INTEGRATED CONTROL OF *Meloidogyne incognita* INFECTION BY CERTAIN ORGANIC AMENDMENTS MIXED WITH *Serratia marcescens*

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ABSTRACT

Integrated control of *Meloidogyne incognita* infecting Balady peach cv. Meet- Gharm was practiced using dried powder leaves of periwinkle, castor alone or combined with *Serratia marcescens* in comparison with Oxamyl under greenhouse conditions. All materials tested relatively improved the plant growth parameters and suppressed nematode population to certain extent. *S. marcescens* plus castor application performed the best results in improving plant growth parameters.

Moreover, application of dried powders of castor or periwinkle accomplished the highest % reduction in the rate of nematode build-up and number of galls on peach roots with values of 0.015 or 0.29 and 1.7 or 2.0%, respectively.

However, peach plants treated with *S. marcescens* plus periwinkle gave the highest % reduction in numbers of egg-masses followed by castor or periwinkle alone with values of 1.6, 1.7 or 3.3%, respectively. Dried powder of periwinkle mixed with *S. marcescens* appeared to be the best treatment tested in suppressing rate of nematode reproduction with value of 0.15% and, in improving plant growth parameters.

**Keywords:** Peach plant, *M. incognita*, periwinkle, castor, *S. marcescens*, Oxamyle, integrated control.

INTRODUCTION

Peach (*Prunus persica*, L. Batsch) is one of the most important commercially deciduous fruit trees in Egypt. The cultivated area of peach was 1016 feddans producing 9848 tons in Dakahlia governorate where Meet- Gharm is the principal commercial peach cultivar grown.

In Egypt, the root-knot nematode, *M. incognita* and *M. javanica* have been considered as serious nematode pests in peach orchards (Oteifa, 1964). Nematode damage has limited the establishment, yield and longevity of peach during the last decades.

Chemical control of the root-knot nematodes has successfully limited the effect of this nematode below damaging levels. However, environmental, health problems, disturbance in the biological balance of nature and high cost of nematicides enhanced scientists to search for another alternatives.

Organic amendments, i.e., dry leaf powders, oil cakes and green manure have been reported to suppress root-knot nematodes infecting vegetable crops, fruit trees field crops and ornamental plants (Burman *et al.*, 1966; Amr and Alam, 1996; Kumar and Vadivelu, 1996; Deka and Phukan, 1997; Youssef and Amin, 1997; Alvarez *et al.*, 1998; Parveen and Alam, 1999; Bertrand and Lizot, 2000; Kheir *et al.*, 2000; Nagesh *et al.*, 2001 and El-Sherif *et al.*, 2001).
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Burman et al. (1995) studied the effect of castor (Ricinus communis), neem (Azadirachta indica), rayada (Brassica juncea) and mustard (Brassica campestris) oil cakes at 3% w/w and carbofuran at 1 kg a.i./ha on M. incognita multiplication, and growth and water relations of egg plant. They revealed that oil cakes, in general, was more efficient in controlling the nematode population, and also in alleviating the adverse effects of nematodes on the growth of egg plants.

In Egypt, Shahda et al. (1998) showed that leaves of castor bean (Ricinus communis) suppressed egg hatching of M. arenaria to certain extents in vitro, depending on the concentrations used.

Kheir et al. (2000) studied the efficacy of 18 ornamental plant powders including periwinkle in controlling M. incognita infecting sunflower, and in improving the plant growth, under greenhouse conditions. Moreover, egg masses and egg production per root were significantly reduced when used such materials. They recorded that the tested powders improved the growth of both shoots and roots of the amended plants.

Rangaswamy et al. (2000) evaluated the efficacy of Pasteuria penetrans, Trichoderma viride, and oil cakes of neem and castor in controlling M. incognita in tomatoes under glasshouse conditions. They found that P. penetrans, alone or in combination with neem cake had parasitized the nematode juveniles and adults, whereas T. viride, alone or in combination with either neem or castor cake was most effective in parasitizing the egg masses of the nematode.

Mukhtar and Ahmad (2000) studied the combined efficacy of Pasteuria penetrans and leaf extracts of Azadirachta indica, Calotropis procera, Datura stramonium, Ricinus communis or Tagetes minuta on M. javanica in pot experiments. They recorded that the combination of P. penetrans and leaf extracts of A. indica, R. communis and T. minuta improved tomato plant growth over the control, while the extracts of C. procera or D. stramonium were phytotoxic when combined with P. penetrans. They also indicated that leaf extracts of all the 5 plants together with P. penetrans reduced the number of galls and egg masses produced by M. javanica.

Nagash et al. (2001) revealed that application of inorganic fertilizers, nitrogen, phosphorus and potassium along with oil cakes of castor (Ricinus communis) or neem (Azadirachta indica) was beneficial to the endozooic antagonistic fungus (Paecilomyces lilacinus) and the plant host (tomento) and also enhanced the antagonistic potential of P. lilacinus against root-knot nematode, M. incognita under nursery conditions.

Therefore, the present investigation was carried out to determine the nematocidal properties of certain plant products, i.e. castor (Ricinus communis) and periwinkle (Catharanthus roseus = Vinca rosea) as organic amendments in combination with S. marcessenea against M. incognita infecting seedlings of Balady peach cv. Meat-Ghamr.
MATERIALS AND METHODS

A greenhouse experiment was conducted in order to determine the impact of dried powdered leaves of periwinkle and castor plants as organic amendments integrated with \textit{S. marcescens} in controlling \textit{M. incognita} infecting peach seedlings.

Fresh leaves of periwinkle, \textit{(Catharanthus roseus = Vinca rosea)} and castor, \textit{(Ricinus communis)} were obtained from the greenhouse of Nematology Research unit and Ornamental greenhouse, Faculty of Agriculture, Mansoura University, sun-dried and powdered.

Twenty four seeds of Balady peach cv. Meet-Gham was stratified for three months in polyethylene bags filled with mixture of peatmoss and sand (1:1, v:v) and kept in refrigerator. Individually, each seed was then planted in plastic pot 25-cm-d. containing steam-sterilized sandy loam soil (1:1, v:v).

Bacterial inocula of \textit{S. marcescens} were prepared (Mostafa et al., 2002). Nine seedlings of peach were inoculated with \textit{S. marcescens}. Bacterial inocula were introduced three times in this experiment at the rate of 100 ml of $10^6$ cfu ml$^{-1}$/pot as follows: the first time with \textit{S. marcescens} strain NRRLB. 959 after 45 days from planting, the second time with strain BJL 200 after 15 days from nematode inoculation, and the third time with strain YPL 1 after 45 days from nematode inoculation.

After 45 days from planting, tested amendments at the rate of 4 g/pot were incorporated into 12 pot soils around the seedlings alone or in combination with bacterial inocula. Pots were then watered to keep soil moist and left for 10 days to facilitate the above materials decomposition. Ten days later, twenty one seedlings were infected with 3000 J$_2$s of \textit{M. incognita} which was obtained from a pure culture of \textit{M. incognita} propagated on coleus plants in the same greenhouse.

Nematodes were extracted from soil by sieving and modified Baermann technique (Goodey, 1957).

Oxamyl (Vydate 10\% G) was applied at the recommended dose (0.6 g/pot) in a single application after 5 days from nematode inoculation. Three seedlings, were kept untreated and uninoculated to serve as control.

Each treatment was replicated three times. Treatments were as follows: 1- \textit{C. roseus} + \textit{S. marcescens} + \textit{M. incognita}, 2- \textit{R. communis} + \textit{S. marcescens} + \textit{M. incognita}, 3- \textit{C. roseus} + \textit{M. incognita}, 4- \textit{R. communis} + \textit{M. incognita}, 5- \textit{S. marcescens} + \textit{M. incognita}, 6- Oxamyl + \textit{M. incognita}, 7- Nematode alone and 8- Plant free of nematode or any treatment (ck).

Pots were randomly arranged on a greenhouse bench at 35 ± 5°C. Plants were received water and a conventional pesticide as needed. After 90 days from inoculation, plants were harvested. Data on lengths, diameters and weights of shoot and root as well as shoot dry weight and number of branches were determined and recorded.

Roots were stained and stages of \textit{M. incognita}, females, galls and egg masses were counted and recorded. \textit{M. incognita} (J$_2$s) were extracted from soil, then counted and recorded. Data were then subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984) followed by Duncan’s multiple range test to compare means (Duncan, 1955).
RESULTS

Data in Table (1) documented growth response of peach seedlings infected with *M. incognita* as influenced by the addition of dried powder leaves of periwinkle, *Catharanthus roseus* (*Vinca rosea*) or castor, *Ricinus communis* alone or in combination with *S. marcescens* in comparison with Oxamyl under greenhouse conditions. It is evident that all materials tested, relatively improved the fresh weight of whole plant as well as shoot dry weight to certain extent. As for single application, *S. marcescens* gave the modest values for percentage of increase of the fresh weight of whole plant (55.19%) and shoot dry weight (59.5%), whereas, Oxamyl performed the least values for the same plant growth parameters which were 4.5% and 29.72%, respectively, followed by periwinkle (17.8% and 12.4%) and castor (18.8% and 35.3%) respectively.

In concomitant treatments, pots receiving *S. marcescens* plus dried powder of castor significantly overwhelmed those receiving *S. marcescens* plus periwinkle with values of 98.5% and 100.3% for the former and with values amounted to 48.1% and 45.7% for the later. Oxamyl significantly increased root length over other treatments. *S. marcescens* plus castor showed significant results in the number of shoot branches and leaves followed by castor, *S. marcescens* then periwinkle, respectively (Table 1).

As a whole, it can be concluded that *S. marcescens* plus castor performed the best result in improving plant fresh weight of Balady peach and shoot dry weight as well followed by single application of *S. marcescens* then *S. marcescens* plus periwinkle.

Data presented in Table (2) show reduction percentages in nematode counts in soil and roots and number of galls as well as egg masses on peach plants. It is evident that total number of nematode was significantly affected by all materials tested when compared with those of the check. Application of dried powders of castor, periwinkle or Oxamyl accomplished the highest reduction percentage in nematode population recording values of the nematode build-up amounted to 0.015, 0.29 and 0.34, respectively. However, *S. marcescens* combined either with castor or periwinkle achieved values of 1.01 and 0.96, respectively followed by that of the single application of *S. marcescens* (0.92) as compared to that of the check treatment where it was 1.49 (Table 2).

Moreover, a significant reduction in number of galls on peach root was achieved with root gall indices ranging from 1.7 to 5.0 (Table 2). Among all materials tested, castor or periwinkle alone significantly decreased numbers of galls on peach roots with root gall index values of 1.7 and 2.0, respectively as compared to that of the nematode alone treatment (5.0). However, *S. marcescens* alone or combined with castor achieved the least percentage of reduction in gall numbers with values of 42.5% and 23.03%, respectively.

Regarding egg mass numbers, a significant reduction was also obtained with all treatments as compared to that of nematode alone (Table 2). It was also evident that peach plants treated with *S. marcescens* plus periwinkle gave the highest reduction in numbers of egg masses over other treatments, followed by castor and periwinkle in single applications with values of 1.6, 1.7 and 3.3, respectively (Table 2).
Table (1): Impact of dried powdered leaves of periwinkle, *Catharanthus roseus* and castor, *Ricinus communis* applied alone or in combination with *Serratia marcescens* on the growth of Batady peach cv. Meet-Ghamr infected with *Meloidogyne incognita* under greenhouse conditions.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Length (cm)</th>
<th>Fresh weight (g)</th>
<th>Diameter (cm)</th>
<th>No. of shoot branches</th>
<th>No. of shoot leaves</th>
<th>Fresh wt. of the whole plant (g)</th>
<th>Increase %</th>
<th>Shoot dry weight (g)</th>
<th>Increase %</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
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<td></td>
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</tr>
<tr>
<td><em>S. marcescens + M. incognita</em></td>
<td>73.7a</td>
<td>40.5c</td>
<td>21.2ab</td>
<td>33.12b</td>
<td>0.39a</td>
<td>6.8ab</td>
<td>4.3cd</td>
<td>12.7ab</td>
<td>4.3ab</td>
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<tr>
<td></td>
<td>47.8b</td>
<td>41.7ab</td>
<td>17.87c</td>
<td>16.11cd</td>
<td>0.32a</td>
<td>5.2bcd</td>
<td>10.67bc</td>
<td>33.88bcd</td>
<td>17.3</td>
</tr>
<tr>
<td></td>
<td>70.9a</td>
<td>49.8c</td>
<td>20.89ab</td>
<td>13.15d</td>
<td>0.35a</td>
<td>4.7bcd</td>
<td>7.7ab</td>
<td>13.57a</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>59.9a</td>
<td>41.3bc</td>
<td>19.11ab</td>
<td>23.95b</td>
<td>0.37a</td>
<td>4.8abcd</td>
<td>3.9cd</td>
<td>9.97cd</td>
<td>4.2ab</td>
</tr>
<tr>
<td></td>
<td>75.1a</td>
<td>41.8bc</td>
<td>27.13a</td>
<td>29.64a</td>
<td>0.48a</td>
<td>5.3abcd</td>
<td>10.0a</td>
<td>18.86a</td>
<td>18.8</td>
</tr>
<tr>
<td><em>S. marcescens + M. incognita</em></td>
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<tr>
<td><em>R. communis + M. incognita</em></td>
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<td><em>S. marcescens + M. incognita</em></td>
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<tr>
<td><em>Oxamyl + M. incognita</em></td>
<td>72.1a</td>
<td>83.1a</td>
<td>15.91b</td>
<td>13.97c</td>
<td>0.55a</td>
<td>4.2bcd</td>
<td>6.7bc</td>
<td>88.6cd</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>72.3a</td>
<td>43.5bc</td>
<td>21.14ab</td>
<td>21.14bc</td>
<td>0.49a</td>
<td>8.0a</td>
<td>6.0bd</td>
<td>19.73cd</td>
<td>4.7bc</td>
</tr>
<tr>
<td></td>
<td>75.0a</td>
<td>44.0bc</td>
<td>15.95b</td>
<td>12.63d</td>
<td>0.33a</td>
<td>3.7d</td>
<td>6.7bc</td>
<td>84.7d</td>
<td>28.5d</td>
</tr>
<tr>
<td><em>Nematode alone (5)</em></td>
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</table>

Each value presented the mean of three replicates.

Means in each column followed by the same letter(s) did not differ at P < 0.01 according to Duncan's multiple-range test.
Table (2): Effect of periwinkle, *Catharanthus roseus* and castor, *Ricinus communis* powders alone or in combination with *Serratia marcescens* on *Meloidogyne incognita* infecting Balady peach cv. Meet-Ghamr under greenhouse conditions.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Nematode population in</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Soil</td>
<td>Root Developental stages</td>
<td>Females</td>
<td>Total</td>
<td>Rate of buildup</td>
<td>Reduction %</td>
<td>Galls</td>
<td>Reduction %</td>
</tr>
<tr>
<td><em>S. marcescens</em></td>
<td>0.0 e</td>
<td>567.7a</td>
<td>2198.7a</td>
<td>2766.3c</td>
<td>0.92</td>
<td>38.2</td>
<td>313.0c</td>
<td>42.46</td>
</tr>
<tr>
<td><em>C. roseus</em></td>
<td>886.0 c</td>
<td>11.0 f</td>
<td>28.0 e</td>
<td>895.0 c</td>
<td>0.29</td>
<td>80.0</td>
<td>5.0 f</td>
<td>99.05</td>
</tr>
<tr>
<td><em>R. communis</em></td>
<td>0.0 e</td>
<td>25.3 ef</td>
<td>19.7 e</td>
<td>45.0 e</td>
<td>0.015</td>
<td>99.9</td>
<td>0.7 f</td>
<td>99.32</td>
</tr>
<tr>
<td><em>C. roseus+S. marcescens</em></td>
<td>1563.0b</td>
<td>246.3 d</td>
<td>1131.0c</td>
<td>2860.7bc</td>
<td>0.96</td>
<td>99.5</td>
<td>164.0d</td>
<td>62.85</td>
</tr>
<tr>
<td><em>R. communis+S. marcescens</em></td>
<td>698.3 d</td>
<td>388.3b</td>
<td>2036.3b</td>
<td>3035.0b</td>
<td>1.01</td>
<td>92.2</td>
<td>418.7b</td>
<td>23.03</td>
</tr>
<tr>
<td>Oxamyl</td>
<td>625.0 d</td>
<td>64.7 e</td>
<td>330.0d</td>
<td>1010.7d</td>
<td>0.34</td>
<td>77.2</td>
<td>71.7 e</td>
<td>86.82</td>
</tr>
<tr>
<td>Nematode alone (ck)</td>
<td>1862.7a</td>
<td>333.3 c</td>
<td>2259.7a</td>
<td>4475.7a</td>
<td>1.49</td>
<td>--</td>
<td>544.0a</td>
<td>--</td>
</tr>
</tbody>
</table>

Each value presented the mean of three replicates.
Means in each column followed by the same letter(s) did not differ at *P* < 0.05 according to Duncan's multiple-range test.
Initial population (Pi) = 3000 J2

*Rate of build-up = Final population (Pf) / Initial population (Pi)*

**Root gall index (RGI) or Egg mass index (EGI)): 0 = no galling or egg masses; 1 = 1-2 galls or egg masses; 2 = 3-10 galls or egg masses; 3 = 11-30 galls or egg masses; 4 = 31-100 galls or egg masses and 5 = more than 100 galls or egg masses.
Data presented in Table (3) recorded that rate of reproduction of *M. incognita* was influenced by most treatments tested. It is clear that all materials reduced rate of reproduction of *M. incognita* as compared with nematode alone except that of periwinkle or castor alone. Dried powder of periwinkle integrated with *S. marcescens* appeared to be the best treatment tested in suppressing rate of reproduction of *M. incognita* infecting peach seedlings with value of 0.15% as compared to that of nematode alone (Table 3).

Table (3): Development of *M. incognita* on Balady peach cv. Meet-Ghamr treated with dried powder of periwinkle, *C. roseus* and castor, *R. communis* alone or in combination with *S. marcescens* under greenhouse conditions.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Young stages</th>
<th>Females</th>
<th>Egg masses</th>
<th><strong>Rate of reproduction (R.R) %</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. marcescens</em></td>
<td>557.7 d</td>
<td>2198.7 a</td>
<td>22.00 b</td>
<td>0.99</td>
</tr>
<tr>
<td><em>C. roseus</em></td>
<td>857.0 c</td>
<td>28.0 e</td>
<td>3.33 c</td>
<td>10.63</td>
</tr>
<tr>
<td><em>R. communis</em></td>
<td>25.33 e</td>
<td>19.7 e</td>
<td>1.71 c</td>
<td>2.17</td>
</tr>
<tr>
<td><em>S. marcescens + C. roseus</em></td>
<td>1749.7 b</td>
<td>1131.0 c</td>
<td>1.61 c</td>
<td>0.15</td>
</tr>
<tr>
<td><em>S. marcescens + R. communis</em></td>
<td>996.7 c</td>
<td>2038.3 b</td>
<td>27.33 b</td>
<td>1.52</td>
</tr>
<tr>
<td>Oxamyl</td>
<td>689.7 d</td>
<td>330.0 d</td>
<td>7.33</td>
<td>2.17</td>
</tr>
<tr>
<td>Nematode alone</td>
<td>2216.0 a</td>
<td>2259.7 a</td>
<td>79.00 a</td>
<td>3.38</td>
</tr>
</tbody>
</table>

Each value presented the mean of three replicates. Means in each column followed by the same letter(s) did not differ at P < 0.05 according to Duncan's multiple-range test.

**Rate of reproduction (R.R) =**

\[
\frac{\text{Count of egg masses}}{\text{Total counts of females} + \text{egg masses}} \times 100
\]

DISCUSSION

With respect to the effect of dried powdered leaves of periwinkle, castor alone or in combination with the prokaryotic bacterium, *S. marcescens* on growth response of Balady peach as well as nematode population. Periwinkle powder integrated with *S. marcescens* appeared to be the best treatment in suppressing nematode reproduction and in improving plant growth parameters. The present results agreed with the findings of El-Sheriff et al. (2001) who reported that the dried powder of periwinkle, *V. rosea* integrated with Oxamyl gave significant increase in sunflower growth parameters as well as reduction in *R. reniformis* population density, rate of build-up and egg-mass numbers over either Oxamyl or *V. rosea* or nematode alone.

Castor alone significantly decreased number of galls and egg masses on peach roots infected with *M. incognita*. The present result confirms the findings of (Patel and Thakur, 1989; Buttol et al., 1998 and Mosiama, 2000) who recorded the nematocidal properties of castor (*Ricinum communis*) on *Tylenchorhynchus vulgaris*, *Tylenchulus semipenetrans*, *M. incognita* and *Meloidogyne spp.*, respectively.
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Apparently, the present investigation proved the potential of the rhizobacterium *S. marcescens* as a biofertilizer and a biocontrol agent for controlling root-knot nematodes infecting peach seedlings and improving the plant growth. Biofertilizers and organic amendments may play an important role in improving soil structure, promoting plant growth and activating different organisms such as bacteria, predators and parasites of the target nematode. The safety of such material and its low cost is one of its advantages. However, additional researches are needed using plant or animal organic amendments and the indigenous bacterium *S. marcescens* in microplot and field experiments to ensure their effective ness in integrated pest management (IPM) programs.

REFERENCES


المكافحة المتكاملة لنيماتودا تعقد الجذور "مليجدوجين إنكوجنيتا" التي تسبب نباتات
الخوخ بمواد عضوية معينة مع بتكتريا "سيراتيا ماركوس".
أحمد جمال الشرف، عبد الفتاح رجب رفاعي، فاطمة عبد الحسن مصطفى
وأحمد حماط نور الدين
وحدة بحوث النباتات - كلية الزراعة - جامعة المنصورة.

أوضح أنها المكافحة المتكاملة لنيماتودا تعقد الجذور "مليجدوجين إنكوجنيتا" التي تسبب نباتات
الخوخ البلدي صحن ميت، عبر دماسات الأوراق الجافة للكن من نباتات الخوخ والبضائع، بمفردها.
أو مع بتكتريا "سيراتيا ماركوس" بالمقارنة مع بين الفاينيت تحت ظروف الصبة أن كل المواد
المختبرة أدت إلى تحسن نسب لكل مقياس نمو النبات كناخففت أعداد نباتات ديمتراجات
مقابلات.

لقد أظهرت المقابلة بالبكتيريا مع مسحوق أوراق الخوخ الجافة أفضل النتائج في تحسين
مقياس نمو النباتات وزيادة على ذلك قد أنتجت مسحوق أوراق الخوخ أو البنوكا بهموفها أعلنت
نسبة خفض في معدل نمو أعداد نباتات ديمتراجات وكذا أعداد العقد نباتات الخوخ على جذور الكرز بين 15.7% 24.9% 1.6% 2.7% على التوالي، لكن نباتات الخوخ المعبأة بالبكتيريا مع البنوكا أظهرت أيضاً
nسلم نقص في معدل نمو النباتات بهبها مسحوق الخوخ ثم البنوكا معهموفها بمقدار 15% 1.6% 1.5% 2.7% على التوالي.

وقد أظهرت مسحوق البنوكا بالبكتيريا أفضل النتائج في خفض معدل تكاثر نباتات ديمتراجات
الخوخ بنسبة 50% مع زيادة ملحوظة في مقياس نباتات الخوخ المختبرة.

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