°C) until the solvent methanol was distilled off and water droplets began to accumulate in the condenser. The concentrated extract from the flask was weighed, transferred to a sample bottle and stored in a refrigerator. Aqueous samples for the pomegranate and roselle extract bioassays were prepared by dissolving 5 g of neat extract (thick paste or liquid) in 5 ml of water, and filtered to create 10 ml of final test solution.

### *RKN culture and inoculum*

*Meloidogyne incognita* Race 1, originally isolated in Maryland, was grown on pepper (*Capsicum annuum* L.) cv. PA-136 in a greenhouse maintained at 24°C to 29°C, with natural lighting. This greenhouse was also used for the experiments. For laboratory assays with RKN and plant extracts, egg masses were hand-picked from roots and immersed in 0.6% sodium hypochlorite for 1 to 3.5 min to separate and surface-sterilize eggs. Eggs were then collected on a 25 µm-diam. mesh sieve, rinsed in sterile deionized water (SDW), and stored overnight at 4°C. To collect previously hatched second-stage juveniles (J2) for direct immersion into extracts, sterilized eggs were placed into a hatching chamber comprised of a Spectra/Mesh Nylon Filter (openings 25 µm in diameter; Spectrum Laboratories Inc., Rancho Dominguez, CA) in an autoclaved dish. To increase hatch, the hatching

chamber was placed on a rotary shaker at 35 rpm to provide aeration. Second-stage juveniles were allowed to pass through the filter for 3 d and then used immediately for assays.

Inoculum used in greenhouse trials was also cultured on pepper. Roots from 3-month-old stock plants were gently rinsed to remove the soil. The roots were rubbed by hand in 0.6% sodium hypochlorite for 1 min to dislodge and break up the egg masses. The egg suspension was poured through nested sieves (250-/45-/25-µm diam.), and the eggs were rinsed with water. Egg suspensions used for assays or to inoculate plants in greenhouse trials contained mixed egg stages, with the "hatchable" eggs (those containing a first-stage juvenile (J1) or J2) counted as the number per ml.

*Laboratory assays of RKN in plant extracts*

Microwell assay procedures were similar to those described in Meyer *et al.* (2006). Each extract was sequentially filtered through 1.0-, 0.45- and 0.20-µm filters. The assays were conducted in 96-well polystyrene plates. The nematode eggs or J2 were suspended in SDW and pipetted into the wells, and then the extracts were added to the wells. Each culture plate was covered with a plastic adhesive sheet (Excel Scientific, Inc., Victorville CA) and incubated at 25°C. Five replicate wells were used per treatment in each trial. For the assays with eggs immersed in extracts, counts were made of the total number of J2 that hatched from eggs, and the number of active and inactive J2. Juveniles that exhibited any body movement were considered active. Specific differences in procedures for assays with and without adjusted pH are described below.

*Laboratory assays with unadjusted pH*

Both roselle leaf and pomegranate rind extracts consisted initially of 5 g in slightly less than 5 ml water and were adjusted to 5 ml with SDW for a concentration of 1 g per ml water. For immersed egg assays, each well received an egg suspension in 10-µl SDW. The suspension contained mixed egg stages with ca. 50 hatchable eggs per well. For assays with previously hatched J2, each well received ca. 20 J2 in 10-µl SDW. Nematodes were added in aliquots and not as individuals, so numbers varied among wells. Each well then received 90 µl of extract treatment or SDW; the latter served as the control treatment. The original concentrations of the pomegranate rind extracts were 0.5%, 1.0%, and 2.0% (volume extract/volume water). After they were added to the egg or J2 suspensions, treatments contained 0% (SDW), 0.45%, 0.9% and 1.8% pomegranate extract. Original concentrations of roselle leaf extract were 0%, 0.5%, 1.0%, 2.0%, 5.0%, 10%, 25%, and 50%. After addition to the egg or J2 suspensions, treatments were 0%, 0.45%, 0.9%, 1.8%, 4.5%, 9.0%, 22.5%, and 45.0% roselle extract. The higher concentrations were not tested with pomegranate because the dark color of the extract and the formation of precipitate even after passage through a 0.20-µm filter made it difficult to see the nematodes.

Each treatment was replicated five times in each of two trials; SDW controls were replicated ten times in each of the two trials. For assays with immersed eggs, counts of egg hatch and activity of hatched J2 were made on days 2, 5, and 7 of incubation in the extracts. For assays with previously hatched, immersed J2, counts of J2 activity were recorded on days 0, 1, 2, and 3 of incubation in the extracts, and J2 viability was recorded on day 4 after a 1-day water rinse. The pH values of the extract treatments were recorded from the second trials of the egg and J2 assays, and a mean pH value calculated for each treatment.

*Laboratory assay with adjusted pH*

The pH values were raised in each extract to determine if the original low pH contributed to the activity against RKN. This also allowed for testing the pomegranate extract at higher concentrations, because less precipitate formed in a higher pH. Roselle leaf and pomegranate rind extracts were each prepared in 10 ml water. The roselle extract was pH 2.6; this was adjusted with KOH to pH 5.0, and a final volume of 20 ml. The pomegranate extract was pH 4.5; this was adjusted to pH 5.9, and a final volume of 20 ml. For immersed eggs, each well received ca. 50 hatchable RKN eggs in 35-µl SDW. For previously hatched J2, a suspension of ca. 20 J2 in 35 µl SDW was pipetted into each well. Each well then received 315-µl extract, resulting in final treatment concentrations of: 0% (SDW), 11.4%, 22.5%, 33.8%, and 45% extract, with 5 replicate wells per treatment. Counts for immersed eggs were recorded on days 1, 2, and 8 of incubation in the extracts. Counts for previously hatched, immersed J2 were made on day 1, and on day 3 after a 1-d water rinse.

*Greenhouse trials with powdered pomegranate amendments in soil*

Cucumber (*Cucumis sativus* L. cv. 'Sweet Slice') seeds were planted into an enriched soil mixture (16 parts sand:9 parts compost, v/v; loamy sand; 85.1% sand, 7.2% silt, 7.6% clay, pH 6.9;

0.6% organic matter) that had been steamed and air-dried. Five days after planting, pomegranate amendment was mixed into new enriched soil, and the amended and control soils were placed into plastic cups with drainage holes. Each cup held ca. 50 g enriched soil or enriched soil plus amendment. The treatments were 0%, 0.25%, 0.5%, 1.0%, 5.0%, and 10% (weight dried, ground pomegranate rinds/ weight dried enriched soil). The cups were placed in water for 30 min to rehydrate the soil. Eggs (mixed egg stages with 500 hatchable eggs in 100-µL SDW) were added to the soil in one location near the perimeter of each cup, and the cups were then marked on that side. Due to phytotoxicity of the soil amendments, seedlings were transplanted into the cups six days after the pomegranate rinds were mixed into the soil. At that time, roots of 11-d-old seedlings were gently rinsed in water, and the seedlings were transplanted into the RKN-inoculated cups, opposite the area where the RKN had been added. Each treatment was replicated in 3 cups in each of two trials. Each trial was arranged in a randomized complete block design. Seedlings were harvested 16 d after inoculation, and shoot heights, shoot and root fresh weights, and gall numbers were recorded. Soil pH was recorded after the second trial.

*Statistical analysis*

Data from the plant extract studies were analyzed with the statistical package JMP 11.2.0 (SAS Institute, Inc., 2015). Differences among treatments were determined by ANOVA, and means were compared using Tukey Kramer's adjustment for multiple comparisons ($P \leq 0.05$). For nonparametric data, a Kruskal-Wallis test with Wilcoxon each pair nonparametric multiple comparisons was used to determine differences ($P \leq 0.05$) among means. The analysis used for each extract treatment is indicated in the footnote of each table. For the greenhouse trials with pomegranate powder, the variables were each analyzed as two-factor mixed models using PROC MIXED (SAS Institute, 2015) with "soil" as the factor and "trial" as a block. The assumptions of the models were checked. The variance grouping technique was used to correct for variance heterogeneity in the variables. Means comparisons were done with Sidak adjusted p-values so that the experiment-wise error was held at 0.05.

## RESULTS

Extracts from pomegranate fruit rinds and roselle leaves were nematotoxic to RKN. In assays without pH adjustment, when eggs were immersed in pomegranate extracts, all of the tested concentrations inhibited hatch of J2 from eggs (Table 1). After 2 d of immersion, egg hatch was suppressed by 74.7% to 82.8% compared with the water control. By day 7, egg hatch was suppressed up to 93.9% (in 1.8% pomegranate extract) compared with the water control. Activity of J2 that hatched from the immersed eggs was also inhibited by pomegranate extract (Table 1). On day 2, J2 activity was suppressed by 59.6% (in 0.9% extract) to 92.4% (in 1.8% extract). After 7 d, the percentage of active J2 was lowest in the two highest pomegranate extract concentrations, with more than 90% suppression of activity in the 1.8% extract.

Roselle leaf extract also suppressed RKN egg hatch and J2 activity (Table 1). This extract was not as nematotoxic as pomegranate extract, as evidenced by the need for higher concentrations to inhibit egg hatch and activity of J2 hatched from immersed eggs. Roselle extract at 1.8% did not affect egg hatch and caused only a minor suppression of J2 activity (Table 1). However, concentrations of 4.5% to 45.0% were all effective at reducing J2 hatch from eggs. In that range of extract concentrations, egg hatch was reduced by 81.6% to 86.2% on day 2, by 90.9% to 95.8% on day 5, and by 93.7% to 97.4% on day 7. Activity of hatched J2 was not affected by the lowest roselle extract concentrations, but extract concentrations of 22.5% and 45.0% resulted in 100% inactive J2 on days 2, 5, and 7.

The pH values of the pomegranate extracts in the egg immersion and previously hatched J2 assays were similar among the three concentrations (Table 2). However, the pH values of the seven roselle extract concentrations decreased with increasing extract concentration.

Pomegranate fruit rind extract suppressed activity in assays with previously hatched J2 (Table 2). The J2 activity was slightly inhibited on day 1. By day 3, the 1.8% extract concentration had the greatest effect, with a 61.9% reduction in J2 activity compared with the water control. There was some J2 recovery on day 4 after the water rinse, indicating nematostatic activity, but higher death in the extracts than in the water control demonstrated nematotoxicity as well (Table 2).

As with the J2 that hatched from immersed eggs, activity of J2 placed directly into roselle leaf extract was suppressed to the greatest degree by extract concentrations of 4.5% and higher on days 1 and 3 (Table 2). However, only the 9.0%, 22.5%, and 45.0% extracts rendered all J2 inactive on those days. The water rinse demonstrated that roselle extract had both nematotoxic and nematostatic effects. While no J2 activity was observed in the

Table 1. *Meloidogyne incognita* egg hatch and second-stage juvenile (J2) activity in extracts of pomegranate (*Punica granatum*) fruit rind and of roselle (*Hibiscus sabdariffa*) leaf. The assays were conducted with eggs immersed in the extracts.

| | Pomegranate extract | | | | | | Roselle extract | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Extract treatment[w] | Day 2 Total egg hatch[x] | Day 2 Percent active J2[y] | Day 5 Total egg hatch[y] | Day 5 Percent active J2[x] | Day 7 Total egg hatch[y] | Day 7 Percent active J2[x] | Day 2 Total egg hatch[x] | Day 2 Percent active J2[x] | Day 5 Total egg hatch[x] | Day 5 Percent active J2[x] | Day 7 Total egg hatch[x] | Day 7 Percent active J2[x] |
| 0% | 8.7 a | 82.5 a | 33.0 a | 96.2 a | 49.6 a | 91.4 a | 8.7 a | 82.5 a | 33.0 a | 96.2 a | 49.6 a | 91.4 a |
| 0.45% | 2.2 b (25.3%)[z] | 29.3 b (35.5%) | 6.3 b (19.1%) | 59.2 b (61.5%) | 8.9 b (17.9%) | 52.7 b (57.7%) | 9.9 a - | 89.3 a - | 33.0 a - | 94.7 a - | 42.9 a - | 93.1 a - |
| 0.9% | 1.5 b (17.2%) | 33.3 b (40.4%) | 2.8 b (8.5%) | 51.3 bc (53.3%) | 3.1 b (6.3%) | 25.5 c (27.9%) | 10.4 a - | 88.1 a - | 30.5 a - | 97.6 a - | 43.6 a - | 91.6 a - |
| 1.8% | 2.1 b (24.1%) | 6.3 b (7.6%) | 2.8 b (8.5%) | 20.3 c (21.1%) | 3.0 b (6.1%) | 8.3 c (9.1%) | 9.2 a - | 84.0 a - | 27.6 a - | 86.8 b (90.2%) | 38.7 a - | 79.9 b (87.4%) |
| 4.5% | - | - | - | - | - | - | 1.4 b (16.1%) | 44.4 a (53.8%) | 1.7 b (5.2%) | 22.2 c (23.1%) | 2.2 b (4.4%) | 13.1 c (14.3%) |
| 9.0% | - | - | - | - | - | - | 1.4 b (16.1%) | 0.0 b (0%) | 3.0 b (9.1%) | 0.7 c (0.7%) | 3.1 b (6.3%) | 1.3 c (1.4%) |
| 22.5% | - | - | - | - | - | - | 1.2 b (13.8%) | 0.0 b (0%) | 1.4 b (4.2%) | 0.0 c (0%) | 1.3 b (2.6%) | 0.0 c (0%) |
| 45.0% | - | - | - | - | - | - | 1.6 b (18.4%) | 0.0 b (0%) | 1.7 b (5.2%) | 0.0 c (0%) | 1.9 b (3.8%) | 0.0 c (0%) |

[w]Volume extract per volume water.
[x]Means within a column followed by the same letter are not significantly different according to a Kruskal-Wallis test with Wilcoxon each pair nonparametric multiple comparisons ($P \leq 0.05$).
[y]Means within a column followed by the same letter are not significantly different according to Tukey's adjustment for multiple comparisons ($P \leq 0.05$).
[z]For treatment means that were lower than the means for the water controls, numbers in parenthesis are percentages of the corresponding water control.

Table 2. *Meloidogyne incognita* second-stage juvenile (J2) activity in extracts of pomegranate (*Punica granatum*) fruit rind and of roselle (*Hibiscus sabdariffa*) leaf. The assays were conducted with previously hatched J2 immersed in the extracts. The pH values were taken from the second trials of egg and J2 assays.

| | Pomegranate extract | | | | Roselle extract | | | |
|---|---|---|---|---|---|---|---|---|
| Extract treatment[w] | Day 1 Percent active J2[x] | Day 3 Percent active J2[x] | Day 4 (1 day water rinse) Percent active J2[x] | pH | Day 1 Percent active J2[y] | Day 3 Percent active J2[y] | Day 4 (1 day water rinse) Percent active J2[y] | pH |
| 0% | 95.9 a | 89.5 a | 88.6 a | - | 95.9 a | 89.5 a | 88.6 a | - |
| 0.45% | 87.8 b (91.6%)[z] | 55.2 b (61.7%) | 60.4 b (68.2%) | 3.8 | 89.8 b (93.6%) | 82.2 b (91.8%) | 75.7 b (85.4%) | 6.3 |
| 0.9% | 83.2 b (86.8%) | 51.6 b (57.7%) | 60.3 b (68.1%) | 3.7 | 91.6 ab - | 86.7 ab - | 63.3 bc (71.4%) | 5.1 |
| 1.8% | 80.2 b (83.6%) | 34.1 c (38.1%) | 57.9 b (65.4%) | 3.4 | 90.4 ab - | 79.1 b (88.4%) | 48.6 d (54.9%) | 4.7 |
| 4.5% | - | - | - | - | 26.1 c (27.2%) | 8.1 c (9.1%) | 90.5 a - | 3.6 |
| 9.0% | - | - | - | - | 0.0 d (0%) | 0.0 d (0%) | 47.8 cd (54.0%) | 3.1 |
| 22.5% | - | - | - | - | 0.0 d (0%) | 0.0 d (0%) | 0.0 e (0%) | 2.9 |
| 45.0% | - | - | - | - | 0.0 d (0%) | 0.0 d (0%) | 0.0 e (0%) | 2.8 |

[w]Volume extract per volume water.
[x]Means within a column followed by the same letter are not significantly different according to Tukey's adjustment for multiple comparisons ($P \leq 0.05$).
[y]Means within a column followed by the same letter are not significantly different according to a Kruskal-Wallis test with Wilcoxon each pair nonparametric multiple comparisons ($P \leq 0.05$).
[z]For treatment means that were lower than the means for the water controls, numbers in parenthesis are percentages of the corresponding water control.

9.0% extract prior to the water rinse, nearly half of the J2 were active again after the water rinse. At the two highest concentrations, J2 did not recover and were therefore considered nonviable.

The extracts were also tested at pH 5.0 for roselle and pH 5.9 for pomegranate. In the egg immersion assay (Table 3), both extracts suppressed J2 hatch from eggs by up to 99.7% (pomegranate) and 97.0% (roselle) by day 8 at the highest tested concentrations. Activity of hatched J2 was 0% in both extracts on day 8. In the assay with previously hatched J2, the water rinse after incubation in 11.4% pomegranate extract resulted in recovery of the J2 on day 3 (Table 4). Results with roselle extract at a higher pH were overall similar to those with nonadjusted pH. All J2 were inactive in the 11.4% to 45.0% extract

Table 3. *Meloidogyne incognita* egg hatch and second-stage juvenile (J2) activity in extracts of pomegranate (*Punica granatum*) fruit rind and of roselle (*Hibiscus sabdariffa*) leaf. The assay was conducted with eggs immersed in the extracts. The stock extracts were adjusted to pH 5.0 (roselle) and pH 5.9 (pomegranate).

| | Pomegranate extract | | | | Roselle extract | | | |
|---|---|---|---|---|---|---|---|---|
| Extract treatment[w] | Day 2 Total egg hatch[x] | Day 2 Percent active J2[y] | Day 8 Total egg hatch[y] | Day 8 Percent active J2[y] | Day 2 Total egg hatch[x] | Day 2 Percent active J2[y] | Day 8 Total egg hatch[x] | Day 8 Percent active J2[y] |
| 0% | 15.0 a | 63.9 a | 81.0 a | 91.1 a | 18.2 a | 79.7 a | 79.0 a | 92.1 a |
| 11.4% | 4.0 b (26.7%)[z] | 0 b (0%) | 4.2 b (5.2%) | 0 b (0%) | 3.8 b (20.9%) | 18.0 b (22.6%) | 8.0 b (10.1%) | 0 b (0%) |
| 22.5% | 3.4 b (22.7%) | 0 b (0%) | 2.8 b (3.5%) | 0 b (0%) | 4.6 b (25.3%) | 0 b (0%) | 3.4 b (4.3%) | 0 b (0%) |
| 33.8% | 1.0 b (6.7%) | 0 b (0%) | 0.2 c (0.03%) | - | 3.2 b (17.6%) | 0 b (0%) | 2.8 b (3.5%) | 0 b (0%) |
| 45.0% | - | - | - | - | 2.8 b (15.4%) | 0 b (0%) | 2.4 b (3.0%) | 0 b (0%) |

[w]Volume extract per volume water.
[x]Means within a column followed by the same letter are not significantly different according to Tukey's adjustment for multiple comparisons ($P \leq 0.05$).
[y]Means within a column followed by the same letter are not significantly different according to a Kruskal-Wallis test with Wilcoxon each pair nonparametric multiple comparisons ($P \leq 0.05$).
[z]For treatment means that were lower than the means for the water controls, numbers in parenthesis are percentages of the corresponding water control.

Table 4. *Meloidogyne incognita* second-stage juvenile (J2) activity in extracts of pomegranate (*Punica granatum*) fruit rind and of roselle (*Hibiscus sabdariffa*) leaf. The assay was conducted with previously hatched J2 immersed in the extracts. The stock extracts were adjusted to pH 5.0 (roselle) and pH 5.9 (pomegranate).

| | Pomegranate extract | | Roselle extract | |
|---|---|---|---|---|
| Extract treatment[x] | Day 1 Percent active J2[y] | Day 3 (1 day water rinse) Percent active J2 | Day 1 Percent active J2 | Day 3 (1 day water rinse) Percent active J2 |
| 0% | 75.7 a | 69.2 a | 72.2 a | 69.1 a |
| 11.4% | 0 b (0%)[z] | 64.9 a - | 0 b (0%) | 50.2 a (72.6%) |
| 22.5% | 0 b (0%) | 0 b (0%) | 0 b (0%) | 0 b (0%) |
| 33.8% | 0 b (0%) | 0 b (0%) | 0 b (0%) | 0 b (0%) |
| 45.0% | NA | 0 b (0%) | 0 b (0%) | 0 b (0%) |

[x]Volume extract per volume water.
[y]Means within a column followed by the same letter are not significantly different according to a Kruskal-Wallis test with Wilcoxon each pair nonparametric multiple comparisons ($P \leq 0.05$).
[z]For treatment means that were lower than the means for the water controls, numbers in parenthesis are percentages of the corresponding water control.

Table 5. 'Sweet Slice' cucumber viability and plant vigor, and root galling caused by *Meloidogyne incognita* in greenhouse trials with dried, powdered pomegranate fruit rind as a soil amendment. The soil pH values were taken from the second trial.

| Pomegranate treatment (percent w/w soil)[x] | Number live/total planted | Shoot height (cm)[y] | Shoot fresh weight (g) | Root fresh weight (g) | Number of galls/root system | Galls/g root fresh weight | Soil pH[z] |
|---|---|---|---|---|---|---|---|
| 0% | 6/6 | 10.8 a | 2.6 a | 2.3 a | 250.3 a | 98.3 a | 7.1 |
| 0.25% | 6/6 | 9.4 b | 2.0 b | 1.8 b | 215.8 ab | 97.7 ab | 6.9 |
| 0.5% | 6/6 | 9.3 b | 1.9 b | 1.9 ab | 184.7 ab | 83.5 ab | 6.8 |
| 1.0% | 6/6 | 8.2 b | 1.7 ab | 1.5 ab | 111.5 b | 65.4 b | 6.5 |
| 5.0% | 1/6 | 6.5 | 0.1 | 0.1 | 1 | 10 | 5.7 |
| 10.0% | 0/6 | - | - | - | - | - | 5.1 |

[x]Weight dried pomegranate rinds/weight dried soil.
[y]For all data, means within a column followed by the same letter are not significantly different. Means comparisons were done with Sidak adjusted p-values so that the experiment-wise error was held at 0.05. The 5.0% and 10.0% data was not included in the analysis due to plant death.
[z]The pH values were determined from the enriched soil + pomegranate amendments used in the second trial.

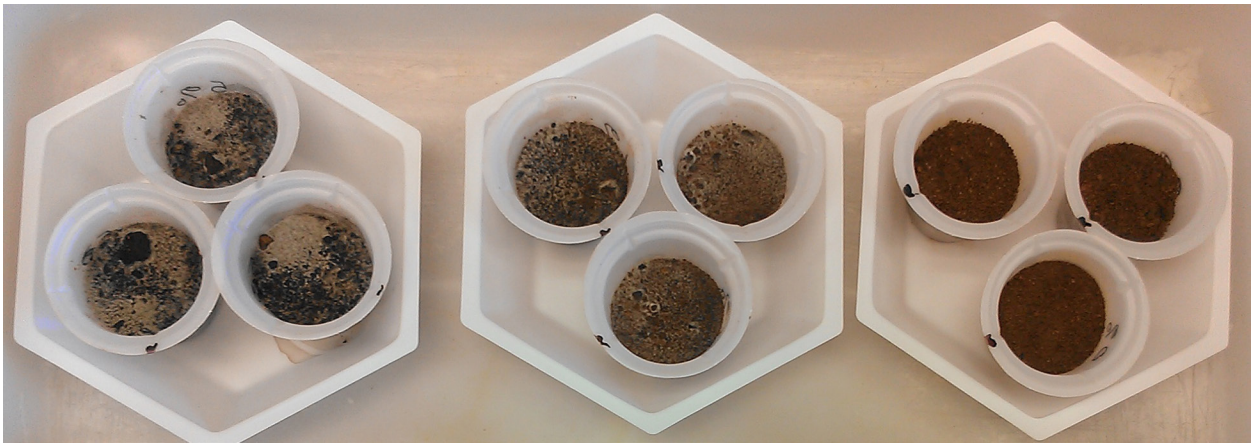

Fig. 1. Growth of the fungi *Rhizopus* sp. and *Aspergillus* sp. on soil amended with pomegranate fruit rind powder. Amendment rates, from left to right: 5.0%, 0.5%, and 0% powder (percent w/w soil).

concentrations on day 1. More of the J2 in the 11.4% extract at pH 5.0 recovered on day 3 after the water rinse than recovered in 9.0% extract at unadjusted pH 3.1. However, all J2 were nonviable at the higher extract concentrations (Table 4).

In the greenhouse trials, the pH of the amended soil decreased with increasing rate of pomegranate powder (Table 5). Powdered pomegranate added to the enriched soil was phytotoxic, resulting in plant death at 5.0% and 10.0% w/w (Table 5). There was stunting and suppressed weights of shoots at the lower application rates of 0.25% to 1.0% (Table 5). Root fresh weights were lower in pomegranate-amended soil, although the difference from the control plants was not always significant. The 1.0% pomegranate application rate significantly suppressed the number of galls per root system by more than half, and the number of galls per g fresh root weight by more than 30% (Table 5).

Following incorporation of the pomegranate powder into enriched soil, fungi grew conspicuously on the surface of the soil in both trials. The fungal growth increased with increasing rates of pomegranate powder (Fig. 1). This fungus growth did not occur in the nonamended soil. Spores were transferred onto potato dextrose agar (PDA; Difco™, Becton, Dickinson and Company, Sparks, MD) for culture, and the fungi were identified as *Rhizopus* (Ehrenb.) sp. and *Aspergillus* (Micheli) sp. Pomegranate powder was also plated onto PDA, and these fungi did not grow on the medium.

## DISCUSSION

Extracts from dried pomegranate rinds and from roselle leaves inhibited RKN egg hatch and the activity of hatched J2. The pomegranate extract was effective even at low concentrations, reducing egg hatch up to 94%. Extract from dried, powdered roselle leaves also suppressed egg hatch, but higher concentrations were needed for this effect. Both extracts were also nematostatic and nematotoxic to J2. Although J2 recovered after a water rinse in lower pomegranate and roselle extract concentrations, all J2 were killed at 22.5% extract and higher concentrations.

The pH of the roselle extract decreased substantially with increasing extract concentration. In fungal culture filtrates containing acetic acid, higher pH values resulted in more active *Meloidogyne* sp. juveniles (Djian *et al.*, 1991). Fungus extract and acetic acid at pH 4.0 and pH 5.0 paralyzed 100% of J2, but fewer J2 were paralyzed at pH 5.5, and no J2 were affected at pH 6.0. The authors suggested that this was because the form of the carboxylic function was altered. In our study, when the pH of each extract was adjusted to a value higher than the original pH, both roselle and pomegranate extracts still reduced viability of RKN. These results indicate that pH was not likely a factor affecting activity of the extracts.

Our results with methanolic pomegranate rind extract correlate with those found in a previous study with water extracts. Pomegranate fruit extracts were prepared with 25 g in 500 ml water (Korayem *et al.*, 1993), which was half the concentration used for our methanolic extracts. Root-knot nematode J2 and the plant-parasitic nematode, *H. dihystera*, were exposed at rates that would have been equivalent to ca. 50%, 25%, and 5.0% in our study. All RKN J2 were inactive, with 5% and 1% recovery at 25% and 50% extract, respectively (5.0% was not tested). Movement of *H. dihystera* was also suppressed, as was acetylcholinesterase activity in this nematode. We tested lower pomegranate extract concentrations with RKN, and observed J2 death in the 0.45% to 1.8% extract treatments. In the earlier study, RKN egg hatch was completely inhibited in the 50% extract, and suppressed by 75% in the 5.0% water extract (Korayem *et al.*, 1993). In our assay, egg hatch was inhibited by 94% in 1.8% extract. Unlike the studies with pomegranate and plant-parasitic nematodes, a blueberry extract fraction with high amounts of proanthocyanidins increased the thermotolerance and life span of *Caenorhabditis elegans* Maupas (Wilson *et al.*, 2006), and pomegranate peel also contains proanthocyanidins (Zam *et al.*, 2012). Our research did not examine thermotolerance of RKN, but did demonstrate that water and methanolic extracts from pomegranate fruit parts reduced RKN egg hatch and were lethal to J2.

An earlier greenhouse study was conducted with dry, crushed pomegranate peels amended into soil at rates of 0.15%, 0.3%, and 0.6% (w/w) (Ismail, 2015). *Meloidogyne javanica* J2 were inoculated into the soil 6 d after tomato seedling transplant. Numbers of root galls were not significantly reduced at any amendment rate when the peels were added 2 wk before transplant, although numbers of egg masses, females, and J2 were suppressed, particularly at 0.6% pomegranate amendment. The 0.3% w/w amendment was also applied at three different times: at transplant, and 1 and 2 wk prior to transplant. The greatest reduction in nematode numbers and galling was with at-transplant application. The pomegranate amendment was not phytotoxic to tomato plants, even when applied the day of transplant. These tested application rates were similar to our lowest tested rates of 0.25% and 0.5%, which did suppress cucumber seedling growth. The soil amendment might be phytotoxic to cucumber but not tomato. While we observed some reduction in galling with those amendment rates, the suppression was only significant at the 1.0% amendment rate. The number of galls per g root was not reported in the earlier study, so those results cannot be directly compared with ours.

Even though the enriched soil was steamed prior to use in our greenhouse trials, *Rhizopus* sp. and *Aspergillus* sp. grew on the pomegranate-amended soil. When pomegranate powder was subsequently placed on PDA, these fungi did not grow on the plates. These fungi must have inoculated the enriched soil, and the pomegranate powder then provided a good substrate for growth. This effect was not reported by Ismail (2015), and therefore may be dependent on the soil and other environmental factors.

We also conducted a preliminary greenhouse test with dried, ground roselle leaves (Meyer, unpublished). The 5.0% and 10.0% roselle (w/w soil) were phytotoxic with at-transplant soil amendment, killing all or most of the seedlings. The lower tested application rates did not kill the plants, but also did not reduce gall indices.

As with all plant-derived extracts and amendments, variability in amounts of active compounds can affect efficacy and is dependent on a number of factors. Amounts of compounds such as ellagic acid and gallagic acid dilactone varied widely in pomegranate, depending on the part of the fruit tested (García-Villalba *et al.*, 2015). The husk (pericarp) and peels (mesocarp) each had roughly 150 times more gallagic acid dilactone, and 5 and 90 times (mesocarp and husk, respectively) more ellagic acid, than the arils containing the seeds. Also, the husk and mesocarp contained punicalin, valoneic

acid dilactone and gallic acid, which were not found in the arils. Chemical components were also affected by growing conditions, plant cultivar, age, and method of storage (Viuda-Martos *et al.*, 2010).

At the application rates and times used in our study, the greenhouse trials did not indicate high efficacy of either pomegranate or roselle powder as a soil amendment for suppressing RKN on cucumber. If efficacious application rates were found, timing of application to soil would need to be planned to avoid phytotoxicity, as is currently done with other soil amendments such as mustard seed meal (Meyer *et al.*, 2011). Pomegranate powder amendment also resulted in overgrowth of saprophytic soil fungi that were already present in the soil. Further research in varying environments would indicate whether this is a common occurrence or merely an anomaly under our particular greenhouse conditions. Extracts from pomegranate rind and roselle leaf demonstrated activity against RKN, indicating that both are potential candidates for future studies of plant-derived compounds that can be applied to suppress plant-parasitic nematode populations.

## ACKNOWLEDGMENTS

Thanks are extended to Shannon Rupprecht, Paroo Chauhan, Meiling Webb, and Dr. Ganga Bhagavathy for technical assistance, to Dr. Megan Romberg (USDA, APHIS) for identification of fungi, and to Mary J. Camp (USDA, ARS, Northeast Area Office of the Director, Statistics Group) for statistical analyses of the greenhouse trials.

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

## LITERATURE CITED

Adeniji, M. O. 1970. Reactions of kenaf and roselle varieties to the root-knot nematodes in Nigeria. Plant Disease Reporter 54:547-549.

Akhtar, S., T. Ismail, D. Fraternale, and P. Sestili. 2015. Pomegranate peel and peel extracts: Chemistry and food features. Food Chemistry 174:417-425.

Baetz, U., and E. Martinoia. 2014. Root exudates: The hidden part of plant defense. Trends in Plant Science 19:90-98.

Boonmasawai, S., S. Sungpradit, C. Jirapattharasate, C. Nakthong, and L. Piasai. 2013. Effects of alcoholic extract from pomegranate (*Punica granatum* L.) peels on gastrointestinal nematode egg counts in doe. Journal of Applied Animal Science 6:27-37.

Da-Costa-Rocha, I., B. Bonnlaender, H. Sievers, I. Pischel, and M. Heinrich. 2014. *Hibiscus sabdariffa* L. – a phytochemical and pharmacological review. Food Chemistry 165:424-443.

Djian, C., L. Pijarowski, M. Ponchet, N. Arpin, and J. Favre-Bonvin. 1991. Acetic acid: A selective nematicidal metabolite from culture filtrates of *Paecilomyces lilacinus* (Thom) Samson and *Trichoderma longibrachiatum* Rifai. Nematologica 37:101-112.

García-Villalba, R., J. C. Espín, K. Aaby, C. Alasalvar, M. Heinonen, G. Jacobs, S. Voorspoels, T. Koivumäki, P. A. Kroon, E. Pelvan, S. Saha, and F. A. Tomás-Barberán. 2015. Validated method for the characterization and quantification of extractable and nonextractable ellagitannins after acid hydrolysis in pomegranate fruits, juices, and extracts. Journal of Agricultural and Food Chemistry 63:6555-6566.

Ismail, A. E. 2015. Management of root-knot nematode *Meloidogyne javanica* on tomato by dry crushed pomegranate *Punica granatum* L. peels as a bio-fumigant in Egypt. Archives of Phytopathology and Plant Protection 48:253-258.

Ismail, T., P. Sestili, and S. Akhtar. 2012. Pomegranate peel and fruit extracts: A review of potential anti-inflammatory and anti-infective effects. Journal of Ethnopharmacology 143:397-405.

Kaplan, I., R. Halitschke, A. Kessler, S. Sardanelli, and R. F. Denno. 2008. Constitutive and induced defenses to herbivory in above- and below-ground plant tissues. Ecology 89:392-406.

Korayem, A. M., S. A. Hasabo, and H. H. Ameen. 1993. Effects and mode of action of some plant extracts on certain plant-parasitic nematodes. Anzeiger fur Schädlingskunde (Journal of Pest Science) 66:32-36.

Meyer, S. L. F., I. A. Zasada, S. B. Orisajo, and M. M. Morra. 2011. Mustard seed meal mixtures: Management of *Meloidogyne incognita* on pepper and potential phytotoxicity. Journal of Nematology 43:7-15.

Meyer, S .L. F., I. A. Zasada, D. P. Roberts, B. T. Vinyard, D. K. Lakshman, J.-K. Lee, D. J. Chitwood, and L. K. Carta. 2006. *Plantago lanceolata* and *Plantago rugelii* extracts are toxic to *Meloidogyne incognita* but not to certain microbes. Journal of Nematology 38:333-338.

Minton, N. A., W. C. Adamson, and G. A. White. 1970. Reaction of kenaf and roselle to three root-knot nematode species. Phytopathology

60:1844-1845.

Mondal, H., H. Hossain, K. Awang, S. Saha, S. M. U. Rashid, M. K. Islam, M. S. Rahman, I. A. Jahan, M. M. Rahman, and J. A. Shilpi. 2015. Anthelmintic activity of ellagic acid, a major constituent of *Alternanthera sessilis* against *Haemonchus contortus*. Pakistan Veterinary Journal 35:58-62.

Ndjonka, D., L. N. Rapado, A. M. Silber, E. Liebau, and C. Wrenger. 2013. Natural products as a source for treating neglected parasitic diseases. International Journal of Molecular Sciences 14:3395-3439.

Ntalli, N. G., and P. Caboni. 2012. Botanical nematicides: A review. Journal of Agricultural and Food Chemistry 60:9929-9940.

Ohri, P., and S. K. Pannu. 2010. Effect of phenolic compounds on nematodes – a review. Journal of Applied and Natural Science 2:344-350.

Rasmann, S., and A. A. Agrawal. 2008. In defense of roots: A research agenda for studying plant resistance to belowground herbivory. Plant Physiology 146:875-880.

SAS Institute, Inc. 2015. SAS/STAT® 14.1 User's Guide, Cary, NC: SAS Institute Inc.

Saxena, K., V. Dube, V. Kushwaha, V. Gupta, M. Lakshmi, S. Mishra, S. Gupta, A. Arora, V. Lakshmi, R. K. Sharma, G. K. Jain, and P. K. Murthy. 2011. Antifilarial efficacy of *Hibiscus sabdariffa* on lymphatic filarial parasite *Brugia malayi.* Medicinal Chemistry Research 20:1594-1602.

Seeram, N., R. Lee, M. Hardy, and D. Heber. 2005. Rapid scale purification of ellagitannins from pomegranate husk, a by-product of the commercial juice industry. Separation and Purification Technology 41:49-55.

Sindi, H. A., L. J. Marshall, and M. R. A. Morgan. 2014. Comparative chemical and biochemical analysis of extracts of *Hibiscus sabdariffa*. Food Chemistry 164:23-29.

Thomsen, H., K. Reider, K. Franke, L. A. Wessjohann, J. Keiser, E. Dagne, and N. Arnold. 2012. Characterization of constituents and anthelmintic properties of *Hagenia abyssinica*. Scientia Pharmaceutica 80:433-446.

Vawdrey, L. L., and G. R. Stirling. 1992. Reaction of kenaf and roselle grown in the Burdekin River irrigation area to root-knot nematodes. Australasian Plant Pathology 21:8-12.

Viuda-Martos, M., J. Fernández-López, and J. A. Pérez-Álvarez. 2010. Pomegranate and its many functional components as related to human health: A review. Comprehensive Reviews in Food Science and Food Safety 9:635-654.

Wilson, F. D., and M. Y. Menzel. 1964. Kenaf (*Hibiscus cannabinus*), roselle (*Hibiscus sabdariffa*). Economic Botany 18:80-91.

Wilson, M. A., B. Shukitt-Hale, W. Kalt, D. K. Ingram, J. A. Joseph, and C. A. Wolkow. 2006. Blueberry polyphenols increase lifespan and thermotolerance in *Caenorhabditis elegans*. Aging Cell 5:59-68.

Wurst, S., R. Wagenaar, A. Biere, and W. H. van der Putten. 2010. Microorganisms and nematodes increase levels of secondary metabolites in roots and root exudates of *Plantago lanceolata*. Plant and Soil 329:117-126.

Zam, W., G. Bashour, W. Abdelwahed, and W. Khayata. 2012. Separation and purification of proanthocyanidins extracted from pomegranate's peels (*Punica granatum*). International Journal of Pharmaceutical Sciences and Nanotechnology 5:1808-1813.

Zhen, J., T. S. Villani, Y. Guo, Y. Qi, K. Chin, M.-H. Pan, C.-T. Ho, J. E. Simon, and Q. Wi. 2016. Phytochemistry, antioxidant capacity, total phenolic content, and anti-inflammatory activity of *Hibiscus sabdariffa* leaves. Food Chemistry 190:673-680.

*Received:* 30/IX/2015
*Recibido:*

*Accepted for publication:* 29/II/2016
*Aceptado para publicación:*