Enhanced biological control of root-knot nematode, *Meloidogyne incognita*, by combined inoculation of cotton or soybean seeds with a plant growth-promoting rhizobacterium and pectin-rich orange peel

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Abstract

LC-MS analysis of plant growth-promoting rhizobacterium (PGPR) *Bacillus velezensis* AP203 supernatants indicated the presence of nematode-inhibiting compounds that increased in abundance when *B. velezensis* AP203 was grown on orange peel. *Meloidogyne incognita* J2 were incubated with *B. velezensis* AP203 spores and orange peel, spores alone, orange peel alone, or with a non-inoculated control, and the combination of *B. velezensis* AP203 with orange peel resulted in 94% mortality of *M. incognita* juveniles (p ≤ 0.05). The J2 mortality rate for *B. velezensis* alone was 53%, compared to 59% mortality with orange peel, and the non-inoculated control exhibited 7% mortality. When tested on soybeans raised in a greenhouse, it was observed that when grown in the presence of orange peel, *B. velezensis* AP203 culture broth, cell suspension or supernatant reduced the numbers of *M. incognita* eggs per g of root at 45 days after planting (DAP) compared to inoculated controls in soybean and cotton (p ≤ 0.05). Likewise, soybean root length and fresh root weight significantly increased after inoculation with *B. velezensis* AP203 amended with orange peel. In cotton, shoot and root length significantly increased after inoculation with cell pellets of *B. velezensis* AP203 amended with orange peel compared to the *M. incognita* inoculated control. These data indicate that *B. velezensis* AP203 responds to growth on pectin-rich orange peel by production of biologically active secondary metabolites that can promote plant growth and inhibit root-knot nematode viability.

Keywords

Biological control, *Meloidogyne incognita*, Orange peel, Pectin, Root-knot nematode.

Cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* L.) are economically important crops in the United States and worldwide. In the U.S. alone, cotton yield in 2018 was 18.4 million bales, and soybean yield was 4.54 billion bushels (Anonymous, 2018). *Meloidogyne incognita* (Kofoid and White) Chitwood, the southern root-knot nematode, is broadly distributed in soils cultivated with cotton (Xiang et al., 2017b) and other crops (Huang et al., 2016), and causes economically significant yield losses annually; for example, in 2018, soybean yield losses due to *M. incognita* in the southern U.S. were estimated at 11.92 million bushels.
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in total, with 70,000 bushels lost in Alabama (Allen et al., 2018).

Multiple methods are used to reduce M. incognita populations in the field, including chemical nematicides (Basony and Abo-Zaid, 2018); however, environmental and health concerns have limited the use of chemical nematicides for controlling plant-parasitic nematodes, and there is a need to develop environmentally friendly methods to manage the pathogen, such as the use of biological control agents (Burkett-Cadena et al., 2008).

Plant growth-promoting rhizobacteria (PGPR) are root-colonizing bacteria that enhance plant growth and biological control against multiple plant pathogens (Olanrewaju et al., 2017). Bacillus velezensis is a Gram-positive, rod-shaped PGPR, with some strains reported to reduce M. incognita populations on cotton (Xiang et al., 2017b). Additionally, B. velezensis PGPR strains are able to utilize pectin as a sole carbon and energy source (Hossain et al., 2015); interestingly, citrus peels which are pectin-rich and an abundant and inexpensive agricultural waste product (Rafiq et al., 2018) have been previously demonstrated to increase the efficacy of soybean growth promotion mediated by B. velezensis PGPR strains when co-inoculated with orange peel powder as a seed treatment (Hassan et al., 2019).

In the presence of different carbohydrates (pectin, sucrose, xylan or galactose), Bacillus spp. produce multiple secondary metabolites that inhibit the growth of multiple plant pathogens. For example, Bacillus amyloliquefaciens SQY 162 grown on pectin-amended media increased surfactin production and inhibit bacterial wilt of tobacco caused byRalstonia solanacearum (Wu et al., 2015). Also supernatants, cell pellet suspensions, and culture broths of B. subtilis strains significantly reduced populations of eggs and J2 of M. incognita under laboratory and greenhouse conditions (Basony and Abo-Zaid, 2018). In addition, previous studies have reported that the combination of separated cow manure and orange peels (SCM-OP) reduced the number of Meloidogyne javanica populations on tomato roots (Raviv et al., 2005). However, the effect of exogenous orange peel amendments has not been evaluated for enhanced PGPR-mediated biological control of plant pathogenic nematodes such as M. incognita.

The overall goal of this study was to evaluate the combined application of B. velezensis AP203 and orange peel for biological control of M. incognita. More specifically, we investigated (i) the effects of B. velezensis AP203, with and without orange peel amendment, on the mortality of second-stage juveniles (J2) of M. incognita under laboratory conditions; (ii) the effect of orange peel or glucose by B. velezensis AP203 on the expression of secondary metabolite(s) predicted to be responsible for reducing M. incognita populations; (iii) evaluate the efficacy of orange peel and/or B. velezensis AP203 amendments in reducing the number of M. incognita populations on the roots of soybean and cotton under greenhouse conditions.

Materials and methods

Evaluation of nematode killing under laboratory conditions

Preparation of B. velezensis spore and orange-peel suspensions

B. velezensis AP203 was obtained from the Biological Control lab in the Department of Entomology and Plant Pathology, Auburn University, Alabama, USA (retired director Prof. Joseph Kloepper). This strain was originally isolated from a cotton rhizosphere. The cryopreserved culture was streaked onto tryptic soy agar (TSA) and incubated at 28°C for 24 hr. A single colony of the strain was transferred into a spore preparation medium (Zhang et al., 2010), and incubated for seven days at 28°C. Sterilized distilled water (20 mL) was added to each petri plate, and the bacterial mass was transferred to a 50 mL centrifuge tube. The B. velezensis AP203 spore preparation was heat-treated for 20 min at 80°C to kill any vegetative cells, serially diluted, and adjusted to 10⁷ colony forming units (CFU) per mL. The non-organic orange peel powder (Citrus Extracts LLC, Fort Pierce, FL USA) was suspended in sterilized distilled water by a magnetic stirrer at a rate of 1.0 g per 100 mL (1.0% w/v) water and was applied as an aqueous suspension.

Preparation of M. incognita inoculum and mortality determination

M. incognita eggs were isolated and extracted from corn plant roots Mycogen 2H273 (Dow AgroScience, Indianapolis, IN) at the Plant Science Research Center (Auburn University, Auburn, AL) using a modified sucrose centrifugation-flotation method (Jenkins, 1964). The eggs were counted using an inverted TS100 Nikon microscope at 40X magnification. The eggs were hatched for a seven-day period at 30°C in an incubator. In total, 10 µL of J2 M. incognita were counted and transferred into a 96-well plate for the J2 mortality test, with 30 to 40 J2 per well. In total, 90 µL of AP203 alone, citrus extract alone, and AP203 with
AP203 was prepared as previously described. A single colony of the PGPR strain was transferred into TSA, Tris-Spizizen Salts medium (TSS), or TSS + orange peel powder (OPP; 0.5% w/v) media and grown for 72 hr in a shaking incubator at 28°C. The *B. velezensis* AP203 cultures were subjected to centrifugation in a Sorvall Legend RT centrifuge (Thermo Scientific, USA) at 10,000 × g for 10 min. The supernatant was collected and passed through a 0.2 µm syringe filter (VWR, Radnor, PA, USA) and was then transferred into a 1 mL microcentrifuge tube for LC-MS analysis.

**LC-MS analysis**

LC-MS analysis was performed at the Auburn University Mass Spectrometry Center using an ultra-performance LC system (ACQUITY, Waters Corp., USA) coupled with a quadrupole time-of-flight mass spectrometer (Q-Tof Premier, Waters) with electrospray ionization (ESI) in positive and negative mode using Masslynx software (V4.1). A 10 µl sample was injected into a C4 column (Aeris Widepore C4, 3.6 µm, 2.1 × 50 mm, Phenomenex) with a 300 µL/min flow rate of the mobile phase. In positive mode, the mobile phase was solution A (0.1% formic acid in water) and solution B (95% acetonitrile, 5% H₂O, and 0.1% formic acid) was initiated beginning at 0% B, held for 2 min, then linear ramping to 50% B in 18 min, followed by ramping to 100% B in 8 min and held at 100% B for 2.5 min, and then back to 0% B in 0.5 min with 4 min of re-equilibration at 0% B. In negative mode, the mobile phase was solution A (2 mM ammonium formate in water) and solution B (100% acetonitrile) beginning at 2% B, held for 2 min, then linear ramping to 50% B in 18 min, followed by ramp to 95% B in 8 min, held at 95% B for 2.5 min, and back to 2% B in 0.5 min with 4 min of re-equilibration at 2% B. The capillary voltage was set at 3.1 kV in positive mode and 2.8 kV in negative mode, the sample cone voltage was 30 V, and the extraction cone was 4.3 V. The source and desolvation temperature were maintained at 105 and 300 °C, respectively, with the desolvation gas flow at 600 L/hr. The Time of Flight Mass Spectrometry (TOF/MS) scan was 1 sec long from 80 to 1400 m/z with a 0.02 sec inter-scan delay using the centroid data format. The lock mass was used to correct instrument accuracy with a 2.5 µg/mL solution of leucine encephalin (Bachem H-2740). The data were converted to mzXML and analyzed with XCMS Online (Huan et al., 2017).

**Greenhouse experiment**

The greenhouse test was to evaluate the combined effects of *B. velezensis* AP203 plus orange peel or glucose for biological control of *M. incognita*. The treatment groups for the greenhouse study included: (i) TSB-CELLS + *M. incognita*, (ii) SUPERNATANT-OPP + *M. incognita*, (iii) SUPERNATANT-Glucose + *M. incognita*, (iv) TSS-CELLS-OPP + *M. incognita*, (v) TSS-CELLS-Glucose + *M. incognita*, (vi) CULTURE-OPP + *M. incognita*, (vii) CULTURE-Glucose + *M. incognita*, (viii) TSS + OPP (no bacteria present) + *M. incognita*, or (ix) TSS + glucose (No bacteria present) + *M. incognita*, (x) a positive control with *M. incognita*, and (xi) a negative control without *M. incognita*.

**Preparation of *B. velezensis* AP203 and orange-peel suspension**

*B. velezensis* AP203 was grown from a cryostock onto Tryptic Soy Agar (TSA) at 28°C for 24 hr. A single colony of this strain was transferred into TSA, TSS + glucose (0.5% w/v), or TSS + OPP (0.5% w/v) media and grown for 48 hr in a shaking incubator at 28°C. The *B. velezensis* cultures were then subjected to centrifugation in a Sorvall Legend RT centrifuge (Thermo Scientific, USA) at 10,000 × g for 10 min and each was then adjusted to approximately 10⁷ CFU/mL based on culture turbidity at an optical density of 600 nm (OD₆₀₀). The TSA grown strain was suspended in TSS broth based on OD₆₀₀ and an aliquot (1 mL containing ~10⁷ CFU) of this sample (TSA-CELLS) was applied to each seed. For *B. velezensis* AP203 grown in TSS media, the carbon source used was either glucose or OPP, and 35 mL of these cultures were subjected to centrifugation at 10,000 × g for 10 min. The supernatant was passed through a 0.2 µm syringe filter (VWR, Radnor, PA, USA) and 1 mL of this sample (SUPERNATANT) was applied to each seed. For the cell pellet suspension, the pellet was suspended in TSS and then subjected to centrifugation again to remove spent media, and
then resuspended in 35 mL of TSS. An aliquot (1 mL containing ~10⁷ CFU) of this sample (TSS-CELLS) was applied to each seed. The TSS broth culture (35 mL) without separating cells and supernatant was also prepared (TSS-CULTURE), and 1 mL of the broth culture was applied to each seed.

**Preparation of M. incognita inoculum**

*M. incognita* eggs were isolated and extracted from corn roots as described previously and 2,000 eggs/mL populations were inoculated into a 2 cm depth of soil in each cone-tainer (Stuewe & Sons, Tangent, OR, USA) during seed planting and covered with field soil. The *M. incognita*-inoculated soybean and cotton seeds were kept at room temperature in the greenhouse for 24 hr before transferring to a greenhouse growth chamber at 25 to 35°C.

**Soil preparation and seed inoculation**

Field soil (Malbis fine sandy loam 59% sand, 31% silt, 10% clay, <1% OM) was collected from the E.V. Smith Research Center of Auburn University (near Shorter, AL) and was mixed with sand at a ratio (2:1). In the greenhouse experiments, the soil/sand mix was placed into each 150 cm³ cone-tainers. Two soybean (DD VSG 75140) and two cotton seeds (DPL1558 NRB2RF) were placed into 2 cm depth of each cone-tainer to ensure seed germination. The different treatment group inocula (i.e. cell pellet suspensions, culture broths, or supernatants) of *B. velezensis* AP203 were applied on the soybean and cotton seed surface. The seeds were then covered with the soil/sand, kept at room temperature for 24 hr, before transferring to a greenhouse growth chamber (25 to 35°C).

**Statistical analyses**

All data collected from the in vitro bioassay and greenhouse tests were analyzed with SAS 9.4 software (SAS Institute, Cary, NC, USA) using the PROC GLIMMIX (Generalized Linear Mixed Models) procedure that performs estimation and statistical inference for GLIMMIX at the $p \leq 0.05$ level of significance. For the in vitro experiments, the mortality percentages of J2 of *M. incognita* were analyzed with four treatments and eight replicates. In the greenhouse experiment, plant height, root length, root and shoot fresh weight, and *M. incognita* eggs/plant data were collected and analyzed. The greenhouse experiments were arranged in a randomized complete block design (RCBD) with eleven treatments and eight replicates. All the in vitro and greenhouse experiments were repeated twice, and the data were pooled.

**Results**

**In vitro antagonistic effects of *B. velezensis* AP203 with orange peel amendment**

A spore preparation of *B. velezensis* AP203 with orange peel amendment was tested in vitro for its ability to kill *M. incognita* J2. The mortality percentage of *M. incognita* J2 ranged from 0 to 100%, with 94% mortality observed for *M. incognita* eggs inoculated with the combination of *B. velezensis* strain AP203 with 1.0% (w/v) OPP, which was significantly greater ($p < 0.05$) than that observed when eggs were incubated with *B. velezensis* AP203 alone (53%), 1.0% OPP (59%), or the negative control (7%) (Fig. 1). Interestingly, a significant increase in *M. incognita* J2 mortality relative to the negative control was also observed for *B. velezensis* AP203 alone (as had been observed previously) as well as inoculation with OPP alone.

**The effects of *B. velezensis* AP203 cultures grown in different media on *M. incognita* populations and cotton and soybean growth under greenhouse conditions**

Soybean root length of plants treated with culture broth from *B. velezensis* AP203 with OPP was significantly greater compared to the glucose treatments and *M. incognita* positive control treatment (Table 1). Soybean root length was also greater in the TSB-growth cells, OPP (supernatant) and OPP (culture) compared to the *M. incognita* control. OPP (culture) also significantly increased root fresh weight as compared to the *M. incognita* control (Table 1). Cotton shoot lengths of plants treated with cell pellets from *B. velezensis* AP203 with OPP was significantly greater compared to the glucose treatments and *M. incognita* positive control treatment (Table 1). Cotton root length was significantly increased by the OPP (supernatant), OPP (cells), and OPP (culture) compared to the *M. incognita* control (Table 2). Supernatant, cell pellet, or culture broth from *B. velezensis* AP203 with OPP had a maximum antagonistic activity against *M. incognita* eggs in soybean (Fig. 2) and cotton (Fig. 3) roots at 45 DAP. The *B. velezensis* AP203 with OPP (supernatant), OPP (cells), and OPP (culture) all reduced *M. incognita* populations as
compared to the *M. incognita* grown on both crop plants without any additives. *B. velezensis* AP203 with glucose amended treatments (supernatant, cell pellet, culture broth from *B. velezensis* AP203 with glucose) did not significantly reduce *M. incognita* populations compared to the *M. incognita* positive

**Table 1. Effect of *B. velezensis* AP203 amended with orange peel powder (OPP) or glucose on soybean growth at 45 days after planting in greenhouse trials.**

| Treatment*   | Shoot length (cm)b | Root length (cm) | Shoot fresh weight (g) | Root fresh weight (g) |
|--------------|---------------------|------------------|------------------------|-----------------------|
| TSB-grown cells | 58.0 a              | 22.5 ab          | 6.51 ab                 | 3.91 ab               |
| OPP (supernatant) | 67.4 a              | 21.9 abc         | 8.12 ab                 | 4.20 ab               |
| Glucose (supernatant) | 66.8 a              | 19.9 cde         | 7.55 ab                 | 3.98 ab               |
| OPP (Cells)   | 72.3 a              | 22.0 abc         | 9.06 ab                 | 4.92 a                |
| Glucose (Cells) | 62.5 a              | 20.1 bcde        | 8.43 ab                 | 3.42 ab               |
| OPP (Culture) | 72.0 a              | 22.6 a           | 10.20 a                 | 4.11 ab               |
| Glucose (Culture) | 60.8 a              | 19.0 e           | 8.25 ab                 | 4.00 ab               |
| OPP           | 62.5 a              | 21.4 abcd        | 8.12 ab                 | 3.65 ab               |
| Glucose       | 67.9 a              | 20.0 cde         | 5.95 ab                 | 2.53 b                |

*M. incognita* nematode

Untreated control

Notes: *B. velezensis* AP203 grown in 1.0% (w/v) OPP or glucose-amended TSS medium. *M. incognita* 2,000 eggs/150 cm³ soil was added to all plants except the untreated control; *means with the same letter are not significantly different at *p* ≤ 0.05 level of significance.
**Table 2. Effect of *B. velezensis* AP203, amended orange peel powder (OPP) or glucose on cotton growth at 45 days after planting in greenhouse trials.**

| Treatment                        | Shoot length (cm) | Root length (cm) | Shoot fresh weight (g) | Root fresh weight (g) |
|----------------------------------|-------------------|------------------|------------------------|-----------------------|
| TSB-grown cells                  | 26.0 ab           | 18.5 abc         | 2.6 a                  | 1.7 a                 |
| OPP (supernatant)                | 27.3 ab           | 20.2 ab          | 2.5 a                  | 1.7 a                 |
| Glucose (supernatant)            | 26.8 ab           | 18.2 abc         | 2.5 a                  | 1.6 a                 |
| OPP (Cells)                      | 30.7 a            | 21.2 ab          | 2.2 a                  | 1.9 a                 |
| Glucose (Cells)                  | 27.0 ab           | 16.1 bc          | 2.7 a                  | 1.6 a                 |
| OPP (Culture)                    | 30.6 ab           | 20.1 ab          | 2.4 a                  | 1.4 a                 |
| Glucose (Culture)                | 28.8 ab           | 18.1 abc         | 2.5 a                  | 1.6 a                 |
| OPP                              | 23.2 ab           | 17.7 abc         | 2.1 a                  | 1.7 a                 |
| Glucose                          | 22.6 b            | 16.1 bc          | 2.3 a                  | 1.1 a                 |
| *M. incognita* nematode          | 20.0 b            | 14.3 c           | 1.7 a                  | 0.9 a                 |
| Untreated control                | 26.2 ab           | 21.8 a           | 2.7 a                  | 1.3 a                 |

Notes: *B. velezensis* AP203 grew in 1.0% (w/v) OPP or glucose-amended TSS medium. *M. incognita* 2,000 eggs/150 cm³ soil was added to all plants except the untreated control; means with the same letter are not significantly different at $p \leq 0.05$ level of significance.

**Figure 2:** Effects of *B. velezensis* AP203 with an orange peel powder (OPP) or glucose amendments on the number of *M. incognita* eggs on the roots of soybean at 45 days after planting in greenhouse trials.
control treatment in soybean or cotton at 45 DAP (Figs. 2 and 3).

**Secretion of secondary metabolites by B. velezensis AP203 is affected by growth on orange peel powder**

Numerous secondary metabolites were detected in the supernatant of *B. velezensis* AP203 amended with orange peel, which revealed a complex mass spec profile even after removing the metabolites present in orange peel powder (data not shown). Among the mass ions predicted to be expressed by *B. velezensis* AP203, and not present in orange peel, were four metabolites that had previously been reported to have nematicidal activity: (i) 1,3-Diphenyl-2-propanone, (ii) p-(3,4-Dihydro-6-methoxy-2-naphthyl) phenol, (iii) (E)-1,1,-(1,2-Diethyl-1,2-ethenediy) bis (4-methoxybenzene), and (iv) 3-(Dimethylamino) propyl benzoate (Table 3) (Huang et al., 2010). The retention times (RT) of these secondary metabolites were 6.69, 6.63, 3.39, and 2.40 min, respectively (Table 3). The product mass to ions charge ratio (m/z) of these secondary metabolites were 211.11, 253.12, 295.17, and 206.12, respectively (Table 3 and Figs. S1-S3). The relative abundances (RA) per colony forming units (CFU) of secondary metabolite 1,3-Diphenyl-2-propanone were 21.11, 25.31, 29.51, and 20.61 (Table 3). The relative abundance (RA) per CFU indicated that (E)-1,1,-(1,2-Diethyl-1,2-ethenediy) bis (4-methoxybenzene) was produced most abundantly under these culture conditions when grown with OP as a carbon source, followed by p-(3,4-Dihydro-6-methoxy-2-naphthyl) phenol, 1,3-Diphenyl-2-propanone, and 3-(Dimethylamino) propyl benzoate in decreasing order of RA/CFU (Table 3). The complete list of metabolites detected from the supernatant of *B. velezensis* AP203 grown on orange peel in TSS minimal medium are listed in Supplementary Table 4.

**Discussion**

This study demonstrated that the PGPR *B. velezensis* AP203 when used in combination with a pectin-rich orange peel amendment resulted in significantly enhanced *M. incognita* J2 mortality. The nematicidal activity observed under laboratory and greenhouse conditions suggests that there are nematicidal secondary metabolites produced by *B. velezensis* AP203 in the presence of a pectin-rich orange peel growth substrate. In the presence of different carbohydrate substrates, the expression of secondary metabolites by PGPR strains has been previously shown to vary considerably (Zhu
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Table 3. Predicted secondary metabolites found in cell-free supernatants of *B. velezensis* AP203 after 48hr growth in 0.5% (w/v) OPP amended Tris Spizizen Salts (TSS) media.

| Treatment | RT (min) | Product ions (m/z) | RA/CFU | Predicted metabolites                   |
|-----------|----------|---------------------|--------|----------------------------------------|
| OPAP203 1 | 6.69 min | 211.11              | 21.11  | 1,3-Diphenyl-2-propanone               |
| OPAP203 2 | 6.63 min | 253.12              | 25.31  | p-(3,4-Dihydro-6-methoxy-2-naphthyl) phenol |
| OPAP203 3 | 3.39 min | 295.17              | 29.51  | (E)-1,1,-(1,2-Diethyl-1,2-ethenediyld) bis (4-methoxybenzene) |
| OPAP203 4 | 2.40 min | 206.12              | 20.61  | 3-(Dimethylamino) propyl benzoate       |
| GluAP203  | 0        | 0                   | 0      | 0                                      |
| OPP        | 0        | 0                   | 0      | 0                                      |
| Glucose    | 0        | 0                   | 0      | 0                                      |

Notes: *a* The in vitro *B. velezensis* AP203 growth test was repeated twice; *b* RT indicates retention time; *c* RA indicates relative abundance/colony forming units.

The results from the greenhouse tests suggested that cell pellet, culture broth, and supernatant of *B. velezensis* AP203 with 1.0% (w/v) orange peel amended media significantly increased soybean and cotton plant growth (root length) compared to the *M. incognita* inoculated positive control. In addition, the numbers of *M. incognita* eggs of cell pellet, culture broth, and supernatant were reduced in the roots of cotton and soybean compared to the *M. incognita* inoculated positive control. However, there were no significant differences between cell pellet, culture broth, or supernatant of *B. velezensis* AP203 amended with orange peel. Previous studies reported that extracts of fresh orange peel significantly reduced *M. incognita* eggs and J2 in planta and in vitro (Faye, 2017; Raviv et al., 2005; Tsai, 2008). Recent studies also showed that *B. velezensis* strains were capable of enhancing cotton and soybean yields and reducing *M. incognita* egg populations in a greenhouse, micro plots, and field experiments (Xiang et al., 2017a, b). This is the first study to evaluate the combination of a PGPR *B. velezensis* strain and an orange peel amendment in antagonizing *M. incognita* populations. Agricultural waste can be an environmental problem, and waste management is an enormous challenge worldwide. The use of orange peel to promote the efficacy of PGPR-mediated plant pathogenic nematode control may reduce the need for chemical nematicides and improve plant and soil health. The present findings indicate the potential for *B. velezensis* AP203 and orange peel amendments to reduce *M. incognita* population density and increase yield under field conditions, thereby providing an alternative option to chemical...
nematicides. In addition to improving plant health and suppressing plant-parasitic nematodes, orange peel amendment to soils may also enhance soil nutrient levels. A recent study showed that agricultural waste orange peel significantly enhanced soil nutrient levels and regenerated tropical forest vegetation in Costa Rica (Treuer et al., 2018). Taken together, the results of this and other studies suggest that this symbiotic treatment may be a cost-effective strategy of benefit to sustainable agricultural practices.

The four metabolites that were specifically produced by *B. velezensis* AP203 when grown on an orange peel substrate may have direct inhibitory effects on *M. incognita* eggs and J2 viability. A previous study reported that the volatile organic compounds phenol, propyl benzene, propanone, and 1-ethenyl-4-methoxy benzene produced by *B. megaterium* YFM3.25 showed nematicidal effects and significantly reduced *M. incognita* eggs in pot experiments (Huang et al., 2010). Orange peel contains pectin, limonene, and phenolic compounds that have antioxidant properties and exerts beneficial effects on plant health (Bocco et al., 1998; Rafiq et al., 2019). The increased *M. incognita* mortality observed in this study may be due to a combination of metabolites producing by *B. velezensis* AP203 and derived from orange peel. These data support the conclusion that the metabolome of *B. velezensis* AP203 is significantly altered when this strain is grown on OPP, resulting in the expression of previously identified nematicidal compounds which were not observed when this strain was grown on glucose as a sole carbon source. No doubt, the chemical milieu of citrus peel includes a greater complexity of carbon sources and organic compounds, relative to growth on glucose alone, may elicit changes in *B. velezensis* gene expression. Further experiments should explore the changes in secondary metabolite expression for *B. velezensis* AP203 and other bioactive PGPR strains within the rhizosphere, as affected by the presence of pectin-rich amendments or other compatible prebiotic complex carbohydrates. This study is the first report that a *B. velezensis* strain in combination with an orange peel prebiotic amendment can inhibit the viability of *M. incognita* eggs and J2, mediated at least in part through the production of bioactive secondary metabolites. Thus, treatments that combine *B. velezensis* strains with nematicidal potential along with an orange peel amendment are predicted to more effectively reduce plant-pathogenic nematode population density in planta.

In conclusion, *B. velezensis* AP203 when combined with an orange peel amendment significantly reduced *M. incognita* populations in vitro and in planta. Hence, the combined use of *B. velezensis* strains and orange peel represents a promising and sustainable biological control technique for plant-parasitic nematodes.

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Table S1. List of predicted secondary metabolites found in cell-free supernatants of *B. velezensis* AP203 after 48 hr growth in 0.5% (w/v) orange peel power (OPP) amended Tris Spizizen Salts (TSS) media.

| Query ID | Query m/z | Name of the bioactive compounds | Formula | Exact mass |
|----------|------------|---------------------------------|---------|------------|
| 1        | 101.0711   | Gyromitrin; Acetaldehyde methylformylhydrazone | C4H8N2O 100.0636629 |
| 2        | 103.0559   | Indoleacetic acid               | C10H9NO2 175.0633285 |
| 3        | 103.0559   | 5-Hydroxyindoleacetaldehyde    | C10H9NO2 175.0633285 |
| 4        | 104.0549   | 2-Ethyl-1-hexanol, 9CI; (Å±)-form, O-Sulfate | C8H18O4S 210.0925798 |
| 5        | 104.0568   | 2-Ethyl-1-hexanol, 9CI; (Å±)-form, O-Sulfate | C8H18O4S 210.0925798 |
| 6        | 104.0585   | Histidinyl-Glycine              | C8H12N4O3 212.0909403 |
| 7        | 104.0585   | D-Glycero-D-galacto-heptitol    | C7H16O7 212.0896029 |
| 8        | 104.0585   | Glycyl-Histidine                | C8H12N4O3 212.0909403 |
| 9        | 104.0707   | N-methyl-beta-alanine           | C4H9NO2 103.0633285 |
| 10       | 104.0707   | Ethyl carbamic acid methyl ester | C4H9NO2 103.0633285 |
| 11       | 104.0707   | N-Methyl-L-alanine              | C4H9NO2 103.0633285 |
| 12       | 104.0707   | HBA                              | C4H9NO2 103.0633285 |
| 13       | 104.0707   | DL-3-aminobutyrate              | C4H9NO2 103.0633285 |
| 14       | 104.0707   | Mefenamic acid                  | C15H15NO2 241.1102787 |
| 15       | 104.0707   | N-[2-(4-Hydroxyphenyl)ethyl]benzamide | C15H15NO2 241.1102787 |
| 16       | 104.0707   | 2-Amino-2-methylpropanoate;2-Aminoisobutyric acid | C4H9NO2 103.0633285 |
| 17       | 104.0707   | (R,S)-3-Amino-2-methylpropanoate | C4H9NO2 103.0633285 |
| 18       | 104.0707   | beta-alanine-methyl-ester       | C4H9NO2 103.0633285 |
| 19       | 104.0707   | N,N-Dimethylglycine;Dimethylglycine | C4H9NO2 103.0633285 |
| 20       | 104.0707   | N-Ethylglycine                  | C4H9NO2 103.0633285 |
| 21       | 104.0707   | (R)-2-Aminobutanoic acid;(S)-2-Aminobutanoate | C4H9NO2 103.0633285 |
| 22       | 104.0707   | 1-nitrobutane                   | C4H9NO2 103.0633285 |
| 23       | 104.0707   | 4-Aminobutanoate;4-Aminobutanoic acid | C4H9NO2 103.0633285 |
| 24       | 105.0366   | 3-methylthiopropanal            | C4H8OS 104.0295856 |
| 25       | 105.0366   | Acutifolane A                   | C16H22O3 262.1569 |
| 26       | 105.0367   | 3-methylthiopropanal            | C4H8OS 104.0295856 |
| 27       | 105.0367   | tetrahydrothiophene 1-oxide     | C4H8OS 104.0295856 |
| 28       | 105.0367   | tetrahydrothiophene 1-oxide     | C4H8OS 104.0295856 |
| 29       | 105.0372   | 3-methylthiopropanal            | C4H8OS 104.0295856 |
| 30       | 105.0372   | tetrahydrothiophene 1-oxide     | C4H8OS 104.0295856 |
| 31       | 105.0376   | 3-methylthiopropanal            | C4H8OS 104.0295856 |
| 32       | 105.0376   | tetrahydrothiophene 1-oxide     | C4H8OS 104.0295856 |
| 33       | 105.0376   | tetrahydrothiophene 1-oxide     | C4H8OS 104.0295856 |
| 34       | 105.044    | 3-cyanopyridine                 | C6H4N2 104.0374481 |
| 35       | 105.044    | 2-Cyanopyridine                 | C6H4N2 104.0374481 |
| No. | Precise Mass (m/z) | Name                                                                 | Formula   | Exact Mass       |
|-----|-------------------|----------------------------------------------------------------------|-----------|------------------|
| 39  | 105.044           | 4-Cyanopyridine                                                       | C6H4N2    | 104.0374481      |
| 40  | 105.0441          | (+)-18-Hydroxy-7,16-sacculatadiene-11,12-dial                        | C20H30O3  | 318.2195         |
| 41  | 105.0441          | ent-7alpha-hydroxykaur-16-en-19-oic acid                            | C20H30O3  | 318.2195         |
| 42  | 105.0441          | 2-Cyanopyridine                                                       | C6H4N2    | 104.0374481      |
| 43  | 105.0441          | Oxymesterone                                                         | C20H30O3  | 318.2194948      |
| 44  | 105.0441          | 4-Cyanopyridine                                                       | C6H4N2    | 104.0374481      |
| 45  | 105.0441          | 3-cyanopyridine                                                       | C6H4N2    | 104.0374481      |
| 46  | 105.0441          | 8-oxo-5E,9Z,11Z,14Z-eicosatetraenoic acid                            | C20H30O3  | 318.2195         |
| 47  | 105.0441          | 9-oxo-5E,7Z,11Z,14Z-eicosatetraenoic acid                            | C20H30O3  | 318.2195         |
| 48  | 105.0441          | 11-oxo-5E8Z,12Z,14Z-Eicosatetraenoic acid                            | C20H30O3  | 318.2195         |
| 49  | 105.0441          | (+)-7beta-Hydroxy-15-beyeren-19-oic acid                            | C20H30O3  | 318.2195         |
| 50  | 105.0557          | Tyrosyl-Tyrosine                                                     | C18H20N2O5| 344.1372218      |
| 51  | 105.0651          | Aminoserine                                                          | C3H8N2O2  | 104.0585775      |
| 52  | 105.0651          | L-2,3-Diaminopropionate                                             | C3H8N2O2  | 104.0585775      |
| 53  | 105.0651          | Hydroxyaminoalanine                                                  | C3H8N2O2  | 104.0585775      |
| 54  | 105.0662          | 2,3-Diaminopropanoic acid                                           | C3H8N2O2  | 104.0585775      |
| 55  | 105.0672          | 4-O-Methylbavachalcone                                              | C22H24O4  | 352.1675         |
| 56  | 105.0672          | Ovalchalcone                                                         | C22H24O4  | 352.1675         |
| 57  | 105.0672          | Pongagallone A                                                       | C22H24O4  | 352.1675         |
| 58  | 105.0672          | Candidone                                                            | C22H24O4  | 352.1675         |
| 59  | 105.0672          | Methylhildgardtol A                                                  | C22H24O4  | 352.1675         |
| 60  | 105.0672          | Methylhildgardtol B                                                  | C22H24O4  | 352.1675         |
| 61  | 105.0672          | Xuulanin                                                             | C22H24O4  | 352.1675         |
| 62  | 105.0715          | Valganciclovir                                                       | C14H22N6O5| 354.1651678      |
| 63  | 105.0733          | 12-hydroxyjasmonic acid                                              | C19H30O8  | 386.1941         |
| 64  | 105.0733          | Citroside A                                                          | C19H30O8  | 386.1940679      |
| 65  | 105.0733          | 6,9-Dihydroxy-4,7-megastigmadien-3-one                               | C19H30O8  | 386.1940679      |
| 66  | 107.0845          | p-Xylene;1,4-Dimethylbenzene;p-Methyltoluene                         | C8H10     | 106.0782503      |
| 67  | 107.0845          | Ethylbenzene;Phenylethane;Ethylbenzol;Ethylbenzene                   | C8H10     | 106.0782503      |
| 68  | 107.0845          | o-Xylene;o-Dimethylbenzene;o-Methyltoluene                          | C8H10     | 106.0782503      |
| 69  | 107.0845          | m-Xylene;1,3-Dimethylbenzene;1,3-Xylene                              | C8H10     | 106.0782503      |
| 70  | 107.0848          | o-Xylene;o-Dimethylbenzene;o-Methyltoluene                          | C8H10     | 106.0782503      |
| 71  | 107.0848          | m-Xylene;1,3-Dimethylbenzene;1,3-Xylene                              | C8H10     | 106.0782503      |
| 72  | 107.0848          | p-Xylene;1,4-Dimethylbenzene;p-Methyltoluene                         | C8H10     | 106.0782503      |
| 73  | 107.0848          | Ethylbenzene;Phenylethane;Ethylbenzol;Ethylbenzene                   | C8H10     | 106.0782503      |
| 74  | 107.0848          | O-6-deoxy-a-L-galactopyranosyl                                        | C20H33NO14| 511.1901048      |
| 75  | 107.0851          | o-Xylene;o-Dimethylbenzene;o-Methyltoluene                          | C8H10     | 106.0782503      |
| 76  | 107.0851          | m-Xylene;1,3-Dimethylbenzene;1,3-Xylene                              | C8H10     | 106.0782503      |
| 77  | 107.0851          | p-Xylene;1,4-Dimethylbenzene;p-Methyltoluene                         | C8H10     | 106.0782503      |
| 78  | 107.0851          | Ethylbenzene;Phenylethane;Ethylbenzol;Ethylbenzene                   | C8H10     | 106.0782503      |
| No. | Mass (Da) | Molecular Formula | Molecular Weight |
|-----|-----------|-------------------|------------------|
| 79  | 107.0858  | p-Xylene; 1,4-Dimethylbenzene; p-Methyltoluene | C₈H₁₀ 106.0782503 |
| 80  | 107.0858  | Ethylbenzene; Phenylethane; Ethylbenzol; Ethylene | C₈H₁₀ 106.0782503 |
| 81  | 107.0858  | o-Xylene; o-Dimethylbenzene; o-Methyltoluene | C₈H₁₀ 106.0782503 |
| 82  | 107.0858  | m-Xylene; 1,3-Dimethylbenzene; 1,3-Xylene | C₈H₁₀ 106.0782503 |
| 83  | 107.0858  | Daunorubicin | C₂₇H₂₉NO₁₀ 527.1791462 |
| 84  | 107.0858  | p-Xylene; 1,4-Dimethylbenzene; p-Methyltoluene | C₈H₁₀ 106.0782503 |
| 85  | 107.0858  | Ethylbenzene; Phenylethane; Ethylbenzol; Ethylene | C₈H₁₀ 106.0782503 |
| 86  | 107.0858  | o-Xylene; o-Dimethylbenzene; o-Methyltoluene | C₈H₁₀ 106.0782503 |
| 87  | 107.0858  | m-Xylene; 1,3-Dimethylbenzene; 1,3-Xylene | C₈H₁₀ 106.0782503 |
| 88  | 109.0287  | 1,2-Benzoquinone | C₆H₄O₂ 108.0211294 |
| 89  | 109.0287  | Quinone; p-Benzquinone; Chinone | C₆H₄O₂ 108.0211294 |
| 90  | 109.0306  | Hordatine B glucoside | C₃₅H₅₀N₈O₁₀ 742.3649899 |
| 91  | 109.0306  | Hydroxymethylmethylsilanediol | C₂H₈O₃Si 108.0242707 |
| 92  | 109.0309  | Hydroxyethylmethylsilanediol | C₂H₈O₃Si 108.0242707 |
| 93  | 109.0309  | Monothioglycerol | C₃H₈O₂S 108.0245002 |
| 94  | 109.0315  | Hydroxyethylmethylsilanediol | C₂H₈O₃Si 108.0242707 |
| 95  | 109.0316  | Hydroxyethylmethylsilanediol | C₂H₈O₃Si 108.0242707 |
| 96  | 109.0321  | Hydroxyethylmethylsilanediol | C₂H₈O₃Si 108.0242707 |
| 97  | 109.0321  | Hydroxyethylmethylsilanediol | C₂H₈O₃Si 108.0242707 |
| 98  | 109.0642  | Benzenemethanol; Phenylmethanol; Phenylcarbinol | C₇H₈O 108.0575149 |
| 99  | 109.0642  | o-Cresol; 2-Hydroxytoluene; o-Methylphenol | C₇H₈O 108.0575149 |
| 100 | 109.0642  | 3-Cresol; m-Cresol; 3-Hydroxytoluene | C₇H₈O 108.0575149 |
| 101 | 109.0642  | 4-Cresol; p-Cresol; 4-Hydroxytoluene | C₇H₈O 108.0575149 |
| 102 | 109.0642  | Anisole; Methoxybenzene; Methyl phenyl ether | C₇H₈O 108.0575149 |
| 103 | 111.0434  | Resorcinol; Resorcin; 1,3-Benzenediol | C₆H₆O₂ 110.0367794 |
| 104 | 111.0434  | Hydroquinone; p-Benzenediol; 1,4-Benzenediol | C₆H₆O₂ 110.0367794 |
| 105 | 111.0434  | 5-Methyl-2-furaldehyde; 5-Methyl-2-furfural | C₆H₆O₂ 110.0367794 |
| 106 | 111.0434  | o-Benzosemiquinone | C₆H₆O₂ 110.0367794 |
| 107 | 111.0434  | Catechol; 1,2-Benzenediol; o-Benzenediol | C₆H₆O₂ 110.0367794 |
| 108 | 111.0434  | Benzosemiquinone; p-Benzosemiquinone | C₆H₆O₂ 110.0367794 |
| 109 | 111.0437  | Resorcinol; Resorcin; 1,3-Benzenediol | C₆H₆O₂ 110.0367794 |
| 110 | 111.0437  | Hydroquinone; p-Benzenediol; 1,4-Benzenediol | C₆H₆O₂ 110.0367794 |
| 111 | 111.0437  | 5-Methyl-2-furaldehyde; 5-Methyl-2-furfural | C₆H₆O₂ 110.0367794 |
| 112 | 111.0437  | Catechol; 1,2-Benzenediol; o-Benzenedio | C₆H₆O₂ 110.0367794 |
| 113 | 111.0437  | Benzosemiquinone; p-Benzosemiquinone | C₆H₆O₂ 110.0367794 |
| 114 | 111.0438  | 2-Furanmethanol | C₅H₆O₂ 98.03677944 |
| 115 | 111.0438  | Benzosemiquinone; p-Benzosemiquinone | C₆H₆O₂ 110.0367794 |
| 116 | 111.0438  | Resorcinol; Resorcin; 1,3-Benzenediol | C₆H₆O₂ 110.0367794 |
| 117 | 111.0438  | Hydroquinone; p-Benzenediol; 1,4-Benzenediol | C₆H₆O₂ 110.0367794 |
| 118 | 111.0438  | 5-Methyl-2-furaldehyde; 5-Methyl-2-furfural | C₆H₆O₂ 110.0367794 |
| 119 | 111.0438  | o-Benzosemiquinone | C₆H₆O₂ 110.0367794 |
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| Line | Mass/Charge | Molecular Formula | Molecular Weight |
|------|-------------|-------------------|------------------|
| 120  | 111.0438    | penta-2,4-dienoic acid; beta-vinyl acrylic acid | C5H6O2 98.0368 |
| 121  | 111.0438    | Catechol; 1,2-Benzenediol; o-Benzenediol | C6H6O2 110.036794 |
| 122  | 111.0438    | 5-Methyl-2(3H)-furanone | C5H6O2 98.0367944 |
| 123  | 111.0447    | o-Benzosemicinone | C6H6O2 110.036794 |
| 124  | 111.0447    | Catechol; 1,2-Benzenediol; o-Benzenediol | C6H6O2 110.036794 |
| 125  | 111.0447    | Benzosemicinone; p-Benzosemicinone | C6H6O2 110.036794 |
| 126  | 111.0447    | Resorcinol; Resorcin; 1,3-Benzenediol; 1,3-Dihydroxybenzene | C6H6O2 110.036794 |
| 127  | 111.0447    | Hydroquinone; p-Benzenediol; 1,4-Benzenedi | C6H6O2 110.036794 |
| 128  | 111.0447    | 5-Methyl-2-furaldehyde; 5-Methyl-2-furfural | C6H6O2 110.036794 |
| 129  | 111.0448    | o-Benzosemicinone | C6H6O2 110.036794 |
| 130  | 111.0448    | Catechol; 1,2-Benzenediol; o-Benzenediol | C6H6O2 110.036794 |
| 131  | 111.0448    | Benzosemicinone; p-Benzosemicinone | C6H6O2 110.036794 |
| 132  | 111.0448    | Resorcinol; Resorcin; 1,3-Benzenediol; 1,3-Dihydroxybenzene | C6H6O2 110.036794 |
| 133  | 111.0448    | Hydroquinone; p-Benzenediol; 1,4-Benzenedi | C6H6O2 110.036794 |
| 134  | 111.0451    | Catechol; 1,2-Benzenediol; o-Benzenediol | C6H6O2 110.036794 |
| 135  | 111.0451    | Benzosemicinone; p-Benzosemicinone | C6H6O2 110.036794 |
| 136  | 111.0451    | Resorcinol; Resorcin; 1,3-Benzenediol; 1,3-Dihydroxybenzene | C6H6O2 110.036794 |
| 137  | 111.0451    | Hydroquinone; p-Benzenediol; 1,4-Benzenedi | C6H6O2 110.036794 |
| 138  | 111.0451    | 5-Methyl-2-furaldehyde; 5-Methyl-2-furfural | C6H6O2 110.036794 |
| 139  | 121.0316    | Dimethylsulfonioacetate | C4H8O2S 120.0245002 |
| 140  | 121.0316    | 3-(Methylthio)propionic acid; 3-Methylthiopropionate | C4H8O2S 120.0245002 |
| 141  | 121.0316    | sulfolane | C4H8O2S 120.0245002 |
| 142  | 121.032     | Dimethylsulfonioacetate | C4H8O2S 120.0245002 |
| 143  | 121.032     | 3-(Methylthio)propionic acid; 3-Methylthiopropionate | C4H8O2S 120.0245002 |
| 144  | 121.032     | sulfolane | C4H8O2S 120.0245002 |
| 145  | 121.0324    | Dimethylsulfonioacetate | C4H8O2S 120.0245002 |
| 146  | 121.0324    | 3-(Methylthio)propionic acid; 3-Methylthiopropionate | C4H8O2S 120.0245002 |
| 147  | 121.0324    | sulfolane | C4H8O2S 120.0245002 |
| 148  | 121.0325    | Dimethylsulfonioacetate | C4H8O2S 120.0245002 |
| 149  | 121.0325    | 3-(Methylthio)propionic acid; 3-Methylthiopropionate | C4H8O2S 120.0245002 |
| 150  | 121.0325    | sulfolane | C4H8O2S 120.0245002 |
| 151  | 121.0325    | Dimethylsulfonioacetate | C4H8O2S 120.0245002 |
| 152  | 121.0325    | 3-(Methylthio)propionic acid; 3-Methylthiopropionate | C4H8O2S 120.0245002 |
| 153  | 121.0325    | sulfolane | C4H8O2S 120.0245002 |
| 154  | 121.0325    | Dimethylsulfonioacetate | C4H8O2S 120.0245002 |
| 155  | 121.0325    | 3-(Methylthio)propionic acid; 3-Methylthiopropionate | C4H8O2S 120.0245002 |
| 156  | 121.0325    | sulfolane | C4H8O2S 120.0245002 |
| Page | M/Z   | Formula           | Molecular Weight   |
|------|-------|-------------------|--------------------|
| 157  | 121.037 | 3-nitro-1-propionate | C\(_3\)H\(_6\)NO\(_4\) | 120.0296827 |
| 158  | 121.0379 | 3-nitro-1-propionate | C\(_3\)H\(_6\)NO\(_4\) | 120.0296827 |
| 159  | 121.05  | 2,3-dihydroxy-2-methyl-propanoic acid | C\(_4\)H\(_8\)O\(_4\) | 120.0422587 |
| 160  | 121.05  | L-(-)-Erythrose;D-threo-Aldose;D-Erythulose | C\(_4\)H\(_8\)O\(_4\) | 120.0422587 |
| 161  | 121.05  | 3-Deoxytetronic acid | C\(_4\)H\(_8\)O\(_4\) | 120.0422587 |
| 162  | 121.05  | 4-Deoxyerythronic acid | C\(_4\)H\(_8\)O\(_4\) | 120.0422587 |
| 163  | 121.05  | L-Erythulose;L-glycero-Tetrolose | C\(_4\)H\(_8\)O\(_4\) | 120.0422587 |
| 164  | 121.05  | 3,4-Dihydroxybutyric acid | C\(_4\)H\(_8\)O\(_4\) | 120.0422587 |
| 165  | 121.05  | D-Threose;D-threo-Tetrose;D-Erythrose | C\(_4\)H\(_8\)O\(_4\) | 120.0422587 |
| 166  | 121.0516 | Purine | C\(_5\)H\(_4\)N\(_4\) | 120.0435961 |
| 167  | 122.0962 | 3,4-DIMETHYLAMINE | C\(_8\)H\(_11\)N | 121.0891494 |
| 168  | 122.0962 | 1-Phenylethylamine;alpha-Phenylethylamine | C\(_8\)H\(_11\)N | 121.0891494 |
| 169  | 122.0962 | N-Ethylaniline;N-Ethylbenzenamine | C\(_8\)H\(_11\)N | 121.0891494 |
| 170  | 122.0962 | Phenethylamine;2-Phenylethylamine;beta-Phenylethylamine | C\(_8\)H\(_11\)N | 121.0891494 |
| 171  | 122.0962 | 2,4-Dimethylaniline;2,4-DMA | C\(_8\)H\(_11\)N | 121.0891494 |
| 172  | 122.0962 | N,N-Dimethylaniline;N,N-Dimethylbenzenamine | C\(_8\)H\(_11\)N | 121.0891494 |
| 173  | 122.0965 | 1-Phenylethylamine;alpha-Phenylethylamine | C\(_8\)H\(_11\)N | 121.0891494 |
| 174  | 122.0965 | 2,6-Dimethylaniline | C\(_8\)H\(_11\)N | 121.0891494 |
| 175  | 122.0965 | N-Ethylaniline;N-Ethylbenzenamine | C\(_8\)H\(_11\)N | 121.0891494 |
| 176  | 122.0965 | 2,5-Dimethylalanine | C\(_8\)H\(_11\)N | 121.0891494 |
| 177  | 122.0965 | 2-Phenylethylamine;beta-Phenylethylamine | C\(_8\)H\(_11\)N | 121.0891494 |
| 178  | 181.0694 | Sorbose;xylo-Hexulose;D-Fructose | C\(_6\)H\(_12\)O\(_6\) | 180.0633881 |
| 179  | 181.0694 | 2-Deoxy-D-gluconate | C\(_6\)H\(_12\)O\(_6\) | 180.0633881 |
| 180  | 181.0694 | Ketose | C\(_6\)H\(_12\)O\(_6\) | 180.0633881 |
| 181  | 181.0996 | Methylphosphonic acid diisopropyl ester | C\(_7\)H\(_17\)O\(_3\)P | 180.0915309 |
| 182  | 229.1235 | 1,1-Bis(4-hydroxyphenyl)propane | C\(_9\)H\(_16\)O\(_3\) | 228.1150298 |
| 183  | 229.1235 | Mansoneone C | C\(_9\)H\(_16\)O\(_3\) | 228.1150298 |
| 184  | 229.1235 | Bisphenol A;2,2-Bis(4-Hydroxyphenyl)propane | C\(_9\)H\(_16\)O\(_3\) | 228.1150298 |
| 185  | 229.1235 | dihydroinosylvin monomethyl ether | C\(_9\)H\(_16\)O\(_3\) | 228.1150298 |
| 186  | 229.1285 | Deoxyguanidinoproclavaminic acid | C\(_9\)H\(_16\)N\(_4\)O\(_3\) | 228.1222404 |
| 187  | 229.1298 | Deoxyamidinoproclavaminate | C\(_9\)H\(_16\)N\(_4\)O\(_3\) | 228.1222404 |
| 188  | 230.058  | Lamivudine;3TC;2',3'-Dideoxy-3'-thiacytidine | C\(_9\)H\(_16\)N\(_3\)O\(_3\)S | 229.0521119 |
| 189  | 230.058  | Carbonylphosphonic acid | C\(_9\)H\(_12\)NO\(_4\)P | 229.0503944 |
| 190  | 230.058  | 2,3-Dihydroxy-2'-carboxybiphenyl | C\(_9\)H\(_12\)O\(_4\) | 229.0500838 |
| 191  | 357.1718 | Rutamarin | C\(_2\)H\(_2\)O\(_5\) | 356.1623739 |
| 192  | 357.1718 | Gingerenone A | C\(_2\)H\(_2\)O\(_5\) | 356.1623739 |
| 193  | 357.1718 | alpha,beta-dihydroxanthohumol | C\(_2\)H\(_2\)O\(_5\) | 356.1623739 |
| 194  | 371.0795 | Kadsurenone;Denudatin B | C\(_2\)H\(_2\)O\(_5\) | 356.1623739 |
| 195  | 371.0795 | Rebamipide | C\(_3\)H\(_4\)Cl\(_2\)N\(_2\)O\(_4\) | 370.0720347 |
| 196  | 371.0795 | Digalacturonate;Digalacturonic acid | C\(_3\)H\(_4\)Cl\(_2\)N\(_2\)O\(_4\) | 370.0747407 |
| 197  | 371.145  | Naphthalene-2-sulfonamide | C\(_4\)H\(_6\)Cl\(_2\)O\(_3\)S | 370.1351133 |
| 198  | 371.1501 | iso-dehydrocycloanthohumol hydrate | C\(_2\)H\(_2\)O\(_5\) | 370.1416384 |
PGPR plus orange peel enhances nematode biocontrol: Hassan et al.

| m/z     | 0.1ppm  | Name                                                   | Molecular Formula | Retention Time |
|---------|--------|--------------------------------------------------------|-------------------|----------------|
| 199     | 371.1501 | xanthohumol D                                           | C_{21}H_{22}O_{6} | 370.1416384    |
| 200     | 371.1501 | 5’-Prenylhomoeriodictyol; Sigmoidin B 3’-methyl ether   | C_{21}H_{22}O_{6} | 370.1416384    |
| 201     | 371.1501 | xanthohumol B                                           | C_{21}H_{22}O_{6} | 370.1416384    |
| 202     | 371.1501 | curcumin                                               | C_{21}H_{22}O_{6} | 370.1416384    |
| 203     | 371.1501 | Alkannin beta, beta-dimethylacrylate                   | C_{21}H_{22}O_{6} | 370.1416384    |
| 204     | 371.1501 | Sophoraisoflavanone A                                  | C_{21}H_{22}O_{6} | 370.1416384    |
| 205     | 371.1509 | iso-dehydrocycloxanthohumol hydrate                    |                   |                |
| 206     | 371.1509 | xanthohumol D                                           | C_{21}H_{22}O_{6} | 370.1416384    |
| 207     | 371.1509 | 5’-Prenylhomoeriodictyol; Sigmoidin B 3’-methyl ether   | C_{21}H_{22}O_{6} | 370.1416384    |
| 208     | 371.1509 | xanthohumol B                                           | C_{21}H_{22}O_{6} | 370.1416384    |
| 209     | 371.1509 | curcumin                                               | C_{21}H_{22}O_{6} | 370.1416384    |
| 210     | 371.1509 | Alkannin beta, beta-dimethylacrylate                   | C_{21}H_{22}O_{6} | 370.1416384    |
| 211     | 371.1509 | Sophoraisoflavanone A                                  | C_{21}H_{22}O_{6} | 370.1416384    |
| 212     | 371.1528 | Galactan; Amylose                                       | C_{14}H_{26}O_{11}| 370.1475117    |
| 213     | 371.1528 | Galactan; Amylose                                       | C_{14}H_{26}O_{11}| 370.1475117    |
| 214     | 371.1541 | Galactan; Amylose                                       | C_{14}H_{26}O_{11}| 370.1475117    |
| 215     | 404.1365 | triflouperazine                                         | C_{21}H_{20}F_{3}N_{3}S | 403.1330029 |
| 216     | 404.1484 | Ampicillin trihydrate                                   | C_{16}H_{25}N_{3}O_{7}S | 403.1413209 |
| 217     | 405.1191 | Spectinomycin dihydrochloride                           | C_{14}H_{26}Cl_{2}N_{2}O_{7} | 404.1117066 |
| 218     | 405.1267 | Sulfinpyrazone; Sulfooxyphenylpyrazolidine               | C_{23}H_{20}N_{2}O_{3}S | 404.1194632 |
| 219     | 405.1269 | Sulfinpyrazone; Sulfooxyphenylpyrazolidine               | C_{23}H_{20}N_{2}O_{3}S | 404.1194632 |

Figure S1: LC-MS/MS spectra of the peaks eluted at 6.69 min (m/z of 211.11).
Figure S2: LC-MS/MS spectra of the peaks eluted at 2.40 min (m/z of 206.12).

Figure S3: LC-MS/MS spectra of the peaks eluted at 6.63 min (m/z of 253.12) and 3.39 min (m/z of 295.17).