University of Zabol

Graduate school

Faculty of Agriculture

Department of Plant Protection

**The Thesis Submitted for the Degree of PhD (in the field of Plant Pathology)**

# Investigation of xylanase and cellulase enzymes in *Trichoderma* spp. and pectinase secretion in *Aspergillus niger* using green algae extract and gamma irradiation

**Supervisors**:

Dr. N. Radman

Dr. S. K. Sabbagh

**Advisors**:

Dr. M. Sarafraz Ardakani

Dr. M. Salari

**By:**

A. M. Heydari Nezhad

January 2022

**Abstract**

Green algae and green-blue algae having pectin, cellulose, xylan hemicellulose, arabinoxylan, etc., in their structures could be a suitable stimulus to produce cellulase, xylanase, and pectinase enzymes in fungi. One of the applications of nuclear technology is the production of fungal mutant isolates with the ability to produce more secondary metabolites. Gamma rays with high energy, penetrating power and the ability to create a variety of mutations by producing ions and excitation in biological molecules. In this study, isolates of *Trichoderma longibrachiatum*, *Trichoderma reesei*, *Trichoderma virens*, *Trichoderma atroviride* and *Aspergillus niger* were selected to measure the production of xylanase, cellulase and pectinase. Algae extract of *Spirogyra* sp. and *Scenedesmus obliquus* at concentrations of 0.5 and 1mg/ml and Carboxymethyl cellulose were used as enzyme inducers. Change in level of enzyme production was followed by *Xyln*, *PgaA* and *Egl1*-related gene expression analysis using spectrophotometry at 235, 540 and 595 nm-Wavelength and quantitative reverse transcription real-time polymerase chain reaction (qRT-PCR) method. New cultures were selected from the isolates and irradiated with a 250g of gamma ray by Gamasel device with a $Co_{60}$ source. According to the existing instructions this dose did not have a significant effect on germination of spores. Mycelium of fungal samples was crushed using liquid nitrogen. RNA extraction was performed using Topaz extraction kit. The primers of *Egl1*, *Xyln2*, and *PgaA* genes were selected and designed from the data available in the NCBI, Expasy and Uniport bioinformatics database using CLC Genomic Workbench 3 and Primer premier 5 software. The maximum amount of enzyme secretion was observed at 1 mg/ml concentration during 8 Day treatment in *T. reesei*. At this concentration and time period, *T. atroviride* showed the lowest amount of enzyme production. Also, gene expression analysis confirmed bio-chemical results so that, the maximum level of gene expression was observed in *T. reesei* species at the similar time period and algae concentration for enzyme production. The highest level of enzyme secretion was observed on the eighth days after culture and in sample that treated with 1000 *µg/ml* algae extract equivalent to 4.28 U/ml. However, the highest expression level of target gene was 10.142 times more than the control sample in four days after culture. Gene expression level of *Egl1* in four species of Trichoderma mutants with control samples showed that transcript level in *T. virens* and *T. reesei* were significantly increased on fourth and eighth day after culture. In *T. reesei*, the expression of the target gene on the fourth and eighth day after culture were 4.74 and 5.26 times higher than the control sample, respectively.

**Keywords**: Fungal Enzymes, Gene expression, Green Algae, RNA extraction, *Trichoderma*.