Effect of media pH on the growth of entomopathogenic fungi isolated from different rhizosphere soils

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Abstract: Many entomopathogenic fungal species are of agricultural importance as safe alternatives for chemical insecticides in controlling the various insect pests of crops. pH is one of the abiotic factors influencing the activity of these fungi in both laboratory survival and field efficacy. The effect of pH of the media on the biomass growth of seven locally isolated entomopathogenic fungal isolates was evaluated in the present study. The isolates in general had a wide pH range for their growth but had maximum biomass at low pH of 4 or 5 and least biomass growth at higher basic pH. Variations in growth among isolates were also noticed. The importance and applications of these fungi was discussed in the present day agricultural as well as industrial field.

Key words: Entomopathogenic fungi; Metarhizium anisopliae; pH tolerance; Lecanicillium fusisporum, Aphanoascus terreus.

Introduction

Use of biological inputs in plant protection started gaining importance as an alternative to chemical pest management practices. Their mode of action and specificity make it difficult for the pest to develop resistance easily and are thus more sustainable than their chemical counterparts. Among the biological inputs, entomopathogenic fungi (EPF) occupy an important place due to their vast host range. They are a polyphyletic fungal group of nearly 750 species (Khachatourians and Sohail, 2008). Their ability of them to infect and kill insect pests is exploited in agriculture for the control of different insect pests of crops thus reducing the dependence on the hazardous chemical pesticides. EPFs like Beauveria bassiana, Metarhizium anisopliae, Nomuraea rileyi, Lecanicillium lecanii and Paecilomyces have been widely researched among entomopathogenic fungi for their bioefficacy and based on that many commercial products have been developed (de Faria and Wraight, 2007).

Like any biological system, these insect pathogenic fungi are affected by biotic and abiotic conditions around them. The survival and pathogenicity of these fungi thus may vary depending on their environment. pH is an important abiotic factor apart from temperature, UV radiation etc. affecting Entomopathogenic fungi. pH of the soil where the fungi inhabit affect their survival whereas the pH of the insect cuticle may influence their pathogenicity against the target pest (St leger et al., 1998 & 1999). pH is also important for the mass production of the entomopathogens in a large scale for use in agriculture. This makes it imperative to evaluate the pH tolerance of different Entomopathogenic fungal strains isolated from the environment. Therefore, the present study was done to assess the effect of media pH on the growth of different entomopathogenic fungal isolates.

Materials and Methods

The fungal isolates: Five isolates of entomopathogenic fungi isolated from different crop rhizosphere soils of four districts of Telangana and Andhra Pradesh were tested for their growth in media at different pH levels. The isolates – three Metarhizium anisopliae and two Aphanoascus terreus isolates were originally isolated using a semi selective media identified using morphological and molecular methods (Chandra Teja and Rahman, 2016) and sub cultured onto Sabouraud’s dextrose Yeast extract Agar (SDAY) media. One isolate of M. anisopliae and one isolate of Lecanicillium fusisporum used in the study were obtained from the lab of the All India Coordinated Research Programme (AICRP) on Biological control of Crop pests and Weeds, Rajendranagar, Hyderabad.

Conidial suspensions of 10⁶/ml concentrations were prepared for each of the seven isolates by firstly using a sterile spatula to scrap the conidia from the surface of the culture plate and mixed in sterile 0.02% tween 80 solution. This solution was passed through two layers of muslin cloth to remove the mycelial strains and conidial clumps. The resulting conidial suspension was checked for its concentration using a haemocytometer. The concentration was adjusted to 10⁵/ml using sterile.
0.02% Tween 80 solution. The conidial suspensions of all the isolates were kept under refrigeration until study.

**pH assay:**
For the pH study, Potato Dextrose Yeast extract (PDBY) liquid media with different pH levels 4, 5, 6, 7, 8 and 9 were prepared with the help of 0.1N HCl or NaOH. About 2,100 ml of media was prepared for each pH and 100 ml was poured into 1000 ml conical flasks before sterilization. This ensured maximum surface area for optimum biomass growth. Three replications of 100 ml each were kept for each isolate in a particular pH. The same was repeated for each of the six pH values making a total of 126 flasks. Upon sterilization, 100 µl of conidial suspension of the respective fungal isolate at the concentration of 10⁶ conidia/ml was inoculated into the flasks. The flasks were incubated at room temperature for ten days without agitation. Mycelial biomass was later collected by filtration and dried at 60°C for 2 days. The biomass dry weight of each replicate was recorded using a sensitive analytical balance. The results were subjected to one way ANOVA (Analysis of Variance) using Microsoft Excel and the LSD (Least Significant Difference) (at p < 0.05) between treatments was calculated.

**Results and Discussion**

pH is an important abiotic factor influencing not only the survival of the Entomopathogenic fungi in the field but also their virulence against the target insect pest (Hallsworth and Magan, 1996; St Leger et al., 1998 & 1999). Yet, little information is available on the role of pH in the growth and pathogenicity of EPFs. Hallsworth and Magan (1996) observed that the growth of some entomopathogenic fungi like Beauveria bassiana, Metarhizium anisopliae and Paecilomyces farinosus was optimal at a pH range of 5 to 8. They noted that in contrary to some other fungi, the entomopathogenic fungal species can grow over a broad range of pH. They attributed this to the ability of entomopathogenic fungal species to regulate their cytosolic pH better than the other species which have optimal growth only at a narrow pH range. Our findings were in agreement with that of Hallsworth and Magan (1996) to the extent that all the isolates studied exhibited growth adaptability in a wide pH range.

![Figure 1: Biomass growth of different entomopathogenic fungal isolates in different pH levels](image)

**Table 1:** Dry weight biomass of different entomopathogenic fungal isolates in media of varied pH levels Units in mg

<table>
<thead>
<tr>
<th>Isolate</th>
<th>pH 4</th>
<th>pH 5</th>
<th>pH 6</th>
<th>pH 7</th>
<th>pH 8</th>
<th>pH 9</th>
<th>LSD at 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>MaAICRP</td>
<td>870± 4.58</td>
<td>827± 6.51</td>
<td>867± 6.51</td>
<td>903± 12.77</td>
<td>767± 4.16</td>
<td>12.79</td>
<td></td>
</tr>
<tr>
<td>KoGrKn</td>
<td>869± 3.51</td>
<td>905± 17.79</td>
<td>842± 5</td>
<td>834± 5.29</td>
<td>827± 7.09</td>
<td>15.82</td>
<td></td>
</tr>
<tr>
<td>LaMa1</td>
<td>998± 4.04</td>
<td>914± 9.07</td>
<td>966± 6.56</td>
<td>934± 5.69</td>
<td>927± 2.08</td>
<td>10.98</td>
<td></td>
</tr>
<tr>
<td>PaCo4</td>
<td>1086± 5.31</td>
<td>923± 2.65</td>
<td>943± 7</td>
<td>839± 5.13</td>
<td>779± 10.59</td>
<td>11.24</td>
<td></td>
</tr>
<tr>
<td>V1 AICRP</td>
<td>851± 3.51</td>
<td>724± 4.36</td>
<td>559± 11.24</td>
<td>530± 9.71</td>
<td>532± 5.57</td>
<td>16.99</td>
<td></td>
</tr>
<tr>
<td>ArCo3</td>
<td>60.6± 1.11</td>
<td>899± 7.21</td>
<td>829± 14.01</td>
<td>817± 9.45</td>
<td>0</td>
<td>25.11</td>
<td></td>
</tr>
<tr>
<td>PRg4</td>
<td>0± 0.02</td>
<td>813± 9.02</td>
<td>805± 23.03</td>
<td>970± 12.66</td>
<td>944± 4.58</td>
<td>26.18</td>
<td></td>
</tr>
</tbody>
</table>

The figures denoted by same alphabet in a column are not significantly different by Duncan’s Multiple Range Test (DMRT) (α = 0.05)

However, and in contrary to Kotwal et al. (2012) who noted that the optimum pH for Entomopathogenic fungi is 5, the majority of the isolates in the present study had 4 as their optimal pH followed by pH 5 (Figure 1). This emphasizes the differences among the individual strains in their pH tolerance. Among the *M. anisopliae* isolates in the present study, the highest growth of biomass was observed in pH 4 for the MaAICRP, KoGrKn and PaCo4 isolates and at 5 for the isolate LaMa1. For the *L. fusisporum* isolate L1AICRP, the optimal pH was similarly 4 and the *A. terreus* isolates had the highest growth in the media with pH 5 with conspicuous decline of biomass at pH 4. While *M. anisopliae* isolates grew the least at pH 9, the other three isolates had least growth at the neutral pH 7. It was noted that the *A. terreus* isolates had a narrow pH growth range compared to the other isolates of the study. The isolate ArCo3 had optimal pH range from 5 to 8 above which it had either negligible or no growth at all. Isolate PRg4 showed no growth at pH 4 (Table 1). The high growth of the fungal species at low pH conditions is advantageous in process of their commercial production as the initial pH of the production media can be reduced avoiding contamination of the media (Hallsworth and Magan, 1996).

St Leger et al. (1999) evaluated the growth characteristics of *M. anisopliae* wild type and mutants in different pH and found that the wild type and the mutants over-producing acid had good growth at wide pH range and
could grow in 6 as well as 8 pH. The mutants that have lost their ability to produce acid had decreased growth at pH 8. They postulated that the acid production by the fungal species increased their ability to grow at higher pH. They also noticed that the acid over-producing mutants of *M. anisopliae* were unable to produce ammonia generally produced in low amino acid conditions for better use of protein nutrients. Production of subtilisin proteases activated at basic pH is also greatly reduced in the mutants.

Secreted enzymes which are important virulence factors in the pathogenicity of the entomopathogenic fungi are also influenced by pH. In a previous study, St Leger et al. (1998) studied the effect of pH on the expression of different cuticle degrading enzymes secreted by *M. anisopliae*. They found that the genes which code for various cuticle degrading enzymes are expressed at the pH optimal for the particular enzyme. They observed that the alkaline pH of the insect cuticle generally triggers the secretion of enzymes like proteases etc. which by degrading the hard surface of the cuticle allows the penetration of the pathogenic fungi. Cuticular pH can also influence the sequence of the enzymes secreted by the fungi (St Leger et al., 1998) playing a vital role in the virulence of an entomopathogenic fungal strain.

In view of the importance of pH as an abiotic environmental factor influencing the growth and virulence of entomopathogenic fungal strains, it is necessary to evaluate the response of newly isolated strains or species to pH variations. Study of a strain's pH tolerance will also help in its efficient commercial production avoiding contaminations. As an agricultural application, strains which can grow and infect at a wide pH range can be highly beneficial as they will be amenable for application over wide soil types with different pH ranges. More research is thus needed to better understand the impact of pH factor on the entomopathogenic fungi.

**Acknowledgements**

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**References**


6. Leger, Raymond J. St, Lokesh Joshi, and Donald Roberts. Ambient pH is a major determinant in the expression of cuticle-degrading enzymes and hydrophobin by *Metarhizium anisopliae*. *Applied and Environmental Microbiology* 64.2 (1998): 709-713.


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