EVALUATION OF BOTANICAL EXTRACTS FOR THE MANAGEMENT OF BRANCH CANKER DISEASE (MACROPHOMA Sp) IN TEA

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Branch canker is a stem disease of tea caused by Macrophoma theicola. The antifungal activity of aqueous extract of Acrous calamus was found to fully inhibit growth of Macrophoma sp. at 10 percent concentration. While the plant extracts of Curcuma longa and Hibiscus rosasinensis showed good results. The inhibitory potential of Psidium guajava and Allamanda cathartica indicated significant effect at 15% concentrations followed by Murraya koenigi, Azadirachta indica and Artemisia nilagirica. Moderate inhibition was noticed by Carica papaya extracts followed by action of Cinnamomum burmannii and Dryopteris linearis extracts. The extracts of Tithonia diversifolia, Conyza ambigua and Adhatoda vasica level at 10% showed lowest control potential.

KEY WORDS: Antifungal-microbial activity, Disease management, Inhibitory potential, plant extracts.

Tea is one of the major non-alcoholic beverages in the world. Tea, being a perennial crop, provides a stable environment for a number of pests and diseases. Pests, pathogens and weeds are important factors limiting the productivity and quality of processed tea. The majority of the tea pathogens are fungal origin and a few diseases are caused by bacteria, virus and algae (Chen and Chen 1990). Collar canker, caused by fungus Phomopsis theae is the most important stem disease of tea in all tea growing countries of the world (Rattan 1993). This disease has been a serious problem in southern India since 1958 (Venkata Ram 1973). Stem diseases like wood rot, collar canker, branch canker and thorny stem blight are predominant in southern India. Among the stem diseases, branch canker is caused by the fungus Macrophoma theicola. It is the most widespread and serious stem disease of the tea plant. Macrophoma theicola has been observed to cause twig die-back of mature tea in Taiwan (Arul pragasam 1992). At present, branch canker disease is controlled by removing the affected stems by adopting rejuvenation pruning and applying Copper oxychloride (COC) paste at cut ends (Premkumar and Baby 2005). Nowadays, plants extracts are being used for seed treatment against bacterial leaf spot of tomato (Ernest et al 2012). Secondary metabolites of plants produce more phytochemical antifungal and microbial activity and can be used for control of plant diseases (Amadioha 2003, Prakash and Rao 1997, Kumar and Parmar 1996, Opara and Wokocha 2008). The present study mainly focused more on selective use of plant extract, because they are generally professed to be ecofriendly. An attempt our research findings were evaluated the plant aqueous extract against branch canker disease under in vitro.

MATERIALS AND METHODS

Isolation of Branch Canker Pathogen: The plant parts were collected from infected portion showing characteristic symptoms of branch canker disease from the field of UPASI –Tea Research Institute, during rainy season of July, 2015, Valparai-642127. The plants parts were examined in microscope to authenticate the presence of respective pathogen Macrophoma sp. This fungus was identified based on the spore morphology and culture characteristics feature by reference book of “Diseases of Tea Bush” (Petch 1923). The infected plant parts were cut to 2-4 mm size of small pieces then surface sterilized with 0.1% mercuric chloride solution for 1minute and washed with sterilized distilled water. The small size of each piece was transferred an individual PDA medium. Each piece of inoculated plates were incubated at room temperature 27±2°C and maintained of four replication in two times.
Finally, the pathogenicity was tested on potted plants according to Koch postulate technique in glass house condition (Agrios 2005). Isolation of genomic DNA amplification, (PCR) was performed to 18S rRNA gene method (Crous and Palm 1999). This fungus was confirmed as *Macrophoma* sp. through molecular technique (NCBI Accession No.KP004441.1) and isolation of genomic DNA amplification, (PCR) was performed to 18S rRNA gene method (Crous and Palm 1999). This fungus was confirmed as *Macrophoma* sp. through molecular technique (NCBI Accession No.KP004441.1).

**Plants Aqueous Extract Preparation:** Total fourteen plants were locally collected from the same areas of tea field at same time, Valparai. The extracts were prepared from leaves which were used as antifungal properties. These plant samples were washed thoroughly under tap water followed by sterilized water. The plant samples were air dried and grinded with the help of pestle and mortar. Stock solution of all the plant extracts were prepared by soaking the grinded plant materials in sterilized water for 2h at room temperature (27-32°C). Each plant extract was filtered through muslin cloth and finally filtered in whatman paper N0.1. The plant extracts were poured in the conical flasks plugged with cotton and heated at 100°C for 10 minutes to avoid microbial contamination (Madavi and Singh 2005). The food poisoned technique was applied for antagonistic level (Nene and Thapilyal 2000). Different concentrations of plant extract were prepared (5 %, 10 % and 15 % W/V) by adding appropriate quantity of sterilized water in to a stock solution. The leaf extracts were poured in to petriplate in appropriate concentration level, the extract was mixed with 20ml/plate containing sterilized PDA medium and as such without addition of plant extracts were served as control plates. The isolated pathogen was grown on PDA medium which was placed at the center of petriplate containing different concentration. For each treatment four replications were maintained at 27±2°C for 7 days. Radial fungal growth (cm) inhibition was calculated in two directions. The percentage of inhibitions were calculated by the formula (Dissanayake 2014)

\[
\text{Inhibition (\%)} = \frac{(C-T)/C} \times 100
\]

Where \( C \) = growth in control plate, \( T \) = growth in treatment plate

**RESULTS AND DISCUSSION**

In this present study, attempts were made to identify the plant aqueous extract to control branch canker disease under *in vitro* condition. Effect of different plant aqueous extracts on radial mycelia growth of pathogen on 7 days of incubation is presented in Table.1. *In vitro* screening of plant extracts against *Macrophoma* sp. showed that six plant extracts out of 14 tested were found to be effective. *Acorus calamus* at 10% concentration showed (100%) inhibition and superior to other plant extracts tested (Table 1).

The maximum inhibitions were found at 15% concentration of plant aqueous extract of *Curcuma longa* (82.05±0.96) and *Hibiscus rosa-sinensis* (81.74±0.75) against branch canker pathogen. Plant extracts of *Psidium guajava* (79.04±2.02) and *Allamanda cathartica* (77.59±1.13) indicated significant control followed by *Murraya koenigii* (73.70±1.22), *Azadirachta indica* (64.44±0.48) and *Artemisia nilagirica* (61.66±1.93). Moderate inhibition was noticed with *Carica papaya* extract (52.77±0.64) followed by *Cinnamomum burmanii* (46.29±1.33) and *Dryopteris linearis* (40.74±0.81). Lowest inhibition was observed in case of *Tithonia diversifolia* (35.37±3.21) followed by *Conyza ambigua* (33.88±4.44) and *Adhatoda vasica* (25.03± 0.58).

In the present study revealed that, the inhibitory potential of *Acorus calamus* extract was 100% effective against *Macrophoma* sp. (Plate 1). Similar results were reported by Jitendiya Devi and Chhetry (2013) the growth inhibition was observed with *Drechslera oryzae* may be due to the presence of antifungal compounds like \( \infty \)-asarone and \( \beta \)-asarone. *Curcuma longa* effectively suppressed the mycelial growth of the *Macrophoma* sp. pathogen (Plate 1) followed...
by *Hibiscus rosasinensis*. These results were in line with Madhiazhagan *et al.* (2002) who found *Curcuma longa* very effective against bacterial blight of rice. Moreover, the antifungal activity of plant extract of *Curcuma longa* showed (100%) growth inhibition against tea pathogens such as *Pestalotipsis theae*, *Colletotrichum camelliae* and *Botryodiplodia theobromae* (Saha *et al.* 2005). Wherever, Raja and Kurucheve (1998) reported that, the *Curcuma aromatica* showed (88.3 %) growth inhibition against *Macrophomina phaseolina*.

Previous finding supported that, the methanol extract of *Hibiscus rosasinensis* was recorded highest growth inhibition with concentration of 100mg/ml against *Aspergillus flavus* (Rathi Sanjesh *et al.* 2012). Generally, the medicinal plant extracts of *Azadirachta indica* and *Catharanthus roseus* have been reported as antifungal activity against *Curvularia lunata* (Bhowmik and Varadhan 1981).

The present investigation revealed that, the inhibitory effect of plant extracts of *Psidium guajava*, *Allamanda cathartica* and *Murraya koenigii* showed good results. *Carica papaya* showed moderate results (Plate 1). The results of the present study were in line with those reported by Neela *et al.* (2014) who reported superior inhibition of ethanol extract of *Psidium*

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**Table 1. In vitro screening of plant aqueous extracts against branch canker (*Macrophoma* sp) pathogen**

<table>
<thead>
<tr>
<th>Different Plant extracts</th>
<th>Family</th>
<th>5%</th>
<th>10%</th>
<th>15%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Curcuma longa</em></td>
<td>Zingiberaceae</td>
<td>59.25±2.09 i</td>
<td>79.44±0.32 bc</td>
<td>82.05±0.96 b</td>
</tr>
<tr>
<td><em>Dryopteris linearis</em></td>
<td>Dryopteridaceae</td>
<td>0.00±0.00 q</td>
<td>10.92±2.25 r</td>
<td>40.74±0.81 1</td>
</tr>
<tr>
<td><em>Cinnamomum burmannii</em></td>
<td>Lauraceae</td>
<td>23.33±1.47 s</td>
<td>39.81±2.73 km</td>
<td>46.29±1.33 k</td>
</tr>
<tr>
<td><em>Carica papaya</em></td>
<td>Caricaceae</td>
<td>24.25±0.37 o</td>
<td>25.92±1.21 o</td>
<td>52.77±0.64 j</td>
</tr>
<tr>
<td><em>Tithonia diversifolia</em></td>
<td>Asteraceae</td>
<td>0.00±0.00 t</td>
<td>9.63±3.22 r</td>
<td>35.37±3.21 s</td>
</tr>
<tr>
<td><em>Allamanda cathartica</em></td>
<td>Apocynaceae</td>
<td>48.33±0.85 k</td>
<td>68.52±1.82 f</td>
<td>77.59±1.13 bde</td>
</tr>
<tr>
<td><em>Conyza ambigua</em></td>
<td>Asteraceae</td>
<td>0.00±0.00 q</td>
<td>3.14±0.49 q</td>
<td>33.88±4.44 n</td>
</tr>
<tr>
<td><em>Psidium guajava</em></td>
<td>Myrtaceae</td>
<td>74.25±1.82 e</td>
<td>74.63±0.98 de</td>
<td>79.04±2.02 bcd</td>
</tr>
<tr>
<td><em>Adhatoda vasica</em></td>
<td>Acanthaceae</td>
<td>0.00±0.00 q</td>
<td>3.51±0.49 q</td>
<td>25.03±0.58 a</td>
</tr>
<tr>
<td><em>Acorus calamus</em></td>
<td>Acoraceae</td>
<td>75.18±0.18 ch</td>
<td>100.00±0.00 a</td>
<td>100.00±0.00 a</td>
</tr>
<tr>
<td><em>Hibiscus rosasinensis</em></td>
<td>Malvaceae</td>
<td>65.26±0.62 g</td>
<td>79.81±1.13 b</td>
<td>81.74±0.75 b</td>
</tr>
<tr>
<td><em>Artemisia nilagirica</em></td>
<td>Asteraceae</td>
<td>23.30±0.62 o</td>
<td>35.86±0.99 m</td>
<td>61.66±1.93 bh</td>
</tr>
<tr>
<td><em>Murraya koenigii</em></td>
<td>Rutaceae</td>
<td>48.51±0.50 heb</td>
<td>60.62±0.44 h</td>
<td>73.70±1.22 e</td>
</tr>
<tr>
<td><em>Azadirachta indica</em></td>
<td>(Neem ernel)</td>
<td>48.70±0.74 k</td>
<td>61.29±0.67 dhi</td>
<td>64.44±0.48 dik</td>
</tr>
</tbody>
</table>

Values are Means ± SE of four replication of three repeated experiments. Means in the same column followed by the same letter are not significantly different at 0.05 level as determined by DMRT.

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![Plate 1. In vitro screening of plant extracts (Curcuma longa and Acorus calamus) against Macrophoma sp. (a) Control, (b) Curcuma longa 5% and (c) Acorus calamus 5%,](attachment:image.png)
guajava and acetone extract of Carica papaya against Fusarium sp. Earlier reports says that, chloroform extract of Allamanda cathartica exhibited promising antifungal activity (Singha et al. 2011) and Murraya koenigii showed strong toxicity and controlled damping-off disease of tomato (67% and 71%) infected with Pythium aphanidermatum and Pythium debaryanum (Pandey and Dubey 1994). Many workers proposed that, (Deo and Shastri 2003, Arima and Danno 2002) guava leaf extract is associated with flavinoids such as mosin glycosides and antimicrobial activity which play a role to resist to fungal attack, while other reported that, Carica papaya leaves exhibited a broad spectrum activity against pathogenic fungi Fusarium sp and Colletotrichum sp (Cha’Vez-Quintal et al. 2011).

From the investigation, it was observed that plant extract of Azadirachta indica (Plate 3) and Artemisia nilagirica effectively inhibited the mycelia growth of the test pathogen. Same results were noticed by Kamalakannan et al. (2001) where they observed greater inhibitory effect of Azadirachta indica against Pyricularia grisea rice blast pathogen. Other scientists have also reported fungitoxic properties of phenolic compound and fungicidal spectrum of Azadirachtin compound in Azadirachta indica (Thapliyal and Nene 1961, Mukerjee and Kundu 1973, Subramanian 1993, Rahejha and Thakore 2002). Our results are in accordance with those put forth by Sati et al. (2013) who reported that plant extract of Artemisia nilagirica has antifungal activity and artimisinin constituents which act against phytopathogenic fungi Rhizoctonia solani, Sclerotium rolfsii and Macrophomina phaseolina. Our findings with Cinnamomum burmannii and Dryopteris linearis is in this agreement with the studies of many scientists (Suresh et al. 1992, Ooi et al. 2006, Chami et al. 2004, Bennis et al. 2004, Pacheco et al. 1993) who reported that Cinnamomum burmannii has a mixture of chemical constituents and possess cinnamaldehyde compound. These compounds have been proved to be active against many pathogenic bacteria, fungi and also possess antibacterial and antifungal activity. Saha et al. (2005) used ethanol and aqueous extract of Dryopteris filix-mas. (L) Schoot and recorded 100% inhibition of spore germination of fungal disease of tea.

The present result indicated that, very low inhibitory level of Tithonia diversifolia, Conyza ambigua and Adathoda vesica. The results are in agreement with those by Dissanayake (2014) and Dissanayake and Jayasinghe (2013) who recorded low activity of Tithonia diversifolia in inhibition of Fusarim oxysporum. The methanol extract of Tithonia diversifolia at all concentrations, showed less inhibition against Colletotrichum musae, Rhizoctonia solani and
Fusarium oxysporum. The results indicated that plant extracts of Tithonia diversifolia very effectively controlled leaf spot diseases such as Fusarium solani, Fusarium lateritium and Cochliobolus lunatus (Ilondu et al. 2014). Tithonia diversifolia has been identified as antifungal activity reported by Ragasa et al. (2008). The antifungal activity of AgNPs synthesis from leaves of Conyza ambigua has also been reported against Aspergillus flavus, Aspergillus niger and Sclerotium rolfsii (Elumalai and Vinothkumar 2013). The phytotoxicity and fungitoxicity of Conyza canadensis is due to the presence of various chemical constituents like, matricaria acid methyl ester, matricaria lactone and lachnophyllum lactone. These lactone compound inhibited growth of the pathogenic fungi Colletotrichum acutatum, Colletotrichum gloeosporioides and Colletotrichum fragariae (Sonia et al 2012).

In the present study very little inhibition was noticed in Adhatoda vesica. Previous information says that, Adhatoda vesica observed 57% reduction in percent of disease inhibition with maximum tannin (Rajeswari 1991). The plant extract of Adhatoda vesica was more efficient in dropping the disease incidence (25.37) (Madhiazhagan et al 2002). Henceforth, the professed of plants extract such as Acorus calamus, Psidium guajava and Allamanda cathartica could be used as biological control against branch canker as well as some other tea pathogens in way of ecological approaches for controlling plant disease.

CONCLUSION

From the above results, the selected plants extract of Acorus calamus, Curcuma longa, Hibiscus rosasinensis, Psidium guajava and Allamanda cathartica have been identified as highest antagonistic potential against branch canker pathogen. Further field study and screening of plants for antifungal and antimicrobial activities may elucidate various options, which may replace the use of chemical fungicides in future.

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