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Environmentally Friendly Management of Root-Knot Disease on Melon with Bioformulated *Pseudomonas chlororaphis* O6

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Abstract

Root-knot nematodes are one of the most serious pests in melon as it is cropped continuously in greenhouses. However, only limited and costly chemical methods are available for nematode control. In previous field work, a biocontrol agent, *Pseudomonas chlororaphis* O6, showed strong efficacy against root-knot nematode. In the summer of 2015, *P. chlororaphis* O6 strain, formulated with freeze-drying agents as a wettable powder form, was applied to melon fields in greenhouse at Gosung in South Korea. The nematocide, Fosthazate, was used for comparison as an authentic chemical pesticide. The melon cultivar Early Elite was transplanted into soil used for three years for melon cultivation; plants in 2014 were highly infected with root-knot nematode. Disease severity in 2015 was rated based on larval mortality, number of galls/plant and plant growth parameters. Root drenches of both the biocontrol and chemical products at transplant stage reduced nematode populations significantly (P<0.01). Reduction was greater (P<0.05) with the biocontrol agent (88 – 93 %) than with applications of Fosthazate (69 %). Drenching with the bioformulation improved (P<0.05) plant height, leaf length, leaf width, and stem diameter when compared with growth of melon treated with the chemical. Fruit yield was 20 % higher. These findings suggest that bioformulation of *P. chlororaphis* O6 provides a sustainable alternative to chemical use for control of root-knot nematode. Applications of the biocontrol agent can become integrated into management strategies for root-knot disease in melon.

Results

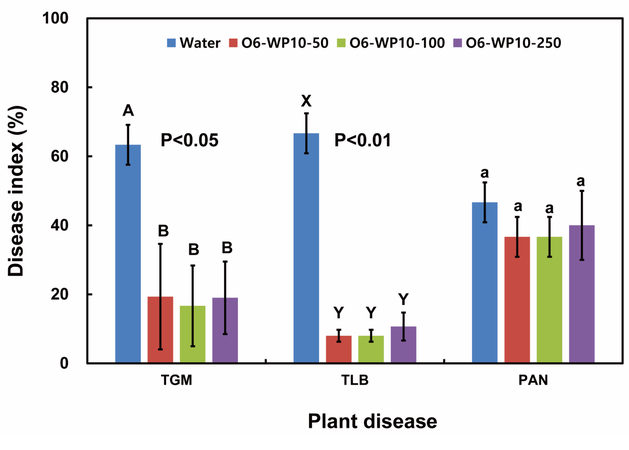

Fig. 1. *In planta* biocontrol efficacies of the formulated O6-WP10 against three vegetable diseases. The O6-WP10 product was suspended with Tween® 20 as a wetting agent (0.025%) at 50 g/L (O6-WP10-50), 10 g/L (O6-WP10-100), or 4 g/L (O6-WP10-250) prior to application. The tomato plants were sprayed to run off with the O6-WP10 dilutions or distilled water plus Tween® 20 was used as the negative control. One day after treatment, plants were inoculated by spraying with spore suspensions of each pathogen. The disease index, rated 3–7 days after pathogen inoculation, was based on the areas of the infected lesion. TGM, tomato gray mold; TLB, tomato late blight; and PAN, pepper anthracnose. Data are means ± standard deviations of three independent experiments with nine plants/treatment. Bars with different letters for each pathosystem have significantly different (P < 0.05 or P < 0.01) levels of disease based on Duncan's multiple range test.

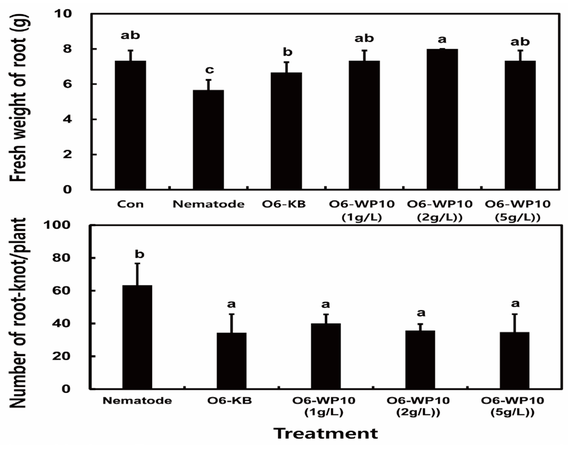

Fig. 2. *In vitro* control by the wettable-powder bioformulated product of *Pseudomonas chlororaphis* O6 of root-knot nematodes in pot-grown tomato seedlings. Each treatment box, containing 2-week-old tomato seedling, was drenched with either 0 ml of a 10-fold diluted fresh culture of the *P. chlororaphis* O6 grown in KB broth, or the O6-WP10 formulation at the designated doses one week before nematode inoculation. Negative controls involved adding 10 ml of sterile water to the boxes. At least 200 *M. hapla* J2 juveniles in 10 ml of sterile water were applied to the test boxes. After 1 month, the roots were collected to measure fresh weight, and the numbers of root-knot galls/root were counted. The study was conducted with three replicates per treatment with 3 tomato plants for each treatment. Different letters within a column represent significant differences at 0.05 probability level according Duncan's multiple range test.

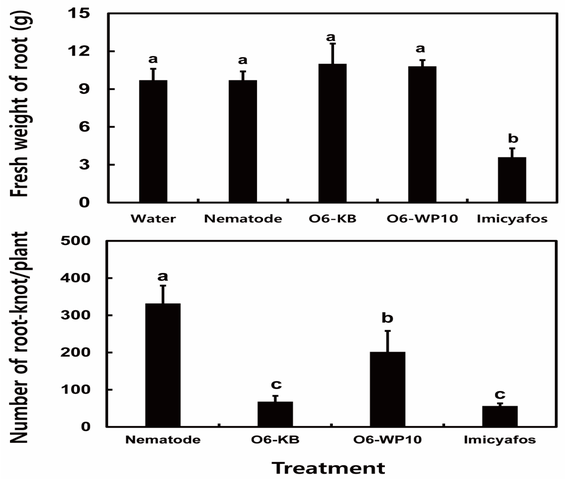

Fig. 3. Curative effects of the wettable powder bio-formulated product (2 g/L) and fresh bacterial cultures of *Pseudomonas chlororaphis* O6 on root-knot nematode in pot grown tomato. The nematicidal potential of the formulated product was determined for potential curative effects. At least 200 *M. hapla* J2 juveniles in 10 ml of sterile water were applied to each Magenta box containing 2-week-old tomato seedling. One week after the root-knot nematode inoculation, each treatment box was drenched with 10 ml of 10-fold diluted fresh culture of *P. chlororaphis* O6 grown in KB broth, or of O6-WP10 formulation at 2 g/L. The chemical nematicide, Imicyafos at 5 ml/20 L was applied as a positive treatment and non-inoculated KB medium diluted 10-fold with sterile water was employed as a negative control. After 1 month, the roots were collected to measure fresh weight, and the numbers of root-knot galls/root were counted. The study was conducted with three replicates per treatment with 3 tomato plants each treatment. Different letters within a column represent significant differences at 0.05 probability level according Duncan's multiple range test.

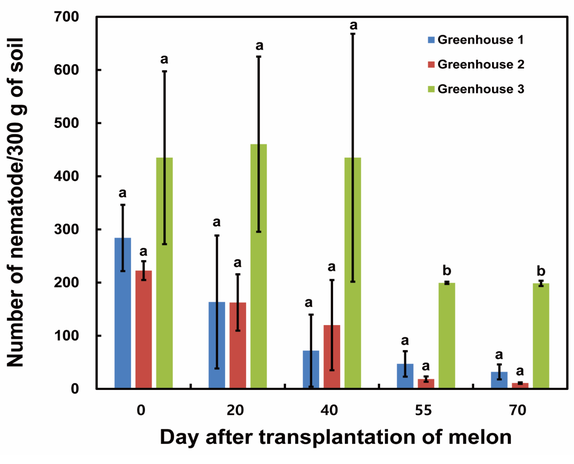

Fig. 4. Control efficacy of the *Pseudomonas chlororaphis* O6 wettable powder, WP-O6-10, against natural infestations of root-knot nematode in melon fields. Melon 'EarlyElite' was transplanted to plots with soil continuously cultivated with the same melon variety for 5 years. Two greenhouses were treated with WP-O6 10 product (greenhouse 1 and 2), whereas Fosthazate GR, was applied to greenhouse 3. The O6-WP10 was applied at a dose of 2 g/L at 2, 20, 40, and 55 days after transplantation. Greenhouse 3 had a single application of Fosthazate GR 1 week before melon transplantation. Soil samples were collected prior to transplantation and at 20 (17 July), 40 (10 Aug), 55 (25 Aug), and 70 (07 Sept) days after transplantation. Nematode density was determined. The data are expressed as the means and standard deviation of three replicates/each time point.

Table 1 Effects of the formulated product on melon growth in commercial cultivation greenhouses heavily infected with root-knot nematodes in Goksung, South Korea in 2015 [a]

Melon growth parameters at days after transplantation (d) [b]	Greenhouse 1 treated with O6-WP10	Greenhouse 2 treated with O6-WP10	Greenhouse 3 treated with Fosthazate GR
Plant height (cm)			
20	13.7 ± 1.5 a	12.7 ± 0.6 a	12.8 ± 0.8 a
40	131.0 ± 5.6 a	124.7 ± 7.2 a	121.3 ± 14.0 a
55	137.7 ± 2.5 a	135.0 ± 6.1 a	132.0 ± 7.8 a
70	146.7 ± 2.9 a	143.3 ± 5.8 a	131.7 ± 7.6 b
Main stem diameter (mm)			
20	6.4 ± 0.4 a	5.7 ± 0.6 a	6.4 ± 0.4 a
40	11.3 ± 1.2 a	11.0 ± 0.7 a	10.7 ± 1.5 a
55	11.3 ± 0.6 ab	11.7 ± 0.6 a	10.3 ± 0.6 b
70	11.7 ± 0.6 a	11.7 ± 0.6 a	10.7 ± 0.6 a
Total number of the harvested fruit (year 2015/2014)	1,000/800	1,240/1,000	1,000/960

[a] Two greenhouses were treated in 2015 with O6-WP10, whereas a third was treated with the nematicide, Fosthazate GR as described in Methods and Materials.

[b] Growth plant height and stem diameter were measured at the defined days after transplantation. The total harvested melon yield of each greenhouse was compared in 2015 after the designated treatments to those of the 2014 in same greenhouses. In 2014, the chemical nematicide, Fosthazate GR was applied in each of the three greenhouses. The data are expressed as the means and standard deviation of three replicates with 50 randomly selected plants/each time point. Different letters within a column represent significant differences at 0.05 probability level according Duncan's multiple range test.

Table 2. Physical and chemical properties of soils used in the commercial greenhouse studies.

Site [a]	pH (1:5)	Organic Matter (g/kg)	Available phosphate (mg/kg)	Exchangeable cations (cmol+/kg)			Electrical conductivity (mS/cm)	Soil texture
				K	Ca	Mg		
G1	6.3	30	198	0.42	6.5	2.0	0.8	Sandy loam
G2	6.8	27	448	0.55	6.4	1.5	1.1	Sandy loam
G3	6.7	30	163	0.74	4.6	1.4	0.7	Sandy loam

[a] Greenhouses (G1, G2, and G3) are located in Gogsung-gun, Jellanamdo Province in South Korea. The physical and chemical properties of the soils were obtained from the soil map (soil.rda.go.kr/) of the Rural Development Administration, Jeonju, South Korea

Acknowledgement

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