ABSTRACT

Attempts were made to establish contamination free cultures of 
Saraca asoca through bioassay and evaluation of suitable explants and medium for micro-propagation of 
S. asoca. During the experiment, fungal contaminants were observed on explants even after complete sterilization. The pure cultures prepared along with contaminants were identified according to their morphological and cultural characteristics. The identified systemic fungus comprised of Fusarium lateritium and Colletotrichum gloeosporoides contaminating the in vitro cultures of Saraca asoca after 2 to 3 weeks. Bioassays were established using three different concentration of fungicides viz. Sectin, Bayleton, Indofil, Bavistin, Copper oxychloride, Propiconazole. The fungicide Propiconazole was found to completely inhibit the growth of the fungal isolates at 0.05% premixed in PDA medium. The pre-sterilization treatment of explants of S.asoca with Propiconazole revealed that maximum contamination free cultures (86.7%) were obtained on the Nistch Medium supplemented with 0.5 BAP, 0.5mg/l plant growth hormone, and 0.25mg/l defol using control.

Key words: Saraca asoca, in vitro culture, fungicide, bioassay, Propiconazole, Nistch medium.

INTRODUCTION

Ashoka, Saraca asoca (Roxb.) De Wilde (family Caesalpinaceae) is one of the important medicinal species known for its variety of pharmacological activities. The species is listed as one of the 32 priority species by National Medicinal Plant Board (NMPB). The species is vulnerable (Taylor, 2000) and is at the verge of extinction due to unscientific harvesting of bark and other plant parts for medicinal uses. The ever
increasing demand of this species and high marketability calls for development of technologies which could be utilized for mass production. Tissue culture is a proven technology for mass multiplication and conservation of many threatened woody species.

Often it is difficult to initiate and maintain cultures of mature woody species due to infestation of systemic fungi. A wide range of microorganisms (filamentous fungi, yeasts, bacteria, viruses and viroids) and micro-arthropods (mites and thrips) have been identified as contaminants in plant tissue cultures. Contaminants may be introduced with the explant, during manipulations in the laboratory, by micro-arthropod vectors (Tanprasert and Reed, 1997; Leifert and Cassells, 2001) or endophytic bacteria (Reed et al., 1995; Pereira et al., 2003). Fungus may arrive with an explants, or airborne, or enter a culture (Babaoglu et al., 2001). Frequently encountered bacterial and fungal contaminations especially in laboratories of commercial micro propagation pose a considerable problem (Reed et al., 1998). Studies on the effect of antibiotics and fungicides on these kinds of contaminants were carried out by George (1993).

Present experiment was framed to identify fungi contaminating cultures and establish bioassays to eradicate the contamination in order to obtain contamination free cultures of *S. asoca* through various fungicides and evaluation of suitable explants.

**MATERIAL AND METHODS**

Explants of *Saraca asoca* were collected from trees (below 10 years age) growing in Forest Research Institute, Dehradun. Collected shoots were kept in plastic cover. After excision of leaves, shoots were cut into 2.5-3.0 cm long apical and nodal shoot segments. To remove dust particles from the explants surface, explants were dipped in Tween-20 (0.1%, v/v) liquid detergent solution for 15 minutes, shaken periodically and washed 3-4 times with double distilled water to remove carryover effect of detergent. In order to minimize fungal contamination, explants were treated with 0.1% (w/v) solution of Bavistin (Carbendazim 50% WP- a systemic fungicide) for 15 min and washed 3-4 times with double distilled water. The treatment of 0.5% streptomycin was given to explants to remove bacterial contaminants. Later on, explants were surface sterilized with 70% (v/v) ethanol for 50 seconds and washed 3-4 times with sterile double distilled water. The two sterilizing agents’ viz. mercuric chloride and sodium hypochlorite were used for final sterilization of explants in laminar airflow.

The contaminated cultures were used to prepare pure cultures for fungal identification through microscopic examinations. Then, the bioassay experiment was carried out to check the inhibition of identified fungi against different concentrations of tested fungicides. In this study, three different concentrations (0.05 pp, 0.1 pp, 0.15 pp) of six fungicides viz. Sectin, Bayleton, Indofil, Bavistin, Copper oxychloride, and Propiconazol were used. The observations were recorded on the basis of growth (diameter in cm.) of
fungal colony on fungicides supplemented PDA. Further the rate of inhibition was calculated by using following formula. The effective fungicide was incorporated in the pre-treatment protocol for sterilization of explants of S. asoca.

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

T = Diameter of fungal colony on fungicide supplemented medium, C = Diameter of fungal colony on control.

**RESULTS AND DISCUSSION:**

*In vitro* protocol for *S. asoca* was standardized at Nistch Medium supplemented with 0.5 BAP, 0.5 mg/l plant growth hormone, and 0.25 mg/l Defol (Himedia) in whole experiment for maximum sprouting response. Fungal characterization and bioassay were required for the sake of establishment of aseptic cultures of *S. asoca*. Maximum percentage of aseptic cultures was obtained at 0.2% HgCl₂ followed by 0.15% HgCl₂ treatment. But the maximum survival % was observed at 0.15% HgCl₂ treatment for 15 minutes (Table. 1).

<table>
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<tr>
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<th>5 minutes</th>
<th>10 minutes</th>
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<tr>
<td></td>
<td>Aseptic cultures (%)</td>
<td>Survival %</td>
<td>Aseptic cultures (%)</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>0.15%</td>
<td>40</td>
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<td>84</td>
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<td>0.20%</td>
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Significance level at 0.05%

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Most of the *in vitro* cultures were contaminated after few weeks due to systemic infection even after complete sterilization with HgCl₂- Bavistin (Figure.1). The pure cultures prepared from the
contaminated cultures showed white and brown colored fungal colonies (Figure 2).

**Figure 1. Cultures contaminated with Fusarium lateritium after 2-3 weeks.**

Microscopic and morphological identification of pure cultures showed that two fungi, *Fusarium lateritium* and *Colletotrichum gloeosporoides*, were found to contaminate the *in vitro* cultures of *S. asoca* after 2 to 3 weeks. Mostly, *Fusarium lateritium* a white cottony slow growing systemic fungus was found on explants inhibiting the growth of *S. asoca* under *in vitro* condition. Reed et al. (1998) also showed internal bacterial contamination in hazelnut shoot cultures and contaminants evident at culture establishment, or became apparent after several subcultures.

**Figure 2. Pure cultures prepare for two systemic funguses on PDA.**

Bioassay experiment showed that out of six fungicides used only Propiconazole completely inhibited the growth of the fungus at all three concentrations used (Figure 3 and 4). Burun et al., (2010) also identified contaminants *in vitro* culture of *Lilium candidum* according to their morphological and cultural characteristics comprising of *Fusarium, Penicillium, Alternaria, Rhizopus, Cylindrocarpon* and *Aspergillus* species. The most effective treatment against fungal contaminations was achieved by utilizing a combination of Benomyl and Nystatin.
Figure 3. Effect of different concentration of fungicide on *F. lateritium*.

Figure 4. Growth inhibition of *F. lateritium* using Propiconazole supplemented PDA medium using control.
Figure 5. Percentage of contaminated cultures after sterilization

Figure 6. *In vitro* cultures after sterilization of explants with Propicanazole
Comparison with HgCl₂- Bavistin (1%) mediated sterilization of explants with HgCl₂- Propiconazole (1%) for 15 minutes showed only 10% contaminated cultures after 60 days. Whereas in HgCl₂- Bavistin protocol survived culture invariably become contaminated (95%) with systemic fungus *F. lateritium* after 60 days (Figure 5). A successful bud break and leaf formation was observed after sterilization with Propiconazole (Figure 6).

**CONCLUSION**

The outcome of the work would lead to development of an efficient protocol for multiplication of this recalcitrant and medicinally important species which otherwise is difficult to propagate due to unavailability and poor viability of seeds. The effective fungicide (Propiconazole) was incorporated in the protocol for sterilization experiment of explants of *S. asoca*. It was found highly effective to act on systemic fungus *Fusarium lateritium* which was previously contaminating the in vitro cultures.

**REFERENCES**


