Influence of Seasonal and Substrate Variation on Seed Germination of Spilanthes acmella Murr.

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Abstract
The seeds of Spilanthes acmella Murr. are known to possess low rates of germination. In the present study the percentage of germination was evaluated considering two parameters i.e the different substrates viz. ½ MS medium, MS medium and soil; and different seasons viz. summer and spring for their germination and growth. Among the three substrates tested, high percentage of seed germination (63%) was observed in the soil in pots, followed by ½ MS medium (50%) and MS medium (30%). Among the two seasons tested, the percentage of germination was high in soil in spring season (63%) whereas very low seed germination (33%) was observed in summer. The results exhibit the soil to be the best substrate for seed germination and also show that the summer season reduced the germination where as the spring season favoured the germination hence bringing forth their seasonal dependency for germination.

Keywords: Spilanthes acmella, seeds, germination, summer, spring, MS, 1/2 MS, Soil.

INTRODUCTION
Spilanthes acmella Murr. (Family : Asteraceae) is an important medicinal plant, found in tropical and subtropical countries mainly India and South America. Traditionally this plant is used in the treatment of dysentery, rheumatism, as a snake bite remedy, to treat stammering in children and many other diseases [1] S.acmella has multiple pharmacological actions, which include antifungal, antipyretic, local
anaesthetic, bioinsecticide, anticonvulsant, pancreatic lipase inhibitor, antimicrobial, antinociception, diuretic, vasorelaxant anti human immunodeficiency virus, toothache relief and anti-inflammatory effects [2]. Presently there is a huge demand for spilanthol isolated from this plant in pharmaceutical, toothpaste and cosmetic industries[3].

*S. acmella* has been reported as a highly endangered plant species [4]. *S.acmella* is conventionally propagated by seeds. Conventional propagation methods of *S. acmella* are not ample for its mass propagation. The major constrains for its large multiplication are its poor vegetative propagation and low rate of seed germination [5]. AOSA (1970) defines germination as the emergence and development from the seed of those essential structures which are indicative of the ability to produce a normal plant under favourable conditions [6]. The conditions that enable germination are the same conditions that the germinant will be exposed to immediately upon germination, and those conditions can have lasting consequences throughout the life of the plant. Indeed, appropriate germination responses to environmental factors are the first requirement for successful growth and adaptation in any life-history trait; no subsequent life-history trait can even be expressed if the plant does not first survive past the germination stage [7]. Hence this study has been undertaken to find out the most suitable season for high seed germination of *S.acmella* that will be useful for its mass cultivation for commercial use.

Many earlier reports have mentioned that *S.acmella* seeds are known to posses low seed germination and viability [8,9]. But their seasonal dependence for germination and evaluation of germination percentages on different substrates has not been performed. This is the first report where the percentage of seed germination of *S.acmella* on different substrates and different seasons has been evaluated.

**METHODOLOGY**

The seeds of *S.acmella* were surface sterilized by washing thoroughly under running tap water followed by treatment with 1% bavistin for 20 minutes, followed by three rinses with distilled water. Then they were treated with Tween 20 (detergent) for 10 minutes and the material was repeatedly washed with sterile water. The seeds were then treated with 0.1 % mercuric chloride (HgCl₂) for 5 minutes under aseptic conditions followed by 3-4 washes with sterilized double water to remove the traces of mercuric chloride.

The seeds of *S.acmella* were sown in soil in two different seasons i.e spring and summer. They were also implanted upon MS and ½ MS medium in vitro and incubated in culture room with 25 ± 2°C temperature and 16 hours photoperiod. The number of days taken for germination of seeds was noted. The percentage of seed germination was calculated using the formula
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\[
\text{% of seed germination} = \frac{\text{Number of seeds showing germination}}{\text{Number of seeds inoculated}} \times 100
\]

RESULTS

The seeds were germinated on all the three substrates tested viz. MS medium and \(\frac{1}{2}\) MS medium and soil in pots with varying frequency (Table 1, Plate 1). Among the three substrates tested, high percentage of seed germination (63%) was observed in the soil in pots, followed by \(\frac{1}{2}\) MS medium (50%). In MS medium very low percentage of seed germination (30%) was observed. The seeds were germinated in \(\frac{1}{2}\) MS medium within 10 days whereas in MS medium it has taken 15 days to germinate.

Table 1: Germination of *Spilanthes acmella* seeds on different substrates and seasons.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>No. of seeds inoculated</th>
<th>No. of seeds showing germination</th>
<th>Seed germination (%)</th>
<th>No. of Days taken for germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS Media</td>
<td>30</td>
<td>9</td>
<td>30</td>
<td>15±0.57</td>
</tr>
<tr>
<td>(\frac{1}{2}) MS Media</td>
<td>30</td>
<td>15</td>
<td>50</td>
<td>10±0.57</td>
</tr>
<tr>
<td>Soil in spring season</td>
<td>30</td>
<td>19</td>
<td>63</td>
<td>7±1.00</td>
</tr>
<tr>
<td>Soil in summer season</td>
<td>30</td>
<td>10</td>
<td>33</td>
<td>16±1.50</td>
</tr>
</tbody>
</table>

Among the two seasons tested, the percentage of germination was high in soil in spring season (63%) whereas very low seed germination (33%) was observed in summer. The seeds germinated in soil in a week in spring and whereas it has taken 16 days in summer season to germinate.
DISCUSSION

The results of this study suggest that there are strong seasonal and substrate effects, influencing the percentage of seed germination of *S.acmella*. Thus drier periods of summer might tend to delay germination and the field moisture in spring might be exerting some control over the successful germination. Plants from temperate regions, the arctic, high mountains and high deserts often germinate best at cool temperatures [10].

There are several reports on effect of substrates on germination on plants like *Jatropha curcas, Gonystylus bancanus* [11,12]. An ideal planting substrate should be sufficiently porous, and be able to retain moisture, which plays an important role in seed germination and growth of seedlings [13,14]. In this study low seed germination was observed in MS medium. Similar results were observed in *Stevia rebaudiana* where lower seed germination was observed in the MS-medium when compared to other substrates [15].
CONCLUSION

The overall objective of this investigation was to explore the better conditions for seed germination of *S.acmella*. The main results showed that both the season and substrate influence the germination capacity of *S.acmella* seeds. This study signifies that more and faster germination occurs in soil in spring when compared to summer. Hence this study is useful for the seed germination, healthy growth and cultivation of *S.acmella* plants.

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