Effect of Three Orobanche spp. Extracts on Some Local Phytopathogens, Agrobacterium and Erwinia

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Abstract: Tissues of the spikes of Orobanche spp. contain phenols and mannitol pathways, and it seems that it is very resistant, or may be immune, to bacterial infection. Therefore extracts of 3 Orobanche species, O. cernua, O. crenata, and O. egyptiaca, were evaluated for their antibacterial activity against local isolates of crown gall (Agrobacterium) and soft rot (Erwinia) phytopathogens. Extract of O. cernua showed the highest activity against both pathogens with clear inhibition zones of 8-24 mm and 12-16 mm in diameters, respectively. When the inhibitory action of 100 mg/ml concentration of the 3 Orobanche species extract on local isolates of Erwinia was compared with each other, data revealed the ability of O. cernua to exhibit more activity than the others and disability of O. egyptiaca to inhibit any of these pathogens. Dilution experiments showed that the minimum inhibitory concentration (MIC) of O. cernua extract was as follows: local isolates of Agrobacterium, No. A91, A201, and A204, 12,500 µg/ml and for all Erwinia isolates-27,800 µg/ml. However, MIC of O. crenata extract was 63,000 µg/ml for all Erwinia isolates. Results showed that the MIC of 12,500 µg/ml of O. cernua is equal to the effect of Streptomycin (10 µg/ml) activity against Agrobacterium isolates No. A91, A201, and A204, Ofloxacin (5 µg/ml) against Agrobacterium isolates No. A91 and A201, Norfloksacin (10 µg/ml) against Agrobacterium isolate No. A204 and Cefotaxime (30 µg/ml) against Agrobacterium isolate No. A95. However, the MIC of O. cernua and O. crenata extract to inhibit all Erwinia isolates is 27,800 and 63,000 µg/ml which is equivalent to the effect of Tobromycin (10 µg/ml). It is concluded that Orobanche tissue could be a potential source for antiphytopathogenic bacterial agents.

Key Words: Bioactivity, Orobanche, crown gall, soft rot

Agrobacterium ve Erwinia Lokal Fitopatojenleri Üzerine Üç Orobanchespp. Özütünün Etkisi

Özet: Orobanche spp. tüy dokularının fenol ve mannitol metabolik yolları vardır ve bakteriyal enfeksiyonlara çok fazla dirençli ya da immune gibi görünmektedirler. Bu nedenle üç Orobanche türü; O. cernua, O. crenata, ve O. egyptiaca öztüleri local Agrobacterium izolatları ile oluşan crown gall hastalığı ve Erwinia fitopatojenlerine üzerine antibakteriyal aktiviteleri incelenmiştir. O. cernua öztü her iki patojene karşı en yüksek aktiviteyi, düzgün inhibisyon zon (8-24 mm) ve (12-16 mm) capları vererek göstermiştir. Üç Orobanche türünün 100 mg/ml derişimlerindeki inhibisyonlar Erwinia local izolatlarında karşılaştırılabildi olarak incelenmiştir. O. cernua diğerlerinden daha aktif bulunmuş ve O. egyptiaca patojenlerin hiçbirini inhibe edemediği gözlemmiştir. Seyretleme denemeleri O. cernua öztünün minimal inhibisyon konsantrasyonu (MIC) Agrobacterium izot No. A91, A201, ve A204, 12,500 µg/ml ve tüm Erwinia izolatlarında 27,800 µg/ml olarak bulunmaktadır. Fakat, O. crenata bitkisinin tüm Erwinia izolatlarına karşı MIC değeri 63,000 µg/ml olmuştur. Sonuç olarak, Agrobacterium izolatları No. A91, A201 ve A204, 12,500 µg/ml olan O cernua öztünün etkisi Streptomisinin (10 µg/ml), No. A91, ve A201, izotamina Ofloksasinin (5 µg/ml), No. A204, izotamina Norfloksaksinin, No. A95, izotatına Sefotaksimin (30 µg/ml) antibiyotiklerinin aktivitelerine eşt bulunmuştur. Fakat, O cernua ve O. crenata öztüleri Erwinia izolatlarının tamamını inhibe ettiği gözlemmiştir. MIC değerleri 27,800 ve 63,000 µg/ml dir bu değerler Tobromisin (10 µg/ml) antibiyotığının etkisine eşiştirilir. Orobanche dokularının anti fitopatojenik bakteriyal aktivite için potansiyeli awakened olduklarını sonucuna varilabilir.

Anahtar Sözcükler: Biyoaktivite, Orobanche, crown gall, soft rot

Introduction

Orobanche species are destructive root parasites of many economic crops that cause serious problems to farmers in Jordan (1) as well as other countries in Europe and Middle East (2). Saadoun and Hameed (3) reported the presence of some bioactive metabolites in the tissue of O. cernua in a recent study. They showed that the tissue of this parasitic seed plant has a remarkable activity
against some pathogenic bacteria. However, the activity against the crown gall and soft rot phytopathogens, *Agrobacterium tumefaciens* and *Erwinia*, respectively, has not been previously studied.

The control of crown gall disease in Jordan has been attempted by in vitro testing the activity of different antibiotic-producing soil *Streptomyces* isolates against this pathogen (4-6). Yet, there are no successful biological control agents against some local soft rot pathogens.

Despite the large amount of research on screening for active compounds from plants in different parts of the world, to date no study has been carried out on the effects of these compounds from the parasitic seed plant (*Orobanche*) against crown gall and soft rot pathogens or other microorganisms. Therefore, the antibacterial activity of its extract was investigated.

**Materials and Methods**

**Plant materials**

The parasitic seed plant *Orobanche cernua*, *O. crenata* and *O. egyptiaca* were collected from several fields in northern Jordan and Jordan Valley. Shoots of mature plants were dried at room temperature then blended with an electrical blender. Tumor samples from infected plant hosts, namely, cherry, apple, grape, and peach were randomly collected from plantation at Jordan University of Science and Technology campus and Ajlun area in northern Jordan. Plant host exhibiting soft rot disease, namely, tomato, carrot, pepper and potato were also collected from different fields in Jordan Valley.

**Sample Processing**

Tumor and vegetable infected plant samples were washed continuously under tap water for 10 min followed by sterile water, surface sterilized in 10% and 5.25% sodium hypochlorite for 10 min and 3 min, respectively.

**Isolation of Bacterial Pathogens**

Tumor samples were macerated in an electrical blender, and then serial dilution of each sample was performed and 0.1 ml of the appropriate dilution was spread by an L-shaped glass rod on the selective medium (King B) (7) and crystal violet pectate medium (8) for isolation of *Agrobacterium* and *Erwinia* pathogens, respectively. The cultures were incubated at 27 °C for 48 h. Colonies of different morphology were further purified and identified based on Bergey's Manual of Determinative Bacteriology (9).

**Preparation of extracts**

100 g blended plant parts (shoots) of each *Orobanche* sp. were placed in a 2-l beaker and then impregnated with 1 l of 96% ethanol. The beaker was covered with a glass dish and left at room temperature for 72 h. After that, the glass cover was removed and the ethanol-impregnated plant was subjected to heat radiation from tungsten lamp to aid in ethanol evaporation. The final extracted material was dissolved in the appropriate volume of sterile distilled water to obtain a concentration of 100 mg/ml and then filter sterilized through 0.45 mm pore size millipore filters (Millipore corp., Bedford, MA).

**Test organisms**

Different *A. tumefaciens* isolates, No. A91, A95, A101, A201, and A204, obtained from different plant tissues (peach, cherry, grape, apple, and peach, respectively) were used as test organisms. *Erwinia* isolates No. E3, E4, and E5 were isolated from different infected plant tissues, namely, carrot, pepper, and potato, respectively, and E1 and E2 from tomato. Cultures of these bacteria were grown in nutrient broth overnight at 27 °C.

**Antimicrobial testing**

Antimicrobial activity of the ethanolic extract of the whole plant was determined by the hole-plate diffusion method, the dilution method (10) and the Bauer-Kirby method (11).

**Hole-plate diffusion method**

Twenty milliliters of nutrient agar were poured into Petri dishes. Plates were inoculated with the appropriate test organism using a sterile swab. Three cores of 6 mm diameter were removed from the agar. Holes were filled up with 50 µl of water-soluble ethanolic extract.

**Dilution method**

Minimum inhibitory concentration (MIC) was determined by the dilution method (12, 13). The stock plant extract (100 mg/ml) was diluted under aseptic conditions in sterile distilled water to obtain different concentrations.

All experiments were carried out in triplicate. The controls (holes) were filled with sterile distilled water. Agar plates with the bacterial pathogens were incubated...
overnight at 27 °C, the results were recorded after 12 h by measuring the zones of growth inhibition surrounding the holes. The following antibiotics were used for comparison: TM10: Tobromycin (10 µg); AMC30: Augmentin (30 µg); Nor10: Norfloxacin (10 µg); S10: Streptomycin (10 µg); OFX5: Ofloxacin (5 µg); CXM30: Cefoxime (30 µg); CTX30: Cefotaxime (30 µg).

Results and Discussion

The extract of Orobanche cernua showed activity against all tested Agrobacterium isolates by forming clear inhibition zones with diameters between 10 and 24 mm (Table 1). Extract MIC for isolates A91, A201 and A204 was 12500 µg /ml. However, isolates No. A95 and A101 showed less sensitivity with extract MIC of 100,000 and 50,000 µg /ml, respectively (Table 1). These concentrations were compared to different standard antibiotics and data indicated that the MIC of 12,500 µg/ml is equal to streptomycin (10 µg) and ofloxacin (5 mg) activity against Agrobacterium isolates No. A91 and A204, norfloxacin (10 µg) activity against isolate No. A204 and cefotaxime (30 µg) activity against isolate No. A95. Results revealed that the activity of 100,000 µg/ml and 50,000 µg/ml of the extract against isolate No. A95 and A101 were equal to its sensitivity to cefotaxime (30 µg) and amoxicillin (30 µg), respectively.

All local Erwinia isolates showed less sensitivity to Orobanche extracts than Agrobacterium isolates with MIC of 27,800 and 63,000 µg/ml of O. cernua and O. crenata extracts, respectively, which is equivalent to the effect of Tobramycin (10 µg) (Table 3).

This study clearly showed an inhibitory effect of the Orobanche cernua extract mainly on Agrobacterium isolates No. A91, A201, and A204 with MIC of 12,500 µg/ml even though its effect against isolates No. A95 and A101 were evident only at high concentrations. However, all Erwinia isolates exhibited less sensitivity than Agrobacterium isolates with higher concentrations of O. cernua and O. crenata extracts needed to inhibit the growth of these pathogens. From this it can be concluded that the extract of this parasitic seed plant has an effective inhibitory action against all local Agrobacterium and Erwinia isolates.

### Table 1. Activity of Orobanche cernua extract by hole diffusion method and standard antibiotics against different bacteria.

<table>
<thead>
<tr>
<th>Agrobacterium isolate</th>
<th>Extract Concentration (µg/ml)</th>
<th>Antibiotic&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100,000</td>
<td>50,000</td>
</tr>
<tr>
<td>A91</td>
<td>24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16</td>
</tr>
<tr>
<td>A95</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>A101</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>A201</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>A204</td>
<td>16</td>
<td>12</td>
</tr>
</tbody>
</table>

<sup>a</sup> S<sub>10</sub>: Streptomycin (10 µg); OFX<sub>5</sub>: Ofloxacin (10 µg); AMC<sub>30</sub>: Amoxicillin (30 µg); CTX<sub>30</sub>: Cefotaxime (30 µg); Nor<sub>10</sub>: Norfloxacin (10 µg).
<sup>b</sup> Source of local Agrobacterium isolate: A91: Peach; A101: Grape; A201: Apple; A204: Peach and A95: Cherry.
<sup>c</sup> Inhibition zone diameters (mm, mean value, N = 3).
<sup>d</sup> Numbers in parenthesis represent the comparison of the MIC (µg/ml) extract activity with the specific standard antibiotic activity as indicated by almost equal inhibition zone diameter.
Acknowledgments

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Table 2. Inhibitory action of 100 mg/ml concentration of the different Orobanche species extract on local isolates of Erwinia relative to standard antibiotics.

<table>
<thead>
<tr>
<th>Erwinia isolate</th>
<th>Diameter of Inhibition Zone (mm)</th>
<th>Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O. cernata</td>
<td>O. cernua</td>
</tr>
<tr>
<td>E1</td>
<td>15</td>
<td>43</td>
</tr>
<tr>
<td>E2</td>
<td>15</td>
<td>47</td>
</tr>
<tr>
<td>E3</td>
<td>18</td>
<td>47</td>
</tr>
<tr>
<td>E4</td>
<td>-</td>
<td>43</td>
</tr>
<tr>
<td>E5</td>
<td>19</td>
<td>43</td>
</tr>
</tbody>
</table>

a: Source of local Erwinia isolate: E1: Tomato; E2: Tomato; E3: Carrot; E4: Pepper; E5: Potato
b: Mean value, N = 3.
c: N. A-30: Nadilixic acid (30 µg); TM10: Tobromycin (10 µg)

Table 3. Minimum concentration of the different Orobanche species extract inhibiting local isolates of Erwinia relative to standard antibiotics.

<table>
<thead>
<tr>
<th>Erwinia isolate</th>
<th>Diameter of Inhibition Zone (mm)</th>
<th>MIC of the Extracts (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O. crenata</td>
<td>O. cernua</td>
</tr>
<tr>
<td>E1</td>
<td>188</td>
<td>94</td>
</tr>
<tr>
<td>E2</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>E3</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>E4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E5</td>
<td>20</td>
<td>18</td>
</tr>
</tbody>
</table>

a: Source of local Erwinia isolate: E1: Tomato; E2: Tomato; E3: Carrot; E4: Pepper; E5: Potato
b: Mean value, N = 3.
c: N. A-30: Nadilixic acid (30 µg); TM10: Tobromycin (10 µg).

References


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