Effect of Neem (Azadirachta indica A. Juss) Seeds and Leaves Extract on Some Plant Pathogenic Fungi

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Abstract: In this study plant pathogenic fungi Alternaria solani, Fusarium oxysporum, Rhizoctonia solani and Sclerotinia sclerotiorum were chosen to study the effect of ethanolic, hexane and methanolic extracts of neem seeds and leaves. Antifungal effects of neem leave and seed extracts obtained by ethanol, hexane and petrolum ether were examined separately in vitro against Fusarium oxysporum, Rhizoctonia solani, Alternaria solani and Sclerotinia sclerotiorum. Results indicated that seeds and leaves extracts could cause growth inhibition of tested fungi, although the rate of inhibition of tested fungi varied with different extracts and concentrations. But all these extracts and concentrations of extract inhibited the growth of pathogenic fungi at a significant level. Azadirachtin, nimonol and exoperoxidase were detected from neem extract by using High Performance Liquid Chromatography (HPLC). We can conclude that neem leave and seed extracts were effective as antifungal against all tested fungi but F. oxysporum and R. solani were the most sensitive fungi.

Key words: Neem extracts, ethanolic, methanolic, hexane, pathogenic fungi

INTRODUCTION

Alternaria solani, Fusarium oxysporum, Rhizoctonia solani and Sclerotinia sclerotiorum are well known as plant pathogens. Alternaria solani causes early blight in tomatoes and potatoes (Haware, 1971; Connath et al., 2003; Reni and Voorrips, 2006) Fusarium oxysporum causes Fusarium wilt disease in different plants (Shanmugan et al., 2007; Floetz, 2000). Rhizoctonia solani and F. solani causes damping off (Yangar et al., 2008), while Sclerotinia sclerotiorum causes stem rot (Mueller et al., 2002; Young et al., 2004). The neem tree is a tropical evergreen plant and is a native plant of India and Burma. Recently it gains importance in the research because of potential of using neem derivatives such as leaf, oil and seed extracts for preparation of environmental friendly herbicides (Verma and Karwar, 2006).

Leaf extract of neem can inhibit the aflatoxin production as well as Aspergillus parasiticus growth (Ghorbanian, 2007; Allameh et al., 2002). Antifungal effects of neem leaf extract also reported from south America against Crinipellis perniciosa and Phytophthora species causing Witches broom and Pot Not of cocoa (Ramos et al., 2007).

Antifungal, antibacterial and anti insecticidal component Azadirachtin, limonoid and terpenoids have been extracted from seeds and leaves of neem (Dai et al., 2001; Nathan et al., 2005; Jarvis and Morgan, 2000). These neem extracts have also been reported to be effective against malaria vector Anopheles stephensi (Nathan et al., 2005; Koul et al., 2004). About ten years back neem trees were imported from India and planted in the Arafat area of Makkah, Saudi Arabia. The aim of this study was to analyzed the antifungal properties of the extracts of leaves and seeds of neem against some plant pathogenic fungi.

MATERIALS AND METHODS

Plant materials: Neem leaves and seeds were obtained from Arafat area of Saudi Arabia.

Plant parts were cleaned with deionized water and dried at 50°C for 24 h. The dried plant parts were ground and then sieved with 80 mesh sieve.

Extraction: The method of Phasuda and Varipat (2004) was adopted for extraction with little modification. Briefly, 20 g portions of the powdered plant materials were soaked separately in solvents (80 mL of each ethanol, hexane and petroleum ether) at ambient temperature for 24 h under shaking condition at 130 rpm. The extract was then filtered using Whatman filter paper No. 1 and re-filtered using 0.22 micro filter paper (Sartorius, Germany). The filtrate was kept in the freezer at -20°C for further study.

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Microorganisms: The microorganisms used included: Fusarium oxysporum, Rhizoctonia solani, Alternaria solani and Sclerotinia sclerotiorum were obtained from Microbiological Resource Center MIRCIN, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Assay for the antifungal effects of the neem leaves and seeds organic extract: To assay the antifungal effects of the organic extracts of neem leaves and seeds using tested microorganisms, measurement of radial growth of the used organisms were made following the technique of Phasuda and Varipat (2007) and Nwachukwu and Umehuruba (2001). The in vitro tests were carried out to measure the effects of the leaf extracts on radial growth of the seed-borne fungi. Potato Dextrose Agar (PDA) medium was used in this study. To every 15 mL of sterile potato dextrose agar medium in Petri dishes, 5 mL of either crude or aqueous extract of each plant sample were added. The solution in each Petri dish was gently swirled and allowed to solidify. The extract-amended medium in the Petri dishes were inoculated each alone at the centre with 5 mm inoculum- disc of each test fungus and incubated at 25±2°C for 14 days. The medium with inoculums disc but without any extract served as control. Percentage inhibition of mycelial growth by the leaf extracts was calculated using the formula:

\[
\% \text{ inhibition of mycelial growth (MG)} = \frac{D_c - D_t}{D_c} \times 100
\]

Where:
\( D_c \) = diameter of control
\( D_t \) = diameter of test

Results and Discussion

In general ethanolic and methanolic leaf extract causing more inhibition of all the fungi as compared to hexane leaf extract except in the case of Sclerotinia sclerotiorum where hexane extract causes more inhibition than methanolic extract but ethanolic extract remains causes the highest inhibition of growth (Sanjeet et al., 2005; Mossini, 2004). At 10% concentration of extracts, R. solani showed the highest inhibition (55.1%) in the case of ethanolic extract and the lowest inhibition of growth was shown by S. sclerotiorum (21.4%) in hexane extract and this trend remain the same at other concentrations of extracts. A complete inhibition (100%) of growth was shown by Fusarium oxysporum and R. solani at 40% level of ethanolic and methanolic extracts. Alternaria solani exhibited the highest percent of inhibition (84.0%) followed by methanolic (78.3%) and hexane (73.1%) at 40% concentrations of leaf extract, while S. sclerotiorum showed the highest inhibition by methanolic (86.1%) followed by hexane (76.5%) and methanolic (67.1%) (Table 1). Results of this study in general, indicates that even at 10% concentration of all types of extract could cause significant inhibition of growth (Gupta and Bansal, 2003; Amadioha, 2004).

Generally similar trends of inhibition were also observed in the case of ethanolic hexane and methanolic neem seed extracts (Table 2) as in the case of leaf extracts. All type of seeds extracts causes higher percentage of inhibition of pathogenic fungi at all concentration used as compared to leaf extracts. Fusarium oxysporum and R. solani showed 100% inhibition at 30% concentrations of ethanolic and methanolic seeds extracts. This is in contrast with leaf extract results where these fungi showed 100% inhibition at 40% concentration of these extracts. Results clearly indicate that F. oxysporum and R. solani were the most sensitive fungi to neem leaf and seed extracts followed by A. solani and S. sclerotiorum. This might be due to production of sclerotia by S. sclerotiorum which obviously more resistant to these extracts. Earlier reports indicates that both leaves and seeds extracts of neem have significant antifungal activities (Sanjeet et al., 2005; Mossini et al., 2004; Amadioha, 2004).

Azadirachtin, Azadiradione, nimonol and epoxy azadiradione were yielded from the organic extract of seeds and leaves of neem (Table 3, 4). Nimomol (82%) look to be a major active component of neem organic extract. All these component extracted and also have been reported as antifungal, antibacterial, anti insecticidal (Dai et al., 2001; Jarvis and Morgan, 2000; Nathan et al., 2005) and could affect the production of aflatoxin by

All experiments were carried out in Plant and Microbiology Department Science Collage, King Saud University during the period from 2007-2009.
Table 1: Effect of ethanolic, hexane and methanolic leaf extracts on the growth of pathogenic fungi (Percent inhibition)

<table>
<thead>
<tr>
<th>Fungi</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>C</th>
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</thead>
<tbody>
<tr>
<td>Alternaria solani</td>
<td>32.0</td>
<td>28.2</td>
<td>26.2</td>
<td>54.8</td>
<td>37.0</td>
<td>35.0</td>
<td>83.0</td>
<td>45.2</td>
<td>50.8</td>
<td>84.0</td>
<td>73.1</td>
<td>78.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>36.2</td>
<td>43.9</td>
<td>30.9</td>
<td>69.1</td>
<td>47.5</td>
<td>36.2</td>
<td>87.9</td>
<td>68.5</td>
<td>64.1</td>
<td>100.0</td>
<td>89.4</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhizoctonia solani</td>
<td>55.1</td>
<td>28.3</td>
<td>40.1</td>
<td>71.1</td>
<td>46.2</td>
<td>45.4</td>
<td>90.9</td>
<td>54.1</td>
<td>72.3</td>
<td>100.0</td>
<td>77.2</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sclerotinia sclerotiorum</td>
<td>45.7</td>
<td>36.3</td>
<td>21.4</td>
<td>55.9</td>
<td>40.3</td>
<td>27.3</td>
<td>85.9</td>
<td>61.5</td>
<td>44.1</td>
<td>86.1</td>
<td>76.5</td>
<td>67.1</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

A: Ethanolic, B: Hexane, C: Methanolic

Table 2: Effects of ethanolic, hexane and methanolic seed extracts on the growth of pathogenic fungi (Percent inhibition)

<table>
<thead>
<tr>
<th>Fungi</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria solani</td>
<td>36.1</td>
<td>31.7</td>
<td>42.9</td>
<td>57.2</td>
<td>40.8</td>
<td>47.8</td>
<td>56.7</td>
<td>53.4</td>
<td>58.0</td>
<td>89.5</td>
<td>80.1</td>
<td>80.7</td>
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<tr>
<td>Fusarium oxysporum</td>
<td>49.8</td>
<td>52.2</td>
<td>43.5</td>
<td>73.6</td>
<td>57.6</td>
<td>64.1</td>
<td>100.0</td>
<td>68.1</td>
<td>100.0</td>
<td>100.0</td>
<td>97.9</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhizoctonia solani</td>
<td>58.9</td>
<td>46.8</td>
<td>48.8</td>
<td>75.3</td>
<td>49.5</td>
<td>69.2</td>
<td>100.0</td>
<td>64.8</td>
<td>100.0</td>
<td>100.0</td>
<td>82.4</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sclerotinia sclerotiorum</td>
<td>49.2</td>
<td>39.7</td>
<td>24.6</td>
<td>59.7</td>
<td>44.1</td>
<td>29.9</td>
<td>87.8</td>
<td>48.7</td>
<td>52.2</td>
<td>92.5</td>
<td>80.5</td>
<td>71.2</td>
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</tr>
</tbody>
</table>

A: Ethanolic, B: Hexane, C: Methanolic

Table 3: High performance liquid chromatographic peaks of ethanolic neem leaf extract

<table>
<thead>
<tr>
<th>Band</th>
<th>Rt (min)</th>
<th>Total peak area detected (%)</th>
<th>Compound (ID)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>67.40</td>
<td>Azadirachtin A (8), B (4), C (9)</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>22.10</td>
<td>Ni</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>57.50</td>
<td>Azadirachtin A (12), B (13), D (3), H (8)6De-acetylnimonin (37)</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>30.50</td>
<td>Ni</td>
</tr>
<tr>
<td>5</td>
<td>37</td>
<td>41.40</td>
<td>Azadiradion (50)</td>
</tr>
<tr>
<td>6</td>
<td>52</td>
<td>65.30</td>
<td>Nimmonol (79.0)</td>
</tr>
<tr>
<td>7</td>
<td>61</td>
<td>75.00</td>
<td>Exoxygenzdrideone (6)</td>
</tr>
<tr>
<td>8</td>
<td>70</td>
<td>31.50</td>
<td>Exoxygenzdrideone (45)</td>
</tr>
<tr>
<td>9</td>
<td>75</td>
<td>33.00</td>
<td>Ni</td>
</tr>
<tr>
<td>10</td>
<td>88</td>
<td>26.50</td>
<td>Ni</td>
</tr>
</tbody>
</table>

Ni: Not Identified. These peaks were detected by HPLC using ethanolic solvent system and found to be complex mixtures. Values in brackets are percentage

Table 4: High performance liquid chromatographic peaks of ethanolic neem seed extract

<table>
<thead>
<tr>
<th>Band</th>
<th>Rt (min)</th>
<th>Total peak area detected (%)</th>
<th>Compound (ID)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>75.50</td>
<td>Azadirachtin A (11), B (9), C (12)</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>30.00</td>
<td>Ni</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>66.00</td>
<td>Azadirachtin A (15), B (16), D (11), H (12)6De-acetylnimonin (41)</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>35.00</td>
<td>Ni</td>
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<tr>
<td>5</td>
<td>37</td>
<td>49.00</td>
<td>Azadiradion (58)</td>
</tr>
<tr>
<td>6</td>
<td>52</td>
<td>74.00</td>
<td>Nimmonol (85.5)</td>
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<tr>
<td>7</td>
<td>61</td>
<td>83.00</td>
<td>Exoxygenzdrideone (14)</td>
</tr>
<tr>
<td>8</td>
<td>70</td>
<td>39.00</td>
<td>Exoxygenzdrideone (48)</td>
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<tr>
<td>9</td>
<td>75</td>
<td>42.00</td>
<td>Ni</td>
</tr>
<tr>
<td>10</td>
<td>88</td>
<td>38.00</td>
<td>Ni</td>
</tr>
</tbody>
</table>

Ni: Not Identified. These peaks were detected by HPLC using ethanolic solvent system and found to be complex mixtures. Values in brackets are percentage

In conclusion the results of this study showed that locally growing neem plants which were imported to be planted have retained the antifungal activities although growing under a very different environment compared to their original native land.

ACKNOWLEDGMENT

Author is thankful to Research Center, College of Science and King Saud University for financial assistance with grant No. Bot.2008/74.

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