Antifungal activity of medicinal plants leaf extracts on growth of *Macrophomina phaseolina*

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ABSTRACT

*Macrophomina phaseolina* (Tassi goid) is a pathogen that causes Charcoal rot of mungbean resulting in to a great economic loss. In the present study effect of *Ocimum sanctum* L., *Calotropis procera* (Ait.) Ait. f. and *Astragalous tribuloides* Delile extract on the survival of *Macrophomina phaseolina* was studied. The variety of different extracts of *Ocimum sanctum* L., *Calotropis procera* (Ait.) Ait. f. and *Astragalous tribuloides* Delile brings various biochemical changes under suitable condition. The alkaline extract of *Ocimum sanctum* L. shows the maximum growth of *Macrophomina phaseolina*. Acidic extract of *Calotropis procera* (Ait.) Ait. f. and alcholic extract of *Astragalous tribuloides* Delile show maximum growth of *Macrophomina phaseolina*. It showed that *Macrophomina phaseolina* was less reactive in these extract. On the other hand, aqueous acidic extract of *Ocimum sanctum* L., alkaline extract of *Calotropis procera* (Ait) Ait. f. and acidic extract of *Astragalous tribuloides* Delile showed minimum growth of *Macrophomina Phaseolina*. It indicated that *Macrophomina phaseolina* was more reactive in these plant extract.

Key words: *Astragalous tribuloides* Delile, *Calotropis procera* (Ait) Ait. f, *Macrophomina phaseolina*, *Ocimum sanctum* L.

INTRODUCTION

*Macrophomina phaseolina* (Tassi goid) is an anamorphic ascomycete of the family Botryosphaeriaceae and causes the disease namely charcoal rot on a broad range of plant in many areas of the world (Reichert and Hellinger; 1947). It causes seedling blight root rot and charcoal rot of more than 500 crops and non crop species (Smith and Carvil; 1977). The perfect stage being *Sclerotium bataticulum* Taub. (Butl.). *Macrophomina phaseolina* infection on sunflower was first reported from Srilanka in 1927 and then reported from Uruguay, Australia and Yugoslavia in 1966, Argentina in 1967, Hungary in 1970, U.S.A in 1971, India 1973, France 1976, Egypt 1980 and Pakistan. *Macrophomina phaseolina* causing charcoal rot is cosmopolitan in distribution and is potential threat to crop production in arid regions (Hoes; 1985). It is one of the most destructive plant pathogens in the tropics and subtropics causing disease in a wide range of host plants (Reuveni *et al*; 1983). *Macrophomina phaseolina* causes infection on soyabean, sunflower, cotton, rice, wheat, maize, cucurbits, okra, *Pisum sativum*, *Macrotyloma uniflorum* verde, *Lens culinaris*, *Phaseolus vulgaris*, *Cicer arietinum*, *Cajanus cajan* (Farr *et al*.1989; Ali and Dennis., 1992). *Macrophomina phaseolina* survives as microsclerotia in the soil and on the infected plant debris. The microsclerotia serve as the primary source of inoculums and have been found to be persisting within the so. Several control measures have been suggested for *Macrophomina phaseolina* however, the seed and seed borne nature of the pathogen makes it difficult to manage (Fakir *et al*.1976; Kumari *et al*; 2012). Resistance to fungicides is a common problem. Out of the several chemical fungicides used, several pose serious concerns of acquired resistance like Bavistin, Vitavax, Brassicola, Allisan, Tospin M (thiophanatemethyl), Rizolex (tolclofos-methyl) (Pande *et al*., 1989; Chattopadhyay *et al*.; 1980). Now a day; plants are being used against many plant pathogenic fungi. The plants serve as eco-friendly and economic biocontrol agents (Swami and Alane; 2013). Different plant extracts shows the antifungal activity for number of fungi (Aqsa Aslam, Farah Naz *et al*., 2010, R. Harish Kumar *et al*., 2009, Zainab Mushtaq Ahmad *et al*.; 2009). Keeping this in view, the present paper is the study of the plant extracts against the fungi isolated from the seeds of mungbean, *Macrophomina phaseolina* (Tassi goid), which is one of the pathogen that causes Charcoal rot of mungbean resulting in great economic loss (khan *et al* 1997). *Ocimum sanctum* L. (S. Sethi *et al*., 2013), *Calotropis procera* (Ait) Ait. f. (Upadhay, 2013) and *Astragalous tribuloides* Delile has antifungal activity. The bioactive compounds isolated from plant pathogenic fungi and they concluded that methanol extraction is more effective than other extraction solvents. They also concluded the antifungal activity of *O. gratissimum* leaf extracts was more effective than *A. melegueta* (Yazdani *et al*., 2011). The present study is an attempt made to evaluate the antifungal activity of *Ocimum*
sanctum L. Calotropis procera (Ait.) Ait f., Astragalous tribuloides Delile leaf extracts on the survival of Macrophomina phaseolina sclerotia. Hence, in nutshell this paper is about use of ecofriendly, nonchemical technique.

MATERIALS AND METHODS

Macrophomina phaseolina was isolated from master culture MTCC (2165). Subculturing was done from single spore to obtain pure culture in sterilized distilled water potato dextrose Broth (PDB, Hi-Media Laboratories Pvt. Ltd., A-406 Bhaveshwar Plaza, Mumbai-400086, India.) were incubated at 35°C and observed daily for emergence of colonies.

The fresh and healthy leaves were collected from Ocimum sanctum L., Calotropis procera (Ait.) Ait. f and Astragalous tribuloides Delile during their vegetative growth stages from the nearby experimental fields. Extraction of plant leaves was done by following the method of (Harborne 1998; Kosar., 2005; Chakraborty and Mandal; 2007). The preparation of extracts was done using followings:

Preparation of extract from leaves of the donor plants:
Alcoholic Extract: 0.2 gm of the leaves sample was crushed in 1ml of 80% aqueous methanol (Rankem, RFCL Limited, 12th floor Pinnacle Claridges Business Tower Shooting Range Road, SurajKund, Faridabad-121009, Haryana, India). The sample were centrifuged at 5000 rpm for 10 minutes and supernatant was collected which is concentrated with vacuum concentrator.

Aqueous Acidic Extract: one gm of leaves were boiled in 0.2 M HCl (Merck Specialists Pvt. Ltd, Shiv Nagar Estate ‘A’,Dr. Annie Besant Road Worli Mumbai-1400018.) for 25-30 minutes. It was filtered with muslin cloth and separated out with ethyl acetate (Rankem, RFCL Limited,12th floor Pinnacle Claridges Business Tower Shooting Range Road,SurajKund,Faridabad-121009,Haryana,India). Shake well and kept it for five minutes and concentrate with vacuum concentrator, this separation is done three times with ethyl acetate. Finally, it was dissolved in 80% aqueous methanol.

Alkaline Extract: 0.2 gm of the leaves were boiled in 0.2 M NaOH (S. d. fine-Chem limited 315-317T.V.Ind.Estate, 248, worli Road, Mumbai-400030.) for overnight. Then, again centrifuged it at 5000 rpm for 10 minutes. Filtered it with muslin cloth and adjust its pH=2.0 with concentrated 1N HCl and separate it out with ethyl acetate and finally dissolve it in 80% methanol.

RESULTS AND DISCUSSION

Macrophomina phaseolina is one of the pathogen that causes Charcoal rot of Vigna radiata L. Wilczek resulting the great economic loss. Hence the different botanicals have been tested as antifungal agents. Ocimum sanctum L. Calotropis procera (Ait.) Ait. f and Astragalous tribuloides Delile inhibited the mycelia growth of fungi with varying degree of sensitivity.

The antifungal activity of screened medicinal plants extract against Macrophomina phaseolina is shown in Table 1

<table>
<thead>
<tr>
<th>Treatment of extracts</th>
<th>Alcoholic</th>
<th>Aqueous Acidic</th>
<th>Alkaline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocimum sanctum</td>
<td>90.1</td>
<td>85.6</td>
<td>16.9</td>
</tr>
<tr>
<td>Calotropis procera</td>
<td>42.2</td>
<td>8.5</td>
<td>18.2</td>
</tr>
<tr>
<td>Astragalous tribuloides</td>
<td>12.5</td>
<td>34.3</td>
<td>0.162</td>
</tr>
</tbody>
</table>

Growth of Macrophomina phaseolina on Potato Dextrose Broth

<table>
<thead>
<tr>
<th>Treatment of extracts</th>
<th>Alcoholic</th>
<th>Aqueous Acidic</th>
<th>Alkaline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocimum sanctum</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Calotropis procera</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Astragalous tribuloides</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

Turbiditory effects of Ocimum sanctum L. Calotropis procera(Ait.)Ait.f and Astragalous tribuloides Delile extracts on Growth of Macrophomina phaseolina

According to figure 1, leaf extracts of Ocimum sanctum L. in alkaline media shows maximum growth of Macrophomina phaseolina. It means alkaline media is less effective of Macrophomina phaseolina as comparison to aqueous acidic and alcoholic extract in comparison to control in which there is no one extracts is added. It means Macrophomina phaseolina is less effective in control condition.

According to figure 2 Calotropis procera (Ait.)Ait. f leaf extracts of aqueous acidic media shows maximum growth of Macrophomina phaseolina. It meant that aqueous acidic media was less effective of Macrophomina phaseolina.
as comparison to alkaline and alcoholic extract in comparison to control in which there is no extract has been added. It meant that *Macrophomina phaseolina* was less effective in control condition.

According to figure 3, leaf extracts of *Astragalous tribuloides* Delile in alkaline media shows maximum growth of *Macrophomina phaseolina*. It meant that alkaline media was less effective of *Macrophomina phaseolina* as comparison to alcoholic and aqueous acidic extract in comparison to control in which no extract was added. It meant *Macrophomina phaseolina* was less effective in control condition.


