The natural populations of insects are controlled by fungal pathogens like Beauveria bassiana and Verticillium lecanii. Thermotolerant mutants of B. bassiana and V. lecanii were obtained after exposing to moist heat and UV treatment. The mutants of B. bassiana (BBMh1) and V. lecanii (VLMh1) were yielded maximum biomass and blastospore at 27°C and 35°C compared to the wild type after moist heat treatment. The mutants obtained at 40°C yielded very less biomass and blastospore. Fungal spore suspension was taken for respective treatment at varying time intervals of 5, 10, 15, 20, and 30 min. The eight mutants were obtained on the basis of thermotolerant characteristics.

Keywords: Beauveria bassiana, Verticillium lecanii, Thermotolerant, Biomass, Blastospores

INTRODUCTION

The natural population of insects is commonly controlled by fungal pathogens. There has been only a limited success in using these organisms as bio-control agents. The fungal mycelium usually invades all body tissues and eventually causes suffocation by blocking the tracheal system of insects (Clarson, 2007). When these fungi are applied in the agricultural field they exhibit poor survival due to higher temperature in the field which results in lower control of the target pests. Simple and expensive methods for obtaining large quantities of entomopathogenic fungus, free from substrate contamination by culturing entomopathogen yields $10^{10}$/g biomass for Tolypocladium cylindrosporum, Verticillium lecanii, and Beauveria bassiana and $10^8$ for Culicinomyces clavisporus (Batist et al., 1988). The conidial germination in three strains each of M. anisopliae and B. bassiana at 25°C and 30°C. The germination rate was reported to be species dependent with M. anisopliae strain having faster germination than that of B. bassiana (Hywel-Jones and Gillespie, 1990). The influence of UV light on the pathogenicity of B. bassiana was observed significantly influencing the mortality effect. The variant SK99C showed the highest level of infectivity. Optimized conidial production of M.
anisopliae var. acridium in mycosed cadavers of Schistocerca gregaria during 10 days incubation at 96°C relative humidity and temperature between 20°C-30°C reported producing 109 conidia per cadaver. Condial yield was reported to be maximal at 25°C, cadaver remain in contact with the dump substrate. Little sporulation occurs at temperature of 15°C and 40°C regardless of RH and there was no sporulation occurred at 10°C and 45°C (Arthur and Thomas, 2001). The report of high variability in conidial thermotolerance was found among the Beauveria spp. isolates after exposure to 45°C for 2 h has evidenced by low (0-20%), medium (20-60%), or high germination (60-80%). The thermal death point (0% germination) for three rather thermotolerant B. bassiana isolates (CG 138, GHA and ARSEF 252) was 46°C for 6 h (Kumar et al., 2007). The investigated effect of temperature on P. lilacinus growth at different temperature (15°C-35°C) were analyzed (Fang et al., 2007). From the above it has been proved that no work found on the development of theromotolerant from B. bassiana and V. lecanii. Isolate of Beauveria and Verticillium that have higher thermotolerance if developed it will be more effective in the field even during summer when temperatures are as high as 35°C-40°C. A thermotolerant isolate can offer effective control in all season.

In the present study the conidia of both B. bassiana and V. lecanii were subjected to physical mutagenesis; with UV irradiation and moist heat treatment. The survivor were tested for their ability to grow either at higher temperature. Development of theromotolerant from entomopathogenic fungi were represented best control on insect pathogen through fungal attack.

MATERIALS AND METHODS

Culture Media

B. bassiana and V. lecanii were obtained from Gokulam Biotech. The cultures were transferred to Czapek-Dox Agar plates incubated at 27°C for 10-12 days. After incubation spore suspensions were prepared in sterile distill water and tenfold serial dilutions were prepared up to 10⁻³.

Test for Change in Characteristics in the New Isolates

Three separate colonies of each fungal strain was taken and inoculated in 10 mL of Czapek-Dox liquid media in test tubes for 2 days at 27°C. This was used as inoculum for 100 mL Czapek-Dox media in duplicate and incubated at room temperature for 4-5 days.

Treatment of B. bassiana and V. lecanii with Moist Heat

Fungal spore dilution 10⁻³/mL were taken for moist heat treatment approximately 2 mL of sample exposed (100°C) for varying time interval of 5 min, 10 min, 20 min and 30 min. Treated samples were incubated at 27°C for 15-17 days on the same media and growth parameters were studied.

Treatment of B. bassiana and V. lecanii with Ultra Violet Light

The 2 mL of spore dilution 10⁻³/mL were taken in sterile petri plates and kept for exposure under UV light (254 nm) for same time intervals mentioned for moist heat. Treated samples were plated on agar plates and were incubated at room temperature for 8 days.

Selection of Mutant Colonies with High Temperature Tolerance

The colonies were screened individually. The
mutant colonies of *B. bassiana* and *V. lecanii* were taken for determination of conidial count and biomass respectively.

**RESULTS AND DISCUSSION**

One mutant of each of *B. bassiana* (BBMh) and *V. lecanii* (VLMh) was selected after treatment with moist heat. When they grew in liquid medium the former species produced higher biomass as well as blastospores where as latter species produced lesser quantity of biomass compared with parent strains. In case of UV treatment, three mutants of *B. bassiana* (BBM1, BBM2, BBM3) and *V. lecanii* (VLM1, VLM2, VLM3) were selected. The *B. bassiana* mutants BBM1 and BBM2 produced higher quantity of biomass where as *B. bassiana* (BBM3) produced lesser quantity of biomass at 27°C as compared to the parent strain. Surprisingly all three mutants of *V. lecanii* VLM1, VLM2, and VLM3 were produced lesser biomass compared to the parent strain.

All the mutants of *B. bassiana* (BBMh) and *V. lecanii* (VLMh) produced higher number of blastospores/mL at 35°C as compared to the parent strain. In case of VLMh mutant produced 7 times biomass as compared to the parent strain. In UV treated mutants of *B. bassiana* (BBM1) 1.2 times higher biomass as compared to the parent strain. BBM2 and BBM3 yielded approximately same and 1.5 times less biomass. In the case of VLM2, mutant of *V. lecanii* yielded 7.02 times higher biomass compared to parent strain. The UV treated mutants of *B. bassiana* BBM1, BBM2, and BBM3 and *V. lecanii* VLM1, VLM2, and VLM3 produced lower biomass compared to the parent strain. The UV light significantly influenced mortality effect as well as infectivity of entomopathogenic fungus *B. bassiana*. High variability in conidial thermostolerance among *B. bassiana* spp. Isolates after exposure to 45°C for 2 h as evidenced by low (0-20%), medium (20-60%), high germination (60-80%) (Everton et al., 2007).

Comparative account of these observations in the present study the mutants of *B. bassiana* and *V. lecanii* produced higher level of biomass and blastospore at 27°C and 35°C.

Insect population controlled by using entomopathogenic fungi.

**CONCLUSION**

In the present study we have focused on isolation of thermotolerant mutants of entomopathogenic fungi, i.e. *Beauveria bassiana* and *Verticillium lecanii*. Mutants isolated by physical mutagenesis; moist heat stress and UV treatment exhibited high degree of control on the insert at versatile temperature range when compared the controlling ability of entomopathogenic fungus between mutant and corresponding parent strain.

**REFERENCES**


insecticides in microorganism as biocontrol agents”, Agro clinic Research center, Kottayam-686 012 India; pp. 36-37.


