Factors influencing growth and sporulation of *Gibbago trianthemae*, a prospective biocontrol agent

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*Gibbago trianthemae* Simmons is a fungal pathogen known as a bioherbicide, particularly against the weed, horse purslane (*Trianthema portulacastrum* L.). Here, we isolated the pathogen from diseased leaves of *T. portulacastrum* and the isolate was considered as a prospective biocontrol agent against *Trianthema* weed after preliminary screening. Extensive work on growth and sporulation of *G. trianthemae* was carried out under various factors to develop bioherbicide approach (spore inoculum treatments) to eradicate the target weed. We evaluate the effect of culture media, temperature, pH and light regimes on growth and sporulation of *G. trianthemae*. Biocontrol efficiency of *G. trianthemae* was tested on various laboratory conditions to develop mycoherbicide potential against target weed.

**Keywords:** Biological control, Bioherbicide, Horse purslane, Sporulation, *Trianthema portulacastrum* L.

Biological control offers a green alternative approach to prevent pests including weeds and also replace the multiple uses of chemical pesticides which can remains as pollutants in agro environment. Microbes such as fungi, bacteria and some insects and nematodes might be useful as biocontrol agents in eco-friendly manner and reduce the extensive use of toxic pesticides\(^\text{1,2}\). Entomopathogenic and phytopathogenic fungi applied as effective natural enemies against insect pests and several weeds of agricultural importance\(^\text{3,4}\). Nowadays, effective weed management practice is a challenging task in view of sustainable agriculture includes agro economy, conservation of natural resources and biodiversity. In this context, mycoherbicides (fungal agents) gain attention because of their specificity, low environmental impact and cost effective\(^\text{5}\).

*Gibbago trianthemae* Simmons, a hyphomycete foliar fungal pathogen has been known for its bioherbicide potential, particularly against the noxious aizoaceae weed horse purslane, *Trianthema portulacastrum* L.\(^\text{6,10}\). *In vitro* studies revealed that the weed populations were highly eliminated by leaf spot disease caused by *G. trianthemae* and it shows the mycoherbicide activity of the isolate\(^\text{8}\). Extensive work on growth and sporulation is required to understand the pathogenicity or virulence, adaptability and survival efficiency of the pathogen for field tests and also development of a commercial mycoherbicide.

Several physical and chemical factors can influence the growth of microbes cultured in an artificial medium. The growth, sporulation (development of spores/conidia) and life cycle of the microbes depend on many factors, such as nutrients, temperature, relative humidity, pH, light regimes and storage periods in laboratory\(^\text{11}\). An individual medium shows a great role in the growth and sporulation of fungi and a suitable growth medium depends upon the specificity of a fungus\(^\text{12}\). The production of abundant inoculum in an artificial culture media is essential for the development of a successful mycoherbicide. *G. trianthemae* has narrow host range and grown abundantly on natural host plant as well as on culture media. It suggests the mass culture of pathogen for mycoherbicidal purpose in future. Therefore, in this study, we tried to determine the impact of the physical and chemical factors on growth and sporulation of *G. trianthemae*. Such details may help selecting suitable media and other cultivation conditions for the mass culture of the pathogen and thereby design potential bioherbicide weed management.

**Materials and Methods**

**Test pathogen**

*Gibbago trianthemae* isolated from infected leaves of horse purslane (*Trianthema portulacastrum* L.), at Visakhapatnam District, Andhra Pradesh was used for *in vitro* studies. The pathogenicity and host specificity of the pathogen was confirmed by Koch’s postulates at plant pathology laboratory, Department of Botany, Andhra University. Foremost, the pathogen was isolated aseptically by the inoculation of leaf bites on potato dextrose agar medium, after surface sterilization. After 3-5 days of incubation, the colony sprouting on nutrient medium was purified and maintained in slant culture for further studies. The

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pathogen was confirmed by its diagnostic characters before in vitro experiments\textsuperscript{6-9, 13-15}.

Factors tested in vitro conditions

*G. trianthermae* was examined on eight solid agar media and four broth media. The range of temperature at 15, 20, 25, 30, and 35°C tested. The range of pH values of 4, 5, 6, 7, 8, and 9 and different light conditions, such as 12 h light/dark, 24 h continuous light and 24 h continuous dark were examined in vitro.

Solid agar media

Different solid agar media namely tap water agar (TWA) (Control), potato dextrose agar (PDA), potato sucrose agar (PSA), cazpek’s dox agar (CDA), sabouraud’s dextrose agar (SDA), potato dextrose-yeast extract agar (PDYA), cazpek’s dox-yeast extract agar (CDYA) and trianthesma extract dextrose agar (TeDA) were applied to examine the volume of biomass and sporulation of the fungus. The cultures were incubated at room temperature for 12 days. Each treatment was maintained in triplicate.

Broth media

Four broth media such as potato dextrose broth (PDB), potato sucrose broth (PSB), cazpek’s dox broth (CDB) and sabouraud’s dextrose broth (SDB) were applied to examine the volume of biomass and sporulation of the fungus. The cultures were incubated at room temperature for 12 days. Each treatment was maintained in triplicate.

Temperature

The cultures of *G. trianthermae* were incubated at 15, 20, 25, 30, and 35°C for 12 days. Each treatment was maintained in triplicate.

<table>
<thead>
<tr>
<th>Solid Media</th>
<th>Radial diameter of colony (mm)</th>
<th>Sporulation</th>
<th>Colony density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incubation period (Days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>WA</td>
<td>8.00±0.58</td>
<td>16.67±3.18</td>
<td>31.00±1.00</td>
</tr>
<tr>
<td>PDA</td>
<td>15.33±0.88</td>
<td>32.00±1.53</td>
<td>63.33±2.60</td>
</tr>
<tr>
<td>PSA</td>
<td>11.33±0.88</td>
<td>26.00±0.58</td>
<td>53.67±1.86</td>
</tr>
<tr>
<td>SDA</td>
<td>12.33±0.67</td>
<td>25.33±0.88</td>
<td>54.67±1.45</td>
</tr>
<tr>
<td>CDA</td>
<td>12.33±0.33</td>
<td>25.00±0.58</td>
<td>53.33±1.76</td>
</tr>
<tr>
<td>PDYA</td>
<td>12.33±0.88</td>
<td>24.33±0.88</td>
<td>54.67±0.33</td>
</tr>
<tr>
<td>CDYA</td>
<td>12.00±0.58</td>
<td>24.00±0.58</td>
<td>52.00±1.15</td>
</tr>
<tr>
<td>TeDA</td>
<td>13.00±0.58</td>
<td>24.33±0.33</td>
<td>54.33±0.67</td>
</tr>
<tr>
<td>F-value</td>
<td>4.53*</td>
<td>5.77*</td>
<td>9.23*</td>
</tr>
</tbody>
</table>

Table 1 — Radial growth of *Gibbago trianthermae* on different solid agar media

[Values are mean ± standard error of three replicates. * = significant at the probability level of *P* <0.05. Sporulation: - = Nil; + = Poor; ++ = Fair; +++ = Good; ++++ = Excellent]
followed by PSA (74.33±0.67), CDYA (73.00±1.53) and CDA (71.33±1.33) while the poor radial growth of the isolate was recorded in TWA (43.00±1.73) used as control of the experiment. The values represented as Mean of three replicates and Standard Error (SE). In contrast to the vegetative growth, sporulation of *G. trianthemae* was greatly affected by nutrient media. The highest sporulation examined on solid media such as CDA and PDA at 20-30°C.

**Effect of liquid growth media**

Out of the four different liquid media selected for the evaluation of biomass of *G. trianthemae*, the maximum vegetative growth was recorded on PDB (994.00±2.91 mg/100 mL) followed by CDB (977.33±1.45), PSB (952.33±1.67) and SDB (950.67±1.67) at 20 days of incubation (Table 2). The data supported that the broth of Potato dextrose and Czapek’s dox were found to be the best medium for culturing *G. trianthemae*.

**Effect of temperature**

The range of temperature at 15, 20, 25, 30, and 35°C was evaluated for the optimum growth and sporulation of *G. trianthemae*. Significantly (*P* <0.05) the maximum radial growth of *G. trianthemae* was recorded at 25°C (84.00±1.00) followed by 30°C (83.00±0.58) in PDA cultures (pH 6) for 12 days of incubation (Table 3). The most suitable temperature for abundant growth and sporulation of *G. trianthemae* on PDA was 25°C.

**Effect of pH**

The pH levels viz., 4, 5, 6, 7, 8 and 9 were tested for optimum growth and sporulation of *G. trianthemae* and the significant (*P* <0.05) maximum growth and sporulation were found at pH 6 (88.67±0.33) followed by pH 7 (84.00±0.58) in PDA cultures at 25°C (Table 4).

**Effect of photoperiod**

The effect of different photoperiods was evaluated for the optimum growth and sporulation of *G. trianthemae* (Table 5). The data revealed that light regimes tested with various levels did not have any effect on growth and sporulation of the pathogen at initial stage, however, at 10-12 days of incubation, the radial growth and production of conidia was higher in culture plates incubated at 12 h light/dark photo period. Significantly (*P* <0.05), the maximum radial growth of the isolate was recorded on PDA cultures incubated at 12 h light/dark (85.00±0.58) followed by 24 h continuous light (82.00±0.58).

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### Table 2 — Dry weight of Gibbago trianthemae in different liquid broth media

<table>
<thead>
<tr>
<th>Media</th>
<th>Dry weight of colony (mg/100 mL)</th>
<th>Sporulation</th>
<th>Mycelial density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>PDA</td>
<td>193.33±1.76</td>
<td>575.00±2.89</td>
<td>785.00±1.20</td>
</tr>
<tr>
<td>PSB</td>
<td>183.33±2.40</td>
<td>542.33±1.45</td>
<td>762.33±4.41</td>
</tr>
<tr>
<td>SDB</td>
<td>181.67±1.20</td>
<td>540.33±1.20</td>
<td>758.33±1.67</td>
</tr>
<tr>
<td>CDB</td>
<td>188.67±2.91</td>
<td>561.67±1.20</td>
<td>781.67±1.45</td>
</tr>
<tr>
<td>F-value</td>
<td>5.76*</td>
<td>25.95*</td>
<td>30.22*</td>
</tr>
</tbody>
</table>

[V]Values are mean ± standard error of three replicates. * = significant at the probability level of *P* <0.05. Sporulation: - = Nil; + = Poor; ++ = Fair; +++ = Good; ++++ = Excellent]

### Table 3 — Radial growth of *Gibbago trianthemae* inoculated on PDA medium at different temperatures

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Radial diameter of colony (mm)</th>
<th>Sporulation</th>
<th>Colony density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>15</td>
<td>14.67±0.33</td>
<td>31.00±1.00</td>
<td>59.67±0.33</td>
</tr>
<tr>
<td>20</td>
<td>15.33±0.07</td>
<td>31.67±1.20</td>
<td>60.00±0.58</td>
</tr>
<tr>
<td>25</td>
<td>18.33±0.33</td>
<td>34.33±0.88</td>
<td>62.67±0.33</td>
</tr>
<tr>
<td>30</td>
<td>16.33±0.88</td>
<td>34.00±0.58</td>
<td>61.33±0.33</td>
</tr>
<tr>
<td>35</td>
<td>15.67±0.67</td>
<td>33.33±0.67</td>
<td>61.00±0.58</td>
</tr>
<tr>
<td>F-value</td>
<td>4.76*</td>
<td>4.06*</td>
<td>5.25*</td>
</tr>
</tbody>
</table>

[V]Values are mean ± standard error of three replicates. * = significant at the probability level of *P* <0.05. Sporulation: - = Nil; + = Poor; ++ = Fair; +++ = Good; ++++ = Excellent]
Pure cultures of *G. trianthemae* were evaluated on different culture media to examine growth and conidial production (Figs 1 & 2). *G. trianthemae* exhibited variable response in terms of growth (mm) and sporulation (density) to various nutrient media (both agar and broth), temperature, pH and photoperiod. The interaction among these physical factors was found to be equally effective for the
growth and sporulation. In earlier studies, a wide range of media were used to evaluate the mycelial growth and sporulation of *G. trianthemae*. Our findings revealed that the optimum radial growth and spore production of the isolate were obtained from colonies grown in solid agar media such as PDA, CDA and TeDA. Temperatures of 25 and 30°C were the most favourable for colony growth and sporulation of the pathogen. Highest sporulation was obtained on PDA and CZA at 12 h light/dark photoperiod. Earlier, the abundant growth and sporulation of *G. trianthemae* was found on different culture media, such as PCA, Hay and V-8 agar incubated at 28°C under a 12 h light/dark regime. The *in vitro* study of previous worker exposed the significant correlation between the growth and sporulation of this pathogen on 10 different culture media. The sporulation of *G. trianthemae* was favoured by temperatures in the range of 25-30°C, while temperatures above 30°C may have an inhibitory effect on sporulation of fungi. The presence of light and temperature may favour the spore production of pathogenic fungi.

The success of mycoherbicide depends on the favourable growth and sporulation of biocontrol agent on synthetic media. *G. trianthemae* can be established readily in culture by means of a single conidium isolates (single cell cultures). Biocontrol studies revealed that *G. trianthemae* has most of the criteria desirable for developing into an effective mycoherbicide i.e. it can be cultured on a cheap medium, it has good sporulation capacity and fast growth rate hence can be mass produces in a short time. The present study may be useful to maintain *G. trianthemae* in the laboratory condition and also to harvest an optimum spore production for mycoherbicide approach. The study revealed that the isolate has been most of the desirable characteristics i.e. easily culturing on a cheap medium and ability to produce sporulating structures. The mass of conidia or mycelial fragments of *G. trianthemae* can be used as infection source to the target weed. The data and knowledge of the present study may helpful to select suitable nutrient and physiological conditions for the mass production of *G. trianthemae*.

**Acknowledgment**

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**References**

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