Inhibitory Effect of Methanolic Extract of *Annona senegalensis* against Seed Germination and Seedling Growth of Four Selected Seeds

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Abstract

We investigated the allelopathic activity of methanol extract of *Annona senegalensis* against four selected seeds. The percentage seed germination was evaluated for 72 hours and percentage inhibition of seedling growth was tested for 7 day and 14 days respectively. The result showed that inhibition activity of the seed germination and seedling growth was in these order; amaranthus, tomato, maize and cowpea. The plant extract showed considerable allelopathic activity and the inhibition effect of the extract against the tested seeds increased with increase in concentration of the extract.

Key words

Allelopathic activity, *Annona senegalensis*, Amaranthus, Tomato, Maize, Cowpea

Materials and Methods

Plant Sample

The plant sample was collected at Akure town in Ondo State and authenticated at Federal Research Institute of Nigeria (FRIN) in Ibadan. Aerial part of the plant was collected. This was air-dried at room temperature under the cool air away from the sun. The dried plant was pulverized and kept in an air-tight polythene bags

Preparation of Extract

500g of dried and pulverized plant material was weighed and poured into 6ltr flat bottom flask. 1.5ltrs of N-Hexane was poured into the flask and this was covered with aluminium foil and made air-tight with Paper tape. This is in a bid to de-fat the plant material. After 24hrs, the supernatant was decanted and the plant...
material air-dried again. The material was thereafter poured again into the flask 2ltr of MeOH added. This was made air-tight and left at room temperature for 72hrs. The supernatant was thereafter decanted and concentrated using a Rotary Evaporator. The yield was 16.65g (3.33%)

**Treatment**

From the crude extracts, three treatments of different concentrations (0.0, 1.0, 2.5, and 5.0% w/w) were prepared in Methanol: two controls were prepared – Methanol and distilled water. The different treatments for each of the organic extracts were obtained from a stock solution which had been previously prepared from each of the raw extracts. The concentrations were 0.0, 1.0, 2.5 and 5.0% v/v for the methanolic extract

**In vitro biotest**

**Seed germination test**

The test was carried out according to the method of Casimiro et al., 2017 with slight modification. Viable seeds were obtained from Agricultural Development Parastatal (ADP) in Akure, Ondo state. Concentration of 5%, 2.5% and 1% (w/v) of the crude extract were used to treat the filter papers placed inside Petri dishes 10 cm in diameter and air-dried at room temperature. 10 seeds per treatment were placed into the each Petri dish. Two control set-ups were prepared in a similar way with pure solvent and distilled water, allowing each to also evaporate fully. The experiment was carried out with three repetitions per treatment. The petri dishes were placed in a dark cupboard with relative humidity and room temperature. Seedling growth was evaluated for 7 days and 14 days after the introduction of the seed. The percentages of inhibition were calculated by comparison with the untreated control, using the following formula: % inhibition = (C − X)/C × 100, where C is the average length of shoot/root in control and X is the average length of the shoot/roots in the test sample

**Seedling Growth Test:**

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**Result and Discussions**

**Percentage Seed Germination**

The result of the percentage seed germination is presented in Table 1. Tomato had the highest percentage seed germination of 23.33%, followed by maize (20.00%), cowpea (13.33%) and amaranthus had no percentage seed germination for 5% (w/v) concentration of the methanolic extract of Annona senegalensis. For 2.5% (w/v) concentration, the percentage seed germination was found to be in the order: tomato (33.33%), maize (26.67%), cowpea (20.00%) and amaranthus (6.67%). For 1% (w/v) concentration of the extract, percentage seed germination was found to be in the order: tomato (40.00%), maize (40.00%), cowpea (33.33) and amaranthus (10.00%). From the study, it showed that the higher the concentration of the extract, the lower the percentage of seed germination. This finding was in agreement with the work of Ines et al., (2014) who reported that the percentage seed germination of Lactuca sativa in the presence of aqueous extract at difference concentration of Tunisian and Indian varieties of Nigella sativa seeds and aerial parts harvested at vegetative, flowering, and fruiting stage, decrease with increase in the concentration of the extract.

<table>
<thead>
<tr>
<th>Percentage seed germination of methanolic extract of Annona senegalensis on four selected seeds.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
</tr>
<tr>
<td>%5</td>
</tr>
<tr>
<td>2.5%</td>
</tr>
<tr>
<td>1%</td>
</tr>
<tr>
<td>Control (MEOH)</td>
</tr>
<tr>
<td>Control (withoutMEOH)</td>
</tr>
</tbody>
</table>
Values are means of triplicate ± standard error. Column means followed by the same superscript letters are not significantly different at $P<0.05$.

**Percentage inhibition of seed germination**
The result of percentage inhibition of seed germination is presented in Table 2. The result showed that the higher the concentration, the higher the inhibition percentage of the seed germination. For 5% (w/v), the inhibition percentage was in these order; amaranthus (100.00%), maize (73.81%), tomato (72.22%) and cowpea (68.05%). For 2.5% (w/v) concentration of the extract, the inhibition percentage was in these order; amaranthus (93.33%), tomato (65.18%), maize (64.88%) and cowpea (59.31). For 1% (w/v) concentration of the extract, the percentage inhibition was in these order; amaranthus (90.00%), tomato (58.55%), maize (47.62%) and cowpea cowpea (45.33%). The overall inhibition effect of the extract against the seeds were in these order; Amaranthus, tomato, maize and cowpea. This result also showed that the higher the concentration of the extract, the higher the concentration of the percentage inhibition of the seed germination and these findings agreed with the previous work of Casimiro et al., (2017) who reported that the allelopathic activity of ethanolic extract of *Arachis hypogaea* on the growth of *L.sativa* increased with increase in the concentration of the extract.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Amaranthus</th>
<th>cowpea</th>
<th>tomato</th>
<th>Maize</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>100.00±0.00</td>
<td>68.05±16.02</td>
<td>72.22±4.00</td>
<td>73.81±1.19</td>
</tr>
<tr>
<td>2.5%</td>
<td>93.33±3.33</td>
<td>59.31±3.77</td>
<td>65.18±4.82</td>
<td>64.88±5.29</td>
</tr>
<tr>
<td>1%</td>
<td>90.00±0.00</td>
<td>45.23±8.58</td>
<td>58.55±1.44</td>
<td>47.62±2.38</td>
</tr>
<tr>
<td>Control</td>
<td>0.00±0.00</td>
<td>8.33±4.17</td>
<td>3.33±0.03</td>
<td>3.70±0.37</td>
</tr>
</tbody>
</table>

Values are means of triplicate ± standard error. Column means followed by the same superscript letters are not significantly different at $P<0.05$.

**Seedling growth bioassay**
The result of the percentage inhibition of the seedling growth is presented in Table 3. For 5% (w/v) and 2.5% (w/v) concentration of the extract, the inhibition activity were higher at 7th day than the 14th day except for the cowpea and maize where the inhibition activity was higher at 14th day than 7th. For 1% (w/v) concentration of the extract, inhibition activity of extract was higher at 14th day than 7th day against the tested seeds. The result showed that in most cases the inhibition *N. sitava* activity against root of the seeds were higher than their corresponding shoot and these was in agreement with the previous by Ines et al., (2014) who reported that the inhibition index of the aqueous extract of Tunisian and Indian varieties of seed and aerial parts against *L.sativa* germination growth had higher value in root length than the shoot length and that half inhibition concentration (IC50) of root growth was lesser than shoot growth. The result was also in agreement with the work of Casimiro et al., (2017) who reported that inhibition activity of *Arachis hypogaea* was higher against rootlet of *L. sativa* than the hypocotyl.

**Conclusions**
The result from this research had showed that methanolic extract of *Annona senegalensis* had allelopathic potential against tested seed, therefore further studies on isolation and characterization of bioactive components in the extract that may be responsible for the allelopathic activity should be carried out.
<table>
<thead>
<tr>
<th>Conc</th>
<th>Tomato 7 days Shoot</th>
<th>Tomato 7 days Root</th>
<th>Amaranthus 7 days Shoot</th>
<th>Amaranthus 7 days Root</th>
<th>Cowpea 7 days Shoot</th>
<th>Cowpea 7 days Root</th>
<th>Maize 7 days Shoot</th>
<th>Maize 7 days Root</th>
<th>Maize 14 days Shoot</th>
<th>Maize 14 days Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>70.68±8.25</td>
<td>86.74±0.96</td>
<td>57.25±5.10</td>
<td>76.51±4.10</td>
<td>89.70±5.69</td>
<td>89.62±6.23</td>
<td>63.22±7.36</td>
<td>75.08±5.73</td>
<td>33.13±2.79</td>
<td>56.52±1.91</td>
</tr>
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<td>2.5%</td>
<td>49.08±2.99</td>
<td>72.48±3.32</td>
<td>54.57±4.22</td>
<td>66.25±2.7</td>
<td>80.96±9.75</td>
<td>82.44±6.23</td>
<td>55.58±7.87</td>
<td>60.60±9.56</td>
<td>26.81±4.42</td>
<td>40.64±2.0</td>
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<td>40.64±2.0</td>
</tr>
<tr>
<td>1%</td>
<td>29.42±3.33</td>
<td>48.56±8.25</td>
<td>31.52±1.28</td>
<td>56.11±4.9</td>
<td>12.16±1.47</td>
<td>29.00±0.13</td>
<td>24.73±2.92</td>
<td>23.92±4.28</td>
<td>10.37±0.84</td>
<td>19.9±1.77</td>
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<tr>
<td>control</td>
<td>9.98±1.42</td>
<td>4.30±0.92</td>
<td>2.42±0.02</td>
<td>1.93±0.0</td>
<td>8.27±0.61</td>
<td>7.09±1.08</td>
<td>2.57±0.42</td>
<td>7.76±0.60</td>
<td>5.12±1.46</td>
<td>7.66±0.72</td>
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References


tetrahydrofuranacetogenins from *Annona senegalensis* and *Annona cherimolia*, *Phytochemistry*, 42 (1) 103-7


