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

Chandra Teja K. N. P.[1]* and S. J. Rahman[2]
[1]Department of Microbiology, Agri Biotech Foundation, PJTSAU campus, Hyderabad and Centre for Environment, Institute of Science and Technology, JNTUH, Hyderabad.
[2]AICRP on Biological control of Crop Pests and Weeds, PJTSAU campus, Hyderabad, India.

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Abstract: Many entomopathgenic fungal species are of agricultural importance as safe alternatives for chemical insecticides in controlling the various insect pests of crops. p^H is one of the abiotic factors influencing the activity of these fungi in both laboratory survival and field efficacy. The effect of p^H 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 p^H range for their growth but had maximum biomass at low p^H of 4 or 5 and least biomass growth at higher basic p^H. 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*; p^H 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*, *Nomorrhea rileyii*, *Lecanicillium lecanii* and *Paecillomyces* 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. p^H is an important abiotic factor apart from temperature, UV radiation etc. affecting Entomopathogenic fungi. p^H 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). p^H 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 p^H tolerance of different Entomopathogenic fungal strains isolated from the environment. Therefore, the present study was done to assess the effect of media p^H 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 10^6/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 10^6/ml using sterile

***Corresponding Author**:
Mr. K. N. P. Chandra Teja,
Department of Microbiology,
Agri Biotech Foundation,
PJTSAU campus, Hyderabad, India.
E-mail: kteja26@gmail.com

0.02% Tween 80 solution. The conidial suspensions of all the isolates were kept under refrigeration until study.

p^H assay:

For the p^H study, Potato Dextrose Yeast extract (PDBY) liquid media with different p^H 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 p^H. The same was repeated for each of the six p^H values making a total of 126 flasks. Upon sterilization, 100 µl of conidial suspension of the respective fungal isolate at the concentration of 10^6 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

p^H 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 p^H 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 *Paecillomyces farinosus* was optimal at a p^H 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 p^H. They attributed this to the ability of entomopathogenic fungal species to regulate their cytosolic p^H better than the other species which have optimal growth only at a narrow p^H 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 p^H range.

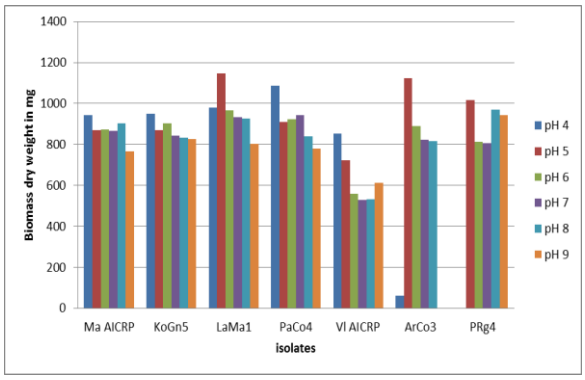

Figure 1: Biomass growth of different entomopathogenic fungal isolates in different pH levels

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

p^H / Isolate	4	5	6	7	8	9	LSD at 0.05
Ma AICRP	$941.3^{d} \pm 4.73$	$870^{e} \pm 4.58$	$872.6^{d} \pm 6.66$	$867^{b} \pm 6.51$	$903^{c} \pm 12.77$	$767^{d} \pm 4.16$	12.79
KoGn5	$949^{c} \pm 6.51$	$869^{e} \pm 3.51$	$903^{c} \pm 17.79$	$842^{c} \pm 5$	$834^{d} \pm 5.29$	$827^{b} \pm 7.09$	15.82
LaMa1	$980^{b} \pm 4.04$	$1147^{a} \pm 9.07$	$966^{a} \pm 6.56$	$934^{a} \pm 5.69$	$927^{b} \pm 2.08$	$803^{c} \pm 7.09$	10.98
PaCo4	$1086^{a} \pm 3.51$	$908^{d} \pm 5.69$	$923^{b} \pm 2.65$	$943^{a} \pm 7$	$839^{d} \pm 5.13$	$779^{d} \pm 10.59$	11.24
Vl AICRP	$851^{e} \pm 3.51$	$724^{f} \pm 4.36$	$559^{f} \pm 11.24$	$530^{e} \pm 9.71$	$532^{f} \pm 5.57$	$611^{e} \pm 16.25$	16.99
ArCo3	$60.6^{f} \pm 1.15$	$1123^{ab} \pm 29.26$	$890^{c} \pm 7.21$	$823^{cd} \pm 14.01$	$817^{e} \pm 9.45$	0^{f}	25.11
PRg4	0^{g}	$1015^{bc} \pm 22.50$	$813^{e} \pm 9.02$	$805^{d} \pm 23.03$	$970^{a} \pm 12.66$	$944^{a} \pm 4.58$	26.18
LSD at 0.05	6.85	25.89	17.14	20.61	14.83	15.03	

The figures denoted by same alphabet in a column are not significantly different by Duncan's Multiple Range Test (DMRT) ($\alpha = 0.05$)

However, and in contrary to Kotwal *et al.* (2012) who noted that the optimum p^H for Entomopathogenic fungi is 5, the majority of the isolates in the present study had 4 as their optimal p^H followed by p^H 5 (Figure 1). This emphasizes the differences among the individual strains in their p^H tolerance. Among the *M. anisopliae* isolates in the present study, the highest growth of biomass was observed in p^H 4 for the MaAICRP, KoGn5 and PaCo4 isolates and at 5 for the isolate LaMa1. For the *L. fusisporum* isolate LlAICRP, the optimal p^H was similarly 4 and the *A. terreus* isolates had the highest growth in the media with p^H 5 with conspicuous decline of biomass at p^H 4. While *M. anisopliae* isolates grew the least at p^H 9, the other three isolates had least growth at the neutral p^H 7. It was noted that the *A. terreus* isolates had a narrow p^H growth range compared to the other isolates of the study. The isolate ArCo3 had optimal p^H range from 5 to 8 above which it had either negligible or no growth at all. Isolate PRg4 showed no growth at p^H 4 (Table 1). The high growth of the fungal species at low p^H conditions is advantageous in process of their commercial production as the initial p^H 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 p^H and found that the wild type and the mutants over-producing acid had good growth at wide p^H range and

could grow in 6 as well as 8 p^H. The mutants that have lost their ability to produce acid had decreased growth at p^H 8. They postulated that the acid production by the fungal species increased their ability to grow at higher p^H. 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 p^H 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 p^H. In a previous study, St Leger *et al.* (1998) studied the effect of p^H 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 p^H optimal for the particular enzyme. They observed that the alkaline p^H 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 p^H 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 p^H as an abiotic environmental factor influencing the growth and virulence of entomopathgenic fungal strains, it is necessary to evaluate the response of newly isolated strains or species to p^H variations. Study of a strain's p^H tolerance will also help in its efficient commercial production avoiding contaminations. As an agricultural application, strains which can grow and infect at a wide p^H range can be highly beneficial as they will be amenable for application over wide soil types with different p^H ranges. More research is thus needed to better understand the impact of p^H factor on the entomopathogenic fungi.

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