ANTIFUNGAL ACTIVITY OF THREE FERN EXTRACTS ON CAUSATIVE AGENTS OF GROUNDNUT EARLY LEAF SPOT AND RUST DISEASES

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Abstract: Antimycotic activity of three fern extracts from Hemionitis arifolia (Burm.f.) Moore., Pteridium aquilinum (Linn.) Kuhn. and Christella parasitica (Linn.) H. Lev. were evaluated against groundnut early leaf spot and rust disease causative agents viz., Puccinia arachidis Sparg., and Phaeoisariopsis personata (Berk & M.A. Cuortis) Vanarx., respectively by mycelial dry weight method. Both the fungi were sensitive to all the three fern extracts tested. Among the extracts, chloroform extract of H. arifolia was found to have maximum antifungal activity against both fungi. Between these two fungi, P. personata was found to be more sensitive to the tested plant extracts than P. arachidis. From this study it is concluded that chloroform extract from H. arifolia can be utilized for managing rust and leaf spots diseases on groundnut.

Key words: antimycotic activity, Christella parasitica, Hemionitis arifolia, Phaeoisariopsis personata, Pteridium aquilinum, Puccinia arachidis

INTRODUCTION

Groundnut is an important oil seed crop particularly for small-scale farmers in semi-arid regions of India (FAO 2006). In India, it is grown annually and about 80% is cultivated under rain fed conditions and the remaining 20% is grown under protected irrigated conditions during summer season (Dwivedi and Verma 2002) with an annual production of about 8.3 million tons. This is contributing about 26.7% to the world groundnut production (Jayaraj 2005). The state of Tamil Nadu has only 14.1% share in area under groundnut cultivation. However, Tamil Nadu produces 23.4% of the total production of the country and the average yield is about 1000kg/ha (Krishnamurthy and Umamaheswari 2004). An important factor contributing to the low groundnut production is diseases attack. The early leaf spot and rust diseases caused by Phaeoisariopsis personata (Berk & M.A. Cuortis) Vanarx., and Puccinia arachidis Sparg., respectively. These diseases are the most destructive diseases, present worldwide causing substantial losses in crop yield in many groundnut-growing regions (Valand et al. 1997; Grichar et al. 1998; Culbreath et al. 2002).

The magnitude of yield losses and percentage of foliation caused by these diseases are very high all over the world, but vary considerably from place to place and between seasons. Subramanyan et al. (1985) and Grichar et al. (1998) reported that applications of fungicide were nevitable to control the groundnut fungal pathogens. Synthetic fungicides give immediate remedy and the farmers use them indiscriminately in field to safeguard their crops it has led to the adverse effects such as pollution and health hazards to the farmers and also the consumers. These effects lead the scientists to look for less persistent and biodegradable alternatives to manage these diseases. In order to reach environmentally safe and economically payable alternative methods, scientist considers the plant products as an alternative to the synthetic fungicides. Few data were available about the fungicidal activity of ferns (Manikam and Irudayaraj 1992; Parihar and Bohra 2002; Singh 2003). However, no serious attempts have been made to use fern extracts against the groundnut diseases management. We evaluated the fungicidal activity of Hemionitis arifolia (Brum.) T., Pteridium aquilinum (L.) Khun. and Christella parasitica (L.) H. Lev. extracts on P. personata and P. arachidis under laboratory condition by mycelial dry weight method.

MATERIALS AND METHODS

Collection of ferns

Hemionitis arifolia (Burm. f.) Moore. (Hemioniitidaceae), Pteridium aquilinum (Linn.) Kuhn (Dennstaedtiaceae) and Christella parasitica (Linn.) H. Lev. (Thelypteridaceae) were collected from Kothiayar hills, Tirunelveli, Tamil Nadu, India. The samples were authenticated by Centre for Biodiversity and Biotechnology, St. Xavier’s College (Autonomous), Palayamkottai, Tamil Nadu, India were the voucher specimens are preserved.

Preparation of ferns extracts

Collected ferns H. arifolia, P. aquilinum and C. parasitica were washed with sterile distilled water, shade dried for
two weeks, whole plant were powdered in a domestic grinder and stored in refrigerator for further use. Known weight (150g) of crushed fern material was extracted in a Soxhlet apparatus using chloroform (40–60°C), hexane and water subsequently. The last trace of solvent was removed under reduced pressure. Then distilled and the crude extract was dried in a vacuum desicator and the final residue was collected, stored in refrigerator at 4°C for further use.

**Isolation of fungal pathogen**

The early leaf spot and rust diseased leaves caused by *P. personata* and *P. arachidis*, respectively were collected from the groundnut field at Sivanthipatti and Kaval Kunru, Tirunelveli District, Tamil Nadu, India and brought to laboratory in a sterile container and used for further isolation of fungal pathogens. The diseased leaves were cut into small pieces (0.5 x 0.5cm), transferred into the Petri plates contained 1.1% HgCl₂ solution and kept for 5 min. Then they were rinsed twice with distilled water and impregnated into the sterile potato dextrose agar (PDA) plates and incubated at room temperature. After 72 hours the grown pathogens were stored in PDA slants for further analysis.

**Antimycotic activity**

*Modified method described by Ghahrafekhi et al. 2003*

For each treatment, 0.1% of respective plant extracts in 10ml of sterile potato dextrose broth was inoculated with a 0.3 x 0.3 cm piece of potato dextrose agar colonized aided by pathogen. Both positive (potato dextrose broth alone) and negative (potato dextrose broth with respective solvents) controls were also maintained for comparison. After 7 days of incubation the pathogen primarily mycelial dry (Primarily mycelium) weight for each treatment was determined separately. The fungus was filtered into 45mm diameter filter paper (Whatman No. 1) in *vacuo* and the medium discarded. Prior to their use the filter paper were held 24 h at 70°C and then weighed. After filtration, the tilter paper with mycelium was held again at 70°C for 24 hours and weighed. Activity index and percentage of inhibition of the fern extracts were calculated by using the following formula:

\[
\text{Activity index} = \frac{\text{Mycelial dry weight of control} - \text{Mycelial dry weight of treatment}}{\text{Mycelial dry weight of treatment}}
\]

\[
\text{Percentage of inhibition} = \left(\frac{\text{Mycelial dry weight of treatment}}{\text{Mycelial dry weight of control}} \times 100\right) - 100
\]

**Statistical analysis**

Mycelial dry weight of the negative control was compared with plant extracts statistically using Duncan’s Multiple Range Test (DMRT) and their significance was expressed at 5% level.

**RESULTS**

*P. arachidis* and *P. personata* were isolated from rust and leaf spot diseased leaves of groundnut. The isolates were identified based on colonial morphology and microscopic appearance. The effect of aqueous, hexane and chloroform extracts of *H. arifolia*, *P. aquilinum* and *C. parasitica* extracts on fungal growth was studied by mycelial dry weight method. The result showed that *P. arachidis* growth was significantly inhibited by chloroform extract of *H. arifolia* followed by hexane extract of *P. aquilinum* and water extract of *H. arifolia* when compared with positive and negative control (p < 0.05) (Table 1). The result further indicated that *P. personata* growth was significantly limited by chloroform extract of *H. arifolia* followed by aqueous extract of *P. aquilinum* and hexane extract of *P. aquilinum* when compare with positive and negative control (p < 0.05) (Table 1). It is noteworthy that all the aqueous fern extracts inhibited the fungus growth significantly when compare to the control. The inhibition of *P. arachidis* and *P. personata* fungal growth were insignificant when we compare *P. aquilinum* with *C. parasitica*. However two fungal pathogens growth inhibition activity was significant between *H. arifolia* with *C. parasitica*.

![Fig. 1. Activity index of hexane (H), chloroform (C) and water (W) extracts of *H. arifolia* (HA), *P. aquilinum* (PA) and *Ch. parasitica* (CP) on *P. arachidis* and *P. personata*](image-url)
DISCUSSION

*P. arachidis* and *P. personata* causing rust and leaf spot diseases, respectively in groundnut ecosystem. The antifungal properties of pteridophytes are widely known (Sen and Nandi 1951; Hansraj 1996). In the present study, the inhibitory effects of *H. arifolia*, *P. aquilinum* and *C. parasitica* against *P. arachidis* and *P. personata* were established. *C. parasitica* was known to possess antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Manikam et al. 2005). Antibacterial activity was also reported in *H. arifolia* (Singh 2003). The obtained results were suitably correlated with results from other workers on rust disease control (Sharma et al. 1980; Usman et al. 1991; Sunkad et al. 2005). Change in hyphal structure and disarrangement in cellular compartments may be responsible for inhibitory effects of fern extract on growth of these two important groundnut fungi. Further studies are needed for confirming this hypothesis. Antifungal activities might be due to the presence of p-coumaric, caffeic, ferulic, p-hydroxybenzoic protocatechuic and vanillic acid in *H. arifolia*; phenolic acids, ponasteroside-A, procyanidin, pteridines, pterins, quercetin, thiaminas-I, tiliroside and xylose (Singh 2003), α and β-ecdysones in *P. aquilinum* (Selvaraj et al. 2005) and kaempferol-3-O-β-D-glucoside (astragalin), kaempferol-3-O-rutinoside in *C. parasitica* (Britto et al. 1996). In addition it was reported that fungicidal activity might be due to the presence of phenolic compounds present in the studied ferns (Singh 1999). More studies are needed to identify the active principles of the tested ferns, their efficacy in the field level and fungal growth inhibiting mechanism(s) of these fern. Now phytochemical study is in progress in our centre.

CONCLUSIONS

Among the examined ferns, chloroform extracts of *H. arifolia* was found to be the most antimycotic activity and can be used for the control of the groundnut rust and leaf spot diseases.

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<table>
<thead>
<tr>
<th>Ferns</th>
<th>Extracts</th>
<th><em>Puccinia arachidis</em></th>
<th><em>Phaeoisariopsis personata</em></th>
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<tbody>
<tr>
<td></td>
<td>hexane</td>
<td>chloroform</td>
<td>water</td>
</tr>
<tr>
<td><em>P. aquilinum</em></td>
<td>124.14*</td>
<td>157.35* (55.77)</td>
<td>228.21* (35.77)</td>
</tr>
<tr>
<td><em>H. arifolia</em></td>
<td>202.23*</td>
<td>(43.66)</td>
<td>208.03* (41.41)</td>
</tr>
<tr>
<td><em>C. parasitica</em></td>
<td>148.11*</td>
<td>(58.31)</td>
<td>201.02* (43.38)</td>
</tr>
<tr>
<td>Negative Control</td>
<td>309.04 (12.96)</td>
<td>315.20 (11.27)</td>
<td>346.14 (2.54)</td>
</tr>
<tr>
<td>Positive Control</td>
<td>355.06 (0.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. aquilinum</em></td>
<td>308.15*</td>
<td>(42.75)</td>
<td>224.08* (58.36)</td>
</tr>
<tr>
<td><em>H. arifolia</em></td>
<td>329.57*</td>
<td>(38.85)</td>
<td>133.19* (75.28)</td>
</tr>
<tr>
<td><em>C. parasitica</em></td>
<td>324.05*</td>
<td>(39.78)</td>
<td>227.21* (57.81)</td>
</tr>
<tr>
<td>Negative Control</td>
<td>465.22 (13.57)</td>
<td>464.09 (13.75)</td>
<td>449.26* (16.54)</td>
</tr>
<tr>
<td>Positive Control</td>
<td>538.13 (0.00)</td>
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Values in parentheses indicate the percentage of growth inhibition * denotes statistical significance at 5% level by DMRT


POLISH SUMMARY

AKTYWNOŚĆ PRZECIWGRZYBOWA TRZECH EKSTRAKTÓW Z PAPROCI W STOSUNKU DO SPRAWCÓW PLAMISTOŚCI I RDZY LIŚCI ORZECHA ZIEMNEGO

Oceniano aktywność przeciwgrzybową trzech ekstraktów z paproci otrzymanych z gatunków Hemionitis arifolia (Burm. f.) Moore., Pteridium aquilium (Linn.) Kuhn. i Christella parasitica (Linn.) H. Lev. w stosunku do sprawców plamistości i rdzy liści orzecha ziemnego, a mianowicie Puccinia arachidis Speg. oraz Phaeoisariopsis personata (Berk & M.A. Curtis) Vanarx., stosując metodę „mycelial dry weight”. Obydwa grzyby były wrażliwe na wszystkie trzy ekstrakty z paproci. Spośród ekstraktów, chloroformowy z H. arifolia posiadał najwyższą aktywność przeciw obydwu grzybom. Z kolei P. personata był wrażliwszy w stosunku do badanych ekstraktów roślinnych niż P. arachidis. Z powyższych badań wynika, że chloroformowy ekstrakt z H. arifolia może być wykorzystany w zwalczaniu plamistości i rdzy liści orzecha ziemnego.